Overview of Cellular Physiology in Medical Physiology

CHAPTER 2

OBJECTIVES

After studying this chapter, you should be able to:

- Name the prominent cellular organelles and state their functions in cells.
- Name the building blocks of the cellular cytoskeleton and state their contributions to cell structure and function.
- Name the intercellular and cellular to extracellular connections.
- Define the processes of exocytosis and endocytosis, and describe the contribution of each to normal cell function.
- Define proteins that contribute to membrane permeability and transport.
- Recognize various forms of intercellular communication and describe ways in which chemical messengers (including second messengers) affect cellular physiology.

INTRODUCTION

The cell is the fundamental working unit of all organisms. In humans, cells can be highly specialized in both structure and function; alternatively, cells from different organs can share features and function. In the previous chapter, we examined some basic principles of biophysics and the catabolism and metabolism of building blocks found in the cell. In some of those discussions, we examined how the building blocks could contribute to basic cellular physiology (eg, DNA replication, transcription, and translation). In this chapter, we will briefly review more of the fundamental aspects of cellular and molecular physiology. Additional aspects that concern specialization of cellular and molecular physiology are considered in the next chapters concerning immune function and excitable cells, and, within the sections that highlight each physiological system.

FUNCTIONAL MORPHOLOGY OF THE CELL

A basic knowledge of cell biology is essential to an understanding of the organ systems and the way they function in the body. A key tool for examining cellular constituents is the microscope. A light microscope can resolve structures as close as 0.2 µm, while an electron microscope can resolve structures as close as 0.002 µm. Although cell dimensions are quite variable, this resolution can give us a good look at the inner workings of the cell. The advent of common access to phase contrast, fluorescent, confocal, and many other microscopy techniques along with specialized probes for both static and dynamic cellular structures further expanded the examination of cell structure and function. Equally revolutionary advances in modern biophysical, biochemical, and molecular biological techniques have also greatly contributed to our knowledge of the cell.

The specialization of the cells in the various organs is considerable, and no cell can be called "typical" of all cells in the body. However, a number of structures **(organelles)** are common to most cells. These structures are shown in **Figure 2–1**. Many of them can be isolated by ultracentrifugation combined with other techniques. When cells are homogenized and the resulting suspension is centrifuged, the nuclei sediment first, followed by the mitochondria. High-speed centrifugation that

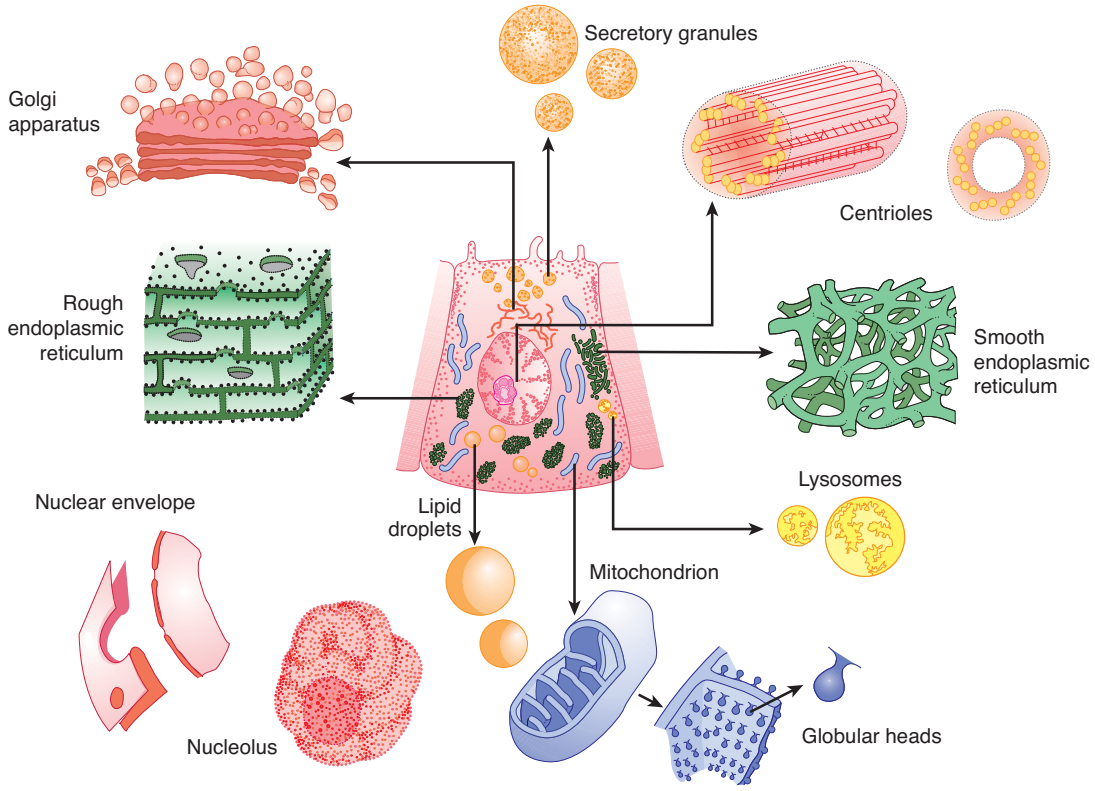


FIGURE 2–1 Diagram showing a hypothetical cell in the center as seen with the light microscope. Individual organelles are expanded for closer examination. (Adapted from Bloom and Fawcett. Reproduced with permission from Junqueira LC, Carneiro J, Kelley RO: *Basic Histology,* 9th ed. McGraw-Hill, 1998.)

generates forces of 100,000 times gravity or more causes a fraction made up of granules called the **microsomes** to sediment. This fraction includes organelles such as the **ribosomes** and **peroxisomes.**

CELL MEMBRANES

The membrane that surrounds the cell is a remarkable structure. It is made up of lipids and proteins and is semipermeable, allowing some substances to pass through it and excluding others. However, its permeability can also be varied because it contains numerous regulated ion channels and other transport proteins that can change the amounts of substances moving across it. It is generally referred to as the **plasma membrane.** The nucleus and other organelles in the cell are bound by similar membranous structures.

Although the chemical structures of membranes and their properties vary considerably from one location to another, they have certain common features. They are generally about 7.5 nm (75 Å) thick. The major lipids are phospholipids such as phosphatidylcholine, phosphotidylserine, and phosphatidylethanolamine. The shape of the phospholipid molecule reflects its solubility properties: the "head" end of the molecule contains the phosphate portion and is relatively soluble in water (polar, **hydrophilic**) and the "tail" ends are relatively insoluble (nonpolar, **hydrophobic**). The possession of both hydrophilic and hydrophobic properties makes the lipid an **amphipathic** molecule. In the membrane, the hydrophilic ends of the molecules are exposed to the aqueous environment that bathes the exterior of the cells and the aqueous cytoplasm; the hydrophobic ends meet in the water-poor interior of the membrane (Figure 2–2). In **prokaryotes** (ie, bacteria in which there is no nucleus), the membranes are relatively simple, but in **eukaryotes** (cells containing nuclei), cell membranes contain various glycosphingolipids, sphingomyelin, and cholesterol in addition to phospholipids and phosphatidylcholine.

Many different proteins are embedded in the membrane. They exist as separate globular units and many pass through or are embedded in one leaflet of the membrane **(integral proteins),** whereas others **(peripheral proteins)** are associated with the inside or outside of the membrane (Figure 2–2). The amount of protein varies significantly with the function of the membrane but makes up on average 50% of the mass of the membrane; that is, there is about one protein molecule per 50 of the much smaller phospholipid molecules. The proteins in the membrane carry out many functions. Some are **cell adhesion molecules (CAMs)** that anchor cells to their neighbors or to basal laminas. Some proteins function

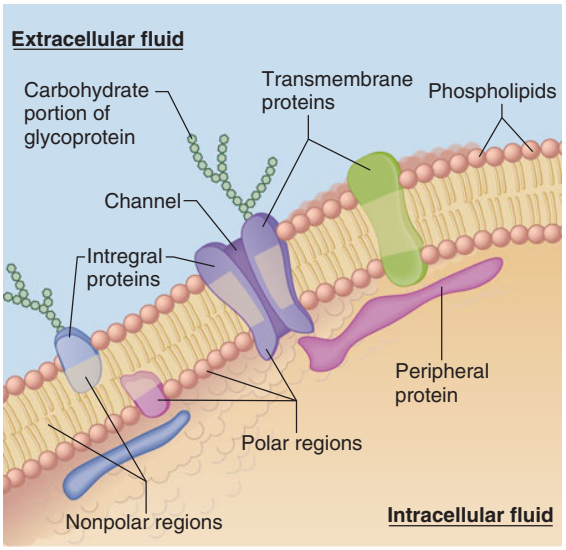


FIGURE 2–2 Organization of the phospholipid bilayer and associated proteins in a biological membrane. The phospholipid molecules each have two fatty acid chains (wavy lines) attached to a phosphate head (open circle). Proteins are shown as irregular colored globules. Many are integral proteins, which extend into the membrane, but peripheral proteins are attached to the inside or outside (not shown) of the membrane. Specific protein attachments and cholesterol commonly found in the bilayer are omitted for clarity. (Reproduced with permission from Widmaier EP, Raff H, Strang K: *Vander's Human Physiology: The Mechanisms of Body Function,* 11th ed. McGraw-Hill, 2008.)

as **pumps,** actively transporting ions across the membrane. Other proteins function as **carriers,** transporting substances down electrochemical gradients by facilitated diffusion. Still others are **ion channels,** which, when activated, permit the passage of ions into or out of the cell. The role of the pumps, carriers, and ion channels in transport across the cell membrane is discussed below. Proteins in another group function as **receptors** that bind **ligands** or messenger molecules, initiating physiologic changes inside the cell. Proteins also function as **enzymes,** catalyzing reactions at the surfaces of the membrane. Examples from each of these groups are discussed later in this chapter.

The uncharged, hydrophobic portions of the proteins are usually located in the interior of the membrane, whereas the charged, hydrophilic portions are located on the surfaces. Peripheral proteins are attached to the surfaces of the membrane in various ways. One common way is attachment to glycosylated forms of phosphatidylinositol. Proteins held by these **glycosylphosphatidylinositol (GPI) anchors** (Figure 2–3) include enzymes such as alkaline phosphatase, various antigens, a number of CAMs, and three proteins that combat cell lysis by complement. Over 45 GPI-linked cell surface proteins have now been described in humans. Other proteins are **lipidated,** that is, they have specific lipids attached to them (Figure 2–3). Proteins may be **myristoylated, palmitoylated,** or **prenylated** (ie, attached to geranylgeranyl or farnesyl groups).

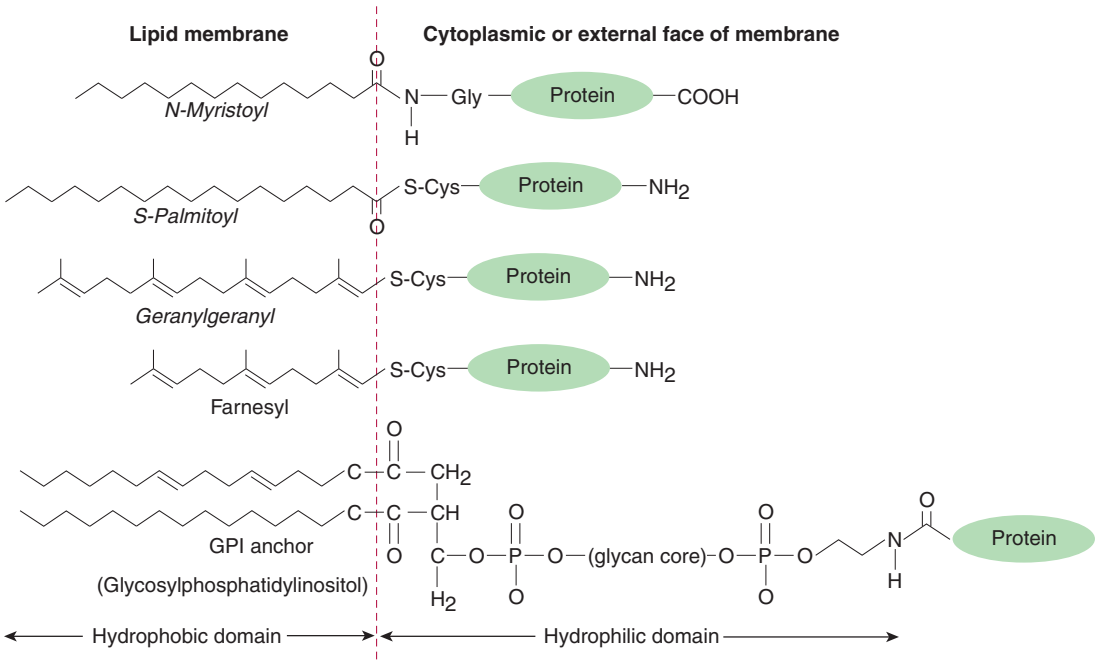


FIGURE 2–3 Protein linkages to membrane lipids. Some are linked by their amino terminals, others by their carboxyl terminals. Many are attached via glycosylated forms of phosphatidylinositol (GPI anchors). (Adapted with permission from Fuller GM, Shields D: *Molecular Basis of Medical Cell Biology*. McGraw-Hill, 1998.)

The protein structure—and particularly the enzyme content—of biologic membranes varies not only from cell to cell, but also within the same cell. For example, some of the enzymes embedded in cell membranes are different from those in mitochondrial membranes. In epithelial cells, the enzymes in the cell membrane on the mucosal surface differ from those in the cell membrane on the basal and lateral margins of the cells; that is, the cells are **polarized.** Such polarization makes directional transport across epithelia possible. The membranes are dynamic structures, and their constituents are being constantly renewed at different rates. Some proteins are anchored to the cytoskeleton, but others move laterally in the membrane.

Underlying most cells is a thin, "fuzzy" layer plus some fibrils that collectively make up the **basement membrane** or, more properly, the **basal lamina.** The basal lamina and, more generally, the extracellular matrix are made up of many proteins that hold cells together, regulate their development, and determine their growth. These include collagens, laminins, fibronectin, tenascin, and various proteoglycans.

MITOCHONDRIA

Over a billion years ago, aerobic bacteria were engulfed by eukaryotic cells and evolved into **mitochondria,** providing the eukaryotic cells with the ability to form the energy-rich compound ATP by **oxidative phosphorylation.** Mitochondria perform other functions, including a role in the regulation of **apoptosis** (programmed cell death), but oxidative phosphorylation is the most crucial. Each eukaryotic cell can have hundreds to thousands of mitochondria. In mammals, they are generally depicted as sausage-shaped organelles (Figure 2–1), but their shape can be quite dynamic. Each has an outer membrane, an intermembrane space, an inner membrane, which is folded to form shelves **(cristae),** and a central matrix space. The enzyme complexes responsible for oxidative phosphorylation are lined up on the cristae (Figure 2–4).

Consistent with their origin from aerobic bacteria, the mitochondria have their own genome. There is much less DNA in the mitochondrial genome than in the nuclear genome, and 99% of the proteins in the mitochondria are the products of nuclear genes, but mitochondrial DNA is responsible for certain key components of the pathway for oxidative phosphorylation. Specifically, human mitochondrial DNA is a double-stranded circular molecule containing approximately 16,500 base pairs (compared with over a billion in nuclear DNA). It codes for 13 protein subunits that are associated with proteins encoded by nuclear genes to form four enzyme complexes plus two ribosomal and 22 transfer RNAs that are needed for protein production by the intramitochondrial ribosomes.

