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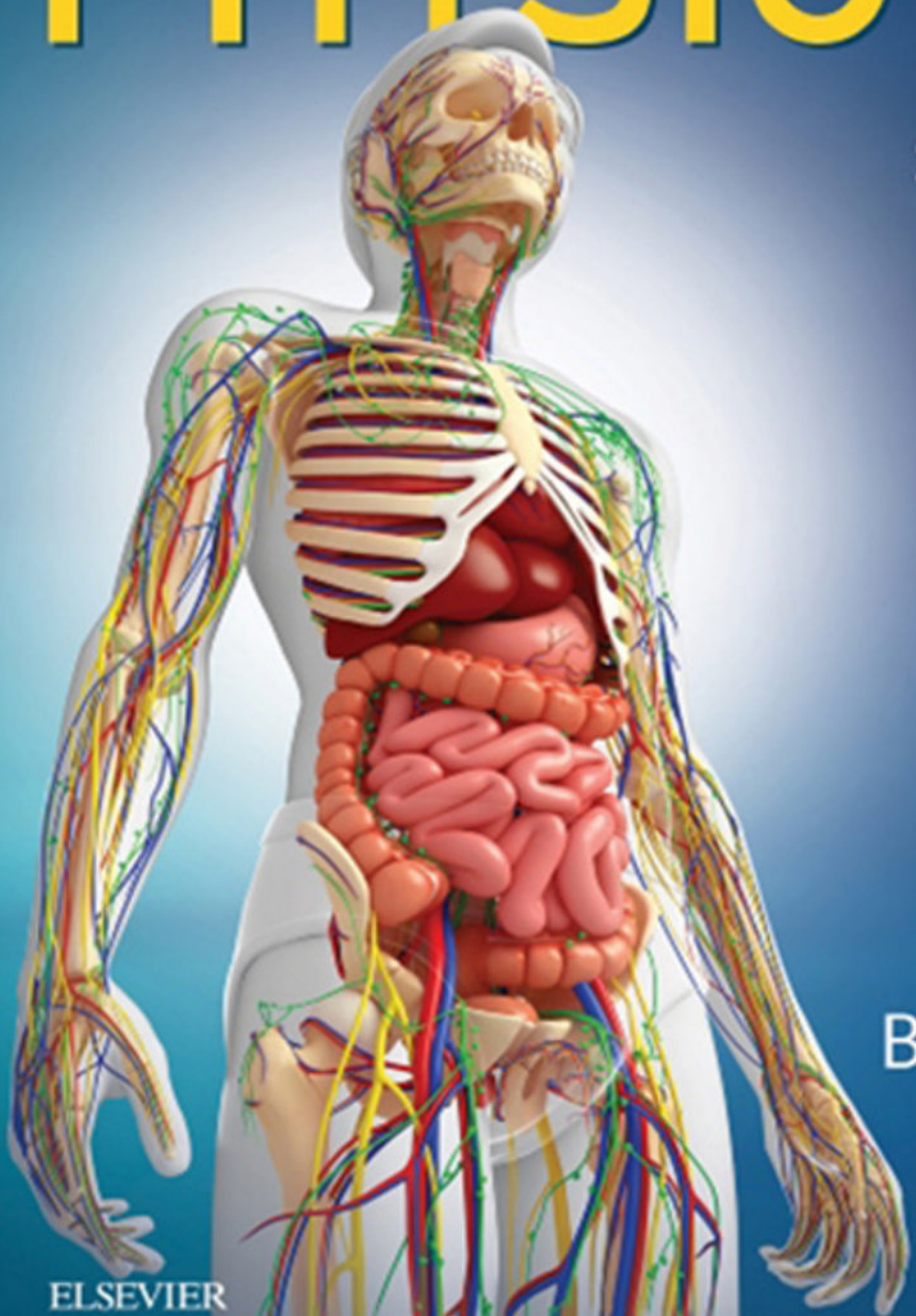

Bruce M. **Koeppen**
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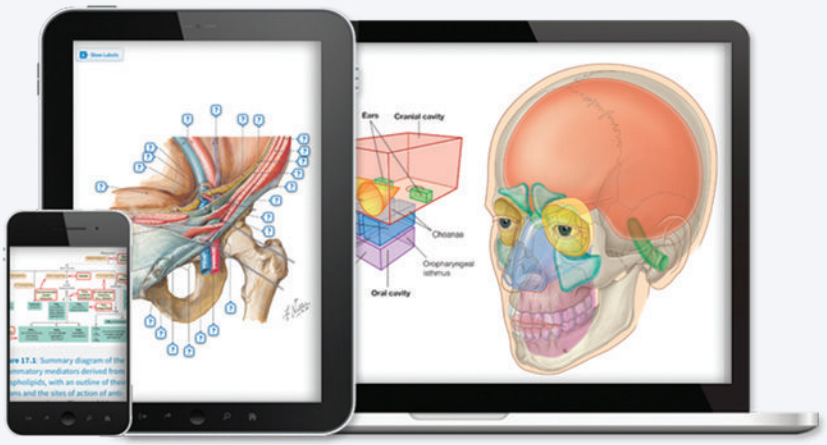

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This seventh edition of Physiology *is dedicated to the many students who have used this textbook to learn and understand the function of the human body.*

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Preface

We are pleased that the following section authors have continued as members of the seventh edition team: Drs. Kalman Rubinson and Eric Lang (nervous system), Dr. James Watras (muscle), Dr. Achilles Pappano (cardiovascular system), Drs. Michelle Cloutier and Roger Thrall (respiratory system), Drs. Kim Barrett and Helen Raybould (gastrointestinal system), and Dr. Bruce White (endocrine and reproductive systems). We also welcome the following authors: Dr. Withrow Gil Wier (cardiovascular system), and Dr. John Harrison (endocrine and reproduction systems).

As in the previous editions of this textbook, we have attempted to emphasize broad concepts and to minimize the compilation of isolated facts. Each chapter has been written to make the text as lucid, accurate, and current as possible. We have included both clinical and molecular information in each section, as feedback on these features has indicated that this information serves to provide clinical context and new insights into physiologic phenomena at the cellular and molecular levels. New to this edition is a list of sources that the reader can consult for further information on the topics covered in each chapter. We hope that you find this a valuable addition to the book.

The human body consists of billions of cells that are organized into tissues (e.g., muscle, epithelia, and nervous tissue) and organ systems (e.g., nervous, cardiovascular, respiratory, renal, gastrointestinal, endocrine, and reproductive). For these tissues and organ systems to function properly and thus allow humans to live and carry out daily activities, several general conditions must be met. First and foremost, the cells within the body must survive. Survival requires adequate cellular energy supplies, maintenance of an appropriate intracellular milieu, and defense against a hostile external environment. Once cell survival is ensured, the cell can then perform its designated or specialized function (e.g., contraction by skeletal muscle cells). Ultimately, the function of cells, tissues, and organs must be coordinated and regulated. All of these functions are the essence of the discipline of physiology and are presented throughout this book. What follows is a brief introduction to these general concepts.

Cells need a constant supply of energy. This energy is derived from the hydrolysis of **adenosine triphosphate** (**ATP**). If not replenished, the cellular ATP supply would be depleted in most cells in less than 1 minute. Thus, ATP must be continuously synthesized. This in turn requires a steady supply of cellular fuels. However, the cellular fuels (e.g., glucose, fatty acids, and ketoacids) are present in the blood at levels that can support cellular metabolism only for a few minutes. The blood levels of these cellular fuels are maintained through the ingestion of precursors (i.e., carbohydrates, proteins, and fats). In addition, these fuels can be stored and then mobilized when ingestion of the precursors is not possible. The storage forms of these fuels are triglycerides (stored in adipose tissue), glycogen (stored in the liver and skeletal muscle), and protein. The maintenance of adequate levels of cellular fuels in the blood is a complex process involving the following tissues, organs, and organ systems:

- *Liver*: Converts precursors into fuel storage forms (e.g., glucose → glycogen) when food is ingested, and converts storage forms to cellular fuels during fasting (e.g., glycogen → glucose and amino acids → glucose).
- *Skeletal muscle*: Like the liver, stores fuel (glycogen and protein) and converts glycogen and protein to fuels (e.g., glucose) or fuel intermediates (e.g., protein → amino acids) during fasting.
- *Gastrointestinal tract*: Digests and absorbs fuel precursors.
- *Adipose tissue*: Stores fuel during feeding (e.g., fatty acids → triglycerides) and releases the fuels during fasting.
- *Cardiovascular system*: Delivers the fuels to the cells and to and from their storage sites.
- *Endocrine system*: Maintains the blood levels of the cellular fuels by controlling and regulating their storage and their release from storage (e.g., insulin and glucagons).
- *Nervous system*: Monitors oxygen levels and nutrient content in the blood and, in response, modulates the cardiovascular, pulmonary, and endocrine systems and induces feeding and drinking behaviors.

In addition to energy metabolism, the cells of the body must maintain a relatively constant intracellular environment to survive. This includes the uptake of fuels needed to produce ATP, the export from the cell of cellular wastes, the maintenance of an appropriate intracellular ionic environment, the establishment of a resting membrane potential, and the maintenance of a constant cellular volume. All of these functions are carried out by specific membrane transport proteins.

The composition of the extracellular fluid (ECF) that bathes the cells must also be maintained relatively constant. In addition, the volume and temperature of the ECF must be regulated. Epithelial cells in the lungs, gastrointestinal tract, and kidneys are responsible for maintaining the volume and composition of the ECF, while the skin plays a major role in temperature regulation. On a daily basis, H_2O and food are ingested, and essential components are absorbed across the epithelial cells of the gastrointestinal tract. This daily intake of solutes and water must be matched by excretion from the body, thus maintaining **steady-state balance**. The kidneys are critically involved in the maintenance of steady-state balance for water and many components of the ECF (e.g., Na^+, K^+, HCO_3^-, pH, Ca^{++}, organic solutes). The lungs ensure an adequate supply of O_2 to "burn" the cellular fuels for the production of ATP and excrete the major waste product of this process (i.e., CO_2). Because CO_2 can affect the pH of the ECF, the lungs work with the kidneys to maintain ECF pH.

Because humans inhabit many different environments and often move between environments, the body must be able to rapidly adapt to the challenges imposed by changes in ambient temperature and availability of food and water. Such adaptation requires coordination of the function of cells in different tissues and organs as well as their regulation. The nervous and endocrine systems coordinate and regulate cell, tissue, and organ function. The regulation of function can occur rapidly (seconds to minutes), as is the case for levels of cellular fuels in the blood, or over much longer periods of time (days to weeks), as is the case for acclimatization when an individual moves from a cool to a hot environment or changes from a high-salt to a low-salt diet.

The function of the human body represents complex processes at multiple levels. This book explains what is currently known about these processes. Although the emphasis is on the normal function of the human body, discussion of disease and abnormal function is also appropriate, as these often illustrate physiologic processes and principles at the extremes.

The authors for each section have presented what they believe to be the most likely mechanisms responsible for the phenomena under consideration. We have adopted this compromise to achieve brevity, clarity, and simplicity.

Bruce M. Koeppen, MD, PhD
Bruce A. Stanton, PhD

Contents

SECTION 1

Cellular Physiology

BRUCE M. KOEPPEN AND BRUCE A. STANTON

1

Principles of Cell and Membrane Function

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. What organelles are found in a typical eukaryotic cell, and what is their function?
2. What is the composition of the plasma membrane?
3. What are the major classes of membrane transport proteins, and how do they transport biologically important molecules and ions across the plasma membrane?
4. What is the electrochemical gradient, and how it is used to determine whether the transport of a molecule or ion across the plasma membrane is active or passive?
5. What are the driving forces for movement of water across cell membrane and the capillary wall?

In addition, the student should be able to define and understand the following properties of physiologically important solutions and fluids:

- Molarity and equivalence
- Osmotic pressure
- Osmolarity and osmolality
- Oncotic pressure
- Tonicity

The human body is composed of billions of cells. Although cells can perform different functions, they share certain common elements. This chapter provides an overview of these common elements and focuses on the important function of the transport of molecules and water into and out of the cell across its plasma membrane.

Overview of Eukaryotic Cells

Eukaryotic cells are distinguished from prokaryotic cells by the presence of a membrane-delimited nucleus. With the exception of mature human red blood cells and cells within the lens of the eye, all cells within the human body contain a nucleus. The cell is therefore effectively divided into two compartments: the nucleus and the cytoplasm. The cytoplasm is an aqueous solution containing numerous organic molecules, ions, cytoskeletal elements, and a number of organelles. Many of the organelles are membrane-enclosed compartments that carry out specific cellular function. An idealized eukaryotic cell is depicted in Fig. 1.1, and the primary function of some components and compartments of the cell are summarized in Table 1-1. Readers who desire a more in-depth presentation of this material are encouraged to consult one of the many textbooks on cell and molecular biology that are currently available.

The Plasma Membrane

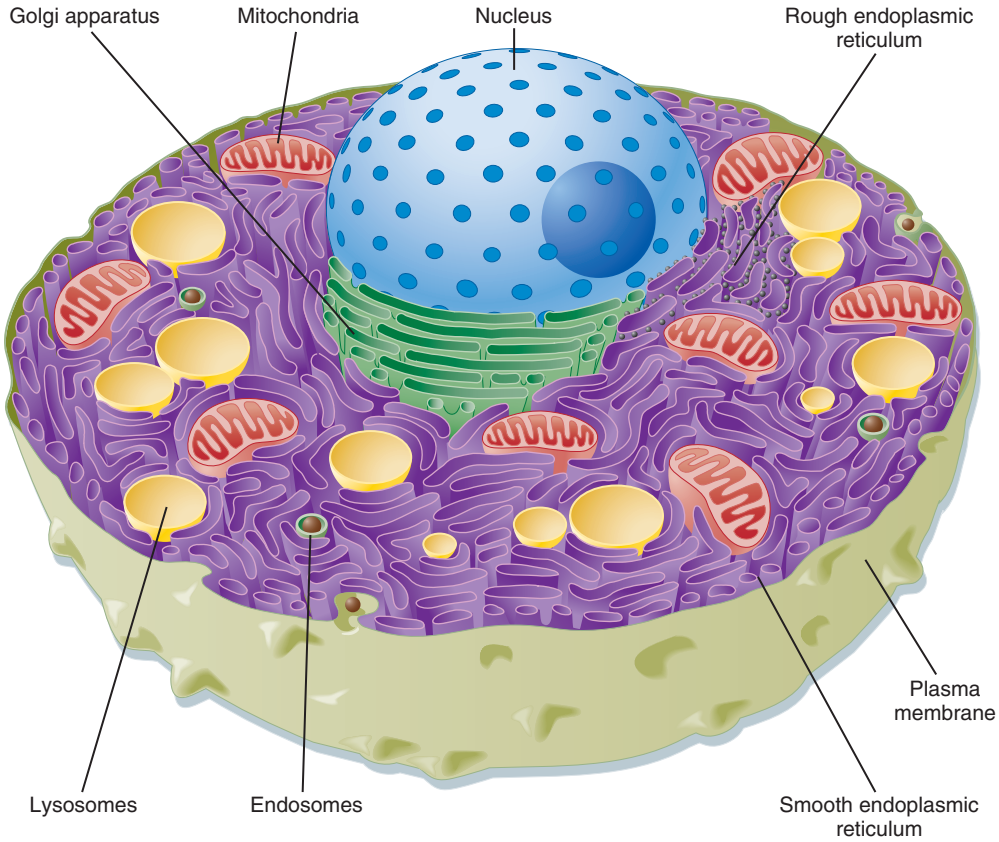
The cells within the body are surrounded by a plasma membrane that separates the intracellular contents from the extracellular environment. Because of the properties of this membrane and, in particular, the presence of specific membrane proteins, the plasma membrane is involved in a number of important cellular functions, including the following:

- Selective transport of molecules into and out of the cell. A function carried out by membrane transport proteins.
- Cell recognition through the use of cell surface antigens.
- Cell communication through neurotransmitter and hormone receptors and through signal transduction pathways.
- Tissue organization, such as temporary and permanent cell junctions, and interaction with the extracellular matrix, with the use of a variety of cell adhesion molecules.
- Membrane-dependent enzymatic activity.
- Determination of cell shape by linkage of the cytoskeleton to the plasma membrane.

In this chapter, the structure and function of the plasma membrane of eukaryotic cells are considered. More specifically, the chapter focuses on the transport of molecules and water across the plasma membrane. Only the principles of membrane transport are presented here. Additional details that relate to specific cells are presented in the various sections and chapters of this book.

Structure and Composition

The plasma membrane of eukaryotic cells consists of a 5-nm-thick lipid bilayer with associated proteins (Fig. 1.2). Some of the membrane-associated proteins are integrated into the lipid bilayer; others are more loosely attached to the



• **Fig. 1.1** Schematic drawing of a eukaryotic cell. The top portion of the cell is omitted to illustrate the nucleus and various intracellular organelles. See text for details.

TABLE 1.1 Primary Functions of Some Eukaryotic Cellular Components and Compartments

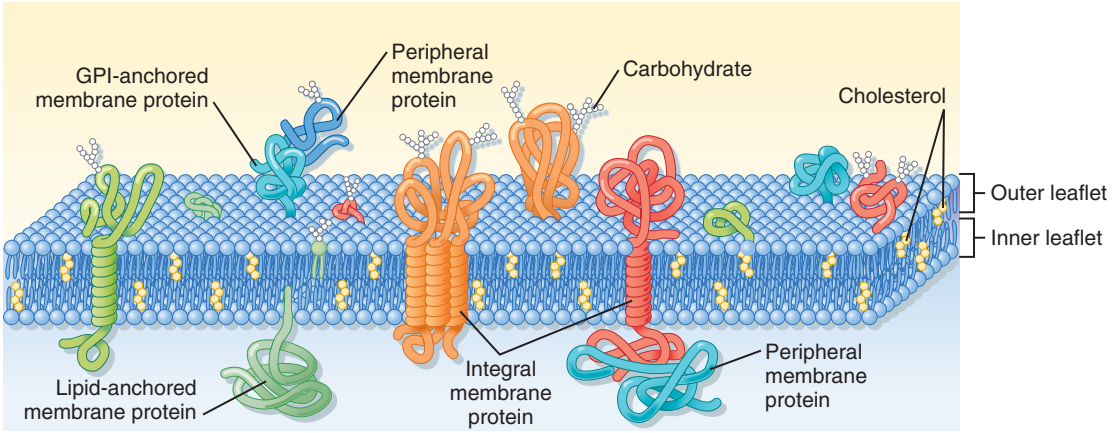
Component	Primary Function
Cytosol	Metabolism, protein synthesis (free ribosomes)
Cytoskeleton	Cell shape and movement, intracellular transport
Nucleus	Genome (22 autosomes and 2 sex chromosomes), DNA and RNA synthesis
Mitochondria	ATP synthesis by oxidative phosphorylation, Ca^{2+} storage
Smooth endoplasmic reticulum	Synthesis of lipids, Ca^{2+} storage
Free ribosomes	Translation of mRNA into cytosolic proteins
Rough endoplasmic reticulum	Translation of mRNA into membrane associated proteins or for secretion out of the cell
Lysosome	Intracellular degradation
Endosome	Cellular uptake of cholesterol, removal of receptors from the plasma membrane, uptake of small molecules and water into the cell, internalization of large particles (e.g., bacteria, cell debris)
Golgi apparatus	Modification, sorting, and packaging of proteins and lipids for delivery to other organelles within the cell or for secretion out of the cell
Proteosome	Degradation of intracellular proteins
Peroxisome	Detoxification of substances

ATP, adenosine triphosphate; mRNA, messenger RNA.

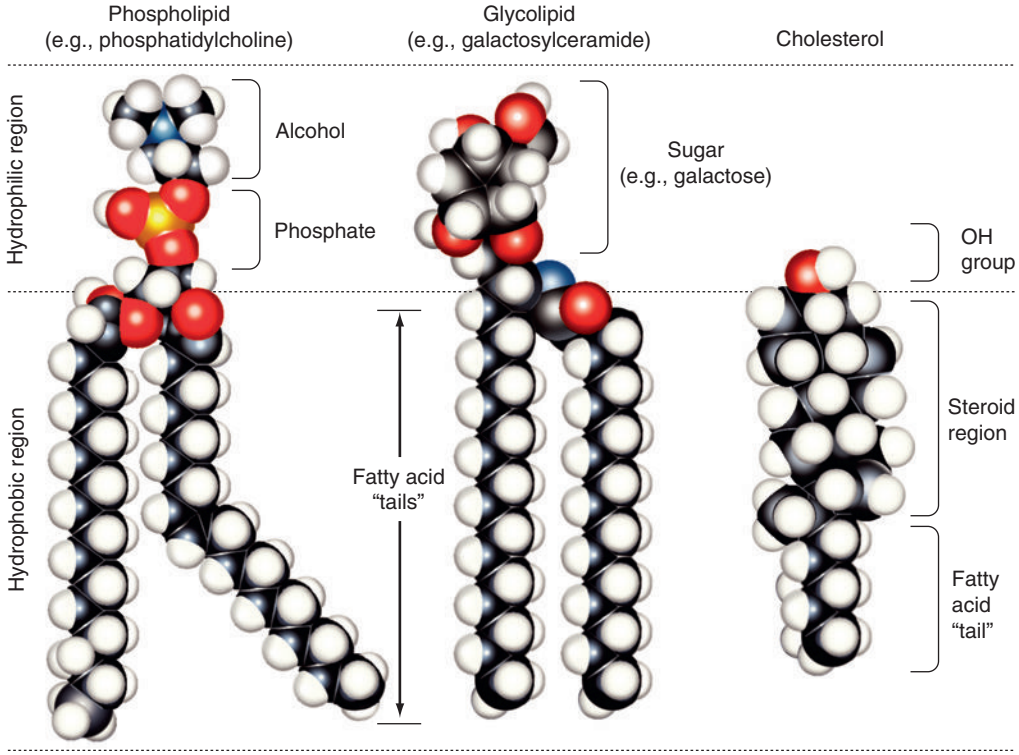
inner or outer surfaces of the membrane, often by binding to the integral membrane proteins.

Membrane Lipids

The major lipids of the plasma membrane are **phospholipids** and **phosphoglycerides.** Phospholipids are amphipathic molecules that contain a charged (or polar) hydrophilic head and two (nonpolar) hydrophobic fatty acyl chains (Fig. 1.3). The amphipathic nature of the phospholipid molecule is critical for the formation of the bilayer: The hydrophobic fatty acyl chains form the core of the bilayer, and the polar head groups are exposed on the surface.



• **Fig. 1.2** Schematic diagram of the cell plasma membrane. Not shown are lipid rafts. See text for details. (Modified from Cooper GM. *The Cell—A Molecular Approach.* 2nd ed. Washington, DC: Sinauer; 2000, Fig. 12.3.)



• **Fig. 1.3** Models of the major classes of plasma membrane lipids, depicting the hydrophilic and hydrophobic regions of the molecules. The molecules are arranged as they exist in one leaflet of the bilayer. The opposing leaflet is not shown. One of the fatty acyl chains in the phospholipid molecule is unsaturated. The presence of this double bond produces a "kink" in the fatty acyl chain, which prevents tight packing of membrane lipids and increases membrane fluidity. (Modified from Hansen JT, Koeppen BM: *Netter's Atlas of Human Physiology.* Teterboro, NJ: Icon Learning Systems; 2002.)

The majority of membrane phospholipids have a glycerol "backbone" to which are attached the fatty acyl chains, and an alcohol is linked to glycerol via a phosphate group. The common alcohols are choline, ethanolamine, serine, inositol, and glycerol. Another important phospholipid, sphingomyelin, has the amino alcohol sphingosine as its "backbone" instead of glycerol. Table 1-2 lists these common phospholipids. The fatty acyl chains are usually 14 to 20 carbons in length and may be saturated or unsaturated (i.e., contain one or more double bonds).

The phospholipid composition of the membrane varies among different cell types and even between the bilayer leaflets. For example, in the erythrocyte plasma membrane, phosphatidylcholine and sphingomyelin are found predominantly in the outer leaflet of the membrane, whereas phosphatidylethanolamine, phosphatidylserine,

TABLE 1.2 Plasma Membrane Lipids

Phospholipid	Primary Location in Membrane
Phosphatidylcholine	Outer leaflet
Sphingomyelin	Outer leaflet
Phospatidylethanolamine	Inner leaflet
Phosphatidylserine	Inner leaflet
Phosphatidylinositol*	Inner leaflet

*Involved in signal transduction.

and phosphatidylinositol are found in the inner leaflet. As described in detail in Chapter 3, phosphatidylinositol plays an important role in signal transduction, and its location in the inner leaflet of the membrane facilitates this signaling role.

The sterol molecule **cholesterol** is also a critical component of the bilayer (see Fig. 1.3). It is found in both leaflets and serves to stabilize the membrane at normal body temperature (37°C). As much as 50% of the lipids found in the membrane can be cholesterol. A minor lipid component of the plasma membrane is **glycolipids.** These lipids, as their name indicates, consist of two fatty acyl chains linked to polar head groups that consist of carbohydrates (see Fig. 1.3). As discussed in the section on membrane proteins, one glycolipid, glycosylphosphatylinositol (GPI), plays an important role in anchoring proteins to the outer leaflet of the membrane. Both cholesterol and glycolipids, like the phospholipids, are amphipathic, and they are oriented with their polar groups on the outer surface of the leaflet in which they are located. Their hydrophobic portion is thus located within the interior of the bilayer.