The enzyme complexes responsible for oxidative phosphorylation illustrate the interactions between the products of the mitochondrial genome and the nuclear genome. For example, complex I, reduced nicotinamide adenine dinucleotide dehydrogenase (NADH), is made up of seven protein subunits coded by mitochondrial DNA and 39 subunits coded by nuclear DNA. The origin of the subunits in the other complexes is shown in Figure 2–4. Complex II, succinate dehydrogenase-ubiquinone oxidoreductase; complex III, ubiquinonecytochrome c oxidoreductase; and complex IV, cytochrome c oxidase, act with complex I, coenzyme Q, and cytochrome c to convert metabolites to CO_2 and water. Complexes I, III, and IV pump protons (H^+) into the intermembrane space during this electron transfer. The protons then flow down their electrochemical gradient through complex V, ATP synthase, which harnesses this energy to generate ATP.

As zygote mitochondria are derived from the ovum, their inheritance is maternal. This maternal inheritance has been used as a tool to track evolutionary descent. Mitochondria have an ineffective DNA repair system, and the mutation rate for mitochondrial DNA is over 10 times the rate for nuclear DNA. A large number of relatively rare diseases have now been traced to mutations in mitochondrial DNA. These include disorders of tissues with high metabolic rates in which energy production is defective as a result of abnormalities in the production of ATP, as well as other disorders (Clinical Box 2–1).

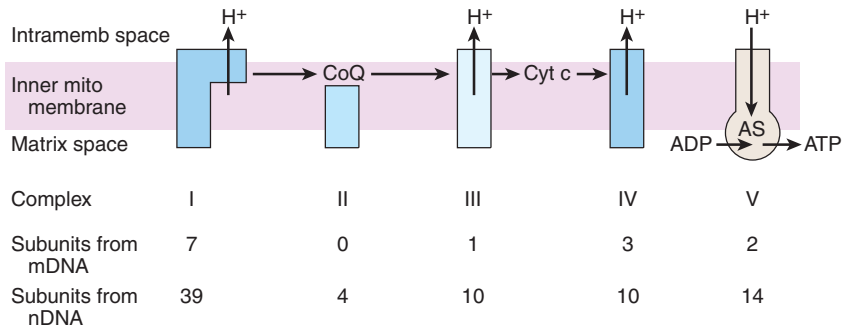


FIGURE 2–4 Components involved in oxidative phosphorylation in mitochondria and their origins. As enzyme complexes I through IV convert 2-carbon metabolic fragments to CO_2 and H_2O, protons (H^+) are pumped into the intermembrane space. The proteins diffuse back to the matrix space via complex V, ATP synthase (AS), in which ADP is converted to ATP. The enzyme complexes are made up of subunits coded by mitochondrial DNA (mDNA) and nuclear DNA (nDNA), and the figures document the contribution of each DNA to the complexes.

CLINICAL BOX 2–1

Mitochondrial Diseases

Mitochondrial diseases encompass at least 40 diverse disorders that are grouped because of their links to mitochondrial failure. These diseases can occur following inheritance or spontaneous mutations in mitochondrial or nuclear DNA that lead to altered functions of the mitochondrial proteins (or RNA). Depending on the target cell and/or tissues affected, symptoms resulting from mitochondrial diseases may include altered motor control, altered muscle output, gastrointestinal dysfunction, altered growth, diabetes, seizures, visual/hearing problems, lactic acidosis, developmental delays, susceptibility to infection or, cardiac, liver and respiratory disease. Although there is evidence for tissue-specific isoforms of mitochondrial proteins, mutations in these proteins do not fully explain the highly variable patterns or targeted organ systems observed with mitochondrial diseases.

THERAPEUTIC HIGHLIGHTS

With the diversity of disease types and the overall importance of mitochondria in energy production, it is not surprising that there is no single cure for mitochondrial diseases and focus remains on treating the symptoms when possible. For example, in some mitochondrial myopathies (ie, mitochondrial diseases associated with neuromuscular function), physical therapy may help to extend the range of movement of muscles and improve dexterity.

LYSOSOMES

In the cytoplasm of the cell there are large, somewhat irregular structures surrounded by membranes. The interior of these structures, which are called **lysosomes,** is more acidic than the rest of the cytoplasm, and external material such as endocytosed bacteria, as well as worn-out cell components, are digested in them. The interior is kept acidic by the action of a **proton pump,** or **H^+ ATPase.** This integral membrane protein uses the energy of ATP to move protons from the cytosol up their electrochemical gradient and keep the lysosome relatively acidic, near pH 5.0. Lysosomes can contain over 40 types of hydrolytic enzymes, some of which are listed in Table 2–1. Not surprisingly, these enzymes are all acid hydrolases, in that they function best at the acidic pH of the lysosomal compartment. This can be a safety feature for the cell; if the lysosomes were to break open and release their contents, the enzymes would not be efficient at the near neutral cytosolic pH (7.2), and thus would be unable to digest cytosolic enzymes they may encounter. Diseases associated with lysosomal dysfunction are discussed in Clinical Box 2–2.

TABLE 2–1 Some of the enzymes found in lysosomes and the cell components that are their substrates.

Enzyme	Substrate
Ribonuclease	RNA
Deoxyribonuclease	DNA
Phosphatase	Phosphate esters
Glycosidases	Complex carbohydrates; glycosides and polysaccharides
Arylsulfatases	Sulfate esters
Collagenase	Collagens
Cathepsins	Proteins

CLINICAL BOX 2–2

Lysosomal Diseases

When a lysosomal enzyme is congenitally absent, the lysosomes become engorged with the material the enzyme normally degrades. This eventually leads to one of the **lysosomal diseases** (also called lysosomal storage diseases). There are over 50 such diseases currently recognized. For example, Fabry disease is caused by a deficiency in α-galactosidase; Gaucher disease is caused by a deficiency in β-galactocerebrosidase and Tay–Sachs disease, which causes mental retardation and blindness, is caused by the loss of hexosaminidase A, a lysosomal enzyme that catalyzes the biodegradation of gangliosides (fatty acid derivatives). Such individual lysosomal diseases are rare, but they are serious and can be fatal.

THERAPEUTIC HIGHLIGHTS

Since there are many different lysosomal disorders, treatments vary considerably and "cures" remain elusive for most of these diseases. Much of the care is focused on managing symptoms of each specific disorder. Enzyme replacement therapy has shown to be effective for certain lysosomal diseases, including Gaucher's disease and Fabry's disease. However, the long-term effectiveness and the tissue specific effects of many of the enzyme replacement treatments have not yet been established. Alternative approaches include bone marrow or stem cell transplantation. Again, these are limited in use and medical advances are necessary to fully combat this group of diseases.

PEROXISOMES

Peroxisomes are 0.5 μm in diameter, are surrounded by a membrane, and contain enzymes that can either produce H_2O_2 **(oxidases)** or break it down **(catalases).** Proteins are directed to the peroxisome by a unique signal sequence with the help of protein chaperones, **peroxins.** The peroxisome membrane contains a number of peroxisome-specific proteins that are concerned with transport of substances into and out of the matrix of the peroxisome. The matrix contains more than 40 enzymes, which operate in concert with enzymes outside the peroxisome to catalyze a variety of anabolic and catabolic reactions (eg, breakdown of lipids). Peroxisomes can form by budding of the endoplasmic reticulum, or by division. A number of synthetic compounds were found to cause proliferation of peroxisomes by acting on receptors in the nuclei of cells. These **peroxisome proliferator-activated receptors (PPARs)** are members of the nuclear receptor superfamily. When activated, they bind to DNA, producing changes in the production of mRNAs. The known effects for PPARs are extensive and can affect most tissues and organs.

CYTOSKELETON

All cells have a **cytoskeleton,** a system of fibers that not only maintains the structure of the cell but also permits it to change shape and move. The cytoskeleton is made up primarily of **microtubules, intermediate filaments,** and **microfilaments** (Figure 2–5), along with proteins that anchor them and tie them together. In addition, proteins and organelles move along microtubules and microfilaments from one part of the cell to another, propelled by molecular motors.

Microtubules (Figure 2–5 and Figure 2–6) are long, hollow structures with 5-nm walls surrounding a cavity 15 nm in diameter. They are made up of two globular protein subunits: α- and β-tubulin. A third subunit, γ-tubulin, is associated with the production of microtubules by the centrosomes. The α and β subunits form heterodimers, which aggregate to form long tubes made up of stacked rings, with each ring usually containing 13 subunits. The tubules interact with GTP to facilitate their formation. Although microtubule subunits can be added to either end, microtubules are polar with assembly predominating at the "+" end and disassembly predominating at the "–" end. Both processes occur simultaneously in vitro. The growth of microtubules is temperature sensitive (disassembly is favored under cold conditions) as well as under the control of a variety of cellular factors that can directly interact with microtubules in the cell.

Because of their constant assembly and disassembly, microtubules are a dynamic portion of the cytoskeleton. They provide the tracks along which several different molecular motors move transport vesicles, organelles such as secretory granules, and mitochondria from one part of the cell to another. They also form the spindle, which moves the chromosomes in mitosis. Cargo can be transported in either direction on microtubules.

There are several drugs available that disrupt cellular function through interaction with microtubules. Microtubule assembly is prevented by colchicine and vinblastine. The

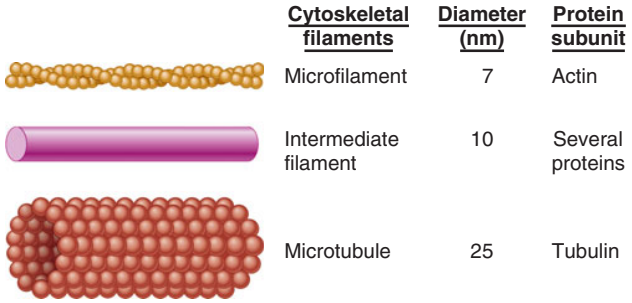


FIGURE 2–5 Cytoskeletal elements of the cell. Artistic impressions that depict the major cytoskeletal elements are shown on the left, with basic properties of these elements on the right. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function,* 11th ed. McGraw-Hill, 2008.)

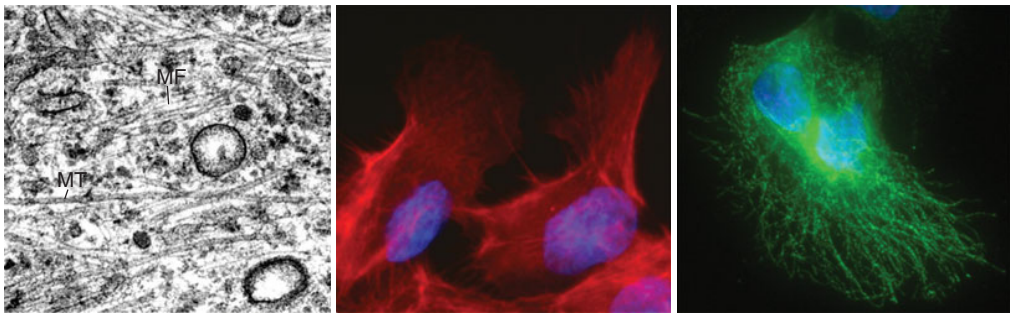


FIGURE 2–6 Microfilaments and microtubules. Electronmicrograph **(Left)** of the cytoplasm of a fibroblast, displaying actin microfilaments (MF) and microtubules (MT). Fluorescent micrographs of airway epithelial cells displaying actin microfilaments stained with phalloidin **(Middle)** and microtubules visualized with an antibody to β-tubulin **(Right).** Both fluorescent micrographs are counterstained with Hoechst dye (blue) to visualize nuclei. Note the distinct differences in cytoskeletal structure. (For left; Courtesy of E Katchburian.)

anticancer drug **paclitaxel (Taxol)** binds to microtubules and makes them so stable that organelles cannot move. Mitotic spindles cannot form, and the cells die.

Intermediate filaments (Figure 2–5) are 8–14 nm in diameter and are made up of various subunits. Some of these filaments connect the nuclear membrane to the cell membrane. They form a flexible scaffolding for the cell and help it resist external pressure. In their absence, cells rupture more easily, and when they are abnormal in humans, blistering of the skin is common. The proteins that make up intermediate filaments are cell-type specific, and are thus frequently used as cellular markers. For example, vimentin is a major intermediate filament in fibroblasts, whereas cytokeratin is expressed in epithelial cells.

Microfilaments (Figures 2–5 and 2–6) are long solid fibers with a 4–6 nm diameter that are made up of **actin.** Although actin is most often associated with muscle contraction, it is present in all types of cells. It is the most abundant protein in mammalian cells, sometimes accounting for as much as 15% of the total protein in the cell. Its structure is highly conserved; for example, 88% of the amino acid sequences in yeast and rabbit actin are identical. Actin filaments polymerize and depolymerize in vivo, and it is not uncommon to find polymerization occurring at one end of the filament while depolymerization is occurring at the other end. **Filamentous (F) actin** refers to intact microfilaments and **globular (G) actin** refers to the unpolymerized protein actin subunits. F-actin fibers attach to various parts of the cytoskeleton and can interact directly or indirectly with membrane-bound proteins. They reach to the tips of the microvilli on the epithelial cells of the intestinal mucosa. They are also abundant in the lamellipodia that cells put out when they crawl along surfaces. The actin filaments interact with integrin receptors and form **focal adhesion complexes,** which serve as points of traction with the surface over which the cell pulls itself. In addition, some molecular motors use microfilaments as tracks.

MOLECULAR MOTORS

The molecular motors that move proteins, organelles, and other cell parts (collectively referred to as "cargo") to all parts of the cell are 100–500 kDa ATPases. They attach to their cargo at one end of the molecule and to microtubules or actin polymers with the other end, sometimes referred to as the "head." They convert the energy of ATP into movement along the cytoskeleton, taking their cargo with them. There are three super families of molecular motors: **kinesin, dynein,** and **myosin.** Examples of individual proteins from each superfamily are shown in Figure 2–7. It is important to note that there is extensive variation among superfamily members, allowing for the specialization of function (eg, choice of cargo, cytoskeletal filament type, and/or direction of movement).

The conventional form of **kinesin** is a doubleheaded molecule that tends to move its cargo toward the "+" ends of microtubules. One head binds to the microtubule and then bends its neck while the other head swings forward and binds, producing almost continuous movement. Some kinesins are associated with mitosis and meiosis. Other kinesins perform different functions, including, in some instances, moving cargo to the "–" end of microtubules. **Dyneins** have two heads, with their neck pieces embedded in a complex of proteins. **Cytoplasmic dyneins** have a function like that of conventional kinesin, except they tend to move particles and membranes to the "–" end of the microtubules. The multiple forms of **myosin** in the body are divided into 18 classes. The heads of myosin molecules bind to actin and produce motion by bending their neck

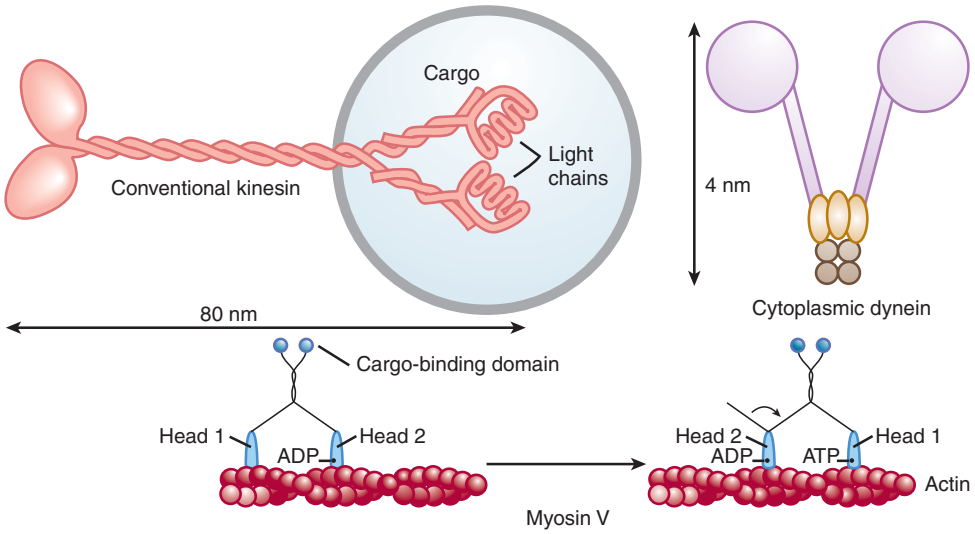


FIGURE 2–7 Three examples of molecular motors. Conventional kinesin is shown attached to cargo, in this case a membrane-bound organelle. The way that myosin V "walks" along a microtubule is also shown. Note that the heads of the motors hydrolyze ATP and use the energy to produce motion.

regions (myosin II) or walking along microfilaments, one head after the other (myosin V). In these ways, they perform functions as diverse as contraction of muscle and cell migration.