The lipid bilayer is not a static structure. The lipids and associated proteins can diffuse within the plane of the membrane. The fluidity of the membrane is determined by temperature and by its lipid composition. As temperature increases, the fluidity of the membrane increases. The presence of unsaturated fatty acyl chains in the phospholipids and glycolipids also increases membrane fluidity. If a fatty acyl chain is unsaturated, the presence of a double bond introduces a "kink" in the molecule (see Fig. 1.3). This kink prevents the molecule from associating closely with surrounding lipids, and, as a result, membrane fluidity is increased. Although the lipid bilayer is "fluid," movement of proteins in the membrane can be constrained or limited. For example, membrane proteins can be anchored to components of the intracellular cytoskeleton, which limits their movement. Membrane domains can also be isolated from one another. An important example of this can be found in epithelial tissues. Junctional complexes (e.g., tight junctions) separate the plasma membrane of epithelial cells into two domains: apical and basolateral (see Chapter 2). The targeted localization of membrane proteins into one or other of these domains allows epithelial cells to carry out vectorial transport of substances from one side of the epithelium to the opposite side. The ability to carry out vectorial transport is crucial for the functioning of several organ systems (e.g., the gastrointestinal tract and kidneys). In addition, some regions of the membrane contain lipids (e.g., sphingomyelin and cholesterol) that aggregate into what are called **lipid rafts.** These lipid rafts often have an association with specific proteins, which diffuse in the plane of the membrane as a discrete unit. Lipid rafts appear to serve a number of functions. One important function of these rafts is to segregate signaling molecules.

Membrane Proteins

As much as 50% of the plasma membrane is composed of proteins. These membrane proteins are classified as integral, lipid-anchored, or peripheral.

Integral membrane proteins are imbedded in the lipid bilayer, where hydrophobic amino acid residues are associated with the hydrophobic fatty acyl chains of the membrane lipids. Many integral membrane proteins span the bilayer; such proteins are termed **transmembrane proteins.** Transmembrane proteins have both hydrophobic and hydrophilic regions. The hydrophobic region, often in the form of an α helix, spans the membrane. Hydrophilic amino acid residues are then exposed to the aqueous environment on either side of the membrane. Transmembrane proteins may pass through the membrane multiple times.

AT THE CELLULAR LEVEL

There is a superfamily of membrane proteins that serve as receptors for many hormones, neurotransmitters, and numerous drugs. These receptors are coupled to heterotrimeric G proteins and are termed *G protein–coupled receptors* (see Chapter 3). These proteins span the membrane with seven α-helical domains. The binding site of each ligand is either on the extracellular portion of the protein (large ligands) or in the membrane-spanning portion (small ligands), whereas the cytoplasmic portion binds to the G protein. This superfamily of membrane proteins makes up the third largest family of genes in humans. Nearly half of all nonantibiotic prescription drugs are targeted toward G protein–coupled receptors.

A protein can also be attached to the membrane via **lipid anchors.** The protein is covalently attached to a lipid molecule, which is then embedded in one leaflet of the bilayer. Glycosylphosphatidylinositol (GPI) anchors proteins to the outer leaflet of the membrane. Proteins can be attached to the inner leaflet via their amino-terminus by fatty acids (e.g., myristate or palmitate) or via their carboxyl-terminus by prenyl anchors (e.g., farnesyl or geranylgeranyl).

Peripheral proteins may be associated with the polar head groups of the membrane lipids, but they more commonly bind to integral or lipid-anchored proteins.

In many cells, some of the outer leaflet lipids, as well as many of the proteins exposed on the outer surface of the

membrane, are glycosylated (i.e., have short chains of sugars, called *oligosaccharides,* attached to them). Collectively, these glycolipids and glycoproteins form what is called the glycocalyx. Depending on the cell these glycolipids and glycoproteins may be involved in cell recognition (e.g., cell surface antigens) and formation of cell-cell interactions (e.g., attachment of neutrophils to vascular endothelial cells).

Membrane Transport

Although plasma membrane proteins perform many important cellular functions, as noted previously, the remainder of this chapter focuses on one group of plasma membrane proteins: the membrane transport proteins, or transporters. It has been estimated that approximately 10% of human genes (≈2000) code for transporters. They are also targets for numerous drugs.

The normal function of cells requires the continuous movement of water and solutes into and out of the cell. The intracellular and extracellular fluids are composed primarily of H_2O, in which solutes (e.g., ions, glucose, amino acids) are dissolved. The plasma membrane, with its hydrophobic core, is an effective barrier to the movement of virtually all of these biologically important solutes. It also restricts the movement of water across the membrane. The presence of specific membrane transporters in the membrane is responsible for the movement of these solutes and water across the membrane.

Membrane Transport Proteins

Membrane transporters have been classified in several different ways. In this chapter, the transporters are divided into four general groups: water channels, ion channels, solute carriers, and adenosine triphosphate (ATP)–dependent transporters. Table 1-3 lists these groups of membrane transporters, their modes of transport, and estimates of the rates at which they transport molecules or ions across the membrane.

Water Channels

Water channels, or **aquaporins (AQPs),** are the main routes for water movement into and out of the cell. They are widely distributed throughout the body (e.g., the brain, lungs, kidneys, salivary glands, gastrointestinal tract, and liver). Cells express different AQP isoforms, and some cells even express multiple isoforms. For example, cells in the collecting ducts of the kidneys express AQP3 and AQP4 in their basolateral membrane and AQP2 in their apical membrane. Moreover, the abundance of AQP2 in the apical membrane is regulated by antidiuretic hormone (also called arginine vasopressin), which is crucial for the ability of the kidneys to concentrate the urine (see Chapter 35).

Although all AQP isoforms allow the passive movement of H_2O across the membrane, some isoforms also provide a pathway for other molecules such as glycerol, urea, mannitol, purines, pyrimidines, CO_2, and NH_3 to cross the membrane. Because glycerol was one of the first molecules identified as crossing the membrane via some AQPs, this group of AQPs is collectively called *aquaglyceroporins* (see also Chapter 34). Regulation of the amount of H_2O that can enter or leave the cell via AQPs occurs primarily by altering the number of AQPs in the membrane.

TABLE 1.3 Major Classes of Plasma Membrane Transporters

Class	Transport Mode	Transport Rate
Pore*	Open (not gated)	Up to 10^9 molecules/sec
Channel	Gated	10^6-10^8 molecules/sec
Solute carrier	Cycle	10^2-10^4 molecules/sec
ATP-dependent	Cycle	10^2-10^4 molecules/sec

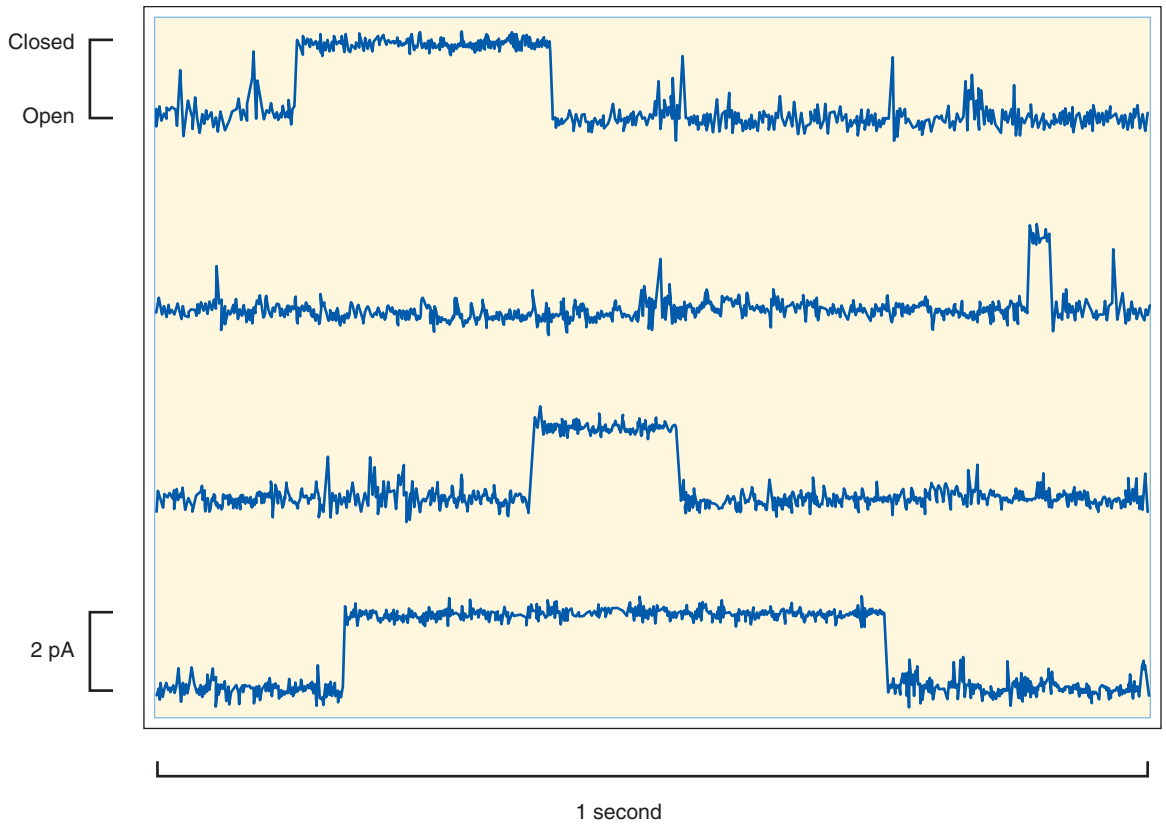
*Examples include porins that are found in the outer membrane of mitochondria, and water channels (i.e., aquaporins) that function as a pore.
ATP, adenosine triphosphate.

AT THE CELLULAR LEVEL

Each AQP molecule consists of six membrane-spanning domains and a central water-transporting pore. Four AQP monomers assemble to form a homotetramer in the plasma membrane, with each monomer functioning as a water channel.

Ion Channels

Ion channels are found in all cells, and are especially important for the function of excitable cells (e.g., neurons and muscle cells). Ion channels are classified by their selectivity, conductance and mechanism of channel gating (i.e., opening and closing). *Selectivity* is defined as the nature of the ions that pass through the channel. At one extreme, ion channels can be highly selective, in that they allow only a specific ion through. At the other extreme, they may be nonselective, allowing all or a group of cations or anions through. *Channel conductance* refers to the number of ions that pass through the channel and is typically expressed in picosiemens (pS). The range of conductance is considerable: Some channels have a conductance of only 1 to 2 pS, whereas others have a conductance of more than 100 pS. For some channels, the conductance varies, depending on the direction in which the ion is moving. For example, if the channel has a larger conductance when ions are moving into the cell than when they are moving out of the cell, the channel is said to be an *inward rectifier.* Moreover, ion channels fluctuate between an open state or a closed state, a process called *gating* (Fig. 1.4). Factors that can control gating include membrane voltage, extracellular agonists or antagonists (e.g., acetylcholine is an extracellular agonist that controls the gating of a cation-selective channel in the motor end plate of skeletal muscle cells; see Chapter 6), intracellular messengers (e.g., Ca^{++}, ATP, cyclic guanosine



● **Fig. 1.4** Recording of current flow through a single ion channel. The channel spontaneously fluctuates between an open state and a closed state. The amplitude of the current is approximately 2 pA (2×10^{-12} amps); that is, 12.5 million ions/second cross the membrane.

monophosphate), and mechanical stretch of the plasma membrane. Ion channels can be regulated by a change in the number of channels in the membrane or by gating of the channels.

Solute Carriers

Solute carriers (denoted *SLCs* by the HUGO Gene Nomenclature Committee) represent a large group of membrane transporters categorized into more than 50 families; almost 400 specific transporters have been identified to date. These carriers can be divided into three groups according to their mode of transport. One group, **uniporters** (or **facilitated transporters**), transports a single molecule across the membrane. The transporter that brings glucose into the cell (glucose transporter 1 [GLUT-1], or SLC2A1) is an important member of this group. The second group, **symporters** (or **cotransporters**), couples the movement of two or more molecules/ions across the membrane. As the name implies, the molecules/ions are transported in the same direction. The Na^+,K^+,$2Cl^-$ (NKCC) symporter found in the kidney (NKCC2, or SLC12A1), which is crucial for diluting and concentrating the urine (see Chapter 34), is a member of this group. The third group, **antiporters** (or **exchange transporters**), also couples the movement of two or more molecules/ions across the membrane; in this case, however, the molecules/ions are transported in opposite directions. The Na^+-H^+ antiporter is a member of this group of solute carriers. One isoform of this antiporter (NHE-1, or SLC9A1) is found in all cells and plays an important role in regulating intracellular pH.

Adenosine Triphosphate–Dependent Transporters

The ATP-dependent transporters, as their name implies, use the energy in ATP to drive the movement of molecules/ions across the membrane. There are two groups of ATP-dependent transporters: the **ATPase ion transporters** and the **ATP-binding cassette (ABC) transporters.** The ATPase ion transporters are subdivided into P-type ATPases and V-type ATPases.[a] The P-type ATPases are phosphorylated during the transport cycle. Na^+,K^+-ATPase is an important example of a P-type ATPase. With the hydrolysis of each ATP molecule, it transports three Na^+ ions out of the cell and two K^+ ions into the cell. Na^+,K^+-ATPase is present in all cells and plays a critical role in establishing cellular ion and electrical gradients, as well as maintaining cell volume (see Chapter 2).

V-type H^+-ATPases are found in the membranes of several intracellular organelles (e.g., endosomes, lysosomes); as a result, they are also referred to as *vacuolar H^+-ATPases.* The

[a]Another type of ATPases, F-type ATPases, is found in the mitochondria, and they are responsible for ATP synthesis. They are not considered in this chapter.

AT THE CELLULAR LEVEL

Na^+,K^+-ATPase (also called the *Na^+,K^+-pump* or just the *Na^+-pump*) is found in all cells and is responsible for establishing the gradients of Na^+ and K^+ across the plasma membrane. These gradients in turn provide energy for several essential cell functions (see Chapter 2). Na^+,K^+-ATPase is composed of three subunits (α, β, and γ), and the protein exists in the membrane with a stoichiometric composition of 1α, 1β, 1γ. The α subunit contains binding sites for Na^+,K^+ and ATP. It is also the subunit that binds cardiac glycosides (e.g., ouabain), which specifically inhibit the enzyme. It has a transmembrane domain and three intracellular domains: phosphorylation (P-domain), nucleotide binding (N-domain), and actuator (A-domain). Although the α subunit is the functional subunit of the enzyme (i.e., it hydrolyzes ATP, binds Na^+ and K^+, and translocates them across the membrane), it cannot function without the β subunit. The β subunit is responsible for targeting the α subunit to the membrane and also appears to modulate the affinity of the Na^+,K^+-ATPase for Na^+ and K^+. The α and β subunits can carry out Na^+ and K^+ transport in the absence of the γ subunit. However, the γ subunit appears to play a regulatory role. The γ subunit is a member of a family of proteins called **FXYD proteins** (so named for the FXYD amino acid sequence found in the protein).

IN THE CLINIC

Cystic fibrosis is an autosomal recessive disease characterized by chronic lung infections, pancreatic insufficiency, and infertility in boys and men. Death usually occurs because of respiratory failure. It is most prevalent in white people and is the most common lethal genetic disease in this population, occurring in 1 per 3000 live births. It is a result of mutations in a gene on chromosome 7 that codes for an ABC transporter. To date, more than 1000 mutations in the gene have been identified. The most common mutation is a deletion of a phenylalanine at position 508 (F508del). Because of this deletion, degradation of the protein by the endoplasmic reticulum in enhanced, and, as a result, the transporter does not reach the plasma membrane. This transporter, called **cystic fibrosis transmembrane conductance regulator (CFTR),** normally functions as a Cl^- channel and also regulates other membrane transporters (e.g., the epithelial Na^+ channel [ENaC]). Thus in individuals with cystic fibrosis, epithelial transport is defective, which is responsible for the pathophysiologic process. For example, in patients not affected by cystic fibrosis, the epithelial cells that line the airway of the lung are covered with a layer of mucus that entraps inhaled particulates and bacteria. Cilia on the epithelial cells then transport the entrapped material out of the lung, a process termed *mucociliary transport* (see Chapter 26 for more details). In patients with cystic fibrosis, the inability to secrete Cl^-, Na^+, and H_2O results in an increase in the viscosity of the airway surface mucus; thus the cilia cannot transport the entrapped bacteria and other pathogens out of the lung. This in turn leads to recurrent and chronic lung infections. The inflammatory process that accompanies these infections ultimately destroys the lung tissue, causing respiratory failure and death. In 2015, the U.S. Food and Drug Administration approved lumacaftor/ivacaftor (Orkambi), a drug that increases the amount of F508del CFTR in the plasma membrane of lung epithelial cells.

H^+-ATPase in the plasma membrane plays an important role in urinary acidification (see Chapter 37).

ABC transporters represent a large group of membrane transporters. They are found in both prokaryotic and eukaryotic cells, and they have amino acid domains that bind ATP (i.e., ABC domains). Seven subgroups of ABC transporters in humans and more than 40 specific transporters have been identified to date. They transport a diverse group of molecules/ions, including Cl^-, cholesterol, bile acids, drugs, iron, and organic anions.

Because biologically important molecules enter and leave cells through membrane transporters, membrane transport is specific and regulated. Although some membrane transporters are ubiquitously expressed in all cells (e.g., Na^+,K^+-ATPase), the expression of many other transporters is limited to specific cell types. This specificity of expression tailors the function of the cell to the organ system in which it is located (e.g., the sodium-glucose–linked transporters SGLT-1 and SGLT-2 in the epithelial cells of the intestines and renal proximal tubules). In addition, the amount of a molecule being transported across the membrane can be regulated. Such regulation can take place through altering the number of transporters in the membrane or altering the rate or kinetics of individual transporters (e.g., the time an ion channel stays in the open versus closed state), or both.

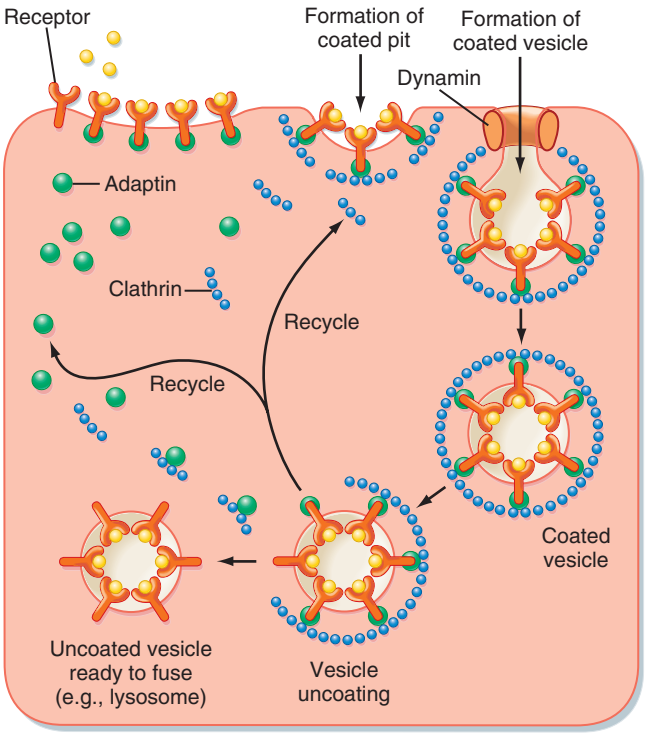
Vesicular Transport

Solute and water can be brought into the cell through a process of **endocytosis** and released from the cell through the process of **exocytosis.** Endocytosis is the process whereby a piece of the plasma membrane pinches off and is internalized into the cell interior, and exocytosis is the process whereby vesicles inside the cell fuse with the plasma membrane. In both of these processes, the integrity of the plasma membrane is maintained, and the vesicles allow for the transfer of the contents among cellular compartments. In some cells (e.g., the epithelial cells lining the gastrointestinal tract), endocytosis across one membrane of the cell is followed by exocytosis across the opposite membrane. This allows the transport of substances inside the vesicles across the epithelium, a process termed **transcytosis.**

Endocytosis occurs in three mechanisms. The first is **pinocytosis,** which consists of the nonspecific uptake of small molecules and water into the cell. Pinocytosis is a prominent feature of the endothelial cells that line capillaries and is responsible for a portion of the fluid exchange that occurs across these vessels. The second form of endocytosis, **phagocytosis,** allows for the cellular internalization of large particles (e.g., bacteria, cell debris). This process is an important characteristic of cells in the immune system (e.g., neutrophils and macrophages). Often, but not always, phagocytosis is a receptor-mediated process. For example,

AT THE CELLULAR LEVEL

Proteins within the plasma membrane of cells are constantly being removed and replaced with newly synthesized proteins. As a result, membrane proteins are constantly being replaced. One mechanism by which membrane proteins are "tagged" for replacement is by the attachment of ubiquitin to the cytoplasmic portion of the protein. Ubiquitin is a 76–amino acid protein that is covalently attached to the membrane protein (usually to lysine) by a class of enzymes called *ubiquitin protein ligases.* One important group of these ligases is the developmentally downregulated protein 4 (Nedd4)/Nedd4-like family. Once a membrane protein is ubiquitinated, it undergoes endocytosis and is degraded either by lysosomes or by the proteosome. Cells also contain deubiquitinating enzymes (DUBs). Thus the amount of time a protein stays in the plasma membrane depends on the rate that ubiquitin groups are added by the ligases versus the rate that they are removed by the DUBs. For example, Na^+ reabsorption by the collecting ducts of the kidneys is stimulated by the adrenal hormone aldosterone (see Chapters 34 and 35). One of the actions of aldosterone is to inhibit Nedd4-2. This prevents ubiquitination of ENaC in the apical membrane of epithelial cells. Thus the channels are retained for a longer period of time in the membrane, and as a result, more Na^+ enters the cell and is thereby reabsorbed.



• **Fig. 1.5** Receptor-mediated endocytosis. Receptors on the surface of the cell bind the ligand. A clathrin-coated pit is formed with adaptin linking the receptor molecules to clathrin. Dynamin, a guanosine triphosphatase (GTPase), assists in separation of the endocytic vesicle from the membrane. Once inside the cell, the clathrin and adaptin molecules dissociate and are recycled. The uncoated vesicle is then ready to fuse with other organelles in the cell (e.g., lysosomes). (Adapted from Ross MH, Pawlina W: *Histology.* 5th ed. Baltimore: Lippincott Williams & Wilkins; 2006.)

macrophages have receptors on their surface that bind the Fc portion of immunoglobulins. When bacteria invade the body they are often coated with antibody, a process called opsonization. These bacteria then attach to the membrane of macrophages via the fragment crystallizable (Fc) portion of the immunoglobulin, undergo phagocytosis, and are destroyed inside the cell. The third mechanism of endocytosis is **receptor-mediated endocytosis,** which allows the uptake of specific molecules into the cell. In this form of endocytosis, molecules bind to receptors on the surface of the cell. Endocytosis involves a number of accessory proteins, including adaptin, clathrin, and the GTPase dynamin (Fig. 1.5).

Exocytosis can be either constitutive or regulated. Constitutive secretion occurs, for example, in plasma cells that are secreting immunoglobulin or in fibroblasts secreting collagen. Regulated secretion occurs in endocrine cells, neurons, and exocrine glandular cells (e.g., pancreatic acinar cells). In these cells, the secretory product (e.g., hormone, neurotransmitter, or digestive enzyme), after synthesis and processing in the rough endoplasmic reticulum and Golgi apparatus, is stored in the cytoplasm in secretory granules until an appropriate signal for secretion is received. These signals may be hormonal or neural. Once the cell receives the appropriate stimulus, the secretory vesicle fuses with the plasma membrane and releases its contents into the extracellular fluid. Fusion of the vesicle with the membrane is mediated by a number of accessory proteins. One important group is the SNARE (**s**oluble *N*-ethylmaleimide sensitive fusion protein [**N**SF] **a**ttachment protein **re**ceptors) proteins. These membrane proteins help target the secretory

IN THE CLINIC

Cholesterol is an important component of cells (e.g., it is a key component of membranes). However, most cells are unable to synthesize cholesterol and therefore must obtain it from the blood. Normally, cholesterol is ingested in the diet, and it is transported through the blood in association with lipoproteins. Low-density lipoproteins (LDLs) in the blood carry cholesterol to cells, where they bind to LDL receptors in the plasma membrane. After the receptors bind LDL, they collect into "coated pits" and undergo endocytosis as clathrin-coated vesicles. Once inside the cell, the endosomes release LDL and then recycle the LDL receptors back to the cell surface. Inside the cell, LDL is then degraded in lysosomes, and the cholesterol is made available to the cell. Defects in the LDL receptor prevent cellular uptake of LDL. Individuals with this defect have elevated levels of blood LDL, often called "bad cholesterol," because it is associated with the development of cholesterol-containing plaques in the smooth muscle layer of arteries. This process, atherosclerosis, is associated with an increased risk for heart attacks as a result of occlusion of the coronary arteries.

vesicle to the plasma membrane. The process of secretion is usually triggered by an increase in the concentration of intracellular Ca^{++} ($[Ca^{++}]$). However, two notable exceptions to this general rule exist: (1) Renin secretion by the juxtaglomerular cells of the kidney occurs with a decrease in intracellular Ca^{++} (see Chapters 34 and 35), as does (2) the secretion of parathyroid hormone by the parathyroid gland (see Chapter 40).

Basic Principles of Solute and Water Transport

As already noted, the plasma membrane, with its hydrophobic core, is an effective barrier to the movement of virtually all biologically important molecules into or out of the cell. Thus membrane transport proteins provide the pathway that allows transport to occur into and out of cells. However, the presence of a pathway is not sufficient for transport to occur; an appropriate driving force is also required. In this section, the basic principles of diffusion, active and passive transport, and osmosis are presented. These topics are discussed in greater depth, as appropriate, in the other sections of the book.

Diffusion

Diffusion is the process by which molecules move spontaneously from an area of high concentration to one of low concentration. Thus wherever a concentration gradient exists, diffusion of molecules from the region of high concentration to the region of low concentration dissipates the gradient (as discussed later, the establishment of concentration gradients for molecules requires the expenditure of energy). Diffusion is a random process driven by the thermal motion of the molecules. **Fick's first law of diffusion** quantifies the rate at which a molecule diffuses from point A to point B:

Equation 1.1

$$J = -DA\frac{\Delta C}{\Delta X}$$

where
J = the flux or rate of diffusion per unit time
D = the diffusion coefficient
A = area across which the diffusion is occurring
ΔC = the concentration difference between point A and B
ΔX = the distance along which diffusion is occurring

The diffusion coefficient takes into account the thermal energy of the molecule, its size, and the viscosity of the medium through which diffusion is taking place. For spherical molecules, D is approximated by the **Stokes-Einstein equation:**

Equation 1.2

$$D = \frac{kT}{6\pi r\eta}$$

where
k = Boltzmann's constant
T = temperature in degrees Kelvin
r = radius of the molecule
η = viscosity of the medium

According to eqs. 1.1 and 1.2, the rate of diffusion will be faster for small molecules than for large molecules. In addition, diffusion rates are high at elevated temperatures, in the presence of large concentration gradients, and when diffusion occurs in a low-viscosity medium. With all other variables held constant, the rate of diffusion is linearly related to the concentration gradient.

Fick's equation can also be applied to the diffusion of molecules across a barrier, such as a lipid bilayer. When applied to the diffusion of a molecule across a bilayer, the diffusion coefficient (D) incorporates the properties of the bilayer and especially the ability of the molecule to diffuse through the bilayer. To quantify the interaction of the molecule with the bilayer, the term *partition coefficient* (β) is used. For a molecule that "dissolves" equally in the fluid bathing the lipid bilayer (e.g., water) and in the lipid bilayer, $\beta = 1$. If the molecule dissolves more easily in the lipid bilayer, $\beta > 1$; and if it dissolves less easily in the lipid bilayer, $\beta < 1$. For a simple lipid bilayer, the more lipid soluble the molecule is, the larger the partition coefficient is, and thus the diffusion coefficient—therefore the rate of diffusion of the molecule across the bilayer—is greater. In this situation, ΔC represents the concentration difference across the membrane, A is the membrane area, and ΔX is the thickness of the membrane.

Another useful equation for quantitating the diffusion of molecules across the plasma membrane (or any membrane) is as follows:

Equation 1.3

$$J = -P(C_i - C_o),$$

where
J = the flux or rate of diffusion across the membrane
P = is the permeability coefficient
C_i = concentration of the molecule inside the cell
C_o = the concentration of the molecule outside the cell

This equation is derived from Fick's equation (eq. 1.1). P incorporates D, ΔX, A, and the partition coefficient (β). P is expressed in units of velocity (e.g., centimeters per second), and C the units of moles/cm^3. Thus the units of flux are moles per square centimeter per second (mol/cm^2/sec). Values for P can be obtained experimentally for any molecule and bilayer.

As noted, the phospholipid portion of the plasma membrane represents an effective barrier to many biologically important molecules. Consequently, diffusion through the lipid phase of the plasma membrane is not an efficient process for movement of these molecules across the membrane. It has been estimated that for a cell 20 μm in diameter, with a plasma membrane composed only of phospholipids, dissipation of a urea gradient imposed across the membrane would take approximately 8 minutes. Similar gradients for glucose and amino acids would take approximately 14 hours to dissipate, whereas ion gradients would take years to dissipate.

As noted previously, the vast majority of biologically important molecules cross cell membranes via specific membrane transporters, rather than by diffusing through the lipid portion of the membrane. Nevertheless, eq. 1.3 can be and has been used to quantitate the diffusion of molecules across many biological membranes. When this is done, the value of the permeability coefficient (P) reflects the properties of the pathway (e.g., membrane transporter or, in some cases, multiple transporters) that the molecule uses to cross the membrane.

Despite the limitations of using diffusion to describe and understand the transport of molecules across cell membranes, it is also important for understanding gas exchange in the lungs (see Chapter 24), the movement of molecules through the cytoplasm of the cell, and the movement of molecules between cells in the extracellular fluid. For example, one of the physiological responses of skeletal muscle to exercise is the recruitment or opening of capillaries that are not perfused at rest. This opening of previously closed capillaries increases capillary density and thereby reduces the diffusion distance between the capillary and the muscle fiber so that oxygen and cellular fuels (e.g., fatty acids and glucose) can be delivered more quickly to the contracting muscle fiber. In resting muscle, the average distance of a muscle fiber from a capillary is estimated to be 40 μm. However, with exercise, this distance decreases to 20 μm or less.

Electrochemical Gradient

The **electrochemical gradient** (also called the **electrochemical potential difference**) is used to quantitate the driving force acting on a molecule to cause it to move across a membrane. The electrochemical gradient for any molecule ($\Delta\mu_x$) is calculated as follows:

Equation 1.4

$$\Delta\mu_x = RT ln \frac{[X]_i}{[X]_o} + z_x F V_m,$$

where
R = the gas constant
T = temperature in degrees Kelvin
Ln = natural logarithm
$[X]_i$ = the concentration of X inside the cell
$[X]_o$ = the concentration of X outside the cell
z_x = the valence of charged molecules
F = the Faraday constant
V_m = the membrane potential ($V_m = V_i - V_o$)[b]

The electrochemical gradient is a measure of the free energy available to carry out the useful work of transporting the molecule across the membrane. It has two components: One component represents the energy in the concentration gradient for X across the membrane **(chemical potential difference).** The second component **(electrical potential difference)** represents the energy associated with moving charged molecules (e.g., ions) across the membrane when a membrane potential exits (i.e., $V_m \neq 0$ mV). Thus for the movement of glucose across a membrane, only the concentrations of glucose inside and outside of the cell need to be considered (Fig. 1.6*A*). However, the movement of K^+ across the membrane, for example, would be determined both from the K^+ concentrations inside and outside of the cell and from the membrane voltage (see Fig. 1.6*B*).

Eq. 1.4 can be used to derive the **Nernst equation** for the situation in which a molecule is at equilibrium across the membrane (i.e., $\Delta\mu = 0$):

Equation 1.5a

$$0 = RT ln \frac{[X]_i}{[X]_o} + z_x F V_m$$

$$-RT ln \frac{[X]_i}{[X]_o} = z_x F V_m$$

$$V_m = -\frac{RT}{z_x F} ln \frac{[X]_i}{[X]_o}$$

Alternatively

Equation 1.5b

$$V_m = \frac{RT}{z_x F} ln \frac{[X]_i}{[X]_o}$$

The value of V_m calculated with the Nernst equation represents the equilibrium condition and is referred to as the **Nernst equilibrium potential** ($\mathbf{E_x}$, the V_m at which there is no net transport of the molecule across the membrane). It should be apparent that the Nernst equilibrium potential quantitates the energy in a concentration gradient and expresses that energy in millivolts. For example, for the cell depicted in Fig. 1.6*B*, the energy in the K^+ gradient (derived from the Nernst equilibrium potential for K^+ [E_{K^+}]) is proportional to 90.8 mV (causing K^+ to move out of the cell). This is opposite to, and of greater magnitude than, the energy in the membrane voltage ($V_m = -60$ mV), which causes K^+ to enter the cell. As a result, the electrochemical gradient is such that the net movement of K^+ across the membrane will be out of the cell. Another way to state this is that the net driving force for K^+ ($V_m - E_{K^+}$) is 30.8 mV (driving K^+ out of the cell). This is described in more detail in Chapter 2.

The Nernst equation, at 37° C, can be written as follows by replacing the natural logarithm function with the base 10 logarithm function:

Equation 1.6a

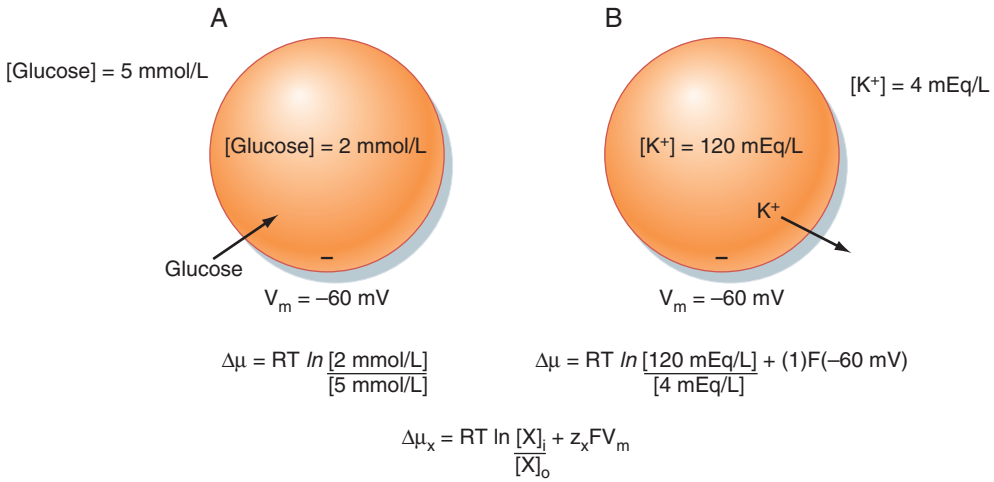
$$E_x = -\frac{61.5\ \text{mV}}{z_x} log \frac{[X]_i}{[X]_o}$$

or

Equation 1.6b

$$E_x = \frac{61.5\ \text{mV}}{z_x} log \frac{[X]_o}{[X]_i}$$

[b]By convention, membrane voltages are determined and reported with regard to the exterior of the cell. In a typical cell, the resting membrane potential (V_m) is negative. Positive V_m values can be observed in some excitable cells at the peak of an action potential.



• **Fig. 1.6** Electrochemical gradients and cellular transport of molecules. **A,** Because glucose is uncharged, the electrochemical gradient is determined solely by the concentration gradient for glucose across the cell membrane. As shown, the glucose concentration gradient would be expected to drive glucose into the cell. **B,** Because K^+ is charged, the electrochemical gradient is determined by both the concentration gradient and the membrane voltage (V_m). The Nernst equilibrium potential for K^+ (E_{K^+}), calculated with eq. 1.5a, is –90.8 mV ($E_{K^+} = V_m$ at equilibrium). The energy in the concentration gradient, which drives K^+ out of the cell, is thus proportional to +90.8 mV. The membrane voltage of –60 mV drives K^+ into the cell. Thus the electrochemical gradient, or net driving force, is 2.97 kJ/mol (equivalent to 30.8 mV), which drives K^+ out of the cell.

These are the most common forms of the Nernst equation in use. In these equations, it is apparent that for a univalent ion (e.g., Na^+, K^+, Cl^-), a 10-fold concentration gradient across the membrane is equivalent in energy to an electrical potential difference of 61.5 mV (at 37° C), and a 100-fold gradient is equivalent to an electrical potential difference of 123 mV. Similarly, for a divalent ion (e.g., Ca^{++}), a 10-fold concentration gradient is equivalent to a 30.7-mV electrical potential difference, because z in eqs. 1.6a and 1.6b is equal to 2.

Active and Passive Transport

When the net movement of a molecule across a membrane occurs in the direction predicted by the electrochemical gradient, that movement is termed **passive transport.** Thus for the examples given in Fig. 1.6, the movement of glucose into the cell and the movement of K^+ out of the cell would be considered passive transport. Transport that is passive is sometimes referred to as either "downhill transport" or "transport with the electrochemical gradient." In contrast, if the net movement of a molecule across the membrane is opposite to that predicted by the electrochemical gradient, that movement is termed **active transport,** a process that requires the input of energy (e.g., ATP). Active transport is sometimes referred to as either "uphill transport" or "transport against the electrochemical gradient."

In the various classes of plasma membrane transport proteins, the movement of H_2O through water channels is a passive process (see later discussion), as is the movement of ions through ion channels and the transport of molecules via uniporters (e.g., transport of glucose via GLUT-1). The ATPase-dependent transporters can use the energy in ATP to drive active transport of molecules (e.g., Na^+,K^+-ATPase, H^+-ATPase, or ABC transporters). Because the transport is directly coupled to the hydrolysis of ATP, it is referred to as **primary active transport.** Solute carriers that couple movement of two or more molecules (e.g., $3Na^+$,Ca^{++} antiporter) often transport one or more molecules (one Ca^{++} molecule in this example) against their respective electrochemical gradient through the use of the energy in the electrochemical gradient of the other molecule or molecules (three Na^+ in this example). When this occurs, the molecule or molecules transported against their electrochemical gradient are said to be transported by **secondary active transport** mechanisms (Fig. 1.7).

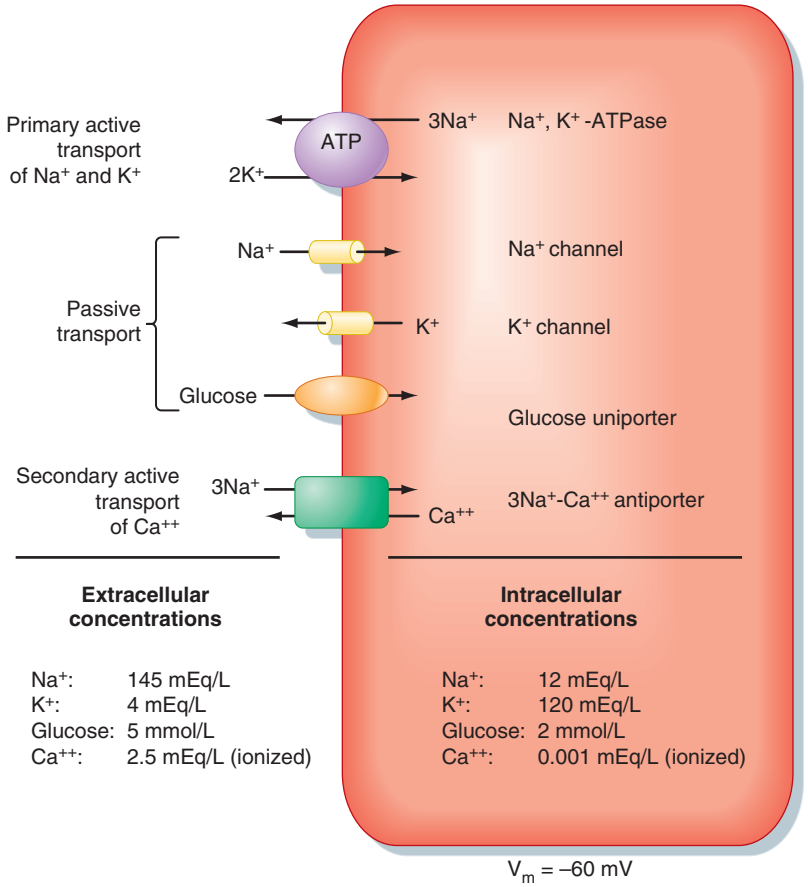
Osmosis and Osmotic Pressure

The movement of water across cell membranes occurs by the process of **osmosis.** The movement of water is passive, with the driving force for this movement being the osmotic pressure difference across the cell membrane. Fig. 1.8 illustrates the concept of osmosis and the measurement of the osmotic pressure of a solution.

Osmotic pressure is determined by the number of solute molecules dissolved in the solution. It is not dependent on such factors as the size of the molecules, their mass, or their chemical nature (e.g., valence). Osmotic pressure (π), measured in atmospheres (atm), is calculated by **van't Hoff's law** as follows:

Equation 1.7

$$\pi = nCRT,$$



• **Fig. 1.7** Examples of several membrane transporters, illustrating primary active, passive, and secondary active transport. See text for details. ATP, adenosine triphosphate.

IN THE CLINIC

Glucose is transported by the epithelial cells that line the gastrointestinal tract (small intestine), and by cells that form the proximal tubules of the kidneys. In the gastrointestinal tract, the glucose is absorbed from ingested food. In the kidney, the proximal tubule reabsorbs the glucose that was filtered across the glomerular capillaries and thereby prevents it from being lost in the urine. The uptake of glucose into the epithelial cell from the lumen of the small intestine and from the lumen of the proximal tubule is a secondary active process involving the sodium-glucose–linked transporters SGLT-1 and SGLT-2. SGLT-2 transports one glucose molecule with one Na^+ ion, and the energy in the electrochemical gradient for Na^+ (into the cell) drives the secondary active uptake of glucose. According to the following equation, for calculating the electrochemical gradient, and if the membrane potential (V_m) is –60 mV and there is a 10-fold $[Na^+]$ gradient across the membrane, an approximate 100-fold glucose gradient could be generated by SGLT-2:

$$\frac{[\text{Glucose}]_i}{[\text{Glucose}]_o} = \frac{[Na^+]_o}{[Na^+]_i} \times 10^{-V_m/61.5\,\text{mV}}$$

Thus, if the intracellular glucose concentration was 2 mmol/L, the cell could lower the extracellular glucose concentration to approximately 0.02 mmol/L. However, by increasing the number of Na^+ ions transported with glucose from one to two, SGLT-1 can generate a nearly 10,000-fold glucose gradient:

$$\frac{[\text{Glucose}]_i}{[\text{Glucose}]_o} = \left(\frac{[Na^+]_o}{[Na^+]_i}\right)^2 \times 10^{-2V_m/61.5\,\text{mV}}$$

Again, if the intracellular glucose concentration is 2 mmol/L, SGLT-1 could remove virtually all glucose from either the lumen of the small intestine or the lumen of the proximal tubule (i.e., the luminal glucose concentration ≅ 0.0002 mmol/L).

where
n = number of dissociable particles per molecule
C = total solute concentration
R = gas constant
T = temperature in degrees Kelvin

For a molecule that does not dissociate in water, such as glucose or urea, a solution containing 1 mmol/L of these molecules at 37°C can exert an osmotic pressure of 2.54×10^{-2} atm, as calculated with eq. 1.7 and the following values:

n = 1
C = 0.001 mol/L
R = 0.082 atm L/mol K
T = 310 °K

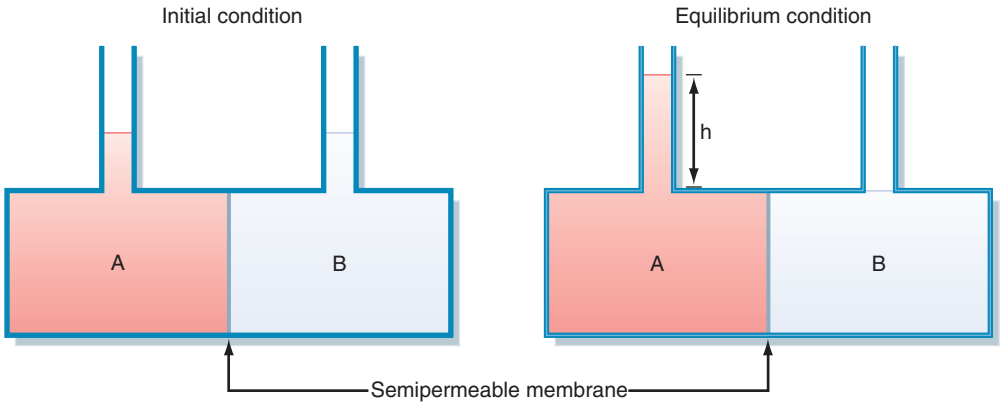


Fig. 1.8 Schematic representation of osmotic water movement and the generation of an osmotic pressure. Compartment A and compartment B are separated by a semipermeable membrane (i.e., the membrane is highly permeable by water but impermeable by solute). Compartment A contains a solute, whereas compartment B contains only distilled water. Over time, water moves by osmosis from compartment B to compartment A. (Note: This water movement is driven by the concentration gradient for water. Because of the presence of solute particles in compartment A, the concentration of water in compartment A is less than that in compartment B. Consequently, water moves across the semipermeable membrane from compartment B to compartment A down its concentration gradient.) This causes the level of fluid to be raised in compartment A and lowered in compartment B. At equilibrium, the hydrostatic pressure exerted by the column of water (h) stops the net movement of water from compartment B to A. Thus at equilibrium, the hydrostatic pressure is equal and opposite to the osmotic pressure exerted by the solute particles in compartment A. (Redrawn from Koeppen BM, Stanton BA. *Renal Physiology.* 4th ed. St. Louis: Mosby; 2006.)

Because 1 atm equals 760 mm Hg at sea level, π for this solution can also be expressed as 19.3 mm Hg. Alternatively, osmotic pressure is expressed in terms of osmolarity (see the following section). Regardless of the molecule, a solution containing 1 mmol/L of the molecule therefore exerts an osmotic pressure proportional to 1 mOsm/L.

For molecules that dissociate in a solution, n of eq. 1.7 will have a value other than 1. For example, a 150-mmol/L solution of NaCl has an osmolarity of approximately 300 mOsm/L because each molecule of NaCl dissociates into a Na^+ and a Cl^- ion (i.e., n = 2).[c] If dissociation of a molecule into its component ions is not complete, n will not be an integer. Accordingly, osmolarity for any solution can be calculated as follows:

Equation 1.8

$$\text{Osmolarity} = \text{concentration} \times \text{number of dissociable particles}$$

$$\text{mOsm/L} = \text{mmol/L} \times \text{number of particles/mole}$$

Osmolarity Versus Osmolality

The terms *osmolarity* and *osmolality* are frequently confused and incorrectly interchanged. *Osmolarity* refers to the osmotic pressure generated by the dissolved solute molecules in 1 L of solvent, whereas osmolality is the number of molecules dissolved in 1 kg of solvent. For a dilute solution, the difference between osmolarity and osmolality is insignificant. Measurements of osmolarity are temperature dependent because the volume of the solvent varies with temperature (i.e., the volume is larger at higher temperatures). In contrast, osmolality, which is based on the mass of the solvent, is temperature independent. For this reason, *osmolality* is the preferred term for biologic systems and is used throughout this book. Because the solvent in biological solutions and bodily fluids is water, and because of the dilute nature of biological solutions and bodily solutions, osmolalities are expressed as milliosmoles per kilogram of water (mOsm/kg H_2O).

Tonicity

The tonicity of a solution is related to the effect of the solution on the volume of a cell. Solutions that do not change the volume of a cell are said to be **isotonic.** A **hypotonic** solution causes a cell to swell, whereas a **hypertonic** solution causes a cell to shrink. Although related to osmolality, tonicity also accounts for the ability of the molecules in solution to cross the cell membrane.

Consider two solutions: a 300-mmol/L solution of sucrose and a 300-mmol/L solution of urea. Both solutions have an osmolality of 300 mOsm/kg H_2O and therefore are said to be **isosmotic** (i.e., they have the same osmolality). When red blood cells—which for the purpose of this illustration also have an intracellular fluid osmolality of 300 mOsm/kg H_2O—are placed in the two solutions, those in the sucrose solution maintain their normal volume, whereas those placed in urea swell and eventually burst. Thus the sucrose solution is isotonic and the urea solution is hypotonic. The differential effect of these solutions on

[c]NaCl does not completely dissociate in water. The value for n is 1.88 rather than 2. However, for simplicity, the value of 2 is most often used.

red blood cell volume is related to the permeability of the red blood cell plasma membrane to sucrose and urea. The red blood cell membrane contains uniporters for urea. Thus urea easily crosses the cell membrane (i.e., the cell is permeable by urea), driven by the concentration gradient (i.e., extracellular urea concentration > intracellular urea concentration). In contrast, the red blood cell membrane does not contain sucrose transporters, and sucrose cannot enter the cell (i.e., the cell is impermeable by sucrose).

To exert an osmotic pressure across a membrane, a molecule must not cross the membrane. Because the red blood cell membrane is impermeable by sucrose, it exerts an osmotic pressure equal and opposite to the osmotic pressure generated by the contents within the red blood cell (in this case, 300 mOsm/kg H_2O). In contrast, urea is readily able to cross the red blood cell membrane, and it cannot exert an osmotic pressure to balance that generated by the intracellular solutes of the red blood cell. Consequently, sucrose is termed an **effective osmole,** whereas urea is an **ineffective osmole.**

To take into account the effect of a molecule's ability to permeate the membrane on osmotic pressure, it is necessary to rewrite eq. 1.7 as follows:

Equation 1.9

$$\Pi_e = \sigma(nCRT),$$

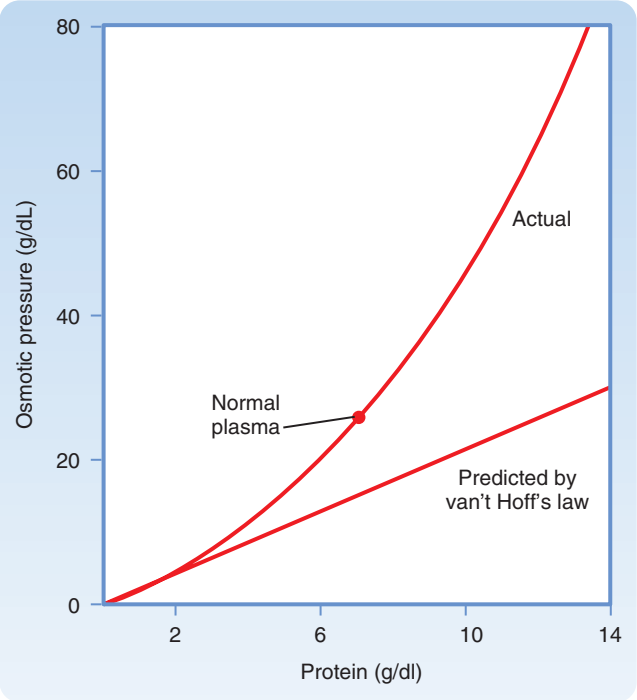
where σ is the **reflection coefficient** (or **osmotic coefficient**) and is a measure of the relative ability of the molecule to cross the cell membrane, and Π_e is the "effective osmotic pressure."