CENTROSOMES

Near the nucleus in the cytoplasm of eukaryotic animal cells is a **centrosome.** The centrosome is made up of two **centrioles** and surrounding amorphous **pericentriolar material.** The centrioles are short cylinders arranged so that they are at right angles to each other. Microtubules in groups of three run longitudinally in the walls of each centriole (Figure 2–1). Nine of these triplets are spaced at regular intervals around the circumference.

The centrosomes are **microtubule-organizing centers (MTOCs)** that contain γ-tubulin. The microtubules grow out of this γ-tubulin in the pericentriolar material. When a cell divides, the centrosomes duplicate themselves, and the pairs move apart to the poles of the mitotic spindle, where they monitor the steps in cell division. In multinucleate cells, a centrosome is near each nucleus.

CILIA

Cilia are specialized cellular projections that are used by unicellular organisms to propel themselves through liquid and by multicellular organisms to propel mucus and other substances over the surface of various epithelia. Additionally, virtually all cells in the human body contain a primary cilium that emanates from the surface. The primary cilium serves as a sensory organelle that receives both mechanical and chemical signals from other cells and the environment. Cilia are functionally indistinct from the eukaryotic flagella of sperm cells. Within the cilium there is an **axoneme** that comprises a unique arrangement of nine outer microtubule doublets and two inner microtubules ("9+2" arrangement). Along this cytoskeleton is **axonemal dynein.** Coordinated dynein–microtubule interactions within the axoneme are the basis of ciliary and sperm movement. At the base of the axoneme and just inside lies the **basal body.** It has nine circumferential triplet microtubules, like a centriole, and there is evidence that basal bodies and centrioles are interconvertible. A wide variety of diseases and disorders arise from dysfunctional cilia (Clinical Box 2–3).

CELL ADHESION MOLECULES

Cells are attached to the basal lamina and to each other by **CAMs** that are prominent parts of the intercellular connections described below. These adhesion proteins have attracted great attention in recent years because of their unique structural and signaling functions found to be important in embryonic development and formation of the nervous system and other tissues, in holding tissues together in adults, in inflammation and wound healing, and in the metastasis of tumors. Many CAMs pass through the cell membrane and are anchored to the cytoskeleton inside the cell. Some bind to like molecules on other cells (homophilic binding), whereas others bind to nonself molecules (heterophilic binding). Many bind to **laminins,** a family of large cross-shaped molecules with multiple receptor domains in the extracellular matrix.

Nomenclature in the CAM field is somewhat chaotic, partly because the field is growing so rapidly and partly because of the extensive use of acronyms, as in other areas of modern biology. However, the CAMs can be divided into four broad families: (1) **integrins,** heterodimers that bind to various receptors; (2) adhesion molecules of the **IgG superfamily** of immunoglobulins; (3) **cadherins,** Ca^{2+}-dependent molecules that mediate cell-to-cell adhesion by homophilic reactions; and (4) **selectins,** which have lectin-like domains that bind carbohydrates. Specific functions of some of these molecules are addressed in later chapters.

The CAMs not only fasten cells to their neighbors, but they also transmit signals into and out of the cell. For example, cells that lose their contact with the extracellular matrix via integrins have a higher rate of apoptosis than anchored cells, and interactions between integrins and the cytoskeleton are involved in cell movement.

CLINICAL BOX 2–3

Ciliary Diseases

Primary ciliary dyskinesia refers to a set of inherited disorders that limit ciliary structure and/or function. Disorders associated with ciliary dysfunction have long been recognized in the conducting airway. Altered ciliary function in the conducting airway can slow the mucociliary escalator and result in airway obstruction and increased infection. Dysregulation of ciliary function in sperm cells has also been well characterized to result in loss of motility and infertility. Ciliary defects in the function or structure of primary cilia have been shown to have effects on a variety of tissues/organs. As would be expected, such diseases are quite varied in their presentation, largely due to the affected tissue and include mental retardation, retinal blindness, obesity, polycystic kidney disease, liver fibrosis, ataxia, and some forms of cancer.

THERAPEUTIC HIGHLIGHTS

The severity in ciliary disorders can vary widely, and treatments targeted to individual organs also vary. Treatment of ciliary dyskinesia in the conducting airway is focused on keeping the airways clear and free of infection. Strategies include routine washing and suctioning of the sinus cavities and ear canals and liberal use of antibiotics. Other treatments that keep the airway from being obstructed (eg, bronchodilators, mucolytics, and steroids) are also commonly used.

INTERCELLULAR CONNECTIONS

Intercellular junctions that form between the cells in tissues can be broadly split into two groups: junctions that fasten the cells to one another and to surrounding tissues, and junctions that permit transfer of ions and other molecules from one cell to another. The types of junctions that tie cells together and endow tissues with strength and stability include **tight junctions,** which are also known as the **zonula occludens** (Figure 2–8). The **desmosome** and **zonula adherens** also help to hold cells together, and the **hemidesmosome** and **focal adhesions** attach cells to their basal laminas. The **gap junction** forms a cytoplasmic "tunnel" for diffusion of small molecules (< 1000 Da) between two neighboring cells.

Tight junctions characteristically surround the apical margins of the cells in epithelia such as the intestinal mucosa, the walls of the renal tubules, and the choroid plexus. They are also important to endothelial barrier function. They are made up of ridges—half from one cell and half from the other—which adhere so strongly at cell junctions that they almost obliterate the space between the cells. There are three main families of transmembrane proteins that contribute to tight junctions: **occludin, junctional adhesion molecules (JAMs),** and **claudins;** and several more proteins that interact from the cytosolic side. Tight junctions permit the passage of some ions and solute in between adjacent cells **(paracellular pathway)** and the degree of this "leakiness" varies, depending in part on the protein makeup of the tight junction. Extracellular fluxes of ions and solute across epithelia at these junctions are a significant part of overall ion and solute flux. In addition, tight junctions prevent the movement of proteins in the plane of the membrane, helping to maintain the different distribution of transporters and channels in the apical and basolateral cell membranes that make transport across epithelia possible.

In epithelial cells, each zonula adherens is usually a continuous structure on the basal side of the zonula occludens, and it is a major site of attachment for intracellular microfilaments. It contains cadherins.

Desmosomes are patches characterized by apposed thickenings of the membranes of two adjacent cells. Attached to the thickened area in each cell are intermediate filaments, some running parallel to the membrane and others radiating away from it. Between the two membrane thickenings the intercellular space contains filamentous material that includes cadherins and the extracellular portions of several other transmembrane proteins.

Hemidesmosomes look like half-desmosomes that attach cells to the underlying basal lamina and are connected intracellularly to intermediate filaments. However, they contain integrins rather than cadherins. Focal adhesions also attach cells to their basal laminas. As noted previously, they are labile structures associated with actin filaments inside the cell, and they play an important role in cell movement.

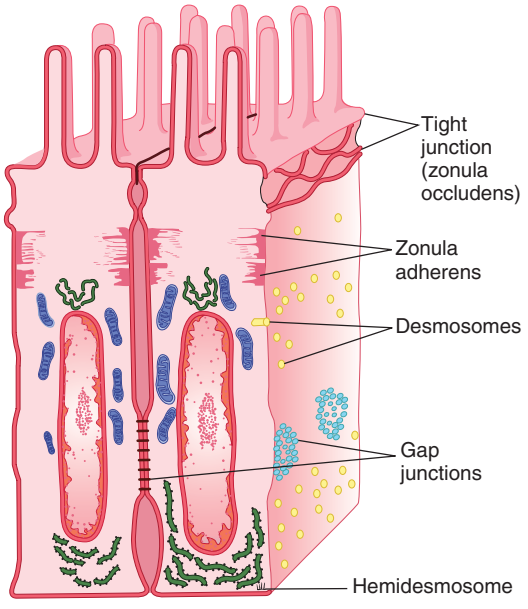


FIGURE 2–8 Intercellular junctions in the mucosa of the small intestine. Tight junctions (zonula occludens), adherens junctions (zonula adherens), desmosomes, gap junctions, and hemidesmosomes are all shown in relative positions in a polarized epithelial cell.

GAP JUNCTIONS

At gap junctions, the intercellular space narrows from 25 to 3 nm, and units called **connexons** in the membrane of each cell are lined up with one another to form the dodecameric gap junction (Figure 2–9). Each connexon is made up of six protein subunits called **connexins.** They surround a channel that, when lined up with the channel in the corresponding connexon in the adjacent cell, permits substances to pass between the cells without entering the ECF. The diameter of the channel is normally about 2 nm, which permits the passage of ions, sugars, amino acids, and other solutes with molecular weights up to about 1000. Gap junctions thus permit the rapid propagation of electrical activity from cell to cell, as well as the exchange of various chemical messengers. However, the gap junction channels are not simply passive, nonspecific conduits. At least 20 different genes code for connexins in humans, and mutations in these genes can lead to diseases that are highly selective in terms of the tissues involved and the type of communication between cells produced (Clinical Box 2–4). Experiments in which particular connexins are deleted by gene manipulation or replaced with different connexins confirm that the particular connexin subunits that make up connexons determine their permeability and selectivity. It should be noted that connexons can also provide a conduit for regulated passage of small molecules between the cytoplasm and the ECF. Such movement can allow additional signaling pathways between and among cells in a tissue.

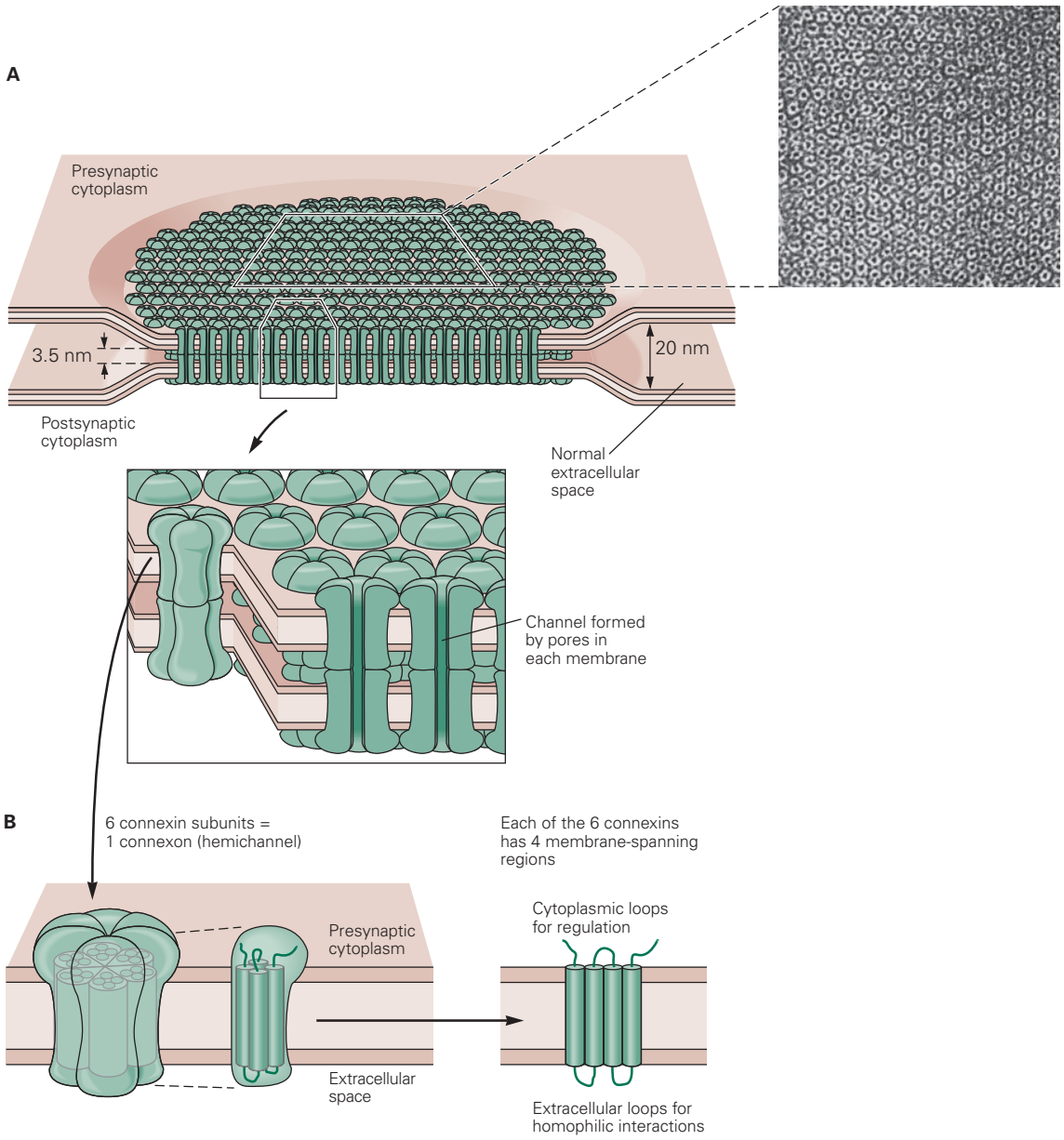


FIGURE 2–9 Gap junction connecting the cytoplasm of two cells. A) A gap junction plaque, or collection of individual gap junctions, is shown to form multiple pores between cells that allow for the transfer of small molecules. Inset is electronmicrograph from rat liver (N. Gilula). **B)** Topographical depiction of individual connexon and corresponding six connexin proteins that traverse the membrane. Note that each connexin traverses the membrane four times. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science*, 4th ed. McGraw-Hill, 2000.)