For a molecule that can freely cross the cell membrane, such as urea in the preceding example, $\sigma = 0$, and no effective osmotic pressure is exerted (e.g., urea is an ineffective osmole for red blood cells). In contrast, $\sigma = 1$ for a solute that cannot cross the cell membrane (in the preceding example, sucrose). Such a substance is said to be an effective osmole. Many molecules are neither completely able nor completely unable to cross cell membranes (i.e., $0 < \sigma < 1$) and generate an osmotic pressure that is only a fraction of what is expected from the molecules' concentration in solution.

Oncotic Pressure

Oncotic pressure is the osmotic pressure generated by large molecules (especially proteins) in solution. As illustrated in Fig. 1.9, the magnitude of the osmotic pressure generated by a solution of protein does not conform to van't Hoff's law. The cause of this anomalous relationship between protein concentration and osmotic pressure is not completely understood, but it appears to be related to the size and shape of the protein molecule. For example, the correlation to van't Hoff's law is more precise with small, globular proteins than with larger protein molecules.

The oncotic pressure exerted by proteins in human plasma has a normal value of approximately 26 to 28 mm Hg. Although this pressure appears to be small in relation to osmotic pressure (28 mm Hg ≅ 1.4 mOsm/kg H_2O), it is an important force involved in fluid movement across capillaries (see Chapter 17).



• **Fig. 1.9** Relationship between the concentration of plasma proteins in solution and the osmotic pressure (oncotic pressure) they generate. Protein concentration is expressed in grams per deciliter. Normal plasma protein concentration is indicated. Note how the actual pressure generated exceeds that predicted by van't Hoff's law.

Specific Gravity

The total concentration of all molecules in a solution can also be measured as specific gravity. Specific gravity is defined as the weight of a volume of solution divided by the weight of an equal volume of distilled water. Thus the specific gravity of distilled water is 1. Because biological fluids contain a number of different molecules, their specific gravities are greater than 1. For example, normal human plasma has a specific gravity in the range of 1.008 to 1.010.

IN THE CLINIC

The specific gravity of urine is sometimes measured in clinical settings and used to assess the urine concentrating ability of the kidneys. The specific gravity of urine varies in proportion to its osmolality. However, because specific gravity depends both on the number of molecules and on their weight, the relationship between specific gravity and osmolality is not always predictable. For example, in patients who have received an injection of radiocontrast dye (molecular weight > 500 g/mole) for x-ray studies, values of urine specific gravity can be high (1.040 to 1.050), even though the urine osmolality is similar to that of plasma (e.g., 300 mOsm/kg H_2O).

Key Points

- The plasma membrane is a lipid bilayer composed of phospholipids and cholesterol, into which are embedded a wide range of proteins. One class of these membrane proteins (membrane transport proteins or transporters) is involved in the selective and regulated transport of molecules into and out of the cell. These transporters include water channels (aquaporins), ion channels, solute carriers, and ATP-dependent transporters.
- The movement of molecules across the plasma membrane through ion channels and solute carriers is driven by chemical concentration gradients and, chemical concentration gradients and electrical potential differences (charged molecules only). The electrochemical gradient is used to quantitate this driving force. ATP-dependent transporters use the energy in ATP to transport molecules across the membrane and often establish the chemical and electrical gradients that then drive the transport of other molecules through channels and by the solute carriers. Water movement through aquaporins is driven by an osmotic pressure difference across the membrane.
- Transport across the membrane is classified as passive or active. Passive transport is the movement of molecules as expected from the electrochemical gradient for that molecule. Active transport represents transport against the electrochemical gradient. Active transport is further divided into primary active and secondary active transport. Primary active transport is directly coupled to the hydrolysis of ATP (e.g., ATP-dependent transporters). Secondary active transport occurs with coupled solute carriers, for which passive movement of one or more molecules drives the active transport of other molecules (e.g., Na^+-glucose symporter, Na^+-H^+ antiporter).

Additional Readings

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2

Homeostasis: Volume and Composition of Body Fluid Compartments

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. What is steady-state balance, and, with water balance as an example, what are the elements needed to achieve steady-state balance?
2. What are the volumes of the body fluid compartments, and how do they change under various conditions?
3. How do the body fluid compartments differ with regard to their composition?
4. What determines the resting membrane potential of cells?
5. How do cells regulate their volume in isotonic, hypotonic, and hypertonic solutions?
6. What are the structural features of epithelial cells, how do they carry out vectorial transport, and what are the general mechanisms by which transport is regulated?

Normal cellular function requires that the intracellular composition—with regard to ions, small molecules, water, pH, and a host of other substances—be maintained within a narrow range. This is accomplished by the transport of many substances and water into and out of the cell via membrane transport proteins, as described in Chapter 1. In addition, each day, food and water are ingested, and waste products are excreted from the body. In a healthy individual, these processes occur without significant changes in either the volume of the body fluid compartments or their composition. The maintenance of constant volume and composition of the body fluid compartments (and their temperature in warm-blooded animals and humans) is termed **homeostasis.** The human body has multiple systems designed to achieve homeostasis, the details of which are explained in the various chapters of this book. In this chapter, the basic principles that underlie the maintenance of homeostasis are outlined. In addition, the volume and composition of the various body fluid compartments are defined.

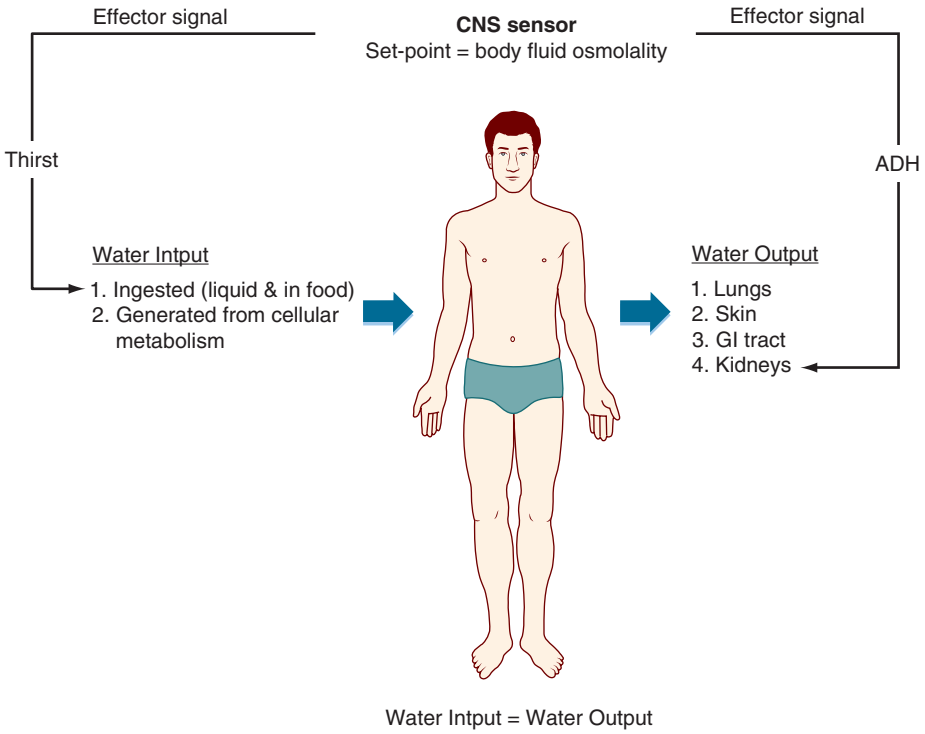
Concept of Steady-State Balance

The human body is an "open system," which means that substances are added to the body each day and, similarly, substances are lost from the body each day. The amounts added to or lost from the body can vary widely, depending on the environment, access to food and water, disease processes, and even cultural norms. In such an open system, homeostasis occurs through the process of **steady-state balance.**

To illustrate the concept of steady-state balance, consider a river on which a dam is built to create a synthetic lake. Each day, water enters the lake from the various streams and rivers that feed it. In addition, water is added by underground springs, rain, and snow. At the same time, water is lost through the spillways of the dam and by the process of evaporation. For the level of the lake to remain constant (i.e., steady-state balance), the rate at which water is added, regardless of source, must be exactly matched by the amount of water lost, again regardless of route. Because the addition of water is not easily controlled and the loss by evaporation cannot be controlled, the only way to maintain a constant level of the lake is to regulate the amount that is lost through the spillways.

To understand steady-state balance as it applies to the human body, the following key concepts are important.

1. There must be a "set point" so that deviations from this baseline can be monitored (e.g., the level of the lake in the preceding example, or setting the temperature in a room by adjusting the thermostat).
2. The sensor or sensors that monitor deviations from the set point must generate "effector signals" that can lead to changes in either input or output, or both, to maintain the desired set point (e.g., electrical signals to adjust the spillway in the dam analogy, or electrical signals sent to either the furnace or air conditioner to maintain the proper room temperature).
3. "Effector organs" must respond in an appropriate way to the effector signals generated by the set point monitor (i.e., the spillway gates must operate, and the furnace or air conditioner must turn on).
4. The sensitivity of the system (i.e., how much of a deviation from the set point is tolerated) depends on several factors, including the nature of the sensor (i.e., how much of a deviation from the set point is needed for the sensor to detect the deviation), the time necessary for generation of the effector signals, and how rapidly the effector organs respond to the effector signals.



• **Fig. 2.1** Whole-Body Steady-State Water Balance. See text for details. ADH, antidiuretic hormone (also called arginine vasopressin); CNS, central nervous system; GI, gastrointestinal.

It is important to recognize that deviations from steady-state balance do occur. When input is greater than output, a state of **positive balance** exists. When input is less than output, a state of **negative balance** exists. Although transient periods of imbalance can be tolerated, prolonged states of positive or negative balance are generally incompatible with life.

Fig. 2.1 illustrates several important concepts for the maintenance of steady-state water balance (details related to the maintenance of steady-state water balance are presented in Chapter 35). As depicted in Fig. 2.1, there are multiple inputs and outputs of water, many of which can vary but nevertheless cannot be regulated. For example, the amount of water lost through the lungs depends on the humidity of the air and the rate of respiration (e.g., low humidity and rapid breathing increase water loss from the lungs). Similarly, the amount of water lost as sweat varies according to ambient temperature and physical activity. Finally, water loss via the gastrointestinal tract can increase from a normal level of 100 to 200 mL/day to many liters with acute diarrhea. Of these inputs and outputs, the only two that can be regulated are increased ingestion of water in response to thirst and alterations in urine output by the kidneys (see Chapter 35).

Water balance determines the osmolality of the body fluids. Cells within the hypothalamus of the brain monitor body fluid osmolality for deviations from the set point (normal range: 280-295 mOsm/kg H_2O). When deviations are sensed, two effector signals are generated. One is neural and relates to the individual's sensation of thirst. The other is hormonal (antidiuretic hormone, also called *arginine vasopressin*), which regulates the amount of water excreted by the kidneys. With appropriate responses to these two signals, water input, water output, or both are adjusted to maintain balance and thereby keep body fluid osmolality at the set point.

Volumes and Composition of Body Fluid Compartments

Unicellular organisms maintain their volume and composition through exchanges with the environment they inhabit (e.g., sea water). The billions of cells that constitute the human body must maintain their volume and composition as well, but their task is much more difficult. This challenge, as well as its solution, was first articulated by the French physiologist Claude Bernard (1813-1878). He recognized that although cells within the body cannot maintain their volume and composition through exchanges with the environment, they can do so through exchanges with the fluid environment that surrounds them (i.e., the extracellular fluid). Bernard referred to the extracellular fluid as the *milieu intérieur* ("the environment within"). He also recognized that the organ systems of the body are designed and function to maintain a constant milieu interieur or a "constant internal environment." This in turn allows all cells to maintain their volume and composition through exchanges with the extracellular fluid as a result of membrane transport (see Chapter 1).

Transport by the epithelial cells of the gastrointestinal tract, kidneys, and lungs are the body's interface with the

external environment and control both the intake and excretion of numerous substances, as well as water. The cardiovascular system delivers nutrients to and removes waste products from the cells and tissues and keeps the extracellular fluid well mixed. Finally, the nervous and endocrine systems provide regulation and integration of these important functions.

To provide background for the study of all organ systems, this chapter presents an overview of the normal volume and composition of the body fluid compartments and describes how cells maintain their intracellular composition and volume. Included is a presentation on how cells generate and maintain a membrane potential, which is fundamental for understanding the function of excitable cells (e.g., neurons and muscle cells). Finally, because epithelial cells are so central to the process of regulating the volume and composition of the body fluids, the principles of solute and water transport by epithelial cells are also reviewed.

Definition and Volumes of Body Fluid Compartments

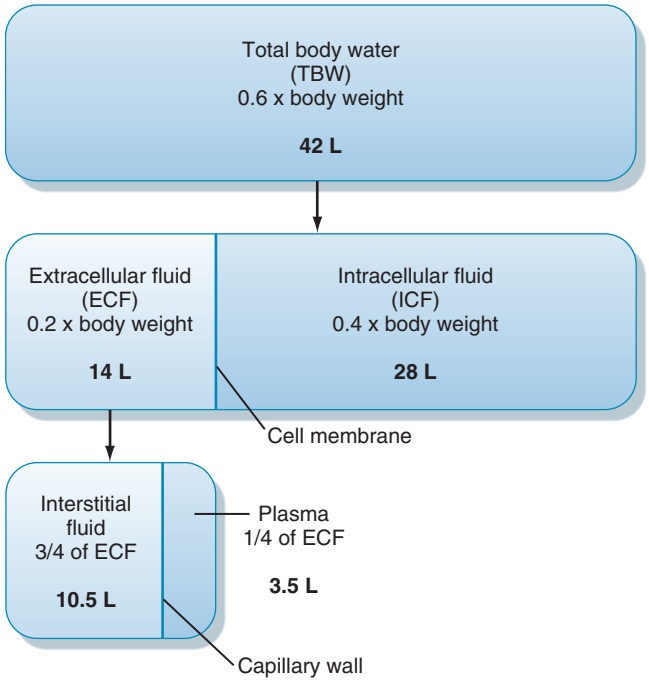
Water makes up approximately 60% of the body's weight; variability among individuals is a function of the amount of adipose tissue. Because the water content of adipose tissue is lower than that of other tissue, increased amounts of adipose tissue reduce the fraction of water in the total body weight. The percentage of body weight attributed to water also varies with age. In newborns, it is approximately 75%. This decreases to the adult value of 60% by the age of 1 year.

As illustrated in Fig. 2.2, **total body water** is distributed between two major compartments, which are divided by the cell membrane.[a] The **intracellular fluid (ICF)** compartment is the larger compartment, and contains approximately two thirds of the total body water. The remaining third is contained in the **extracellular fluid (ECF)** compartment. Expressed as percentages of body weight, the volumes of total body water, ICF, and ECF are as follows:

$$\text{Total body water} = 0.6 \times (\text{body weight})$$
$$\text{ICF} = 0.4 \times (\text{body weight})$$
$$\text{ECF} = 0.2 \times (\text{body weight})$$

The ECF compartment is further subdivided into interstitial fluid and plasma. The ECF also includes fluid contained within bone and dense connective tissue, as well as the cerebrospinal fluid. The interstitial fluid surrounds the cells in the various tissues of the body and makes up three fourths of the ECF volume. Plasma is contained within the vascular compartment and represents the remaining fourth of the ECF. In some pathological conditions, additional fluid may accumulate in what is referred to as a *third space.* Third-space collections of fluid are part of the ECF; an example is the accumulation of fluid in the peritoneal cavity **(ascites)** of individuals with liver disease.

[a]In these and all subsequent calculations, it is assumed that 1 L of fluid (e.g., ICF and ECF) has a mass of 1 kg. Although, 1 L of the ICF and ECF has a mass of slightly more than 1 kg, this simplification allows conversion from measurements of body weight to volume of body fluids.



• **Fig. 2.2** Relationship Between the Volumes of the Various Body Fluid Compartments. The actual values shown are for an individual weighing 70 kg. (Modified from Levy MN, Koeppen BM, Stanton BA. *Berne & Levy's Principles of Physiology*. 4th ed. St. Louis: Mosby; 2006.)

Movement of Water Between Body Fluid Compartments

As depicted in Fig. 2.2, water moves between the ICF and ECF compartments across the plasma membranes of cells, and it moves between the vascular (plasma) and interstitial compartments across capillary walls. The pathways and driving forces for this water movement are different across cell membranes, in comparison to the capillary walls.

Movement of water between the ICF and ECF compartments, across cell membranes, occurs through aquaporins expressed in the plasma membrane (see Chapter 1). The driving force for this water movement is an osmotic pressure difference. The osmotic pressure of both the ICF and ECF is determined by the molecules/ions present in these fluids. For simplicity, these can be divided into (1) molecules of low molecular weight (e.g., glucose) and ions (e.g., Na^+) and (2) macromolecules (e.g., proteins). The osmotic pressures of both the ICF and ECF are in the range of 280 to 295 mOsm/kg H_2O. For the ECF, the low-molecular-weight molecules and ions account for nearly all of this pressure because the osmotic pressure contributed by proteins is only 1 to 2 mOsm/kg H_2O. The molecules/ions contributing to the osmotic pressure within the cell are less well understood, but they also include low-molecular-weight molecules (e.g., glucose), ions (e.g., Na^+), and macromolecules (e.g., proteins). The fact that cell

volume remains constant when ECF osmolality is constant means that the osmotic pressure inside the cells is equal to that of the ECF. If an osmotic pressure difference did exist, the cells would either swell or shrink, as described in the section "Nonisotonic Cell Volume Regulation."

Movement of water between the vascular (plasma) compartment and the interstitial fluid compartment occurs across the capillary wall. The amount of water that moves across the capillary wall, and the mechanism of the water movement varies depending on the capillary. For example, in the capillary sinusoids of the liver, endothelial cells are often separated by large gaps (discontinuous capillary). As a result, water and all components of the plasma (and some cellular elements) can pass easily across the wall. Other capillaries are lined by endothelial cells that contain fenestrations that are up to 80 to 100 nm in diameter (e.g., in the kidneys). These fenestrations allow all components of the plasma (only cellular elements of blood cannot pass through the fenestrations) to move across the capillary wall. Some capillaries (e.g., in the brain) form a relatively tight barrier to water and small molecules and ions, and water movement occurs through small pores on the endothelial cell surface or through clefts between adjacent endothelial cells. These pores and clefts allow water and molecules smaller than 4 nm to pass. In addition, a small amount of water traverses the capillary wall via pinocytosis by endothelial cells.

The driving forces for fluid (water) movement across the capillary wall are hydrostatic pressure and oncotic pressure (i.e., osmotic pressure generated by proteins). Collectively, these are called the *Starling forces*. Capillary fluid movement is discussed in detail in Chapter 17; in brief, hydrostatic pressure within the capillary (as a result of the pumping of the heart and the effect of gravity on the column of blood in the vessels feeding a capillary) is a force that causes fluid to move out of the capillary. Hydrostatic pressure in the surrounding interstitial tissue opposes the effect of the capillary hydrostatic pressure. The oncotic pressure of the plasma in the capillary tends to draw fluid from the interstitium into the capillary. The oncotic pressure of the interstitial fluid opposes this. Thus the amount of fluid moving across the wall of the capillary is determined as follows:

Equation 2.1

$$\text{Fluid flow}\,(Q_f) = K_f\,[(P_c - P_i) - (\pi_c - \pi_i)]$$

or

$$\text{Fluid flow}\,(Q_f) = K_f\,[(P_c + \pi_i) - (P_i + \pi_c)]$$

where

Q_f = fluid movement
K_f = filtration constant (measure of surface area + intrinsic permeability)
P_c = capillary hydrostatic pressure
P_i = interstitial fluid hydrostatic pressure
π_c = plasma oncotic pressure
π_i = interstitial fluid oncotic pressure.

Depending on the magnitude of these forces, fluid may move out of the capillary or into the capillary.

The compositions of the various body fluid compartments differ; however, as described later, the osmolalities of the fluid within these compartments are essentially identical.[b] Thus the compartments are in "osmotic equilibrium." In addition, any change in the osmolality of one compartment quickly causes water to redistribute across all compartments, which brings them back into osmotic equilibrium. Because of this rapid redistribution of water, measuring the osmolality of plasma or serum,[c] which is easy to do, reveals the osmolality of the other body fluid compartments (i.e., interstitial fluid and intracellular fluid).

As described later, Na^+ is a major constituent of the ECF. Because of its high concentration in comparison with other molecules and ions, Na^+ (and its attendant anions, primarily Cl^- and HCO_3^-) is the major determinant of the osmolality of this compartment. Accordingly, it is possible to obtain an approximate estimate of the ECF osmolality by simply doubling the sodium concentration $[Na^+]$. For example, if a blood sample is obtained from an individual, and the $[Na^+]$ of the serum is 145 mEq/L, its osmolality can be estimated as follows:

Equation 2.2

$$\text{Plasma Osmolality} = 2(\text{serum}\,[Na^+]) = 290\ \text{mOsm/kg}\ H_2O$$

In contrast to water, the movement of ions across cell membranes is more variable from cell to cell and depends on the presence of specific membrane transport proteins (see the section "Composition of Body Fluid Compartments"). Consequently, in trying to understand the physiology of fluid shifts between body fluid compartments, it can be assumed that while water moves freely between the compartments, there is little net movement of solutes. For most situations, this is a reasonable assumption.

To illustrate the physiologic characteristics of fluid shifts, consider what happens when solutions containing various amounts of NaCl are added to the ECF.[d]

Example 1: Addition of Isotonic Sodium Chloride to the Extracellular Fluid

Addition of an isotonic NaCl solution (e.g., intravenous infusion of 0.9% NaCl: osmolality ≈ 290 mOsm/kg H_2O)[e]

[b]Some exceptions do exist. The cerebrospinal fluid is part of the ECF, but its osmolality is slightly higher than that of the ECF elsewhere in the body. Also, regions within the kidney can have osmolalities that are either less than or greater than that of the ECF. However, these volumes are small (≈150 mL) in comparison with the total volume of the ECF (≥12 L).

[c]Serum is derived from clotted blood. Thus serum differs from plasma by the absence of clotting factors. With regard to osmolality and the concentrations of other molecules and ions, the osmolality and concentrations in plasma and serum are virtually identical.

[d]Fluids are usually administered intravenously. When electrolyte solutions are infused by this route, equilibration between plasma and interstitial fluid is rapid (i.e., minutes) because of the high permeability of many capillary walls for water and electrolytes. Thus these fluids are essentially added to the entire ECF.

[e]A 0.9% NaCl solution (0.9 g NaCl/100 mL) contains 154 mmol/L of NaCl. Because NaCl does not dissociate completely in solution (i.e., 1.88 Osm/mol), the osmolality of this solution is 290 mOsm/kg H_2O, which is very similar to that of normal ECF.

IN THE CLINIC

In some clinical situations, it is possible to obtain a more accurate estimate of the serum osmolality, and thus the osmolalities of the ECF and ICF, by also considering the osmoles contributed by glucose and urea, as these are the next most abundant solutes in the ECF (the other components of the ECF contribute only a few additional milliosmoles). Accordingly, serum osmolality can be estimated as follows:

$$\text{Serum osmolality} = 2(\text{serum } [Na^+]) + \frac{[\text{glucose}]}{18} + \frac{[\text{urea}]}{2.8}$$

The glucose and urea concentrations are expressed in units of milligrams per deciliter (dividing by 18 for glucose and 2.8 for urea* allows conversion from the units of milligrams per deciliter to millimoles per liter and thus to milliosmoles per kilogram of H_2O). This estimation of serum osmolality is especially useful in treating patients who have an elevated serum glucose concentration secondary to diabetes mellitus, and in patients with chronic renal failure, whose serum urea concentration is elevated because of reduced renal excretion.

As discussed in Chapter 1, the ability of a substance to cause water to move across the plasma membrane of a cell depends on whether the substance itself crosses the membrane. Recall Eq. 1.9:

$$\Pi_e = \sigma(nCRT)$$

where Π_e = the effective osmotic pressure and σ = the reflection coefficient for the substance. For many cells, glucose and urea cross the cell membrane. Although they contribute to serum osmolality, as measured by a laboratory osmometer where all molecules are "effective osmoles," they are ineffective osmoles for water movement across many, but not all, cell membranes. In contrast, Na^+ is an "effective osmole" for water movement across the plasma membrane of virtually all cells. Eq. 2.2 gives the best estimate of the effective osmolality of the serum.

*The urea concentration in plasma is measured as the nitrogen in the urea molecule, or blood urea nitrogen (BUN).

IN THE CLINIC

Neurosurgical procedures and cerebrovascular accidents (strokes) often result in the accumulation of interstitial fluid in the brain (i.e., edema) and swelling of the neurons. Because the brain is enclosed within the skull, edema can raise intracranial pressure and thereby disrupt neuronal function, which leads to coma and death. The blood-brain barrier, which separates the cerebrospinal fluid and brain interstitial fluid from blood, can be permeated freely by water but not by most other substances. As a result, excess fluid in brain tissue can be removed by imposing an osmotic gradient across the blood-brain barrier. Mannitol can be used for this purpose. Mannitol is a sugar (molecular weight, 182 g/mol) that does not readily cross the blood-brain barrier and membranes of cells (neurons and other cells in the body). Therefore, mannitol is an effective osmole, and intravenous infusion results in the movement of interstitial fluid out of the brain by osmosis.

to the ECF increases the volume of this compartment by the volume of fluid administered. Because this fluid has the same osmolality as does the ECF, and therefore the ICF, there is no driving force for fluid movement between these compartments, and the volume of the ICF remains unchanged. Although Na^+ can cross cell membranes, it is effectively restricted to the ECF by the activity of the Na^+,K^+-ATPase, which is present in the plasma membrane of all cells (see the section "Ionic Composition of Cells"). Therefore, there is no net movement of the infused isotonic NaCl solution into cells.

Example 2: Addition of Hypotonic Sodium Chloride to the Extracellular Fluid

Addition of a hypotonic NaCl solution to the ECF (e.g., intravenous infusion of 0.45% NaCl; osmolality ≅ 145 mOsm/kg H_2O) decreases the osmolality of this fluid compartment, which results in the movement of water into the ICF. After osmotic equilibration, the osmolalities of the ICF and ECF are again equal but lower than before the infusion, and the volume of each compartment is increased. The increase in ECF volume is greater than the increase in ICF volume.

Example 3: Addition of Hypertonic Sodium Chloride to the Extracellular Fluid

Addition of a hypertonic NaCl solution to the ECF (e.g., intravenous infusion of 3% NaCl: osmolality ≅ 1000 mOsm/kg H_2O) increases the osmolality of this compartment, which results in the movement of water out of cells. After osmotic equilibration, the osmolalities of the ECF and ICF are again equal but higher than before the infusion. The volume of the ECF is increased, whereas that of the ICF is decreased.

Composition of Body Fluid Compartments

The compositions of the ECF and ICF differ considerably. The ICF has significantly more proteins and macromolecules than the ECF. There are also differences in the concentrations of many ions. The composition of the ICF is maintained by the action of a number of specific cell membrane transport proteins. Principal among these transporters is the Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase), which converts the energy in ATP into ion and electrical gradients, which can in turn be used to drive the transport of other ions and molecules by means of ion channels and solute carriers (e.g., symporters and antiporters).

The compositions of the plasma and interstitial fluid compartments of the ECF are similar because those compartments are separated only by the capillary endothelium, a barrier that ions and small molecules can permeate. The major difference between the interstitial fluid and plasma is that the latter contains significantly more protein. Although this differential concentration of protein can affect the

IN THE CLINIC

Fluid and electrolyte disorders are observed commonly in clinical practice (e.g., in patients with vomiting or diarrhea, or both). In most instances, these disorders are self-limited, and correction of the disorder occurs without need for intervention. However, more severe or prolonged disorders may necessitate fluid replacement therapy. Such therapy may be administered orally, with special electrolyte solutions, or intravenously, with fluid.

Intravenous solutions are available in many formulations. The type of fluid administered to a particular patient is dictated by the patient's need. For example, if an increase in the patient's vascular volume is necessary, a solution containing substances that do not readily cross the capillary wall is infused (e.g., 5% protein or dextran solutions). The oncotic pressure generated by the albumin molecules causes fluid to be retained in the vascular compartment, which expands its volume. Expansion of the ECF is accomplished most often with isotonic saline solutions (e.g., 0.9% NaCl or lactated Ringer solution). As already noted, administration of an isotonic NaCl solution does not result in the development of an osmotic pressure gradient across the plasma membrane of cells. Therefore, the entire volume of the infused solution remains in the ECF.

Patients whose body fluids are hyperosmotic need hypotonic solutions. These solutions may be hypotonic NaCl (e.g., 0.45% NaCl) or 5% dextrose in water (D_5W). Administration of the D_5W solution is equivalent to the infusion of distilled water because the dextrose is metabolized to CO_2 and water. Administration of these fluids increases the volumes of both the ICF and ECF. In addition, patients whose body fluids are hypotonic need hypertonic solutions. These are typically NaCl-containing solutions (e.g., 3% or 5% NaCl). These solutions expand the volume of the ECF but decrease the volume of the ICF. Other constituents, such as electrolytes (e.g., K^+) or drugs, can be added to intravenous solutions to tailor the therapy to the patient's fluid, electrolyte, and metabolic needs.

distribution of cations and anions between these two compartments by the Gibbs-Donnan effect (see the section "Isotonic Cell Volume Regulation" for details), this effect is small, and the ionic compositions of the interstitial fluid and plasma can be considered to be identical.

Maintenance of Cellular Homeostasis

Normal cellular function requires that the ionic composition of the ICF be tightly controlled. For example, the activity of some enzymes is pH dependent; therefore, intracellular pH must be regulated. In addition, the intracellular composition of other electrolytes is similarly held within a narrow range. This is necessary for the establishment of the membrane potential, a cell property especially important for the normal function of excitable cells (e.g., neurons and muscle cells) and for intracellular signaling (e.g., intracellular $[Ca^{++}]$; see Chapter 3 for details). Finally, the volume of cells must be maintained because shrinking or swelling of cells can lead to cell damage or death. The regulation of intracellular composition and cell volume is accomplished through the activity of specific transporters in the plasma membrane of the cells. This section is a review of the mechanisms by which cells maintain their intracellular ionic environment and their membrane potential and by which they control their volume.

TABLE 2.1 Ionic Composition of a Typical Cell

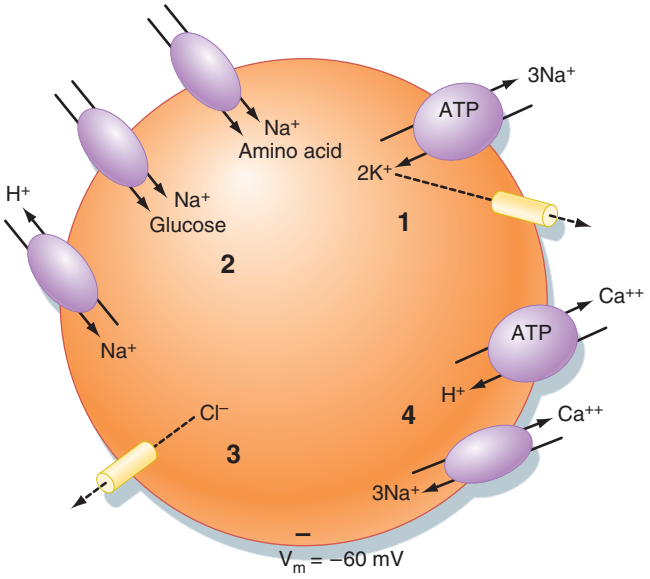
Ion	Extracellular Fluid	Intracellular Fluid
Na^+	135-147 mEq/L	10-15 mEq/L
K^+	3.5-5.0 mEq/L	120-150 mEq/L
Cl^-	95-105 mEq/L	20-30 mEq/L
HCO_3^-	22-28 mEq/L	12-16 mEq/L
*Ca^{++}	2.1-2.8 (total) mmol/L	
	1.1-1.4 (ionized) mmol/L	≈10^{-7} M (ionized) mmol/L
*Pi	1.0-1.4 (total) mmol/L	
	0.5-0.7 (ionized) mmol/L	0.5-0.7 (ionized) mmol/L

*Ca^{++} and Pi ($H_2PO_4^-/HPO_4^{-2}$) are bound to proteins and other organic molecules. In addition, large amounts of Ca^{++} can be sequestered within cells. Large amounts of Pi are present in cells as part of organic molecules, such as adenosine triphosphate (ATP).

Ionic Composition of Cells

The intracellular ionic composition of cells varies from tissue to tissue. For example, the intracellular composition of neurons is different from that of muscle cells, both of which differ from that of blood cells. Nevertheless, there are similar patterns, and these are presented in Table 2-1. In comparison with the ECF, the ICF is characterized by a low $[Na^+]$ and a high $[K^+]$. This is the result of the activity of the Na^+,K^+-ATPase, which transports 3 Na^+ ions out of the cell and 2 K^+ ions into the cell for each ATP molecule hydrolyzed. As discussed later in this chapter, the activity of the Na^+,K^+-ATPase not only is important for establishing the cellular Na^+ and K^+ gradients but also is involved in determining, indirectly, the cellular gradients for many other ions and molecules. Of importance is that the cellular K^+ gradient generated by the activity of the Na^+,K^+-ATPase is a major determinant of the membrane voltage because of the leak of K^+ out of the cell through K^+-selective channels (see the section "Membrane Potential"). Thus the Na^+,K^+-ATPase converts the energy in ATP into ion gradients (i.e., Na^+ and K^+), and a voltage gradient (i.e., membrane voltage).

The Na^+,K^+-ATPase–generated ion and electrical gradients are used to drive the transport of other ions and molecules into or out of the cell (Fig. 2.3). For example, as described in Chapter 1, a number of solute carriers couple the transport of Na^+ to that of other ions or molecules. The Na^+-glucose and Na^+–amino acid symporters use the energy in the Na^+ electrochemical gradient, directed to bring Na^+



• **Fig. 2.3** Cell Model Depicting How Cellular Gradients and the Membrane Potential (V_m) Are Established. (1) The Na^+,K^+-ATPase decreases the intracellular [Na^+] and increases the intracellular [K^+]. Some K^+ exits the cell via K^+-selective channels and generates the V_m (cell's interior is electrically negative). (2) The energy in the Na^+ electrochemical gradient drives the transport of other ions and molecules through the use of various solute carriers. (3) The V_m drives Cl^- out of the cell via Cl^--selective channels. (4) The Ca^{++}-ATPase and the $3Na^+$-Ca^{++} antiporters maintain the low intracellular [Ca^{++}].

into the cell, to drive the secondary active cellular uptake of glucose and amino acids. Similarly, the inwardly directed Na^+ gradient drives the secondary active extrusion of H^+ from the cell and thus contributes to the maintenance of intracellular pH. The $3Na^+$-Ca^{++} antiporter, along with the plasma membrane Ca^{++}-ATPase, extrudes Ca^{++} from the cell and thus contributes to the maintenance of a low intracellular [Ca^{++}].[f] In addition, the membrane voltage drives Cl^- out of the cell through Cl^--selective channels, thus lowering the intracellular concentration below that of the ECF.

Membrane Potential

As described previously, the Na^+,K^+-ATPase and K^+-selective channels in the plasma membrane are important determinants of the membrane potential (V_m) of the cell. For all cells within the body, the resting V_m is oriented with the interior of the cell electrically negative in relation to the ECF. However, the magnitude of the V_m can vary widely.

To understand what determines the magnitude of the V_m, it is important to recognize that any transporter that transfers charge across the membrane has the potential to influence the V_m. Such transporters are said to be **electrogenic.** As might be expected, the contribution of various electrogenic transporters to the V_m is highly variable from cell to cell. For example, the Na^+,K^+-ATPase channel transports three Na^+ and two K^+ ions and thus transfers one net positive charge across the membrane. However, the direct contribution of the Na^+,K^+-ATPase to the V_m of most cells is only a few millivolts at the most. Similarly, the contribution of other electrogenic transporters, such as the $3Na^+$-Ca^{++} antiporter and the Na^+-glucose symporter is minimal. The major determinants of the V_m are ion channels. The type (e.g., selectivity), number, and activity (e.g., gating) of these channels determine the magnitude of the V_m. As described in Chapter 5, rapid changes in ion channel activity underlies the action potential in neurons and other excitable cells, such as those of skeletal and cardiac muscle (see Chapters 12 and 13).

As ions move across the membrane through a channel, they generate a current. As described in Chapter 1, this current can be measured, even at the level of a single channel. By convention, the current generated by the movement of cations into the cell, or the movement of anions out of the cell, is defined as negative current. Conversely, the movement of cations out of the cell, or the movement of anions into the cell, is defined as positive current. Also by convention, the magnitude of the V_m is expressed in relation to the outside of the cell; thus for a cell with a V_m of −80 mV, the interior of the cell is electrically negative in relation to the outside of the cell.

The current carried by ions moving through a channel depends on the driving force for that ion and on the conductance of the channel. As described in Chapter 1, the driving force is determined by the energy in the concentration gradient for the ion across the membrane (E_i), as calculated by the Nernst equation (Eq. 1.5a) and the V_m:

Equation 2.3

$$\text{Driving force} = V_m - E_i.$$

Thus as defined by **Ohm's law,** the ion current through the channel (I_i) is determined as follows:

Equation 2.4

$$I_i = (V_m - E_i) \times g_i$$

where g_i is the conductance of the channel. For a cell, the conductance of the membrane to a particular ion (G_i) is determined by the number of ion channels in the membrane and by the amount of time each channel is in the open state.

As illustrated in Fig. 2.4, the V_m is the voltage at which there is no net ion flow into or out of the cell. Thus for a cell that has ion channels selective for Na^+, K^+, and Cl^-,

Equation 2.5

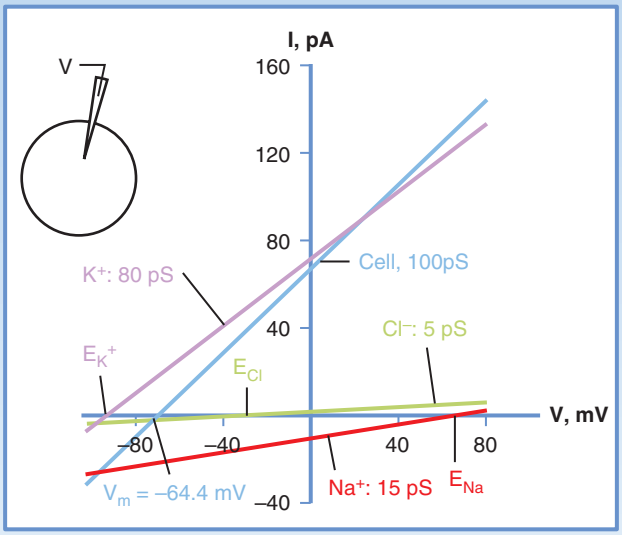
$$I_{Na^+} + I_{K^+} + I_{Cl^-} = 0$$

or

Equation 2.6

$$[(V_m - E_{Na^+}) \times G_{Na^+}] + [(V_m - E_{K^+}) \times G_{K^+}] + [(V_m - E_{Cl^-}) \times G_{Cl^-}] = 0.$$

[f] In muscle cells, in which contraction is regulated by the intracellular [Ca^{++}], the maintenance of a low intracellular [Ca^{++}] during the relaxed state involves not only the activity of the plasma membrane $3Na^+$-Ca^{++} antiporter and the Ca^{++}-ATPase but also a Ca^{++}-ATPase molecule located in the smooth endoplasmic reticulum (see Chapters 12 to 14).



	Intracellular [ ] mEq/L	Extracellular [ ] mEq/L	Nernst potential E_i, mV
Na^+	12	145	66.6
K^+	120	4	–90.8
Cl^-	30	105	–33.5

• **Fig. 2.4** Current-Voltage Relationship of a Hypothetical Cell Containing Na^+-, K^+-, and Cl^--Selective Channels. Membrane currents are plotted over a range of membrane voltages (i.e., current-voltage relationships). Each ion current is calculated with the use of Ohm's law, the Nernst equilibrium potential for the ion (E_{Cl}, E_K, and E_{Na}), and the membrane conductance for the ion. The current-voltage relationship for the whole cell is also shown. Total cell current (I_{cell}) was calculated with the chord conductance equation (see Eq. 2.7). Because 80% of cell conductance is due to K^+, the resting membrane voltage (V_m) of –64.4 mV is near to that of the Nernst equilibrium potential for K^+.

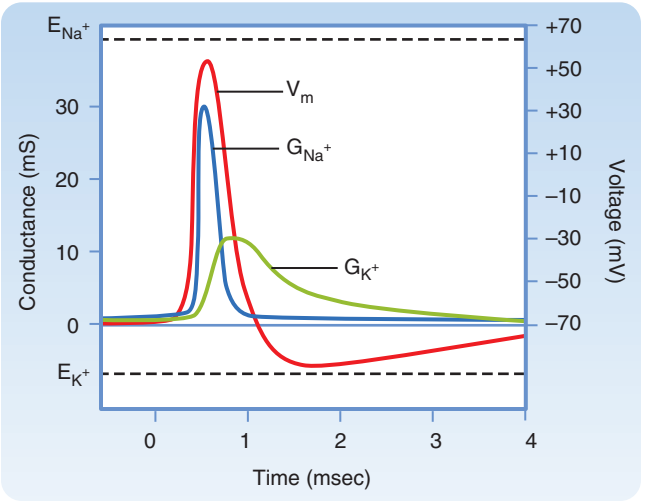
Solving for V_m yields

Equation 2.7

$$V_m = E_{Na^+}\frac{G_{Na^+}}{\Sigma G} + E_{K^+}\frac{G_{K^+}}{\Sigma G} + E_{Cl^-}\frac{G_{Cl^-}}{\Sigma G}$$

where $\Sigma G = G_{Na^+} + G_{K^+} + G_{Cl^-}$.

Inspection of Eq. 2.7, which is often called the **chord conductance equation,** reveals that the V_m will be near to the Nernst equilibrium potential of the ion to which the membrane has the highest conductance. In Fig. 2.4, 80% of the membrane conductance is attributable to K^+; as a result, V_m is near to the Nernst equilibrium potential for K^+ (E_{K^+}). For most cells at rest, the membrane has a high conductance to K^+, and thus the V_m approximates E_{K^+}. Moreover, the V_m is greatly influenced by the magnitude of E_{K^+}, which in turn is greatly influenced by changes in the $[K^+]$ of the ECF. For example, if the intracellular $[K^+]$ is 120 mEq/L and the extracellular $[K^+]$ is 4 mEq/L, E_{K^+} has a value of –90.8 mV. If the extracellular $[K^+]$ is increased to 7 mEq/L, E_{K^+} would be –79.9 mV. This change in E_{K^+} **depolarizes** the V_m (i.e., V_m is less negative). Conversely, if the extracellular $[K^+]$ is decreased to 2 mEq/L, E_{K^+} becomes –109.4 mV, and the V_m **hyperpolarizes** (i.e., V_m is more negative).



• **Fig. 2.5** Nerve Action Potential Showing the Changes in Na^+ and K^+ Conductances (G_{Na^+} and G_{K^+}, Respectively) and the Membrane Potential (V_m). At rest, the membrane has a high K^+ conductance, and V_m is near the Nernst equilibrium potential for K^+ (E_{K^+}). With the initiation of the action potential, there is a large increase in the Na^+ conductance of the membrane, and the V_m approaches the Nernst equilibrium potential for Na^+ (E_{Na^+}). The increase in Na^+ conductance is transient, and the K^+ conductance then increases above its value before the action potential. This hyperpolarizes the cell as V_m approaches E_{K^+}. As the K^+ conductance returns to its baseline value, V_m returns to its resting value of –70 mV. (Modified from Levy MN, Koeppen BM, Stanton BA. *Berne & Levy's Principles of Physiology.* 4th ed. St. Louis: Mosby; 2006.)

IN THE CLINIC

Changes in the extracellular $[K^+]$ can have important effects on excitable cells, especially those of the heart. A decrease in extracellular $[K^+]$ **(hypokalemia)** hyperpolarizes the V_m of cardiac myocytes and, in so doing, makes initiating an action potential more difficult, because a larger depolarizing current is needed to reach threshold (see Chapter 16). If severe, hypokalemia can lead to cardiac arrhythmias, and eventually the heart can stop contracting **(asystole).** An increase in the extracellular $[K^+]$ **(hyperkalemia)** can be equally deleterious to cardiac function. With hyperkalemia, the V_m is depolarized, and it is easier to initiate an action potential. However, once the action potential fires the channels become inactivated, and are unable to initiate another action potential, until they are reactivated by normal repolarization of the V_m. Because the V_m is depolarized in hyperkalemia, the channels stay in an inactivated state. Thus depolarization of the V_m with hyperkalemia can lead to cardiac arrhythmias and loss of cardiac muscle contraction.

Eq. 2.7 also defines the limits for the membrane potential. In the example depicted in Fig. 2.4, it is apparent that the V_m cannot be more negative than E_{K^+} (–90.8 mV), as would be the case if the membrane were only conductive to K^+. Conversely, the V_m could not be more positive than E_{Na^+} (66.6 mV); such a condition would be met if the membrane were conductive only to Na^+. The dependence of the V_m on the conductance of the membrane to specific ions is the basis by which action potentials in excitable cells are generated (Fig. 2.5). As noted previously, in all excitable cells, the

membrane at rest is conductive predominantly to K^+, and thus V_m is near E_{K^+}. When an action potential is initiated, Na^+-channels open and the membrane is now conductive predominantly to Na^+. As a result, V_m now approaches E_{Na^+}. The generation of action potentials is discussed in more detail in Chapter 5.

AT THE CELLULAR LEVEL

The establishment of the V_m requires the separation of charge across the plasma membrane. However, the number of ions that must move across the membrane is a tiny fraction of the total number of ions in the cell. For example, consider a spherical cell with a diameter of 20 μm and a V_m of –80 mV. Furthermore, assume that this V_m of –80 mV is the result of the diffusion of K^+ out of the cell and that the intracellular [K^+] is 120 mmol/L. The amount of K^+ that would have to diffuse out of the cell to establish the V_m of –80 mV is then calculated as follows.

First the charge separation across the membrane needs to be calculated. This is done with the knowledge that the plasma membrane behaves electrically like a capacitor, the capacitance (C) of which is approximately 1 μF/cm^2, and

$$C = \frac{Q}{V}$$

where Q = charge and is expressed in units of coulombs. If the surface area of the cell is $4\pi r^2$ or 1.26×10^{-5} cm^2, the capacitance of the cell is calculated as follows:

$$1\times10^{-6}\ \text{F/cm}^2 \times 1.26\times10^{-5}\ \text{cm}^2 = 1.26\times10^{-11}\ \text{F}.$$

Thus the charge separation across the membrane is calculated as follows:

$$Q = C\times V_m = 1.26\times10^{-11}\ \text{F}\times 0.08\ \text{volts}$$
$$= 1.01\times10^{-12}\ \text{coulombs}.$$

Because 1 mole of K^+ contains 96,480 coulombs, the amount of K^+ that had to diffuse across the membrane to establish the V_m of –80 mV is calculated as follows:

$$\frac{1.01\times10^{-12}\ \text{coulombs}}{96{,}480\ \text{coulombs/mole}} = 1.05\times10^{-17}\ \text{mole of } K^+$$

With a cell volume of 4.19×10^{-12} L (volume = $4\pi r^3/3$) and an intracellular [K^+] of 120 mmol/L, the total intracellular K^+ content is

$$4.19\times10^{-12}\times0.12\ \text{mol/L} = 5.03\times10^{-13}\ \text{moles}$$

Therefore, the diffusion of 1.05×10^{-17} moles of K^+ out of the cell represents only a 0.002% change in the intracellular K^+ content:

$$\frac{1.05\times10^{-13}\ \text{moles}}{5.03\times10^{-13}\ \text{moles}} \approx 0.002\%$$

Thus the intracellular [K^+] of the cell is not appreciably altered by the diffusion of K^+ out of the cell.

Regulation of Cell Volume

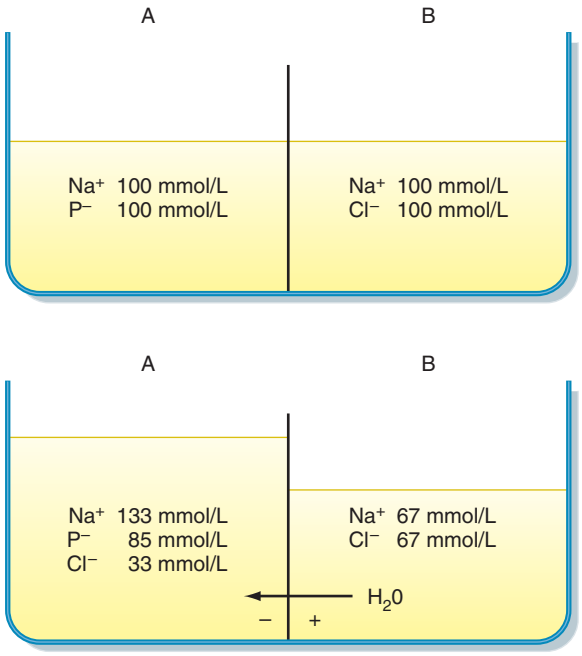
As already noted, changes in cell volume can lead to cell damage and death. Cells have developed mechanisms to regulate their volume. Most cells are highly permeable by water because of the presence of aquaporins in their plasma membranes. As discussed in Chapter 1, osmotic pressure gradients across the cell membrane that are generated by effective osmoles cause water to move either into or out of the cell, which result in changes in cell volume. Thus cells swell when placed in hypotonic solutions and shrink when placed in hypertonic solutions (see the section "Nonisotonic Cell Volume Regulation"). However, even when a cell is placed in an isotonic solution, the maintenance of cell volume is an active process requiring the expenditure of ATP and specifically the activity of the Na^+,K^+-ATPase.