NUCLEUS & RELATED STRUCTURES

A nucleus is present in all eukaryotic cells that divide. The nucleus is made up in large part of the **chromosomes,** the structures in the nucleus that carry a complete blueprint for all the heritable species and individual characteristics of the animal. Except in germ cells, the chromosomes occur in pairs, one originally from each parent. Each chromosome is made up of a giant molecule of **DNA.** The DNA strand is about 2 m long, but it can fit in the nucleus because at intervals it is wrapped around a core of histone proteins to form a **nucleosome.** There are about 25 million nucleosomes in each nucleus. Thus, the structure of the chromosomes has been likened to a string of beads. The beads are the nucleosomes, and the linker DNA between them is the string. The whole complex of DNA and proteins is called **chromatin.** During cell division, the coiling around histones is loosened, probably by acetylation of

CLINICAL BOX 2–4

Connexins in Disease

In recent years, there has been an explosion of information related to the in vivo functions of connexins, growing out of work on connexin knock-outs in mice and the analysis of mutations in human connexins. The mouse knock-outs demonstrated that connexin deletions lead to electrophysiological defects in the heart and predisposition to sudden cardiac death, female sterility, abnormal bone development, abnormal growth in the liver, cataracts, hearing loss, and a host of other abnormalities. Information from these and other studies has allowed for the identification of several connexin mutations now known to be responsible for almost 20 different human diseases. These diseases include several skin disorders such as Clouston Syndrome (a connexin 30 (Cx30) defect) and erythrokeratoderma variabilis (Cx30.3 and Cx31); inherited deafness (Cx26, Cx30, and Cx31); predisposition to myoclonic epilepsy (Cx36), predisposition to arteriosclerosis (Cx37); cataract (Cx46 and Cx50); idiopathic atrial fibrillation (Cx40); and X-linked Charcot-Marie-Tooth disease (Cx32). It is interesting to note that each of these target tissues for disease contain other connexins that do not fully compensate for loss of the crucial connexins in disease development. Understanding how loss of individual connexins alters cell physiology to contribute to these and other human diseases is an area of intense research.

the histones, and pairs of chromosomes become visible, but between cell divisions only clumps of chromatin can be discerned in the nucleus. The ultimate units of heredity are the **genes** on the chromosomes). As discussed in Chapter 1, each gene is a portion of the DNA molecule.

The nucleus of most cells contains a **nucleolus** (Figure 2–1), a patchwork of granules rich in **RNA.** In some cells, the nucleus contains several of these structures. Nucleoli are most prominent and numerous in growing cells. They are the site of synthesis of ribosomes, the structures in the cytoplasm in which proteins are synthesized.

The interior of the nucleus has a skeleton of fine filaments that are attached to the **nuclear membrane,** or **envelope** (Figure 2–1), which surrounds the nucleus. This membrane is a double membrane, and spaces between the two folds are called **perinuclear cisterns.** The membrane is permeable only to small molecules. However, it contains **nuclear pore complexes.** Each complex has eightfold symmetry and is made up of about 100 proteins organized to form a tunnel through which transport of proteins and mRNA occurs. There are many transport pathways; many proteins that participate in these pathways, including **importins** and **exportins** have been isolated and characterized. Much current research is focused on transport into and out of the nucleus, and a more detailed understanding of these processes should emerge in the near future.

ENDOPLASMIC RETICULUM

The **endoplasmic reticulum** is a complex series of tubules in the cytoplasm of the cell (Figure 2–1; Figure 2–10; and Figure 2–11). The inner limb of its membrane is continuous with a segment of the nuclear membrane, so in effect this part of the nuclear membrane is a cistern of the endoplasmic reticulum. The tubule walls are made up of membrane. In **rough,** or **granular, endoplasmic reticulum,** ribosomes are attached to the cytoplasmic side of the membrane, whereas in **smooth,** or **agranular, endoplasmic reticulum,** ribosomes are absent. Free ribosomes are also found in the cytoplasm. The granular endoplasmic reticulum is concerned with protein synthesis

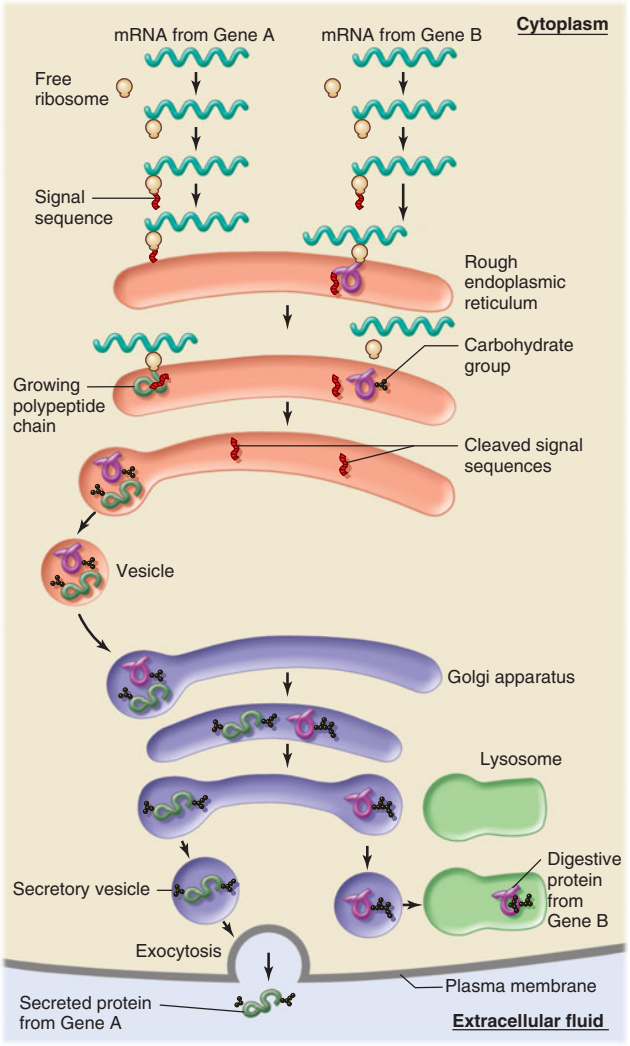


FIGURE 2–10 Rough endoplasmic reticulum and protein translation. Messenger RNA and ribosomes meet up in the cytosol for translation. Proteins that have appropriate signal peptides begin translation, and then associate with the endoplasmic reticulum (ER) to complete translation. The association of ribosomes is what gives the ER its "rough" appearance. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. McGraw-Hill, 2008.)

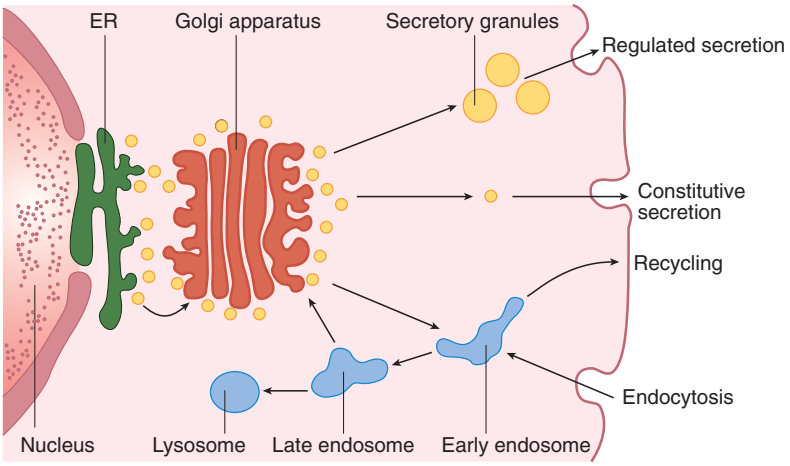


FIGURE 2–11 Cellular structures involved in protein processing. See text for details.

and the initial folding of polypeptide chains with the formation of disulfide bonds. The agranular endoplasmic reticulum is the site of steroid synthesis in steroid-secreting cells and the site of detoxification processes in other cells. A modified endoplasmic reticulum, the sarcoplasmic reticulum, plays an important role in skeletal and cardiac muscle. In particular, the endoplasmic or sarcoplasmic reticulum can sequester Ca^{2+} ions and allow for their release as signaling molecules in the cytosol.

RIBOSOMES

The ribosomes in eukaryotes measure approximately 22 × 32 nm. Each is made up of a large and a small subunit called, on the basis of their rates of sedimentation in the ultracentrifuge, the 60S and 40S subunits. The ribosomes are complex structures, containing many different proteins and at least three ribosomal RNAs. They are the sites of protein synthesis. The ribosomes that become attached to the endoplasmic reticulum synthesize all transmembrane proteins, most secreted proteins, and most proteins that are stored in the Golgi apparatus, lysosomes, and endosomes. These proteins typically have a hydrophobic **signal peptide** at one end (Figure 2–10). The polypeptide chains that form these proteins are extruded into the endoplasmic reticulum. The free ribosomes synthesize cytoplasmic proteins such as hemoglobin and the proteins found in peroxisomes and mitochondria.

GOLGI APPARATUS & VESICULAR TRAFFIC

The Golgi apparatus is a collection of membrane-enclosed sacs (cisternae) that are stacked like dinner plates (Figure 2–1). One or more Golgi apparati are present in all eukaryotic cells, usually near the nucleus. Much of the organization of the Golgi is directed at proper glycosylation of proteins and lipids. There are more than 200 enzymes that function to add, remove, or modify sugars from proteins and lipids in the Golgi apparatus.

The Golgi apparatus is a polarized structure, with cis and trans sides (Figures 2–1; 2–10; 2–11). Membranous vesicles containing newly synthesized proteins bud off from the granular endoplasmic reticulum and fuse with the cistern on the cis side of the apparatus. The proteins are then passed via other vesicles to the middle cisterns and finally to the cistern on the trans side, from which vesicles branch off into the cytoplasm. From the trans Golgi, vesicles shuttle to the lysosomes and to the cell exterior via constitutive and nonconstitutive pathways, both involving **exocytosis.** Conversely, vesicles are pinched off from the cell membrane by **endocytosis** and pass to endosomes. From there, they are recycled.

Vesicular traffic in the Golgi, and between other membranous compartments in the cell, is regulated by a combination of common mechanisms along with special mechanisms that determine where inside the cell they will go. One prominent feature is the involvement of a series of regulatory proteins controlled by GTP or GDP binding **(small G proteins)** associated with vesicle assembly and delivery. A second prominent feature is the presence of proteins called SNAREs (for soluble N-ethylmaleimide-sensitive factor attachment receptor). The v- (for vesicle) SNAREs on vesicle membranes interact in a lock-and-key fashion with t- (for target) SNAREs. Individual vesicles also contain structural protein or lipids in their membrane that help to target them for specific membrane compartments (eg, Golgi sacs, cell membranes).

QUALITY CONTROL

The processes involved in protein synthesis, folding, and migration to the various parts of the cell are so complex that it is remarkable that more errors and abnormalities do not occur. The fact that these processes work as well as they do is because of mechanisms at each level that are responsible

for "quality control." Damaged DNA is detected and repaired or bypassed. The various RNAs are also checked during the translation process. Finally, when the protein chains are in the endoplasmic reticulum and Golgi apparatus, defective structures are detected and the abnormal proteins are degraded in lysosomes and proteasomes. The net result is a remarkable accuracy in the production of the proteins needed for normal body function.

APOPTOSIS

In addition to dividing and growing under genetic control, cells can die and be absorbed under genetic control. This process is called **programmed cell death,** or **apoptosis** (Gr. apo "away" + ptosis "fall"). It can be called "cell suicide" in the sense that the cell's own genes play an active role in its demise. It should be distinguished from necrosis ("cell murder"), in which healthy cells are destroyed by external processes such as inflammation.

Apoptosis is a very common process during development and in adulthood. In the central nervous system (CNS), large numbers of neurons are produced and then die during the remodeling that occurs during development and synapse formation. In the immune system, apoptosis gets rid of inappropriate clones of immunocytes and is responsible for the lytic effects of glucocorticoids on lymphocytes. Apoptosis is also an important factor in processes such as removal of the webs between the fingers in fetal life and regression of duct systems in the course of sexual development in the fetus. In adults, it participates in the cyclic breakdown of the endometrium that leads to menstruation. In epithelia, cells that lose their connections to the basal lamina and neighboring cells undergo apoptosis. This is responsible for the death of the enterocytes sloughed off the tips of intestinal villi. Abnormal apoptosis probably occurs in autoimmune diseases, neurodegenerative diseases, and cancer. It is interesting that apoptosis occurs in invertebrates, including nematodes and insects. However, its molecular mechanism is much more complex than that in vertebrates.

One final common pathway bringing about apoptosis is activation of **caspases,** a group of cysteine proteases. Many of these have been characterized to date in mammals; 11 have been found in humans. They exist in cells as inactive proenzymes until activated by the cellular machinery. The net result is DNA fragmentation, cytoplasmic and chromatin condensation, and eventually membrane bleb formation, with cell breakup and removal of the debris by phagocytes (see Clinical Box 2–5).

TRANSPORT ACROSS CELL MEMBRANES

There are several mechanisms of transport across cellular membranes. Primary pathways include exocytosis, endocytosis, movement through ion channels, and primary and secondary active transport. Each of these are discussed below.

CLINICAL BOX 2–5

Molecular Medicine

Fundamental research on molecular aspects of genetics, regulation of gene expression, and protein synthesis has been paying off in clinical medicine at a rapidly accelerating rate.

One early dividend was an understanding of the mechanisms by which antibiotics exert their effects. Almost all act by inhibiting protein synthesis at one or another of the steps described previously. Antiviral drugs act in a similar way; for example, acyclovir and ganciclovir act by inhibiting DNA polymerase. Some of these drugs have this effect primarily in bacteria, but others inhibit protein synthesis in the cells of other animals, including mammals. This fact makes antibiotics of great value for research as well as for treatment of infections.

Single genetic abnormalities that cause over 600 human diseases have now been identified. Many of the diseases are rare, but others are more common and some cause conditions that are severe and eventually fatal. Examples include the defectively regulated Cl^- channel in cystic fibrosis and the unstable **trinucleotide repeats** in various parts of the genome that cause Huntington's disease, the fragile X syndrome, and several other neurologic diseases. Abnormalities in mitochondrial DNA can also cause human diseases such as Leber's hereditary optic neuropathy and some forms of cardiomyopathy. Not surprisingly, genetic aspects of cancer are probably receiving the greatest current attention. Some cancers are caused by **oncogenes,** genes that are carried in the genomes of cancer cells and are responsible for producing their malignant properties. These genes are derived by somatic mutation from closely related **proto-oncogenes,** which are normal genes that control growth. Over 100 oncogenes have been described. Another group of genes produce proteins that suppress tumors, and more than 10 of these **tumor suppressor genes** have been described. The most studied of these is the p53 gene on human chromosome 17. The p53 protein produced by this gene triggers apoptosis. It is also a nuclear transcription factor that appears to increase production of a 21-kDa protein that blocks two cell cycle enzymes, slowing the cycle and permitting repair of mutations and other defects in DNA. The p53 gene is mutated in up to 50% of human cancers, with the production of p53 proteins that fail to slow the cell cycle and permit other mutations in DNA to persist. The accumulated mutations eventually cause cancer.

EXOCYTOSIS

Vesicles containing material for export are targeted to the cell membrane (Figure 2–11), where they bond in a similar manner to that discussed in vesicular traffic between Golgi stacks, via the v-SNARE/t-SNARE arrangement. The area of fusion then breaks down, leaving the contents of the vesicle outside and the cell membrane intact. This is the Ca^{2+}-dependent process of **exocytosis** (Figure 2–12).

Note that secretion from the cell occurs via two pathways (Figure 2–11). In the **nonconstitutive pathway,** proteins from the Golgi apparatus initially enter secretory granules, where processing of prohormones to the mature hormones occurs before exocytosis. The other pathway, the **constitutive pathway,** involves the prompt transport of proteins to the cell membrane in vesicles, with little or no processing or storage. The nonconstitutive pathway is sometimes called the **regulated pathway,** but this term is misleading because the output of proteins by the constitutive pathway is also regulated.

ENDOCYTOSIS

Endocytosis is the reverse of exocytosis. There are various types of endocytosis named for the size of particles being ingested as well as the regulatory requirements for the particular process. These include **phagocytosis, pinocytosis, clathrin-mediated endocytosis, caveolae-dependent uptake,** and **nonclathrin/noncaveolae endocytosis.**

Phagocytosis ("cell eating") is the process by which bacteria, dead tissue, or other bits of microscopic material are engulfed by cells such as the polymorphonuclear leukocytes of the blood. The material makes contact with the cell membrane, which then invaginates. The invagination is pinched off, leaving the engulfed material in the membrane-enclosed vacuole and the cell membrane intact. **Pinocytosis** ("cell drinking") is a similar process with the vesicles much smaller in size and the substances ingested are in solution. The small size membrane that is ingested with each event should not be misconstrued; cells undergoing active pinocytosis (eg, macrophages) can ingest the equivalent of their entire cell membrane in just 1 h.