Isotonic Cell Volume Regulation

The importance of the Na^+,K^+-ATPase in isotonic cell volume regulation can be appreciated by the observation that red blood cells swell when chilled (i.e., reduced ATP synthesis) or when the Na^+,K^+-ATPase is inhibited by cardiac glycosides (e.g., ouabain, digoxin [Lanoxin]). The necessity for energy expenditure to maintain cell volume in an isotonic solution is the result of the effect of intracellular proteins on the distribution of ions across the plasma membrane: the so-called **Gibbs-Donnan effect** (Fig. 2.6).

The Gibbs-Donnan effect occurs when a membrane separating two solutions can be permeated by some but not all of the molecules in solution. As noted previously, this effect accounts for the small differences in the ionic compositions of the plasma and the interstitial fluid. In this case, the capillary endothelium represents the membrane, and the plasma proteins are the molecules whose ability to permeate across the capillary is restricted. For cells, the membrane is the plasma membrane, and the impermeant molecules are the intracellular proteins and organic molecules.

As depicted in Fig. 2.6, the presence of impermeant molecules (e.g., protein) in one compartment results over time in the accumulation of permeant molecules/ions in the same compartment. This increases the number of osmotically active particles in the compartment containing the impermeant anions, which in turn increases the osmotic pressure, and water thereby enters that compartment. For cells, the Gibbs-Donnan effect would increase the number of osmotically active particles in the cell, and result in cell swelling. However, the activity of the Na^+,K^+-ATPase counteracts the Gibbs-Donnan effect by actively extruding cations (three Na^+ ions are extruded, whereas two K^+ ions are brought into the cell). In addition, the K^+ gradient established by the Na^+,K^+-ATPase allows for the development of the V_m (in which the cell's interior is electrically negative), that in turn drives Cl^- and other anions out of the cell. Thus through the activity of the Na^+,K^+-ATPase, the number of intracellular osmotically active particles is



• **Fig. 2.6** The Gibbs-Donnan Effect. **Top,** Two solutions are separated by a membrane that is permeable by Na^+, Cl^-, and H_2O but not permeable by protein (P^-). The osmolality of solution A is identical to that of solution B. **Bottom,** Cl^- diffuses from compartment B to compartment A down its concentration gradient. This causes compartment A to become electrically negative with regard to compartment B. The membrane voltage then drives the diffusion of Na^+ from compartment B to compartment A. The accumulation of additional Na^+ and Cl^- in compartment A increases its osmolality and causes water to flow from compartment B to compartment A (Note: the increase volume of compartment A results in a lower [P^-]). If the container containing the two solutions were sealed at the top so that water could not move from compartment B to compartment A, the pressure in compartment A would increase as the number of osmotically active particles increases in that compartment.

reduced from what would be caused by the Gibbs-Donnan effect, and cell volume is maintained in isotonic solutions.

Nonisotonic Cell Volume Regulation

Most cells throughout the body are bathed with isotonic ECF, the composition of which is tightly regulated (see Chapter 35). However, certain regions within the body are not isotonic (e.g., the medulla of the kidney), and with disorders of water balance, the ECF can become either hypotonic or hypertonic. When this occurs, cells either swell or shrink. Cell swelling or shrinkage can result in cell damage or death, but many cells have mechanisms that limit the degree to which the cell volume changes. These mechanisms are particularly important for neurons, in which swelling within the confined space of the skull can lead to serious neurological damage.

In general, when a cell is exposed to nonisotonic ECF, volume-regulatory responses are activated within seconds to minutes to restore cell volume (Fig. 2.7). With cell swelling, a regulatory volume decrease response transports osmotically active particles (osmolytes) out of the cell, reducing the intracellular osmotic pressure and thereby restoring cell volume to normal. Conversely with cell shrinking a regulatory volume increase response transports osmolytes into the cell, raising the intracellular osmotic pressure and thereby restoring cell volume to normal. These osmolytes include ions and organic molecules such as polyols (sorbitol and myo-inositol), methylamines (glycerophosphorylcholine and betaine), and some amino acids (taurine, glutamate, and β-alanine). If the cell is exposed to the nonisotonic ECF for an extended period of time, the cell alters the intracellular levels of the organic osmolytes through metabolic processes.

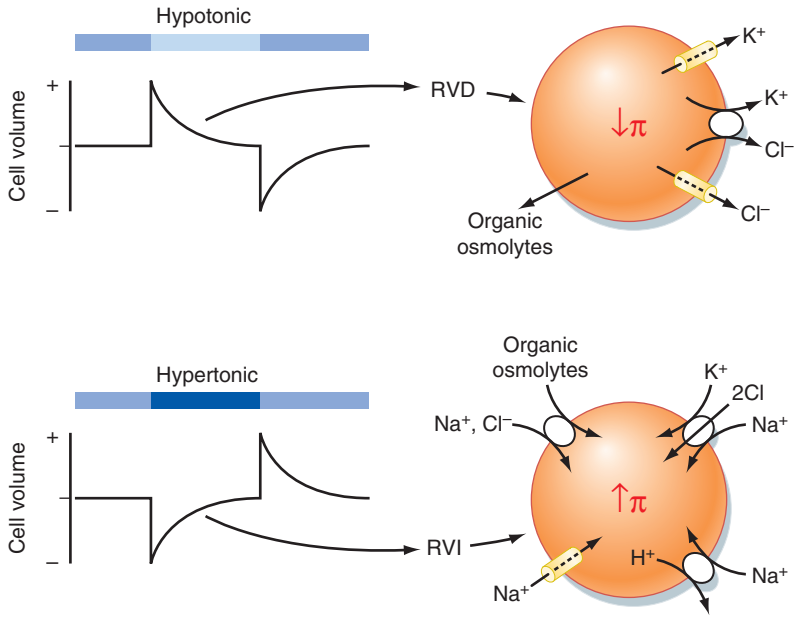
IN THE CLINIC

The ECF of individuals with disorders in water balance may be either hypotonic (positive water balance) or hypertonic (negative water balance). With a decrease in ECF osmolality, neurons and glial cells swell as water enters the cell. To minimize this swelling, the neurons and glial cells reduce intracellular osmolytes. If the ECF osmolality is corrected (i.e., increased) too quickly, the neurons and glial cells then shrink because of the reduced number of intracellular osmolytes. This response to a rapid correction of ECF osmolality can lead to cell damage. Damage to the glial cells that synthesize myelin within the brain can result in demyelinization. This demyelinization response, termed *osmotic demyelinization syndrome,* can affect any of the white matter of the brain, but especially regions of the pons. These effects are often irreversible. Therefore, correction of disorders of water balance is usually accomplished slowly to avoid this serious neurological complication.

The regulatory volume increase response results in the rapid uptake of NaCl and a number of organic osmolytes. To increase cell volume there is an activation of the Na^+-H^+ antiporter (NHE-1), the $1Na^+,1K^+,2Cl^-$ symporter (NKCC-1), and a number of cation-selective channels, which together bring NaCl into the cell. The Na^+,K^+-ATPase then extrudes the Na^+ in exchange for K^+, so that ultimately the KCl content of the cell is increased. Several organic osmolyte transporters are also activated to increase cell volume. These include a $3Na^+,1Cl^-$-taurine symporter, a $3Na^+,2Cl^-$-betaine symporter, a $2Na^+$–myo-inositol symporter, and a Na^+–amino acid symporter. These transporters use the energy in the Na^+ and Cl^- gradients to drive the secondary active uptake of these organic osmolytes into cells.

The regulatory volume decrease response results in the loss of KCl and organic osmolytes from the cell. The loss of KCl occurs through the activation of a wide range of K^+-selective, Cl^--selective, and anion-selective channels (the specific channels involved vary depending on the cell), as well as through activation of K^+-Cl^- symporters. Some of the organic osmolytes appear to leave the cell via anion channels (e.g., volume-sensitive organic osmolyte-anion channels).

Several mechanisms are involved in activation of these various transporters during the volume regulatory



• **Fig. 2.7** Volume Regulation of Cells in Hypotonic and Hypertonic Media. **Top,** When cells are exposed to a hypotonic medium, they swell and then undergo a volume-regulatory decrease (RVD). The RVD involves loss of KCl and organic osmolytes from the cell. The decrease in cellular KCl and organic osmolytes causes intracellular osmotic pressure to decrease, water leaves the cell, and the cell returns to nearly its original volume. **Bottom,** When cells are exposed to a hypertonic medium, they shrink and then undergo a volume-regulatory increase (RVI). During the RVI, NaCl and organic osmolytes enter the cell. The increase in the activity of Na^+,K^+-ATPase (not depicted) enhances the exchange Na^+ for K^+ so that the K^+ (and Cl^-) content of the cell is increased. The increase in cellular KCl, along with a rise in intracellular organic osmolytes, increases intracellular osmotic pressure, which brings water back into the cell, and the cell volume returns to nearly its original volume. π, the oncotic pressure inside the cell.

responses. Changes in cell volume appear to monitored by the cytoskeleton, by changes in macromolecular crowding and ionic strength of the cytoplasm, and by channels whose gating is influenced, either directly or indirectly, by stretch of the plasma membrane (e.g., stretch-activated cation channels). A number of second messenger systems may also be involved in these responses (e.g., intracellular [Ca^{++}], calmodulin, protein kinase A, and protein kinase C), but the precise mechanisms have not been defined completely.

Principles of Epithelial Transport

Epithelial cells are arranged in sheets and provide the interface between the external world and the internal environment (i.e., ECF) of the body. Depending on their location, epithelial cells serve many important functions, such as establishing a barrier to microorganisms (lungs, gastrointestinal tract, and skin), prevention of the loss of water from the body (skin), and maintenance of a constant internal environment (lungs, gastrointestinal tract, and kidneys). This latter function is a result of the ability of epithelial cells to carry out regulated vectorial transport (i.e., transport from one side of the epithelial cell sheet to the opposite side). In this section, the principles of epithelial transport are reviewed. The transport functions of specific epithelial cells are discussed in the appropriate chapters throughout this book.

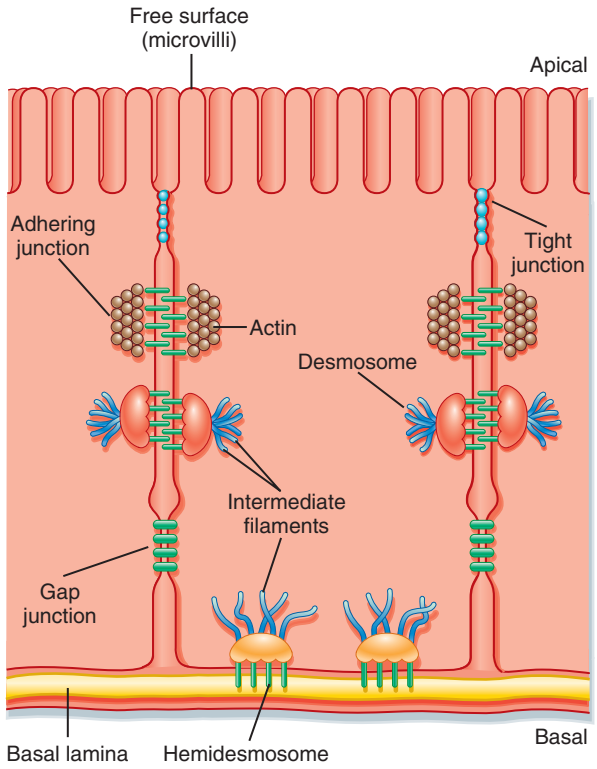
Epithelial Structure

Fig. 2.8 shows a schematic representation of an epithelial cell. The free surface of the epithelial layer is referred to as the *apical membrane.* It is in contact with the external environment (e.g., air within the alveoli and larger airways of the lungs and the contents of the gastrointestinal tract) or with extracellular fluids (e.g., glomerular filtrate in the nephrons of the kidneys and the secretions of the ducts of the pancreas or sweat glands). The basal side of the epithelium rests on a basal lamina, which is secreted by the epithelial cells, and this in turn is attached to the underlying connective tissue.

Epithelial cells are connected to one another and to the underlying connective tissue by a number of specialized junctions (see Fig. 2.8). The **adhering junction, desmosomes,** and **hemidesmosomes** provide mechanical adhesion by linking together the cytoskeleton of adjacent cells (adhering junction and desmosome) or to the underlying connective tissue (hemidesmosome). The **gap junction** and **tight junction** play important physiological roles.

Gap junctions provide low-resistance connections between cells.[g] The functional unit of the gap junction is the **connexon.** The connexon is composed of six integral

[g]Gap junctions are not limited to epithelial cells. A number of other cells also have gap junctions (e.g., cardiac myocytes and smooth muscle cells).



• **Fig. 2.8** Schematic of an Epithelial Cell, Illustrating the Various Adhering Junctions. The tight junction separates the apical membrane from basolateral membrane (see text for details).

membrane protein subunits called **connexins.** A connexon in one cell is aligned with the connexon in the adjacent cell, forming a channel. The channel may be gated, and when it is open, it allows the movement of ions and small molecules between cells. Because of their low electrical resistance, they effectively couple electrically one cell to the adjacent cell.

The tight junction serves two main functions. It divides the cell into two membrane domains (apical and basolateral) and, in so doing, restricts the movement of membrane lipids and proteins between these two domains. This so-called fence function allows epithelial cells to carry out vectorial transport from one surface of the cell to the opposite surface by segregating membrane transporters to one or other of the membrane domains. They also serve as a pathway for the movement of water, ions, and small molecules across the epithelium. This pathway between the cells is referred to as the **paracellular pathway,** as opposed to the **transcellular pathway** through the cells.

The apical surface of epithelial cells may have specific structural features. One such feature is **microvilli** (Fig. 2.9*A*). Microvilli are small (typically 1 to 3 µm in length), nonmotile projections of the apical plasma membrane that serve to increase surface area. They are commonly located on cells that must transport large quantities of ions, water, and molecules (e.g., epithelial cells lining the small intestine and cells of the renal proximal tubule). The core of the microvilli is composed of actin filaments and a number of accessory proteins. This actin core is connected to the cytoskeleton of the cell via the terminal web (a network of actin fibers at

AT THE CELLULAR LEVEL

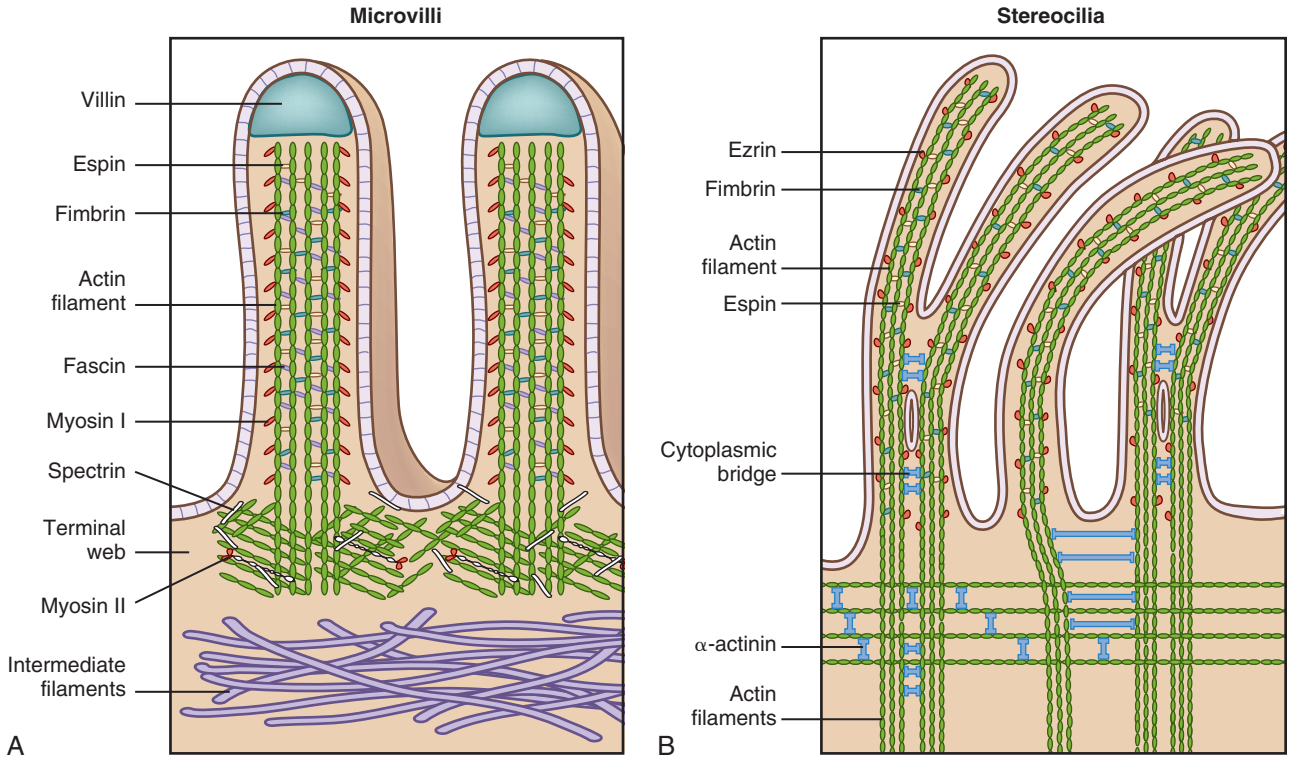
Epithelial cell tight junctions (also called **zonula occludens**) are composed of several integral membrane proteins, including **occludins, claudins,** and several members of the immunoglobulin superfamily (e.g., the **junctional adhesion molecule [JAM]**). Occludins and claudins are transmembrane proteins that span the membrane of one cell and link to the extracellular portion of the same molecule in the adjacent cell. Cytoplasmic linker proteins (e.g., tight junction protein [ZO-1, ZO-2, and ZO-3]) then link the membrane spanning proteins to the cytoskeleton of the cell.

Of these junctional proteins, claudins appear to be important in determining the permeability characteristics of the tight junction, especially with regard to cations and anions. To date, 27 mammalian claudin genes have been identified, and 26 are found in the human genome (the gene for claudin 13 is not found in humans). Certain claudins serve as barrier proteins that restrict the movement of ions through the tight junction, whereas others form a "pore" that facilitates the movement of ions through the junction. Thus the permeability characteristics of the tight junction of an epithelium are determined by the complement of claudins expressed by the cell. For example, the proximal tubule of the kidney is termed a "leaky" epithelium, in which water and solutes (e.g., Na^+) move through the junction. Claudin 4 and claudin 10 are expressed in the tight junction of proximal tubule cells. In contrast, the collecting duct of the kidney is considered a "tight" epithelium, with restricted movement of ions through the tight junction. Collecting duct cells express claudins 3, 4, 7, 8, 10, and 18.

The function of claudins can be regulated at several levels, including gene expression, posttranslational modification, interactions with cytoplasmic scaffolding proteins, and interactions with other claudins in the same membrane (*cis*-interaction), as well as with claudins of adjacent cells (*trans*-interaction). The mineralocorticoid hormone aldosterone stimulates Na^+ reabsorption by distal segments of the renal nephron (see Chapters 34 and 35). In addition to the hormone's effect on Na^+ transporters in the cell, aldosterone also upregulates expression of claudin 8 in the tight junction. The increased expression of claudin 8 reduces the ability of Na^+ to permeate the tight junction, which then reduces the backwards leak of Na^+ from the interstitium into the tubule lumen, thereby allowing more efficient Na^+ reabsorption by the epithelium.

IN THE CLINIC

Mutations in the gene that codes for claudin 16 result in the autosomal recessive condition know as *familial hypomagnesemia, hypercalcuria, and nephrocalcinosis* (FHHNC). Claudin 16 is found in the tight junction of the thick ascending portion of Henle's loop in the kidneys and serves as a route for the paracellular reabsorption of Ca^{++} and Mg^{++} from the tubular fluid. Individuals with FHHNC lack functional copies of claudin 16, and reabsorption of these divalent ions is thus reduced, which leads to hypomagnesemia, hypercalcuria, and nephrocalcinosis.



• **Fig. 2.9** Illustration of Apical Membrane Specializations of Epithelial Cells (Not Drawn to Scale). **A,** Microvilli 1 to 3 μm in length serve to increase the surface area of the apical membrane (e.g., those of the epithelial cells of the small intestine). **B,** Stereocilia can be up to 120 μm in length (e.g., those of the epididymis of the male reproductive tract). Both microvilli and stereocilia have a core structure composed primarily of actin, with a number of associated proteins. Both are nonmotile. (Redrawn from Pawlina, W. *Histology: A Text and Atlas, with Correlated Cell and Molecular Biology.* 7th ed. Philadelphia: Wolters Kluwer Health, 2016.)

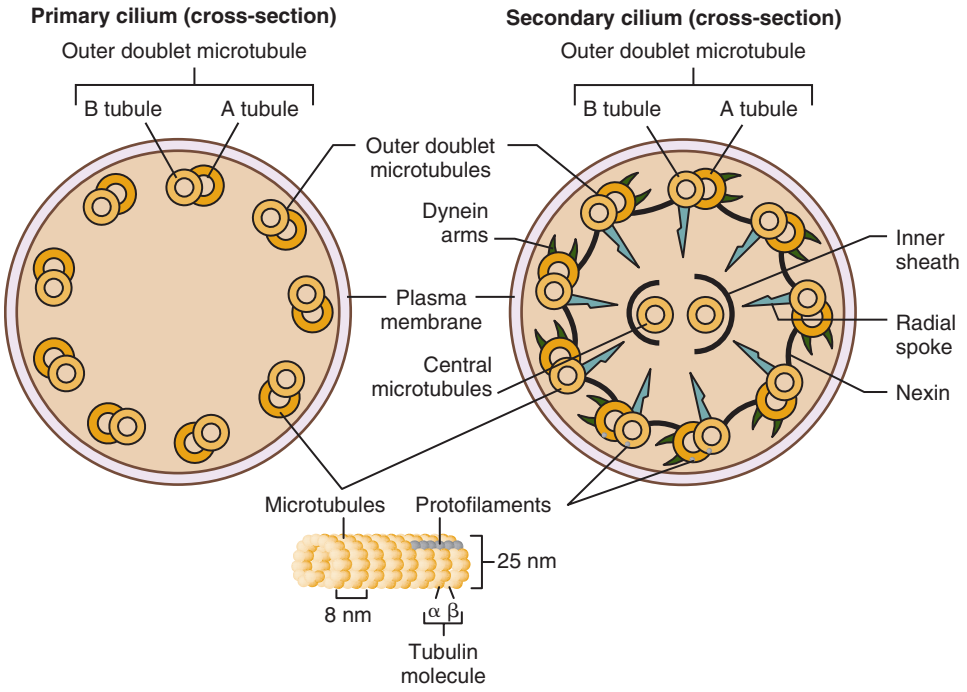
the base of the microvilli) and provides structural support for the microvilli. Another surface feature is stereocilia (see Fig. 2.9*B*). Stereocilia are long (up to 120 μm), nonmotile membrane projections that, like microvilli, increase the surface area of the apical membrane. They are found in the epididymis of the testis and in the "hair cells" of the inner ear. Their core also contains actin filaments and accessory proteins.

A third apical membrane feature is **cilia** (Fig. 2.10). Cilia may be either motile (called *secondary cilia*) or nonmotile (called *primary cilia*). The motile cilia contain a microtubule core arranged in a characteristic "9+2" pattern (nine pairs of microtubules around the circumference of the cilium, and one pair of microtubules in the center). Dynein is the molecular motor that drives the movement of the cilium. Motile cilia are characteristic features of the epithelial cells that line the respiratory tract. They pulsate in a synchronized manner and serve to transport mucus and inhaled particulates out of the lung, a process termed **mucociliary transport** (see Chapter 26). Nonmotile cilia serve as mechanoreceptors and are involved in determining left-right asymmetry of organs during embryological development, as well as sensing the flow rate of fluid in the nephron of the kidneys (see Chapter 33). Only a single nonmotile cilium is found in the apical membrane of cells. Nonmotile cilia have a microtubule core ("9+0" arrangement) and lack a motor protein.

As noted previously, the tight junction effectively divides the plasma membrane of an epithelial cell into two domains: an apical surface and a basolateral surface. The basolateral membrane of many epithelial cells is folded or invaginated. This is especially so for epithelial cells that have high transport rates. These invaginations serve to increase the membrane surface area to accommodate the large number of membrane transporters (e.g., Na^+,K^+-ATPase) needed in the membrane.

Vectorial Transport

Because the tight junction divides the plasma membrane into two domains (i.e., apical and basolateral), epithelial cells are capable of vectorial transport, whereby an ion or molecule can be transported from one side of the epithelial sheet to the opposite side (Fig. 2.11). The accomplishment of vectorial transport requires that specific membrane transport proteins be targeted to and remain in one or the other of the membrane domains. In the example shown in Fig. 2.11, the Na^+ channel is present only in the apical

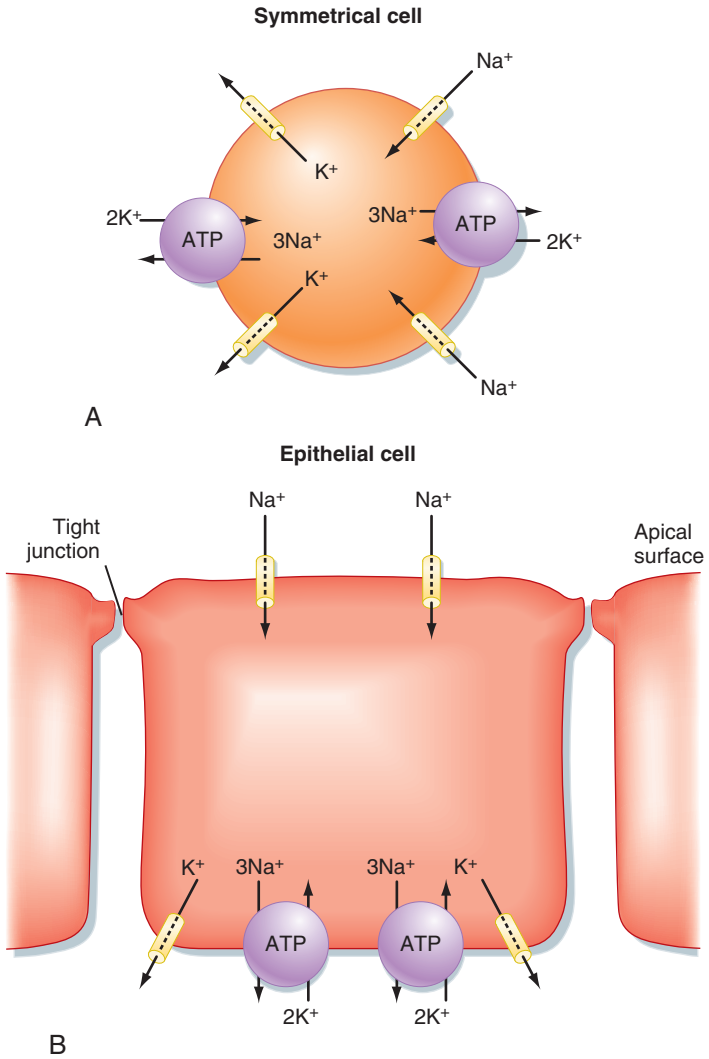


• **Fig. 2.10** Cilia are apical membrane specializations of some epithelial cells. Cilia are 5 to 10 µm in length and contain arrays of microtubules, as depicted in these cross-section diagrams. **Left,** The primary cilium has nine peripheral microtubule arrays. It is nonmotile and serves as a mechanoreceptor (e.g., cells of the renal collecting duct). Cells that have a primary cilium have only a single cilium. **Right,** The secondary cilium has a central pair of microtubules in addition to the nine peripheral microtubule arrays. Also in the secondary cilium, the motor protein dynein is associated with the microtubule arrays and therefore is motile. A single cell can have thousands of secondary cilia on its apical surface (e.g., epithelial cells of the respiratory tract). (Redrawn from Rodat-Despoix L, Delmas P. Ciliary functions in the nephron. *Pflugers Archiv*. 2009;458:179.)

membrane, whereas the Na^+,K^+-ATPase and the K^+ channels are confined to the basolateral membrane. The operation of the Na^+,K^+-ATPase channel and the leakage of K^+ out of the cell across the basolateral membrane sets up a large electrochemical gradient for Na^+ to enter the cell across the apical membrane through the Na^+ channel (intracellular [Na^+] < extracellular [Na^+], and V_m which is oriented with the cell's interior electrically negative with respect to the cell's exterior). The Na^+ is then pumped out of the cell by the Na^+,K^+-ATPase, and vectorial transport from the apical side of the epithelium to the basolateral side of the epithelium occurs. Transport from the apical side to the basolateral side of an epithelium is termed either **absorption** or **reabsorption:** For example, the uptake of nutrients from the lumen of the gastrointestinal tract is termed *absorption,* whereas the transport of NaCl and water from the lumen of the renal nephrons is termed *reabsorption.* Transport from the basolateral side of the epithelium to the apical side is termed **secretion.**

As noted previously, the Na^+,K^+-ATPase and K^+-selective channels play an important role in establishing cellular ion gradients for Na^+ and K^+ and in generating the V_m. In all epithelial cells except the choroid plexus and retinal pigment epithelium,[h] the Na^+,K^+-ATPase channel is located in the basolateral membrane of the cell. Numerous K^+-selective channels are in epithelial cells and may be located in either membrane domain. Through the establishment of these chemical and voltage gradients, the transport of other ions and solutes can be driven (e.g., Na^+-glucose symporter, Na^+-H^+ antiporter, $1Na^+$,$1K^+$,$2Cl^-$ symporter, $1Na^+$-$3HCO_3^-$ symporter). The direction of transepithelial transport (reabsorption or secretion) depends simply on which membrane domain the transporters are located. Because of the dependence on the Na^+,K^+-ATPase, epithelial transport requires the expenditure of energy. Other ATP-dependent transporters, such as the H^+-ATPase, H^+,K^+-ATPase, and a host of ABC transporters—such as P-glycoprotein (PGP) and multidrug resistance-associated protein 2 (MRP2), which transport xenobiotics (drugs), and cystic fibrosis transmembrane conductance regulator (CFTR), which transports Cl^-—are involved in epithelial transport.

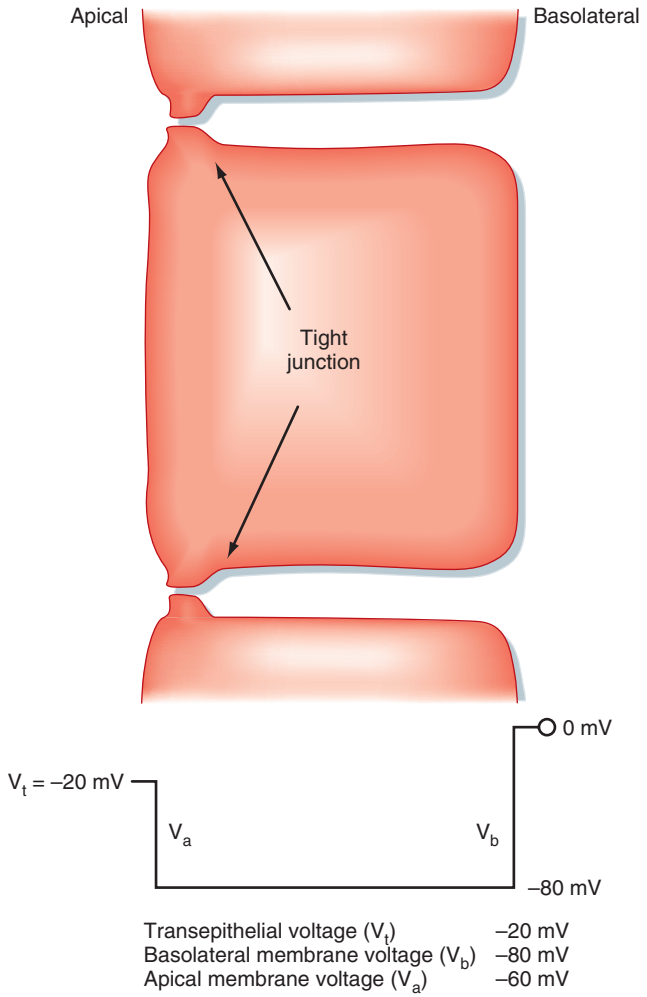
[h]The choroid plexus is located in the ventricles of the brain and secretes the cerebrospinal fluid. The Na^+,K^+-ATPase channel is located in the apical membrane of these cells.



• **Fig. 2.11** In symmetrical cells (**A;** e.g., red blood cells), membrane transport proteins are distributed over the entire surface of the cell. Epithelial cells **(B),** in contrast, are asymmetrical and target various membrane transport proteins to either the apical or the basolateral membrane. When the transporters are confined to a membrane domain, vectorial transport can occur. In the cell depicted, Na^+ is transported from the apical surface to the basolateral surface. ATP, adenosine triphosphate.

Solutes and water can be transported across an epithelium by traversing both the apical and basolateral membranes **(transcellular transport)** or by moving between the cells across the tight junction **(paracellular transport).** Solute transport via the transcellular route is a two-step process, in which the solute molecule is transported across both the apical and basolateral membrane. Uptake into the cell, or transport out of the cell, may be either a passive or an active process. Typically, one of the steps is passive, and the other is active. For the example shown in Fig. 2.11*B*, the uptake of Na^+ into the cell across the apical membrane through the Na^+-selective channel is passive and driven by the electrochemical gradient for Na^+. The exit of Na^+ from the cell across the basolateral membrane is primary active transport via the Na^+,K^+-ATPase channel. Because a transepithelial gradient for Na^+ can be generated by this process (i.e., the [Na^+] in the apical compartment can be reduced below that of the basolateral compartment, the overall process of transepithelial Na^+ transport is said to be active). Any solute that is actively transported across an epithelium must be transported via the transcellular pathway.

Depending on the epithelium, the paracellular pathway is an important route for transepithelial transport of solute and water. As noted, the permeability characteristics of the paracellular pathway are determined, in large part, by the specific claudins that are expressed by the cell. Thus the tight junction can have low permeability for solutes, water, or both, or it can have a high permeability. For epithelia in which there are high rates of transepithelial transport,



• **Fig. 2.12** The Electrical Profile Across an Epithelial Cell. The magnitude of the membrane voltages, and the transepithelial voltage are determined by the various membrane transport proteins in the apical and basolateral membranes. The transepithelial voltage is equal to the sum of the apical and basolateral membrane voltages (see text for details).

the tight junctions typically have a high permeability (i.e., are leaky). Examples of such epithelia include the proximal tubule of the renal nephron and the early segments of the small intestine (e.g., duodenum and jejunum). If the epithelium must establish large transepithelial gradients for solutes, water, or both, the tight junctions typically have low permeability (i.e., are tight). Examples of this type of epithelium include the collecting duct of the renal nephron, the urinary bladder, and the terminal portion of the colon. In addition, the tight junction may be selective for certain solutes (e.g., cation versus anion selective).

All solute transport that occurs through the paracellular pathway is passive in nature. The two driving forces for this transport are the transepithelial concentration gradient for the solute and, if the solute is charged, the transepithelial voltage (Fig. 2.12). The transepithelial voltage may be oriented with the apical surface electrically negative in relation to the basolateral surface as shown in Fig. 2.12, or it may be oriented with the apical surface electrically positive in relation to the basolateral surface. The polarity and magnitude of the transepithelial voltage is determined by the specific membrane transporters in the apical and basolateral membranes, as well as by the permeability characteristics of the tight junction.

It is important to recognize that transcellular transport processes set up the transepithelial chemical and voltage gradients, which in turn can drive paracellular transport. This is illustrated in Fig. 2.13 for an epithelium that reabsorbs NaCl and for an epithelium that secretes NaCl. In both epithelia, the transepithelial voltage is oriented with the apical surface electrically negative in relation to the basolateral surface. For the NaCl-reabsorbing epithelium, the transepithelial voltage is generated by the active, transcellular reabsorption of Na^+. This voltage in turn drives Cl^- reabsorption through the paracellular pathway. In contrast, for the NaCl-secreting epithelium, the transepithelial voltage is generated by the active transcellular secretion of Cl^-. Na^+ is then secreted passively via the paracellular pathway, driven by the negative transepithelial voltage.

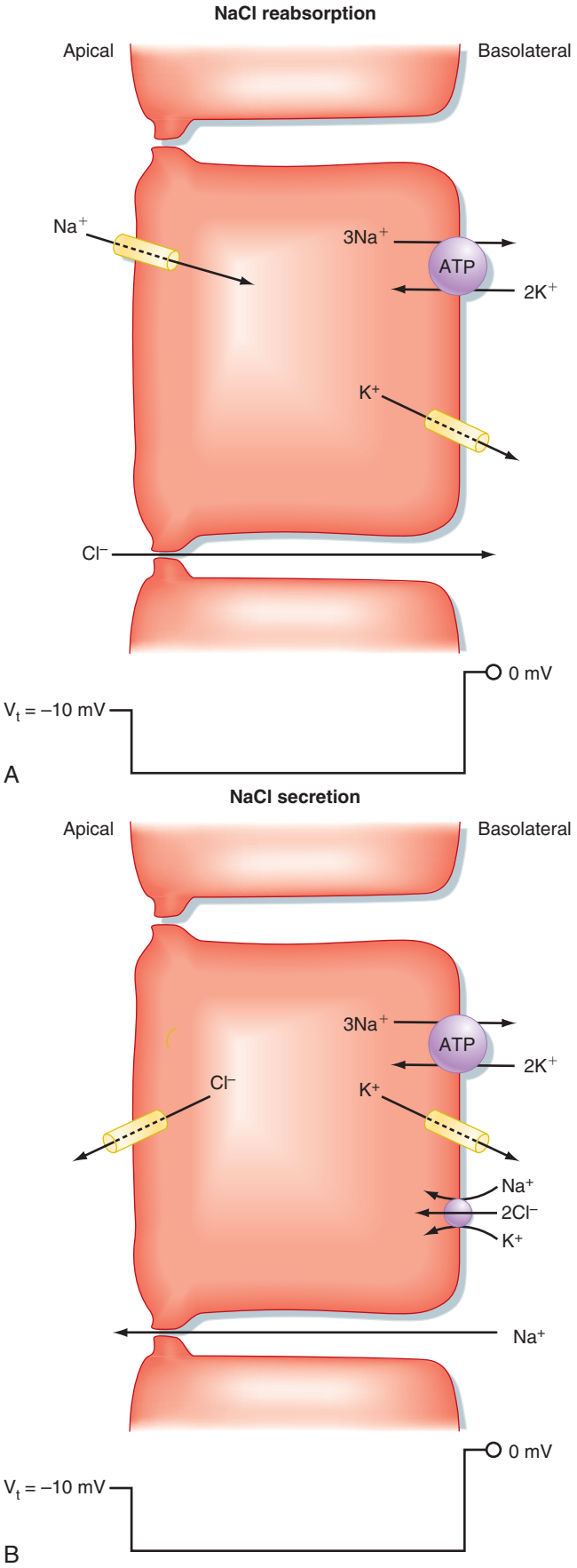
Transepithelial Water Movement

Water movement across epithelia is passive and driven by transepithelial osmotic pressure gradients. Water movement can occur by a transcellular route involving aquaporins in both the apical and basolateral membranes.[i] In addition, water may also move through the paracellular pathway. In the NaCl-reabsorbing epithelium depicted in Fig. 2.13*A*, the reabsorption of NaCl from the apical compartment lowers the osmotic pressure in that compartment, whereas the addition of NaCl to the basolateral compartment raises the osmotic pressure in that compartment. As a result, a transepithelial osmotic pressure gradient is established that drives the movement of water from the apical to the basolateral compartment (i.e., reabsorption). The opposite occurs with NaCl-secreting epithelia (see Fig. 2.13*B*), in which the transepithelial secretion of NaCl establishes a transepithelial osmotic pressure gradient that drives water secretion.

In some epithelia (e.g., proximal tubule of the renal nephron), the movement of water across the epithelium via the paracellular pathway can drive the movement of additional solute. This process is termed **solvent drag** and reflects the fact that solutes dissolved in the water will traverse the tight junction with the water.

As is the case with the establishment of transepithelial concentration and voltage gradients, the establishment of transepithelial osmotic pressure gradients requires transcellular transport of solutes by the epithelial cells.

[i]Different aquaporin isoforms are often expressed in the apical and basolateral membrane. In addition, multiple isoforms may be expressed in one or more of the membrane domains.



• **Fig. 2.13** The Role of the Paracellular Pathway in Epithelial Transport. **A,** Na^+ transport through the cell generates a transepithelial voltage that then drives the passive movement of Cl^- through the tight junction. NaCl reabsorption results. **B,** Cl^- transport through the cell generates a transepithelial voltage that then drives the passive transport of Na^+ through the tight junction. NaCl secretion results.

Regulation of Epithelial Transport

Epithelial transport must be regulated to meet the homeostatic needs of the individual. Depending on the epithelium, this regulation involves neural or hormonal mechanisms, or both. For example, the enteric nervous system of the gastrointestinal tract regulates solute and water transport by the epithelial cells that line the intestine and colon. Similarly, the sympathetic nervous system regulates transport by the epithelial cells of the renal nephron. Aldosterone, a steroid hormone produced by the adrenal cortex (see Chapter 43), is an example of a hormone that stimulates NaCl transport by the epithelial cells of the colon, renal nephron, and sweat ducts. Epithelial cell transport can also be regulated by locally produced and locally acting substances, a process termed **paracrine regulation.** The stimulation of HCl secretion in the stomach by histamine is an example of this process. Cells that are located near the epithelial cells of the stomach release histamine, which acts on the HCl-secreting cells of the stomach (parietal cells) and stimulates them to secrete HCl.

When acted upon by a regulatory signal, the epithelial cell may respond in several different ways, including:

- Retrieval of transporters from the membrane, by endocytosis, or insertion of transporters into the membrane from an intracellular vesicular pool, by a process called *exocytosis*
- Change in activity of membrane transporters (e.g., channel gating)
- Synthesis of specific transporters, and their insertion into the membrane

The first two mechanisms can occur quite rapidly (seconds to minutes), but the synthesis of transporters takes additional time (minutes to days).

Key Concepts

- The body maintains steady-state balance for water and a number of important solutes. This occurs when input into the body equals output from the body. For each solute and water, there is a normal set point. Deviations from this set point are monitored (i.e., when input ≠ output), and effector mechanisms are activated that restore balance. This balance is achieved by adjustment of either intake or excretion of water and solutes. Thereafter, input and output are again equal to maintain balance.
- The Na^+,K^+-ATPase and K^+-selective channels are critically important in establishing and maintaining the intracellular composition, the membrane potential (V_m), and cell volume. Na^+,K^+-ATPase converts the energy in ATP into potential energy of ion gradients and the membrane potential. The ion and electrical gradients created by this process are then used to drive the transport of other ions and other molecules, especially by solute carriers (i.e., symporters and antiporters).
- Epithelial cells constitute the interface between the external world and the internal environment of the body. Vectorial transport of solutes and water across epithelia helps maintain steady-state balance for water and a number of important solutes. Because the external environment constantly changes, and because dietary intake of food and water is highly variable, transport by epithelia is regulated to meet the homeostatic needs of the individual.

Additional Readings

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3

Signal Transduction, Membrane Receptors, Second Messengers, and Regulation of Gene Expression

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. How do cells communicate with each other?
2. What are the four classes of receptors, and what signal transduction pathways are associated with each class of receptors?
3. How do steroid and thyroid hormones, cyclic adenosine monophosphate, and receptor tyrosine kinases regulate gene expression?

The human body is composed of billions of cells, each with a distinct function. However, the function of cells is tightly coordinated and integrated by external chemical signals, including hormones, neurotransmitters, growth factors, odorants, and products of cellular metabolism that serve as chemical messengers and provide cell-to-cell communication. Mechanical and thermal stimuli and light are physical external signals that also coordinate cellular function. Chemical and physical messengers interact with receptors located in the plasma membrane, cytoplasm, and nucleus. Interaction of these messengers with receptors initiates a cascade of signaling events that mediate the response to each stimulus. These signaling pathways ensure that the cellular response to external messengers is specific, amplified, tightly regulated, and coordinated. This chapter provides an overview of how cells communicate via external messengers and a discussion of the signaling pathways that process external information into a highly coordinated cellular response. In subsequent chapters, details on signaling pathways in the nervous system, muscular system, cardiovascular system, respiratory system, gastrointestinal system, renal system, and endocrine system are discussed in greater detail.

IN THE CLINIC

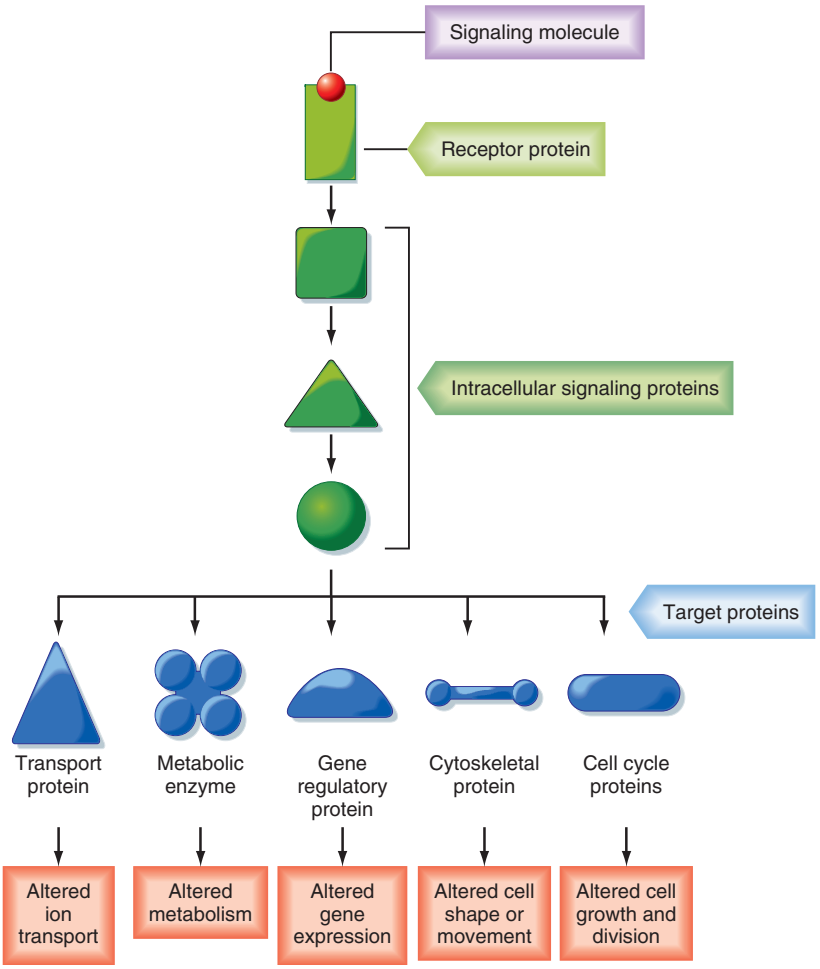
The significance of signaling pathways in medicine is illustrated by the following short list of popular drugs that act by regulating signaling pathways. Details on these pathways are presented later in this and other chapters.

- **Aspirin,** the first pharmaceutical (1899), inhibits cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2) and therefore is antithrombotic (i.e., reduces the formation of blood clots).
- **β-Adrenergic receptor agonists and antagonists** are used to treat a variety of medical conditions. β_1-Agonists increase cardiac contractility and heart rate in patients with low blood pressure. β_2-Agonists dilate bronchi and are used to treat asthma and chronic obstructive lung disease. In contrast, β-adrenergic antagonists are used to treat hypertension, angina, cardiac arrhythmias, and congestive heart failure (see Chapter 18).
- **Fluoxetine (Prozac)** is an antidepressant medication that inhibits reuptake of the neurotransmitter serotonin into the presynaptic cell, which results in enhanced activation of serotonin receptors (see Chapter 6).
- Several monoclonal antibodies are used to treat cancer caused by the activation of growth factor receptors in cancer cells. For example, **trastuzumab (Herceptin)** is a monoclonal antibody used to treat metastatic breast cancer in women who overexpress **HER2/neu,** a member of the family of epidermal growth factor (EGF) receptors, which stimulate cell growth and differentiation. **Cetuximab (Erbitux)** and **bevacizumab (Avastin)** are monoclonal antibodies that are used to treat metastatic colorectal cancer and cancers of the head and neck. These antibodies bind to and inhibit the EGF receptor and thereby inhibit EGF-induced cell growth in cancer cells.
- Drugs that inhibit cyclic guanosine monophosphate (cGMP)–specific phosphodiesterase type 5, such as **sildenafil (Viagra), tadalafil (Cialis),** and **vardenafil (Levitra),** prolong the vasodilatory effects of nitric oxide and are used to treat erectile dysfunction and pulmonary arterial hypertension (see Chapter 17).

Cell-to-Cell Communication

An overview of how cells communicate with each other is presented in Fig. 3.1. Cells communicate by releasing extracellular signaling molecules (e.g., **hormones and neurotransmitters**) that bind to **receptor** proteins located in the plasma membrane, cytoplasm, or nucleus. This signal is transduced into the activation, or inactivation, of one or more intracellular messengers by interacting with receptors. Receptors interact with a variety of intracellular signaling proteins, including **kinases, phosphatases,** and guanosine triphosphate (GTP)–binding proteins **(G proteins)**. These signaling proteins interact with and regulate the activity of target proteins and thereby modulate cellular function. Target proteins include, but are not limited to, ion channels and other transport proteins, metabolic enzymes, cytoskeletal proteins, gene regulatory proteins, and cell cycle proteins that regulate cell growth and division. Signaling pathways are characterized by (1) multiple, hierarchical steps; (2) amplification of the signal-receptor binding event, which magnifies the response; (3) activation of multiple pathways and regulation of multiple cellular functions; and (4) antagonism by constitutive and regulated feedback mechanisms, which minimize the response and provide tight regulatory control over these signaling pathways. A brief description of how cells communicate follows. Readers who desire a more in-depth presentation of this material are encouraged to consult one of the many cellular and molecular biology textbooks currently available.