Clathrin-mediated endocytosis occurs at membrane indentations where the protein **clathrin** accumulates. Clathrin molecules have the shape of triskelions, with three "legs" radiating from a central hub (Figure 2–13). As endocytosis progresses, the clathrin molecules form a geometric array that surrounds the endocytotic vesicle. At the neck of the vesicle, the GTP binding protein **dynamin** is involved, either directly or indirectly, in pinching off the vesicle. Once the complete vesicle is formed, the clathrin falls off and the three-legged proteins recycle to form another vesicle. The vesicle fuses with and dumps its contents into an **early endosome** (Figure 2–11). From the early

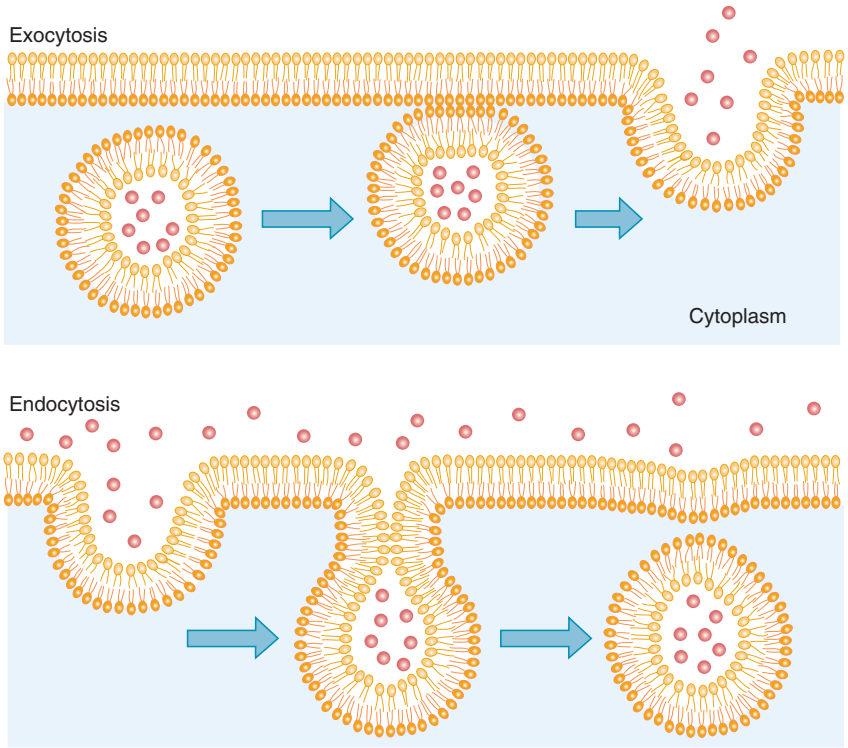


FIGURE 2–12 Exocytosis and endocytosis. Note that in exocytosis the cytoplasmic sides of two membranes fuse, whereas in endocytosis two noncytoplasmic sides fuse.

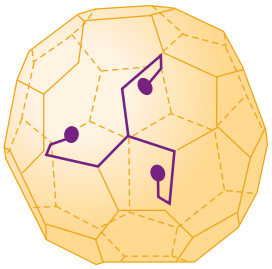


FIGURE 2–13 Clathrin molecule on the surface of an endocytotic vesicle. Note the characteristic triskelion shape and the fact that with other clathrin molecules it forms a net supporting the vesicle.

endosome, a new vesicle can bud off and return to the cell membrane. Alternatively, the early endosome can become a **late endosome** and fuse with a lysosome (Figure 2–11) in which the contents are digested by the lysosomal proteases. Clathrin-mediated endocytosis is responsible for the internalization of many receptors and the ligands bound to them—including, for example, nerve growth factor (NGF) and low-density lipoproteins. It also plays a major role in synaptic function.

It is apparent that exocytosis adds to the total amount of membrane surrounding the cell, and if membrane were not removed elsewhere at an equivalent rate, the cell would enlarge. However, removal of cell membrane occurs by endocytosis, and such exocytosis–endocytosis coupling maintains the surface area of the cell at its normal size.

RAFTS & CAVEOLAE

Some areas of the cell membrane are especially rich in cholesterol and sphingolipids and have been called **rafts.** These rafts are probably the precursors of flask-shaped membrane depressions called **caveolae** (little caves) when their walls become infiltrated with a protein called **caveolin** that resembles clathrin. There is considerable debate about the functions of rafts and caveolae, with evidence that they are involved in cholesterol regulation and transcytosis. It is clear, however, that cholesterol can interact directly with caveolin, effectively limiting the protein's ability to move around in the membrane. Internalization via caveolae involves binding of cargo to caveolin and regulation by dynamin. Caveolae are prominent in endothelial cells, where they help in the uptake of nutrients from the blood.

COATS & VESICLE TRANSPORT

It now appears that all vesicles involved in transport have protein coats. In humans, over 50 coat complex subunits have been identified. Vesicles that transport proteins from the trans Golgi to lysosomes have **assembly protein 1 (AP-1)** clathrin coats, and endocytotic vesicles that transport to endosomes have AP-2 clathrin coats. Vesicles that transport between the endoplasmic reticulum and the Golgi have coat proteins I and II (COPI and COPII). Certain amino acid sequences or attached groups on the transported proteins target the proteins for particular locations. For example, the amino acid sequence Asn–Pro–any amino acid–Tyr targets transport from the cell surface to the endosomes, and mannose-6-phosphate groups target transfer from the Golgi to mannose-6-phosphate receptors (MPR) on the lysosomes.

Various small G proteins of the Rab family are especially important in vesicular traffic. They appear to guide and facilitate orderly attachments of these vesicles. To illustrate the complexity of directing vesicular traffic, humans have 60 Rab proteins and 35 SNARE proteins.

MEMBRANE PERMEABILITY & MEMBRANE TRANSPORT PROTEINS

An important technique that has permitted major advances in our knowledge about transport proteins is **patch clamping.** A micropipette is placed on the membrane of a cell and forms a tight seal to the membrane. The patch of membrane under the pipette tip usually contains only a few transport proteins, allowing for their detailed biophysical study (Figure 2–14). The cell can be left intact **(cell-attached patch clamp).** Alternatively, the patch can be pulled loose from the cell, forming an **inside-out patch.** A third alternative is to suck out the patch with the micropipette still attached to the rest of the cell membrane, providing direct access to the interior of the cell **(whole cell recording).**

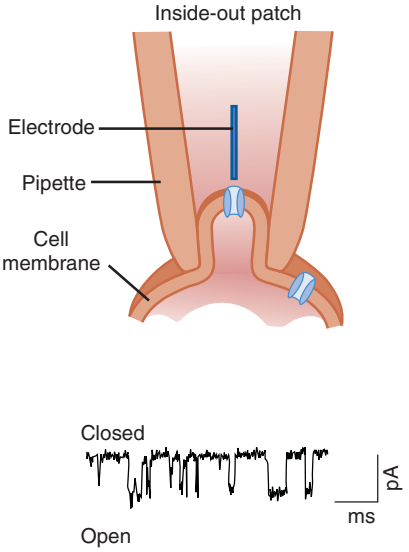


FIGURE 2–14 Patch clamp to investigate transport. In a patch clamp experiment, a small pipette is carefully maneuvered to seal off a portion of a cell membrane. The pipette has an electrode bathed in an appropriate solution that allows for recording of electrical changes through any pore in the membrane (shown below). The illustrated setup is termed an "inside-out patch" because of the orientation of the membrane with reference to the electrode. Other configurations include cell attached, whole cell, and outside-out patches. (Modified from Ackerman MJ, Clapham DE: Ion channels: Basic science and clinical disease. *N Engl J Med* 1997;336:1575.)

Small, nonpolar molecules (including O_2 and N_2) and small uncharged polar molecules such as CO_2 diffuse across the lipid membranes of cells. However, the membranes have very limited permeability to other substances. Instead, they cross the membranes by endocytosis and exocytosis and by passage through highly specific **transport proteins,** transmembrane proteins that form channels for ions or transport substances such as glucose, urea, and amino acids. The limited permeability applies even to water, with simple diffusion being supplemented throughout the body with various water channels **(aquaporins).** For reference, the sizes of ions and other biologically important substances are summarized in Table 2–2.

Some transport proteins are simple aqueous **ion channels,** though many of these have special features that make them selective for a given substance such as Ca^{2+} or, in the case of aquaporins, for water. These membrane-spanning proteins (or collections of proteins) have tightly regulated pores that can be **gated** opened or closed in response to local changes (Figure 2–15). Some are gated by alterations in membrane potential **(voltage-gated),** whereas others are opened or closed in response to a ligand **(ligand-gated).** The ligand is often external (eg, a neurotransmitter or a hormone). However, it can also be internal; intracellular Ca^{2+}, cAMP, lipids, or one of the G proteins produced in cells can bind directly to channels and activate them. Some channels are also opened by mechanical stretch, and these mechanosensitive channels play an important role in cell movement.

Other transport proteins are **carriers** that bind ions and other molecules and then change their configuration, moving the bound molecule from one side of the cell membrane to the other. Molecules move from areas of high concentration to areas of low concentration (down their **chemical gradient**), and cations move to negatively charged areas whereas anions move to positively charged areas (down their **electrical gradient**). When carrier proteins move substances in the direction of their chemical or electrical gradients, no energy input is required and the process is called **facilitated diffusion.** A typical example is glucose transport by the glucose transporter, which moves glucose down its concentration gradient from the ECF to the cytoplasm of the cell. Other carriers transport

TABLE 2–2 Size of hydrated ions and other substances of biologic interest.

Substance	Atomic or Molecular Weight	Radius (nm)
Cl^-	35	0.12
K^+	39	0.12
H_2O	18	0.12
Ca^{2+}	40	0.15
Na^+	23	0.18
Urea	60	0.23
Li^+	7	0.24
Glucose	180	0.38
Sucrose	342	0.48
Inulin	5000	0.75
Albumin	69,000	7.50

Data from Moore EW: *Physiology of Intestinal Water and Electrolyte Absorption.* American Gastroenterological Association, 1976.

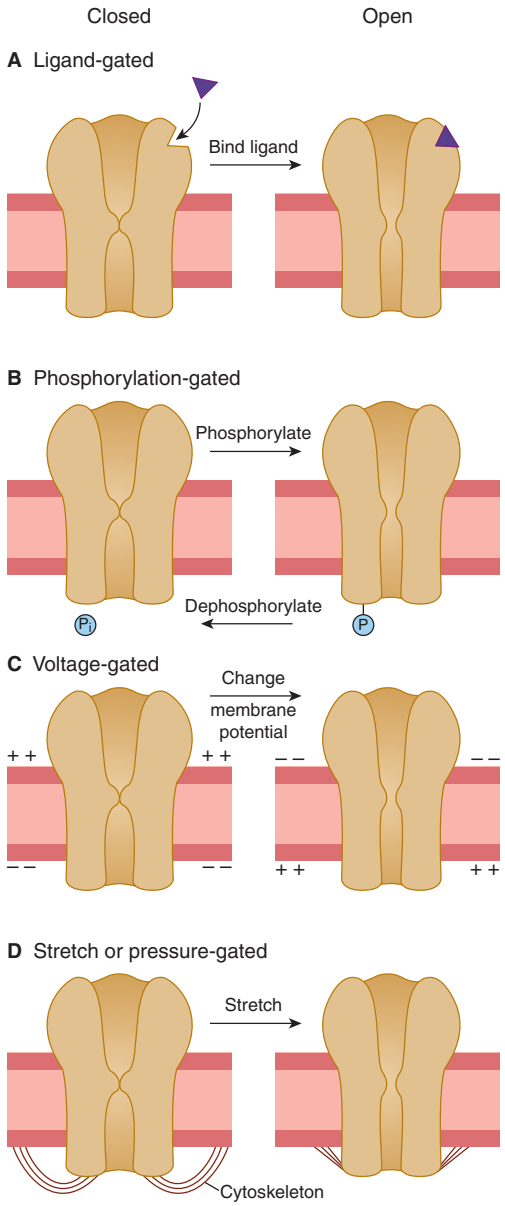


FIGURE 2–15 Regulation of gating in ion channels. Several types of gating are shown for ion channels. **A)** Ligand-gated channels open in response to ligand binding. **B)** Protein phosphorylation or dephosphorylation regulate opening and closing of some ion channels. **C)** Changes in membrane potential alter channel openings. **D)** Mechanical stretch of the membrane results in channel opening. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science,* 4th ed. McGraw-Hill, 2000.)

substances against their electrical and chemical gradients. This form of transport requires energy and is called **active transport.** In animal cells, the energy is provided almost exclusively by hydrolysis of ATP. Not surprisingly, therefore, many carrier molecules are ATPases, enzymes that catalyze the hydrolysis of ATP. One of these ATPases is **sodium–potassium adenosine triphosphatase (Na, K ATPase),** which is also known as the **Na, K pump.** There are also H, K ATPases in the gastric mucosa and the renal tubules. Ca^{2+} ATPase pumps Ca^{2+} out of cells. Proton ATPases acidify many intracellular organelles, including parts of the Golgi complex and lysosomes.

Some of the transport proteins are called **uniports** because they transport only one substance. Others are called **symports** because transport requires the binding of more than one substance to the transport protein and the substances are transported across the membrane together. An example is the symport in the intestinal mucosa that is responsible for the cotransport of Na^+ and glucose from the intestinal lumen into mucosal cells. Other transporters are called **antiports** because they exchange one substance for another.

ION CHANNELS

There are ion channels specific for K^+, Na^+, Ca^{2+}, and Cl^-, as well as channels that are nonselective for cations or anions. Each type of channel exists in multiple forms with diverse properties. Most are made up of identical or very similar subunits. Figure 2–16 shows the multiunit structure of various channels in diagrammatic cross-section.

Most K^+ channels are tetramers, with each of the four subunits forming part of the pore through which K^+ ions pass. Structural analysis of a bacterial voltage-gated K^+ channel indicates that each of the four subunits have a paddle-like extension containing four charges. When the channel is closed, these extensions are near the negatively charged interior of the cell. When the membrane potential is reduced, the paddles containing the charges bend through the membrane to its exterior surface, causing the channel to open. The bacterial K^+ channel is very similar to the voltage-gated K^+ channels in a wide variety of species, including mammals. In the acetylcholine ion channel and other ligand-gated cation or anion channels, five subunits make up the pore. Members of the ClC family of Cl^- channels are dimers, but they have two pores, one in each subunit. Finally, aquaporins are tetramers with a water pore in each of the subunits. Recently, a number of ion channels with intrinsic enzyme activity have been cloned. More than 30 different voltage-gated or cyclic nucleotide-gated Na^+ and Ca^{2+} channels of this type have been described. Representative Na^+, Ca^{2+}, and K^+ channels are shown in extended diagrammatic form in Figure 2–17.

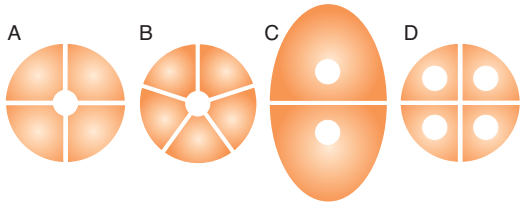


FIGURE 2–16 Different ways in which ion channels form pores. Many K^+ channels are tetramers **A),** with each protein subunit forming part of the channel. In ligand-gated cation and anion channels **B)** such as the acetylcholine receptor, five identical or very similar subunits form the channel. Cl^- channels from the ClC family are dimers **C),** with an intracellular pore in each subunit. Aquaporin water channels **(D)** are tetramers with an intracellular channel in each subunit. (Reproduced with permission from Jentsch TJ: Chloride channels are different. Nature 2002;415:276.)

Another family of Na^+ channels with a different structure has been found in the apical membranes of epithelial cells in the kidneys, colon, lungs, and brain. The **epithelial sodium channels (ENaCs)** are made up of three subunits encoded by three different genes. Each of the subunits probably spans the membrane twice, and the amino terminal and carboxyl terminal are located inside the cell. The α subunit transports Na^+, whereas the β and γ subunits do not. However, the addition of the β and γ subunits increases Na^+ transport through the α subunit. ENaCs are inhibited by the diuretic amiloride, which binds to the α subunit, and they used to be called **amiloride inhibitable Na^+ channels.** The ENaCs in the kidney play an important role in the regulation of ECF volume by aldosterone. ENaC knockout mice are born alive but promptly die because they cannot move Na^+, and hence water, out of their lungs.

Humans have several types of Cl^- channels. The ClC dimeric channels are found in plants, bacteria, and animals, and there are nine different ClC genes in humans. Other Cl^- channels have the same pentameric form as the acetylcholine receptor; examples include the γ-aminobutyric acid A ($GABA_A$) and glycine receptors in the CNS. The cystic fibrosis transmembrane conductance regulator (CFTR) that is mutated in cystic fibrosis is also a Cl^- channel. Ion channel mutations cause a variety of **channelopathies**—diseases that mostly affect muscle and brain tissue and produce episodic paralyses or convulsions, but are also observed in nonexcitable tissues (Clinical Box 2–6).

Na, K ATPase

As noted previously, Na, K ATPase catalyzes the hydrolysis of ATP to adenosine diphosphate (ADP) and uses the energy to extrude three Na^+ from the cell and take two K^+ into the cell for each molecule of ATP hydrolyzed. It is an **electrogenic pump** in that it moves three positive charges out of the cell for each two that it moves in, and it is therefore said to have a **coupling ratio** of 3:2. It is found in all parts of the body. Its activity is inhibited by ouabain and related digitalis glycosides used in the treatment of heart failure. It is a heterodimer made up of an α subunit with a molecular weight of approximately 100,000 and a β subunit with a molecular weight of approximately 55,000. Both extend through the cell membrane

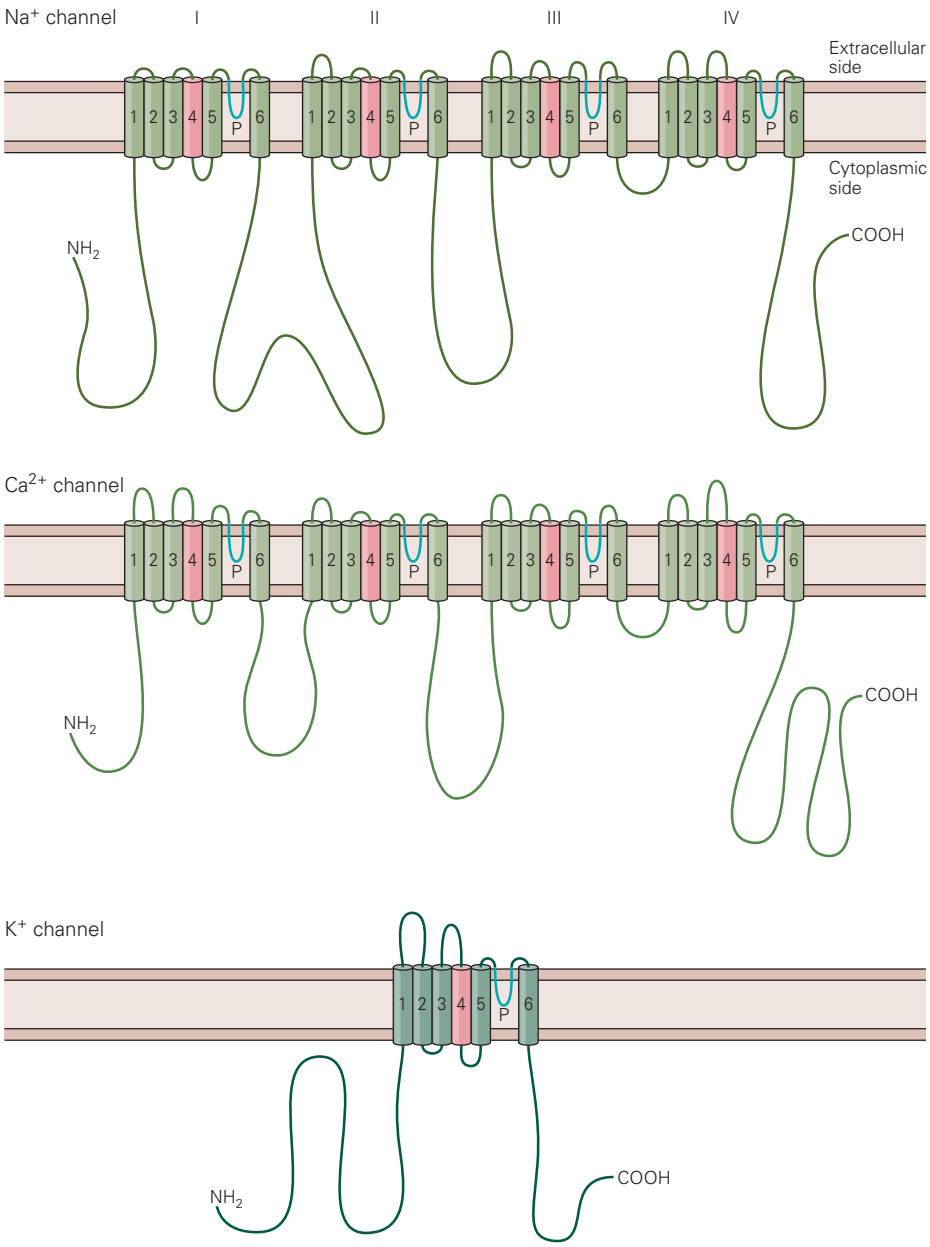


FIGURE 2–17 Diagrammatic representation of the pore-forming subunits of three ion channels. The α subunit of the Na^+ and Ca^{2+} channels traverse the membrane 24 times in four repeats of six membrane-spanning units. Each repeat has a "P" loop between membrane spans 5 and 6 that does not traverse the membrane. These P loops are thought to form the pore. Note that span 4 of each repeat is colored in red, representing its net "+" charge. The K^+ channel has only a single repeat of the six spanning regions and P loop. Four K^+ subunits are assembled for a functional K^+ channel. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science,* 4th ed. McGraw-Hill, 2000.)

(Figure 2–18). Separation of the subunits eliminates activity. The β subunit is a glycoprotein, whereas Na^+ and K^+ transport occur through the α subunit. The β subunit has a single membrane-spanning domain and three extracellular glycosylation sites, all of which appear to have attached carbohydrate residues. These residues account for one third of its molecular weight. The α subunit probably spans the cell membrane 10 times, with the amino and carboxyl terminals both located intracellularly. This subunit has intracellular Na^+- and ATP-binding sites and a phosphorylation site; it also has extracellular binding sites for K^+ and ouabain. The endogenous ligand of the ouabain-binding site is unsettled. When Na^+ binds to the α subunit, ATP also binds and is converted to ADP, with a phosphate being transferred to Asp 376, the phosphorylation site. This causes a change in the configuration of the protein, extruding Na^+ into the ECF. K^+ then binds extracellularly,

CLINICAL BOX 2–6

Channelopathies

Channelopathies include a wide range of diseases that can affect both excitable (eg, neurons and muscle) and nonexcitable cells. Using molecular genetic tools, many of the pathological defects in channelopathies have been traced to mutations in single ion channels. Examples of channelopathies in excitable cells include periodic paralysis (eg, Kir2.1, a K^+ channel subunit, or $Na_v2.1$, a Na^+ channel subunit), myasthenia (eg, nicotinic Acetyl Choline Receptor, a ligand gated nonspecific cation channel), myotonia (eg, Kir1.1, a K^+ channel subunit), malignant hypothermia (Ryanodine Receptor, a Ca^{2+} channel), long QT syndrome (both Na^+ and K^+ channel subunit examples) and several other disorders. Examples of channelopathies in nonexcitable cells include the underlying cause for Cystic fibrosis (CFTR, a Cl^- channel) and a form of Bartter's syndrome (Kir1.1, a K^+ channel subunit). Importantly, advances in treatment of these disorders can come from the understanding of the basic defect and tailoring drugs that act to alter the mutated properties of the affected channel.

dephosphorylating the α subunit, which returns to its previous conformation, releasing K^+ into the cytoplasm.

The α and β subunits are heterogeneous, with α_1, α_2, and α_3 subunits and β_1, β_2, and β_3 subunits described so far. The α_1 isoform is found in the membranes of most cells, whereas α_2 is present in muscle, heart, adipose tissue, and brain, and α_3 is present in heart and brain. The β_1 subunit is widely distributed but is absent in certain astrocytes, vestibular cells of the inner ear, and glycolytic fast-twitch muscles. The fast-twitch muscles contain only β_2 subunits. The different α and β subunit structures of Na, K ATPase in various tissues probably represent specialization for specific tissue functions.

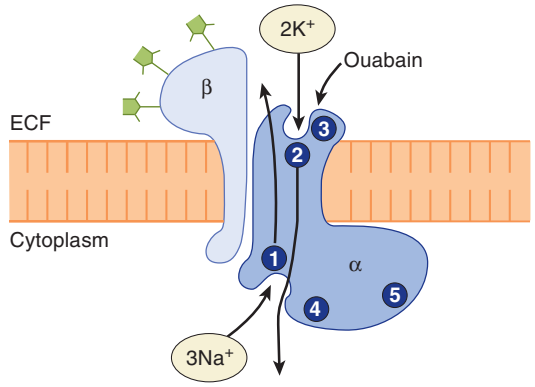


FIGURE 2–18 **Na, K ATPase.** The intracellular portion of the α subunit has a Na^+-binding site **(1),** a phosphorylation site **(4),** and an ATP-binding site **(5).** The extracellular portion has a K^+-binding site **(2)** and an ouabain-binding site **(3).** (From Horisberger J-D et al: Structure–function relationship of Na, K ATPase. Annu Rev Physiol 1991;53:565. Reproduced with permission from the *Annual Review of Physiology,* vol. 53. Copyright 1991 by Annual Reviews.)

REGULATION OF Na, K ATPase

The amount of Na^+ normally found in cells is not enough to saturate the pump, so if the Na^+ increases, more is pumped out. Pump activity is affected by second messenger molecules (eg, cAMP and diacylglycerol [DAG]). The magnitude and direction of the altered pump effects vary with the experimental conditions. Thyroid hormones increase pump activity by a genomic action to increase the formation of Na, K ATPase molecules. Aldosterone also increases the number of pumps, although this effect is probably secondary. Dopamine in the kidney inhibits the pump by phosphorylating it, causing a natriuresis. Insulin increases pump activity, probably by a variety of different mechanisms.

SECONDARY ACTIVE TRANSPORT

In many situations, the active transport of Na^+ is coupled to the transport of other substances **(secondary active transport).** For example, the luminal membranes of mucosal cells in the small intestine contain a symport that transports glucose into the cell only if Na^+ binds to the protein and is transported into the cell at the same time. From the cells, the glucose enters the blood. The electrochemical gradient for Na^+ is maintained by the active transport of Na^+ out of the mucosal cell into ECF. Other examples are shown in **Figure 2–19**. In the heart, Na, K ATPase indirectly affects Ca^{2+} transport. An antiport in the membranes of cardiac muscle cells normally exchanges intracellular Ca^{2+} for extracellular Na^+.

Active transport of Na^+ and K^+ is one of the major energy-using processes in the body. On the average, it accounts for about 24% of the energy utilized by cells, and in neurons it accounts for 70%. Thus, it accounts for a large part of the basal metabolism. A major payoff for this energy use is the establishment of the electrochemical gradient in cells.

TRANSPORT ACROSS EPITHELIA

In the gastrointestinal tract, the pulmonary airways, the renal tubules, and other structures lined with polarized epithelial cells, substances enter one side of a cell and exit another, producing movement of the substance from one side of the epithelium to the other. For transepithelial transport to occur, the cells need to be bound by tight junctions and, obviously, have different ion channels and transport proteins in different parts of their membranes. Most of the instances of secondary active transport cited in the preceding paragraph involve transepithelial movement of ions and other molecules.

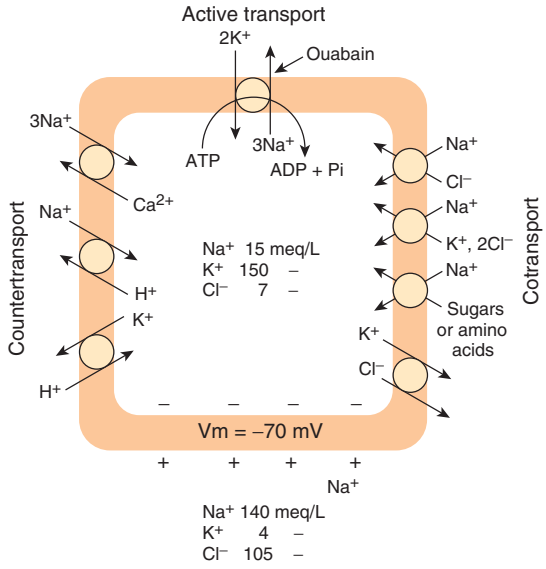


FIGURE 2–19 Composite diagram of main secondary effects of active transport of Na^+ and K^+. Na,K ATPase converts the chemical energy of ATP hydrolysis into maintenance of an inward gradient for Na^+ and an outward gradient for K^+. The energy of the gradients is used for countertransport, cotransport, and maintenance of the membrane potential. Some examples of cotransport and countertransport that use these gradients are shown. (Reproduced with permission from Skou JC: The Na–K pump. News Physiol Sci 1992;7:95.)

SPECIALIZED TRANSPORT ACROSS THE CAPILLARY WALL

The capillary wall separating plasma from interstitial fluid is different from the cell membranes separating interstitial fluid from intracellular fluid because the pressure difference across it makes **filtration** a significant factor in producing movement of water and solute. By definition, filtration is the process by which fluid is forced through a membrane or other barrier because of a difference in pressure on the two sides.

The structure of the capillary wall varies from one vascular bed to another. However, near skeletal muscle and many other organs, water and relatively small solutes are the only substances that cross the wall with ease. The apertures in the junctions between the endothelial cells are too small to permit plasma proteins and other colloids to pass through in significant quantities. The colloids have a high molecular weight but are present in large amounts. Small amounts cross the capillary wall by vesicular transport, but their effect is slight. Therefore, the capillary wall behaves like a membrane impermeable to colloids, and these exert an osmotic pressure of about 25 mm Hg. The colloid osmotic pressure due to the plasma colloids is called the **oncotic pressure.** Filtration across the capillary membrane as a result of the hydrostatic pressure head in the vascular system is opposed by the oncotic pressure. The way the balance between the hydrostatic and oncotic pressures controls exchanges across the capillary wall is considered in detail in Chapter 31.