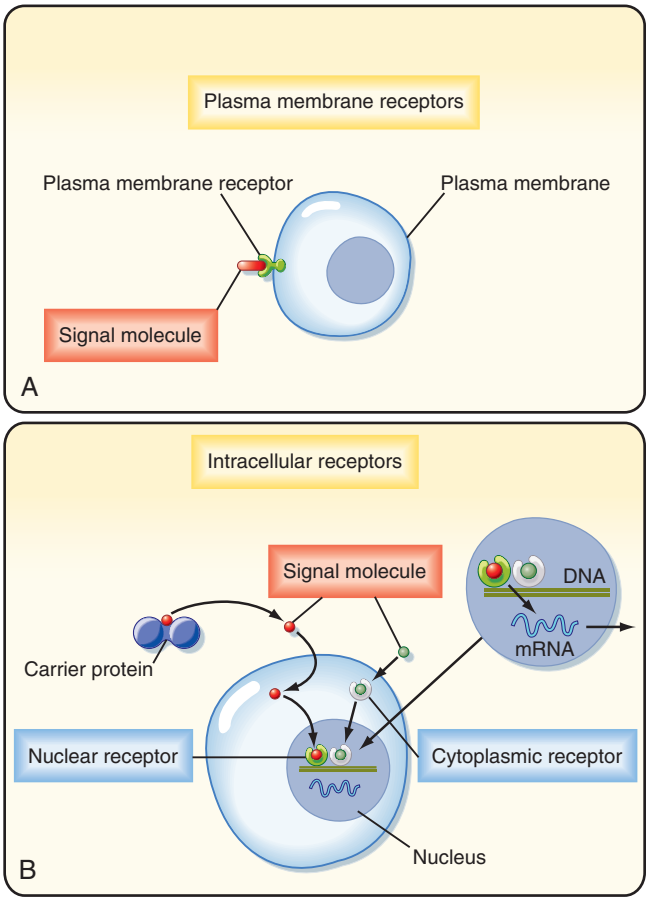
Cells in higher animals release into the extracellular space hundreds of chemicals, including (1) **peptides and proteins** (e.g., insulin); **(2) amines** (e.g., epinephrine and norepinephrine); (3) **steroid hormones** (e.g., aldosterone, estrogen); and (4) **small molecules,** including amino acids, nucleotides, ions (e.g., Ca^{++}), and gases, such as nitric oxide and carbon dioxide. Secretion of signaling molecules is



• **Fig. 3.1** An Overview of How Cells Communicate. A signaling molecule (i.e., hormone or neurotransmitter) binds to a receptor, which may be in the plasma membrane, cytosol, or nucleus. Binding of ligand to a receptor activates intracellular signaling proteins, which interact with and regulate the activity of one or more target proteins to change cellular function. Signaling molecules regulate cell growth, division, and differentiation and influence cellular metabolism. In addition, they modulate the intracellular ionic composition by regulating the activity of ion channels and transport proteins. Signaling molecules also control cytoskeleton-associated events, including cell shape, division, and migration and cell-to-cell and cell-to-matrix adhesion. (Redrawn from Alberts B, et al: *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

cell-type specific. For example, beta cells in the pancreas release insulin, which stimulates glucose uptake into cells. The ability of a cell to respond to a specific signaling molecule depends on the expression of receptors that bind the signaling molecule with high affinity and specificity. Receptors are located in the plasma membrane, the cytosol, and the nucleus (Fig. 3.2).

Signaling molecules can act over long or short distances and can require cell-to-cell contact or very close cellular proximity (Fig. 3.3). **Contact-dependent signaling,** in which a membrane-bound signaling molecule of one cell binds directly to a plasma membrane receptor of another cell, is important during development, in immune responses, and in cancer (see Fig. 3.3*A*). Molecules that are released and act locally are called **paracrine** (see Fig. 3.3*B*) or **autocrine** (see Fig. 3.3*C*) **hormones.** Paracrine signals are released by one type of cell and act on another type; they are usually taken up by target cells or rapidly degraded (within minutes) by enzymes. For example, enterochromaffin-like cells in the stomach secrete histamine, which stimulates the production of acid by neighboring parietal cells (see Chapter 27 for details). Autocrine signaling involves the release of a molecule that affects the same cell or other cells of the same type (e.g., cancer cells). In **synaptic signaling** (see Fig. 3.3*D*), neurons transmit electrical signals along their axons and release neurotransmitters at synapses that affect the function of other neurons or cells that are distant from the neuron cell body. The close physical relationship between the nerve terminal and the target cell ensures that the neurotransmitter is delivered to a specific cell. Details on synaptic signaling are discussed in Chapter 6. **Endocrine** signals are hormones that are secreted into the blood and are widely dispersed in the body (see Fig. 3.3*E*). Details on endocrine signaling are discussed in Chapter 38.



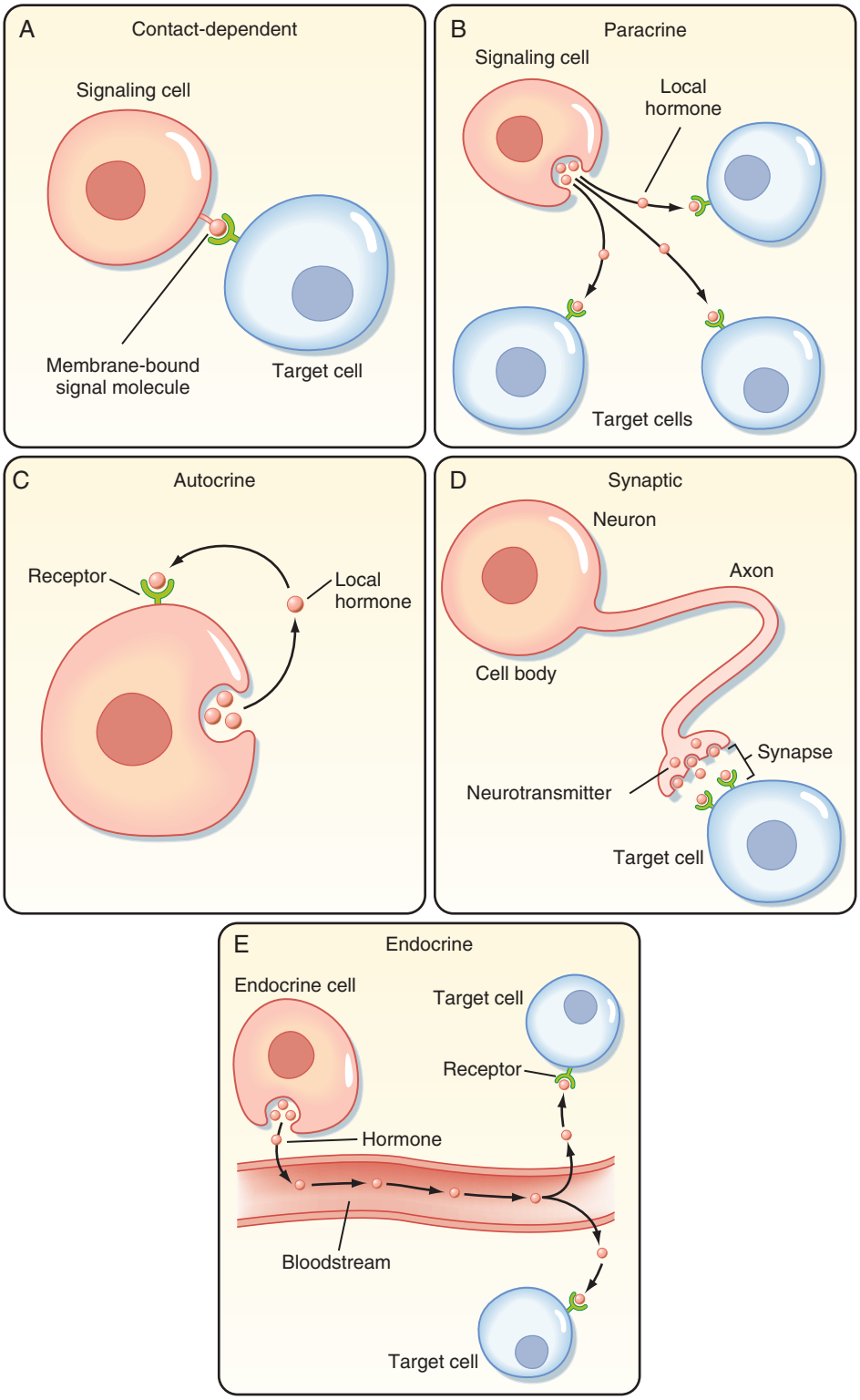
• **Fig. 3.2** Signaling molecules, especially ones that are hydrophilic and cannot cross the plasma membrane, bind directly to their cognate receptors in the plasma membrane **(A).** Other signaling molecules—including steroid hormones, triiodothyronines, retinoic acids, and vitamin D—bind to carrier proteins in blood and readily diffuse across the plasma membrane, where they bind to cognate nuclear receptors in the cytosol or nucleus **(B).** Still other signaling molecules, including nitric oxide, can diffuse without carrier proteins and cross the membrane to act on intracellular protein targets **(B).** Both classes of receptors, when ligand bound, regulate gene transcription. mRNA, messenger RNA. (Redrawn from Alberts B, et al: *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

In addition to paracrine, autocrine, endocrine, and synaptic signaling, cell-to-cell communication also occurs via **gap junctions** that form between adjacent cells (see Chapter 2). Gap junctions are specialized junctions that allow intracellular signaling molecules, generally less than 1200 D in size, to diffuse from the cytoplasm of one cell to an adjacent cell. The permeability of gap junctions is regulated by cytosolic [Ca^{++}], [H^{+}], and cyclic adenosine monophosphate (cAMP) and by the membrane potential. Gap junctions also allow cells to be electrically coupled, which is vitally important for the coordinated activity of cardiac and smooth muscle cells (see Chapters 13 and 14).

The speed of a response to an extracellular signal depends on the mechanism of delivery. Endocrine signals are relatively slow (seconds to minutes) because time is required for diffusion and blood flow to the target cell, whereas synaptic signaling is extremely fast (milliseconds). If the response involves changes in the activity of proteins in the cell, the response may occur in milliseconds to seconds. However, if the response involves changes in gene expression and the de novo synthesis of proteins, the response may take hours to occur, and a maximal response may take days. For example, the stimulatory effect of aldosterone on sodium transport by the kidneys requires days to develop fully (see Chapter 35).

The response to a particular signaling molecule also depends on the ability of the molecule to reach a particular cell, on expression of the cognate receptor (i.e., receptors that recognize a particular signaling molecule or ligand with a high degree of specificity), and on the cytoplasmic signaling molecules that interact with the receptor. Thus signaling molecules frequently have many different effects that are dependent on the cell type. For example, the neurotransmitter acetylcholine stimulates contraction of skeletal muscle but decreases the force of contraction in heart muscle. This is because skeletal muscle and heart cells express different acetylcholine receptors.[a]

[a]The acetylcholine receptor in skeletal muscle is termed *nicotinic* because nicotine can mimic this action of the neurotransmitter. In contrast, the acetylcholine receptor in cardiac muscle is termed *muscarinic* because this effect is mimicked by muscarine, an alkaloid derived from the mushroom *Amanita muscaria.*



• **Fig. 3.3** Cell-to-cell communication is mediated by five basic mechanisms: contact-dependent **(A),** paracrine **(B),** autocrine **(C),** synaptic **(D),** and endocrine signaling **(E).** These mechanisms are described in detail in the text. (Redrawn from Alberts B, et al: *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

TABLE 3.1 Classes of Membrane Receptors

Receptor Class	Ligand	Signal Transduction Pathway/Target
Ligand-gated ion channels	**Extracellular ligand:**	**Membrane currents:**
	GABA	**Cl^-**
	ACh (muscle)	**Na^+, K^+, Ca^{++}**
	ATP	**Ca^{++}, Na^+, K^+**
	Glutamate: NMDA	**Na^+, K^+, Ca^{++}**
	Intracellular ligand:	
	cAMP (olfaction)	**K^+**
	cGMP (vision)	**Na^+, K^+**
	InsP3	**Ca^{++}**
G protein–coupled receptors	**Neurotransmitters (ACh)** **Peptides (PTH, oxytocin)** **Odorants** **Cytokines, lipids**	**βγ Subunits activate ion channels** **α Subunit activates enzymes:** Cyclases that generate cAMP, cGMP, phospholipases that generate InsP3 and diacylglycerol, and phospholipases that generate arachidonic acid and its metabolites. **Monomeric G proteins**
Enzyme-linked receptors	**ANP**	**Receptor guanylyl cyclase**
	TGF-β	**Receptor serine/threonine kinase**
	Insulin, EGF	**Receptor tyrosine kinase**
	Interleukin-6, erythropoietin	**Tyrosine kinase–associated receptor**
Nuclear receptors	**Steroid hormones:** Mineralocorticoids Glucocorticoids Androgens Estrogens Progestins	**Bind to regulatory sequences in DNA and increase or decrease gene transcription**
	Miscellaneous hormones: Thyroid Vitamin D Retinoic acid Prostaglandins	**Bind to regulatory sequences in DNA and increase or decrease gene transcription**

ACh, acetylcholine; ANP, atrial natriuretic peptide; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; EGF, epidermal growth factor; GABA, gamma-aminobutyric acid; InsP3, inositol 1,4,5-triphosphate; NMDA, *N*-methyl-D-aspartate; PTH, parathyroid hormone; TGF, transforming growth factor.

Receptors

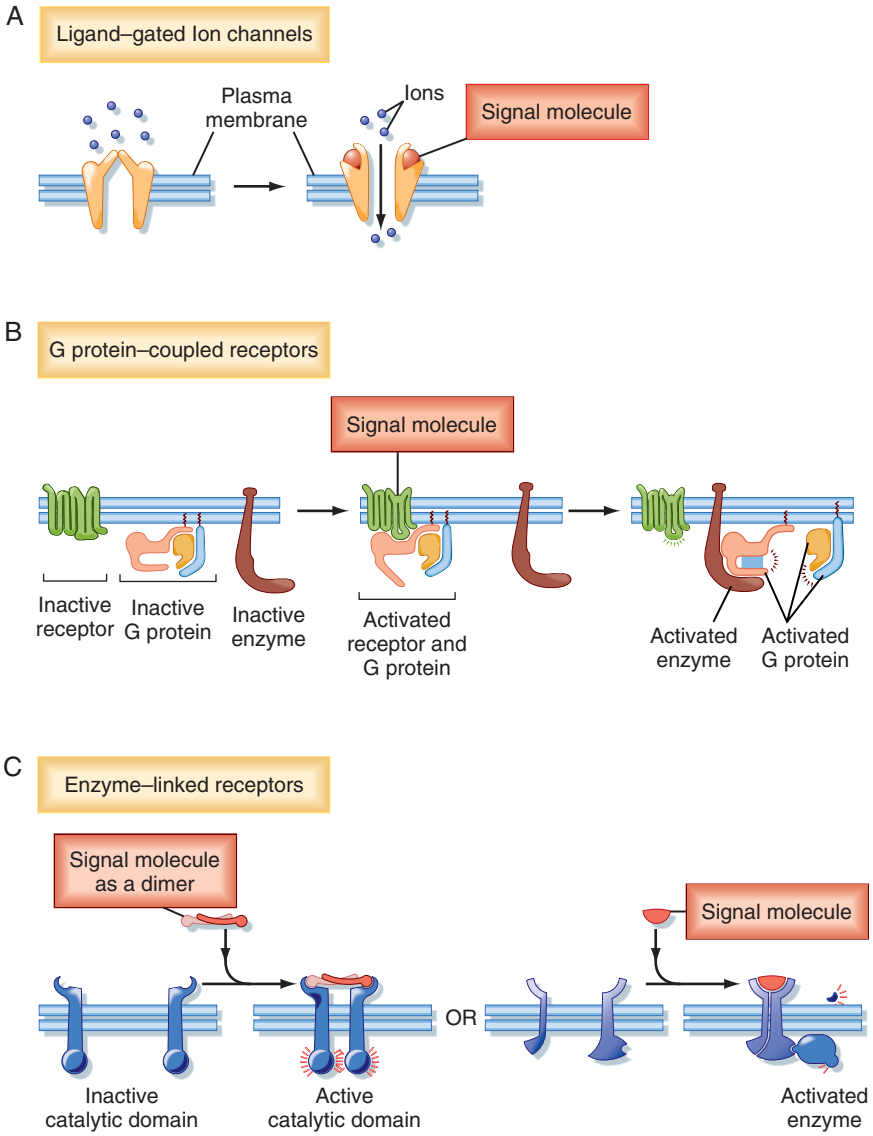
All signaling molecules bind to specific receptors that act as signal transducers, thereby converting a ligand-receptor binding event into intracellular signals that affect cellular function. Receptors can be divided into four basic classes on the basis of their structure and mechanism of action: (1) **ligand-gated ion channels,** (2) **G protein–coupled receptors (GPCRs),** (3) **enzyme-linked receptors,** and (4) **nuclear receptors** (Table 3.1; Figs. 3.4 and 3.5).

Ligand-gated ion channels mediate direct and rapid synaptic signaling between electrically excitable cells (see Fig. 3.4*A*). Neurotransmitters bind to receptors and either open or close ion channels, thereby changing the ionic permeability of the plasma membrane and altering the membrane potential. For examples and more details, see Chapter 6.

GPCRs regulate the activity of other proteins, such as enzymes and ion channels (see Fig. 3.4*B*). In the example in Fig. 3.4*B,* the interaction between the receptor and the target protein is mediated by heterotrimeric G proteins, which are composed of α, β, and γ subunits. Stimulation of G proteins by ligand-bound receptors activates or inhibits downstream target proteins that regulate signaling pathways if the target protein is an enzyme or changes membrane ion permeability if the target protein is an ion channel.

Enzyme-linked receptors either function as enzymes or are associated with and regulate enzymes (see Fig. 3.4*C*). Most enzyme-linked receptors are protein kinases or are associated with protein kinases, and ligand binding causes the kinases to phosphorylate a specific subset of proteins on specific amino acids, which in turn activates or inhibits protein activity.

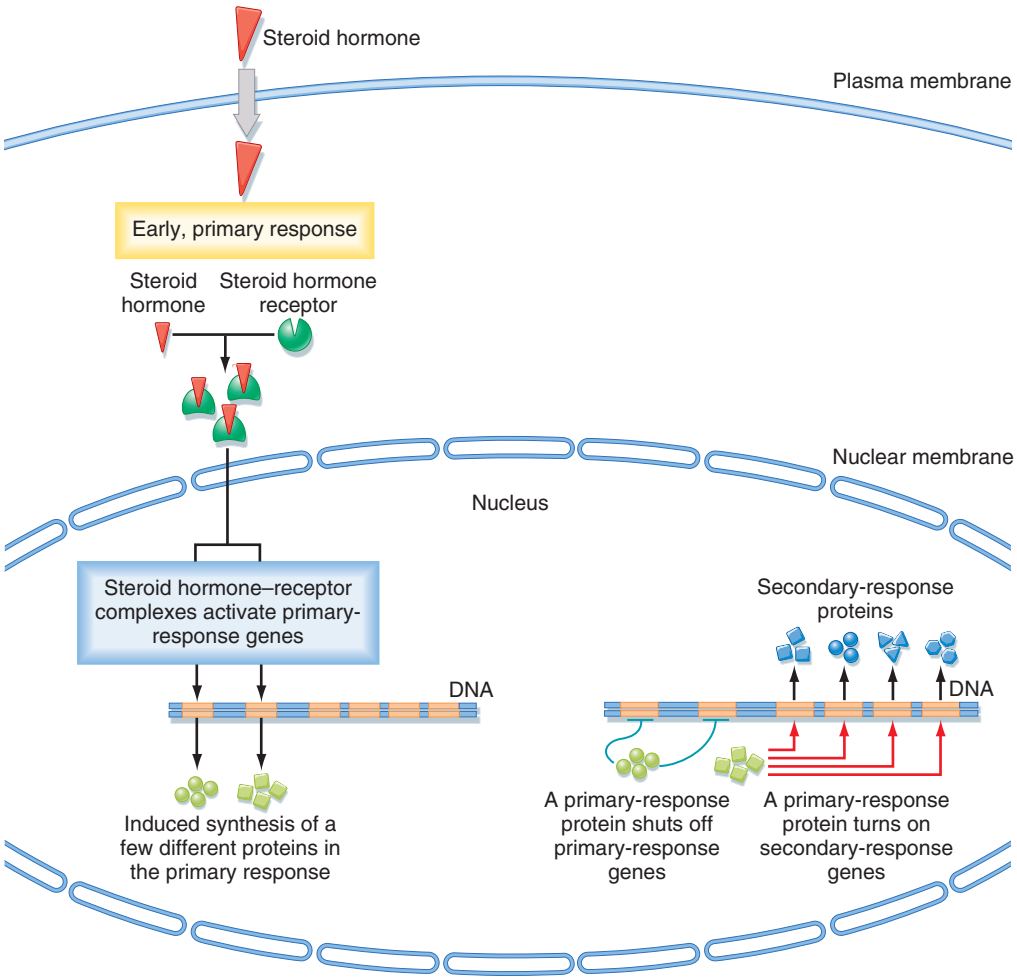
Nuclear receptors are small hydrophobic molecules, including steroid hormones, thyroid hormones, retinoids, and vitamin D, that have a long biological half-life (hours to days), diffuse across the plasma membrane, and bind to nuclear receptors or to cytoplasmic receptors that, once bound to their ligand, translocate to the nucleus (see Fig. 3.5). Some nuclear receptors, such as those that bind cortisol



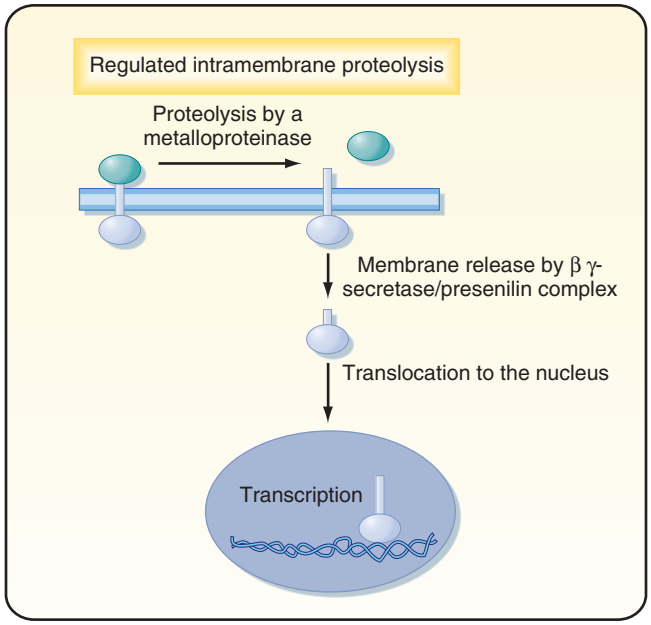
• **Fig. 3.4** Three of the Four Classes of Plasma Membrane Receptors. See text for details. (Redrawn from Alberts B, et al: *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

and aldosterone, are located in the cytosol and enter the nucleus after binding to hormone, whereas other receptors, including the thyroid hormone receptor, are located in the nucleus. In both cases, inactive receptors are bound to inhibitory proteins, and binding of hormone results in dissociation of the inhibitory complex. Hormone binding causes the receptor to bind coactivator proteins that activate gene transcription. Once activated, the hormone-receptor complex regulates the transcription of specific genes. Activation of specific genes usually occurs in two steps: an early primary response (≈30 minutes), which activates genes that stimulate other genes to produce a delayed (hours to days) secondary response (see Fig. 3.5). Each hormone elicits a specific response that is based on cellular expression of the cognate receptor, as well as on cell type–specific expression of gene regulatory proteins that interact with the activated receptor to regulate the transcription of a specific set of genes (see Chapter 38 for more details). In addition to steroid receptors that regulate gene expression, evidence also suggests the existence of membrane and juxtamembrane steroid receptors that mediate the rapid, nongenomic effects of steroid hormones.

Some membrane proteins do not fit the classic definition of receptors, but they subserve a receptor-like function in that they recognize extracellular signals and transduce the signals into an intracellular second messenger that has a biological effect. For example, on activation by a ligand, some membrane proteins undergo **regulated intramembrane proteolysis (RIP),** which elaborates a cytosolic peptide fragment that enters the nucleus and regulates gene expression (Fig. 3.6). In this signaling pathway, binding of ligand to a plasma membrane receptor leads to ectodomain shedding, facilitated by members of the metalloproteinase-disintegrin family, and produces a carboxy-terminal fragment that is the substrate for γ-secretase. γ-Secretase induces RIP, thereby causing the release of an intracellular domain of



• **Fig. 3.5** Steroid Hormones Stimulate the Transcription of Early-Response Genes and Late-Response Genes. See text for details. (Redrawn from Alberts B, et al: *Molecular Biology of Cell.* 6th ed. New York: Garland Science; 2015.)



• **Fig. 3.6** Regulated Intramembrane Proteolysis. See text for details. (Redrawn from Alberts B, et al: *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

the protein that enters the nucleus and regulates transcription (see Fig. 3.6). The best characterized example of RIP is the sterol regulatory element–binding protein (SREBP), a transmembrane protein expressed in the membrane of the endoplasmic reticulum. When cellular cholesterol levels are low, SREBP undergoes RIP, and the proteolytically cleaved fragment is translocated into the nucleus, where it transcriptionally activates genes that promote cholesterol biosynthesis.

Receptors and Signal Transduction Pathways

When hormones bind to plasma membrane receptors, signals are relayed to effector proteins via intracellular signaling pathways. When hormones bind to nuclear or cytosolic receptors, they relay signals primarily through regulation of gene expression. Signaling pathways can amplify and integrate signals but can also downregulate and desensitize signals, reducing or terminating the response, even in the continued presence of hormone.