TRANSCYTOSIS

Vesicles are present in the cytoplasm of endothelial cells, and tagged protein molecules injected into the bloodstream have been found in the vesicles and in the interstitium. This indicates that small amounts of protein are transported out of capillaries across endothelial cells by endocytosis on the capillary side followed by exocytosis on the interstitial side of the cells. The transport mechanism makes use of coated vesicles that appear to be coated with caveolin and is called **transcytosis, vesicular transport,** or **cytopempsis.**

INTERCELLULAR COMMUNICATION

Cells communicate with one another via chemical messengers. Within a given tissue, some messengers move from cell to cell via gap junctions without entering the ECF. In addition, cells are affected by chemical messengers secreted into the ECF, or by direct cell–cell contacts. Chemical messengers typically bind to protein receptors on the surface of the cell or, in some instances, in the cytoplasm or the nucleus, triggering sequences of intracellular changes that produce their physiologic effects. Three general types of intercellular communication are mediated by messengers in the ECF: (1) **neural communication,** in which neurotransmitters are released at synaptic junctions from nerve cells and act across a narrow synaptic cleft on a postsynaptic cell; (2) **endocrine communication,** in which hormones and growth factors reach cells via the circulating blood or the lymph; and (3) **paracrine communication,** in which the products of cells diffuse in the ECF to affect neighboring cells that may be some distance away (Figure 2–20). In addition, cells secrete chemical messengers that in some situations bind to receptors on the same cell, that is, the cell that secreted the messenger **(autocrine communication).** The chemical messengers include amines, amino acids, steroids, polypeptides, and in some instances, lipids, purine nucleotides, and pyrimidine nucleotides. It is worth noting that in various parts of the body, the same chemical messenger can function as a neurotransmitter, a paracrine mediator, a hormone secreted by neurons into the blood (neural hormone), and a hormone secreted by gland cells into the blood.

An additional form of intercellular communication is called **juxtacrine communication.** Some cells express multiple repeats of growth factors such as **transforming growth factor alpha (TGFα)** extracellularly on transmembrane proteins that provide an anchor to the cell. Other cells have TGFα receptors. Consequently, TGFα anchored to a cell can bind to a TGFα receptor on another cell, linking the two. This could be important in producing local foci of growth in tissues.

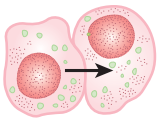
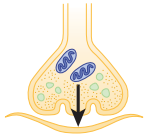
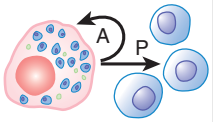
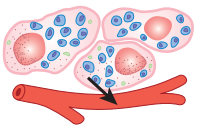
	GAP JUNCTIONS	SYNAPTIC	PARACRINE AND AUTOCRINE	ENDOCRINE
				
Message transmission	Directly from cell to cell	Across synaptic cleft	By diffusion in interstitial fluid	By circulating body fluids
Local or general	Local	Local	Locally diffuse	General
Specificity depends on	Anatomic location	Anatomic location and receptors	Receptors	Receptors

FIGURE 2–20 **Intercellular communication by chemical mediators.** A, autocrine; P, paracrine.

RECEPTORS FOR CHEMICAL MESSENGERS

The recognition of chemical messengers by cells typically begins by interaction with a receptor at that cell. There have been over 20 families of receptors for chemical messengers characterized. These proteins are not static components of the cell, but their numbers increase and decrease in response to various stimuli, and their properties change with changes in physiological conditions. When a hormone or neurotransmitter is present in excess, the number of active receptors generally decreases **(down-regulation),** whereas in the presence of a deficiency of the chemical messenger, there is an increase in the number of active receptors **(up-regulation).** In its actions on the adrenal cortex, angiotensin II is an exception; it increases rather than decreases the number of its receptors in the adrenal. In the case of receptors in the membrane, receptor-mediated endocytosis is responsible for down-regulation in some instances; ligands bind to their receptors, and the ligand–receptor complexes move laterally in the membrane to coated pits, where they are taken into the cell by endocytosis **(internalization).** This decreases the number of receptors in the membrane. Some receptors are recycled after internalization, whereas others are replaced by de novo synthesis in the cell. Another type of down-regulation is **desensitization,** in which receptors are chemically modified in ways that make them less responsive.

MECHANISMS BY WHICH CHEMICAL MESSENGERS ACT

Receptor–ligand interaction is usually just the beginning of the cell response. This event is transduced into secondary responses within the cell that can be divided into four broad categories: (1) ion channel activation, (2) **G-protein** activation, (3) activation of enzyme activity within the cell, or (4) direct activation of transcription. Within each of these groups, responses can be quite varied. Some of the common mechanisms by which chemical messengers exert their intracellular effects are summarized in Table 2–3. Ligands such as acetylcholine bind directly to ion channels in the cell membrane, changing their conductance. Thyroid and steroid hormones, 1,25-dihydroxycholecalciferol, and retinoids enter cells and act on one or another member of a family of structurally related cytoplasmic or nuclear receptors. The activated receptor binds to DNA and increases transcription of selected mRNAs. Many other ligands in the ECF bind to receptors on the surface of cells and trigger the release of intracellular mediators such as cAMP, IP_3, and DAG that initiate changes in cell function. Consequently, the extracellular ligands are called **"first messengers"** and the intracellular mediators are called **"second messengers."** Second messengers bring about many short-term changes in cell function by altering enzyme function, triggering exocytosis, and so on, but they also can lead to the alteration of transcription of various genes. A variety of enzymatic changes, protein–protein interactions, or second messenger changes can be activated within a cell in an orderly fashion following receptor recognition of the primary messenger. The resulting **cell signaling pathway** provides amplification of the primary signal and distribution of the signal to appropriate targets within the cell. Extensive cell signaling pathways also provide opportunities for feedback and regulation that can fine-tune the signal for the correct physiological response by the cell.

The most predominant posttranslation modification of proteins, phosphorylation, is a common theme in cell signaling pathways. Cellular phosphorylation is under the control of two groups of proteins: **kinases,** enzymes that catalyze the phosphorylation of tyrosine or serine and threonine residues in proteins (or in some cases, in lipids); and **phosphatases,** proteins that remove phosphates from proteins (or lipids). Some of the larger receptor families are themselves kinases. Tyrosine kinase receptors initiate phosphorylation on tyrosine residues on complementary receptors following ligand binding. Serine/threonine kinase receptors initiate phosphorylation on serines or threonines in complementary receptors following ligand binding. Cytokine receptors are directly

TABLE 2–3 Common mechanisms by which chemical messengers in the ECF bring about changes in cell function.

Mechanism	Examples
Open or close ion channels in cell membrane	Acetylcholine on nicotinic cholinergic receptor; norepinephrine on K^+ channel in the heart
Act via cytoplasmic or nuclear receptors to increase transcription of selected mRNAs	Thyroid hormones, retinoic acid, steroid hormones
Activate phospholipase C with intracellular production of DAG, IP_3, and other inositol phosphates	Angiotensin II, norepinephrine via α_1-adrenergic receptor, vasopressin via V_1 receptor
Activate or inhibit adenylyl cyclase, causing increased or decreased intracellular production of cAMP	Norepinephrine via β_1-adrenergic receptor (increased cAMP); norepinephrine via α_2-adrenergic receptor (decreased cAMP)
Increase cGMP in cell	Atrial natriuretic peptide; nitric oxide
Increase tyrosine kinase activity of cytoplasmic portions of transmembrane receptors	Insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), monocyte colony-stimulating factor (M-CSF)
Increase serine or threonine kinase activity	TGFβ, activin, inhibin

TABLE 2–4 Sample protein kinases.

Phosphorylate serine or threonine residues, or both
Calmodulin-dependent
Myosin light-chain kinase
Phosphorylase kinase
Ca^{2+}/calmodulin kinase I
Ca^{2+}/calmodulin kinase II
Ca^{2+}/calmodulin kinase III
Calcium-phospholipid-dependent
Protein kinase C (seven subspecies)
Cyclic nucleotide-dependent
cAMP-dependent kinase (protein kinase A; two subspecies)
cGMP-dependent kinase
Phosphorylate tyrosine residues
Insulin receptor, EGF receptor, PDGF receptor, and M-CSF receptor

associated with a group of protein kinases that are activated following cytokine binding. Alternatively, second messenger changes can lead to phosphorylation further downstream in the signaling pathway. More than 500 protein kinases have been described. Some of the principal ones that are important in mammalian cell signaling are summarized in Table 2–4. In general, addition of phosphate groups changes the conformation of the proteins, altering their functions and consequently the functions of the cell. The close relationship between phosphorylation and dephosphorylation of cellular proteins allows for a temporal control of activation of cell signaling pathways. This is sometimes referred to as a **"phosphate timer."** The dysregulation of the phosphate timer and subsequent cellular signaling in a cell can lead to disease (Clinical Box 2–7).

STIMULATION OF TRANSCRIPTION

The activation of transcription, and subsequent translation, is a common outcome of cellular signaling. There are three distinct pathways for primary messengers to alter transcription of cells. First, as is the case with steroid or thyroid hormones, the primary messenger is able to cross the cell membrane and bind to a nuclear receptor, which then can directly interact with DNA to alter gene expression. A second pathway to gene transcription is the activation of cytoplasmic protein kinases that can move to the nucleus to phosphorylate a latent transcription factor for activation. This pathway is a common endpoint of signals that go through the **mitogen activated protein (MAP) kinase** cascade. MAP kinases can be activated following a variety of receptor–ligand interactions through second messenger signaling. They comprise a series of three kinases that coordinate a stepwise phosphorylation to activate each protein in series in the cytosol. Phosphorylation of the last MAP kinase in series allows it to migrate to the nucleus where it phosphorylates a latent transcription factor. A third common pathway is the activation of a latent transcription factor in the cytosol, which then migrates to the nucleus and alters transcription. This pathway is shared by a diverse set of transcription factors that include **nuclear factor kappa B (NFκB;** activated following tumor necrosis family receptor binding and others), and **signal transducers of activated transcription (STATs;** activated following cytokine receptor binding). In all cases, the binding of the activated transcription factor to DNA increases (or in some cases, decreases) the transcription of mRNAs encoded by the gene to which it binds. The mRNAs are translated in the ribosomes, with the production of increased quantities of proteins that alter cell function.

INTRACELLULAR Ca^{2+} AS A SECOND MESSENGER

Ca^{2+} regulates a very large number of physiological processes that are as diverse as proliferation, neural signaling, learning, contraction, secretion, and fertilization, so regulation of intracellular Ca^{2+} is of great importance. The free Ca^{2+} concentration in the cytoplasm at rest is maintained at about 100 nmol/L. The Ca^{2+} concentration in the interstitial fluid is about 12,000 times the cytoplasmic concentration (ie, 1,200,000 nmol/L), so there is a marked inwardly directed concentration

CLINICAL BOX 2–7

Kinases in Cancer: Chronic Myeloid Leukemia

Kinases frequently play important roles in regulating cellular physiology outcomes, including cell growth and cell death. Dysregulation of cell proliferation or cell death is a hallmark of cancer. Although cancer can have many causes, a role for kinase dysregulation is exemplified in Chronic myeloid leukemia (CML). CML is a pluripotent hematopoietic stem cell disorder characterized by the Philadelphia (Ph) chromosome translocation. The Ph chromosome is formed following a translocation of chromosomes 9 and 22. The resultant shortened chromosome 22 (Ph chromosome). At the point of fusion a novel gene (BCR-ABL) encoding the active tyrosine kinase domain from a gene on chromosome 9 (Abelson tyrosine kinase; c-Abl) is fused to novel regulatory region of a separate gene on chromosome 22 (breakpoint cluster region; bcr). The BCR-ABL fusion gene encodes a cytoplasmic protein with constitutively active tyrosine kinase. The dysregulated kinase activity in BCR-ABL protein effectively limits white blood cell death signaling pathways while promoting cell proliferation and genetic instability. Experimental models have shown that translocation to produce the fusion BCR-ABL protein is sufficient to produce CML in animal models.

THERAPEUTIC HIGHLIGHTS

The identification of BCR-ABL as the initial transforming event in CML provided an ideal target for drug discovery. The drug imatinib (Gleevac) was developed to specifically block the tyrosine kinase activity of the BCR-ABL protein. Imatinib has proven to be an effective agent for treating chronic phase CML.

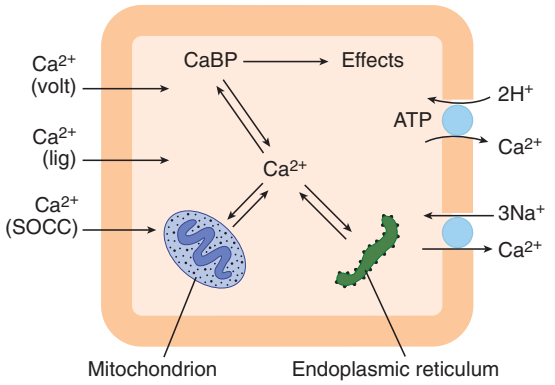


FIGURE 2–21 Ca^{2+} handling in mammalian cells. Ca^{2+} is stored in the endoplasmic reticulum and, to a lesser extent, mitochondria and can be released from them to replenish cytoplasmic Ca^{2+}. Calcium-binding proteins (CaBP) bind cytoplasmic Ca^{2+} and, when activated in this fashion, bring about a variety of physiologic effects. Ca^{2+} enters the cells via voltage-gated (volt) and ligand-gated (lig) Ca^{2+} channels and store-operated calcium channels (SOCCs). It is transported out of the cell by Ca, Mg ATPases (not shown), Ca, H ATPase and a Na, Ca antiport. It is also transported into the ER by Ca ATPases.

gradient as well as an inwardly directed electrical gradient. Much of the intracellular Ca^{2+} is stored at relatively high concentrations in the endoplasmic reticulum and other organelles (Figure 2–21), and these organelles provide a store from which Ca^{2+} can be mobilized via ligand-gated channels to increase the concentration of free Ca^{2+} in the cytoplasm. Increased cytoplasmic Ca^{2+} binds to and activates calcium-binding proteins. These proteins can have direct effects in cellular physiology, or can activate other proteins, commonly protein kinases, to further cell signaling pathways.

Ca^{2+} can enter the cell from the extracellular fluid, down its electrochemical gradient, through many different Ca^{2+} channels. Some of these are ligand-gated and others are voltage-gated. Stretch-activated channels exist in some cells as well.

Many second messengers act by increasing the cytoplasmic Ca^{2+} concentration. The increase is produced by releasing Ca^{2+} from intracellular stores—primarily the endoplasmic reticulum—or by increasing the entry of Ca^{2+} into cells, or by both mechanisms. IP_3 is the major second messenger that causes Ca^{2+} release from the endoplasmic reticulum through the direct activation of a ligand-gated channel, the IP_3 receptor. In effect, the generation of one second messenger (IP_3) can lead to the release of another second messenger (Ca^{2+}). In many tissues, transient release of Ca^{2+} from internal stores into the cytoplasm triggers opening of a population of Ca^{2+} channels in the cell membrane **(store-operated Ca^{2+} channels; SOCCs).** The resulting Ca^{2+} influx replenishes the total intracellular Ca^{2+} supply and refills the endoplasmic reticulum. Recent research has identified the physical relationships between SOCCs and regulatory interactions of proteins from the endoplasmic reticulum that gate these channels.

As with other second messenger molecules, the increase in Ca^{2+} within the cytosol is rapid, and is followed by a rapid decrease. Because the movement of Ca^{2+} outside of the cytosol (ie, across the plasma membrane or the membrane of the internal store) requires that it move up its electrochemical gradient, it requires energy. Ca^{2+} movement out of the cell is facilitated by the plasma membrane Ca^{2+} ATPase. Alternatively, it can be transported by an antiport that exchanges three Na^+ for each Ca^{2+} driven by the energy stored in the Na^+ electrochemical gradient. Ca^{2+} movement into the internal stores is through the action of the **sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase,** also known as the **SERCA pump.**

CALCIUM-BINDING PROTEINS

Many different Ca^{2+}-binding proteins have been described, including **troponin, calmodulin,** and **calbindin.** Troponin is the Ca^{2+}-binding protein involved in contraction of skeletal muscle (Chapter 5). Calmodulin contains 148 amino acid

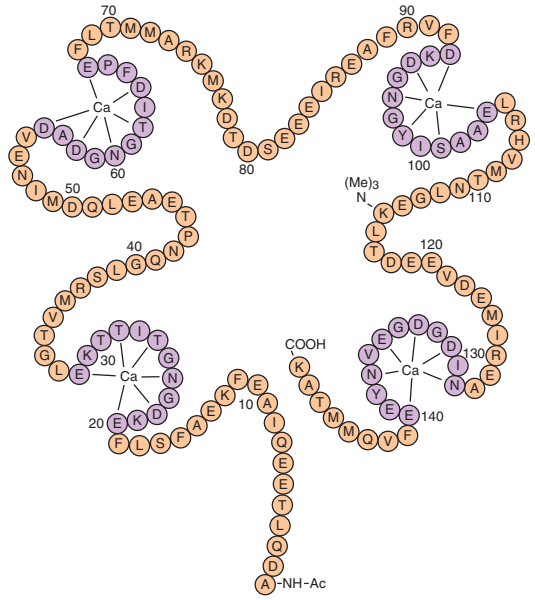


FIGURE 2–22 Secondary structure of calmodulin from bovine brain. Single-letter abbreviations are used for the amino acid residues. Note the four calcium domains (purple residues) flanked on either side by stretches of amino acids that form α-helices in tertiary structure. (Reproduced with permission from Cheung WY: Calmodulin: An overview. Fed Proc 1982;41:2253.)

residues (Figure 2–22) and has four Ca^{2+}-binding domains. It is unique in that amino acid residue 115 is trimethylated, and it is extensively conserved, being found in plants as well as animals. When calmodulin binds Ca^{2+}, it is capable of activating five different calmodulin-dependent kinases (CaMKs; Table 2–4), among other proteins. One of the kinases is **myosin light-chain kinase,** which phosphorylates myosin. This brings about contraction in smooth muscle. CaMKI and CaMKII are concerned with synaptic function, and CaMKIII is concerned with protein synthesis. Another calmodulin-activated protein is **calcineurin,** a phosphatase that inactivates Ca^{2+} channels by dephosphorylating them. It also plays a prominent role in activating T cells and is inhibited by some immunosuppressants.

MECHANISMS OF DIVERSITY OF Ca^{2+} ACTIONS

It may seem difficult to understand how intracellular Ca^{2+} can have so many varied effects as a second messenger. Part of the explanation is that Ca^{2+} may have different effects at low and at high concentrations. The ion may be at high concentration at the site of its release from an organelle or a channel **(Ca^{2+} sparks)** and at a subsequent lower concentration after it diffuses throughout the cell. Some of the changes it produces can outlast the rise in intracellular Ca^{2+} concentration because of the way it binds to some of the Ca^{2+}-binding proteins. In addition, once released, intracellular Ca^{2+} concentrations frequently oscillate at regular intervals, and there is evidence that the frequency and, to a lesser extent, the amplitude of those oscillations codes information for effector mechanisms. Finally, increases in intracellular Ca^{2+} concentration can spread from cell to cell in waves, producing coordinated events such as the rhythmic beating of cilia in airway epithelial cells.

G PROTEINS

A common way to translate a signal to a biologic effect inside cells is by way of nucleotide regulatory proteins that are activated after binding GTP **(G proteins).** When an activating signal reaches a G protein, the protein exchanges GDP for GTP. The GTP–protein complex brings about the activating effect of the G protein. The inherent GTPase activity of the protein then converts GTP to GDP, restoring the G protein to an inactive resting state. G proteins can be divided into two principal groups involved in cell signaling: **small G proteins** and **heterotrimeric G proteins.** Other groups that have similar regulation and are also important to cell physiology include elongation factors, dynamin, and translocation GTPases.

There are six different families of small G proteins (or **small GTPases**) that are all highly regulated. **GTPase activating proteins (GAPs)** tend to inactivate small G proteins by encouraging hydrolysis of GTP to GDP in the central binding site. **Guanine exchange factors (GEFs)** tend to activate small G proteins by encouraging exchange of GDP for GTP in the active site. Some of the small G proteins contain lipid modifications that help to anchor them to membranes, while others are free to diffuse throughout the cytosol. Small G proteins are involved in many cellular functions. Members of the Rab family regulate the rate of vesicle traffic between the endoplasmic reticulum, the Golgi apparatus, lysosomes, endosomes, and the cell membrane. Another family of small GTP-binding proteins, the Rho/Rac family, mediates interactions between the cytoskeleton and cell membrane; and a third family, the Ras family, regulates growth by transmitting signals from the cell membrane to the nucleus.

Another family of G proteins, the larger **heterotrimeric G proteins,** couple cell surface receptors to catalytic units that catalyze the intracellular formation of second messengers or couple the receptors directly to ion channels. Despite the knowledge of the small G proteins described above, the heteromeric G proteins are frequently referred to in the shortened "G protein" form because they were the first to be identified. Heterotrimeric G proteins are made up of three subunits designated α, β, and γ (Figure 2–23). Both the α and the γ subunits have lipid modifications that anchor these proteins to the plasma membrane. The α subunit is bound to GDP. When a ligand binds to a G protein-coupled receptor (GPCR, discussed below), this GDP is exchanged for GTP and the α subunit separates from the combined β and γ subunits. The separated α subunit brings about many biologic effects. The β and γ subunits are tightly bound in the cell and together

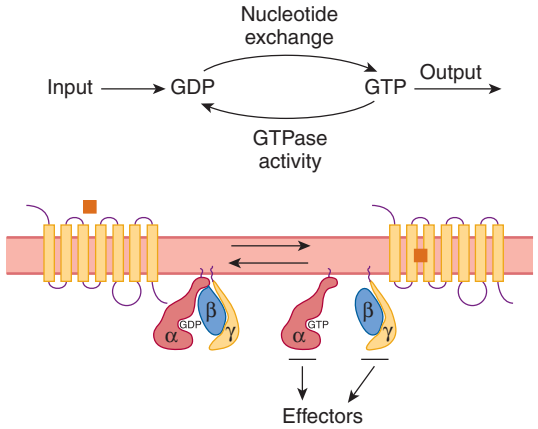


FIGURE 2–23 **Heterotrimeric G proteins. Top:** Summary of overall reaction that occurs in the Gα subunit. **Bottom:** When the ligand (square) binds to the G protein-coupled receptor in the cell membrane, GTP replaces GDP on the α subunit. GTP-α separates from the βγ subunit and GTP-α and βγ both activate various effectors, producing physiologic effects. The intrinsic GTPase activity of GTP-α then converts GTP to GDP, and the α, β, and γ subunits reassociate.

form a signaling molecule that can also activate a variety of effectors. The intrinsic GTPase activity of the α subunit then converts GTP to GDP, and this leads to re-association of the α with the βγ subunit and termination of effector activation. The GTPase activity of the α subunit can be accelerated by a family of **regulators of G protein signaling (RGS).**

Heterotrimeric G proteins relay signals from over 1000 GPCRs, and their effectors in the cells include ion channels and enzymes. There are 20 α, 6 β, and 12 γ genes, which allow for over 1400 α, β, and γ combinations. Not all combinations occur in the cell, but over 20 different heterotrimeric G proteins have been well documented in cell signaling. They can be divided into five families, each with a relatively characteristic set of effectors.

G PROTEIN-COUPLED RECEPTORS

All the **GPCRs** that have been characterized to date are proteins that span the cell membrane seven times. Because of this structure they are alternatively referred to as **seven-helix receptors** or **serpentine receptors.** A very large number have been cloned, and their functions are multiple and diverse. This is emphasized by the extensive variety of ligands that target GPCRs (Table 2–5). The structures of four GPCRs are shown in Figure 2–24. These receptors assemble into a barrel-like structure. Upon ligand binding, a conformational change activates a resting heterotrimeric G protein associated with the cytoplasmic leaf of the plasma membrane. Activation of a single receptor can result in 1, 10, or more active heterotrimeric G proteins, providing amplification as well as transduction of the first messenger. Bound receptors can be inactivated to limit the amount of cellular signaling. This frequently occurs through phosphorylation of the cytoplasmic side of the receptor. Because of their diversity and importance in cellular signaling pathways, GPCRs are prime targets for drug discovery (Clinical Box 2–8).

TABLE 2–5 **Examples of ligands for G-protein coupled receptors.**

Class	Ligand
Neurotransmitters	Epinephrine
	Norepinephrine
	Dopamine
	5-Hydroxytryptamine
	Histamine
	Acetylcholine
	Adenosine
	Opioids
Tachykinins	Substance P
	Neurokinin A
	Neuropeptide K
Other peptides	Angiotensin II
	Arginine vasopressin
	Oxytocin
	VIP, GRP, TRH, PTH
Glycoprotein hormones	TSH, FSH, LH, hCG
Arachidonic acid derivatives	Thromboxane A_2
Other	Odorants
	Tastants
	Endothelins
	Platelet-activating factor
	Cannabinoids
	Light

INOSITOL TRISPHOSPHATE & DIACYLGLYCEROL AS SECOND MESSENGERS

The link between membrane binding of a ligand that acts via Ca^{2+} and the prompt increase in the cytoplasmic Ca^{2+} concentration is often **inositol trisphosphate (inositol 1,4,5-trisphosphate; IP_3).** When one of these ligands binds to its receptor, activation of the receptor produces activation of phospholipase C (PLC) on the inner surface of the membrane. Ligands bound to GPCR can do this through the G_q heterotrimeric G proteins, while ligands bound to tyrosine kinase receptors can do this through other cell signaling pathways. PLC has at least eight isoforms; PLC_β is activated by heterotrimeric G proteins, while PLCγ forms are activated through tyrosine kinase receptors. PLC isoforms can catalyze the hydrolysis

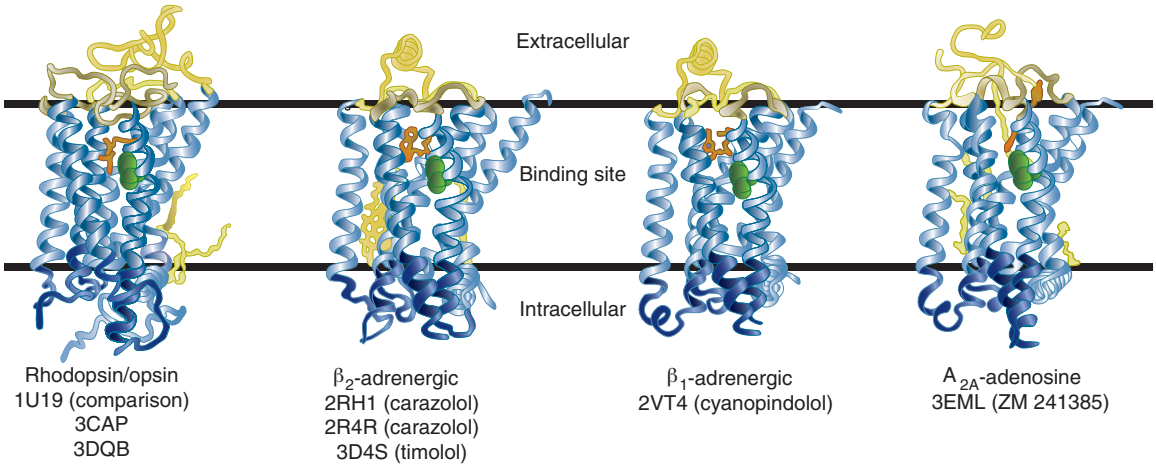


FIGURE 2–24 Representative structures of four G protein-coupled receptors from solved crystal structures. Each group of receptors is represented by one structure, all rendered with the same orientation and color scheme: transmembrane helices are colored light blue, intracellular regions are colored darker blue, and extracellular regions are brown. Each ligand is colored orange and rendered as sticks, bound lipids are colored yellow, and the conserved tryptophan residue is rendered as spheres and colored green. This figure highlights the observed differences seen in the extracellular and intracellular domains as well as the small differences seen in the ligand binding orientations among the four GPCRs various ligands. (Reproduced with permission from Hanson MA, Stevens RC: Discovery of new GPCR biology: one receptor structure at a time. Structure 1988 Jan 14;17(1):8–14.)

of the membrane lipid phosphatidylinositol 4,5-diphosphate (PIP_2) to form IP_3 and **DAG** (Figure 2–25). The IP_3 diffuses to the endoplasmic reticulum, where it triggers the release of Ca^{2+} into the cytoplasm by binding the IP_3 receptor, a ligand-gated Ca^{2+} channel (Figure 2–26). DAG is also a second messenger; it stays in the cell membrane, where it activates one of several isoforms of **protein kinase C.**

CYCLIC AMP

Another important second messenger is cyclic adenosine 3',5'-monophosphate (**cyclic AMP** or **cAMP;** Figure 2–27). Cyclic AMP is formed from ATP by the action of the enzyme **adenylyl cyclase** and converted to physiologically inactive 5'AMP by the action of the enzyme **phosphodiesterase.** Some of the

CLINICAL BOX 2–8

Drug Development: Targeting the G-Protein Coupled Receptors (GPCRs)

GPCRs are among the most heavily investigated drug targets in the pharmaceutical industry, representing approximately 40% of all the drugs in the marketplace today. These proteins are active in just about every organ system and present a wide range of opportunities as therapeutic targets in areas including cancer, cardiac dysfunction, diabetes, central nervous system disorders, obesity, inflammation, and pain. Features of GPCRs that allow them to be drug targets are their specificity in recognizing extracellular ligands to initiate cellular response, the cell surface location of GPCRs that make them accessible to novel ligands or drugs, and their prevalence in leading to human pathology and disease.

Specific examples of successful GPCR drug targets are noted with two types of **Histamine Receptors.**

Histamine-1 Receptor (H1-Receptor) antagonists: allergy therapy. Allergens can trigger local mast cells or basophils to release histamine in the airway. A primary target for histamine is the H1-Receptor in several airway cell types and this can lead to transient itching, sneezing, rhinorrhea, and nasal congestion. There are a variety of drugs with improved peripheral H1 receptor selectivity that are currently used to block histamine activation of the H1-Receptor and thus limit allergen effects in the upper airway. Current H1-Receptor antagonists on the market today include loratadine, fexofenadine, cetirizine, and desloratadine. These "second" an "third" generation anti H1-Receptor drugs have improved specificity and reduced adverse side effects (eg, drowsiness and central nervous system dysfunction) associated with some of the "first" generation drugs first introduced in the late 1930's and widely developed over the next 40 years.

Histamine-2 Receptor (H2-Receptor) antagonists: treating excess stomach acid. Excess stomach acid can result in gastroesophageal reflux disease or even peptic ulcer symptoms. The parietal cell in the stomach can be stimulated to produce acid via histamine action at the H2-Receptor. Excess stomach acid results in heartburn. Antagonists or H2-Receptor blockers, reduce acid production by preventing H2-Receptor signaling that leads to production of stomach acid. There are several drugs (eg, ranitidine, famotidine, cimetidine, and nizatidine) that specifically block the H2-receptor and thus reduce excess acid production.

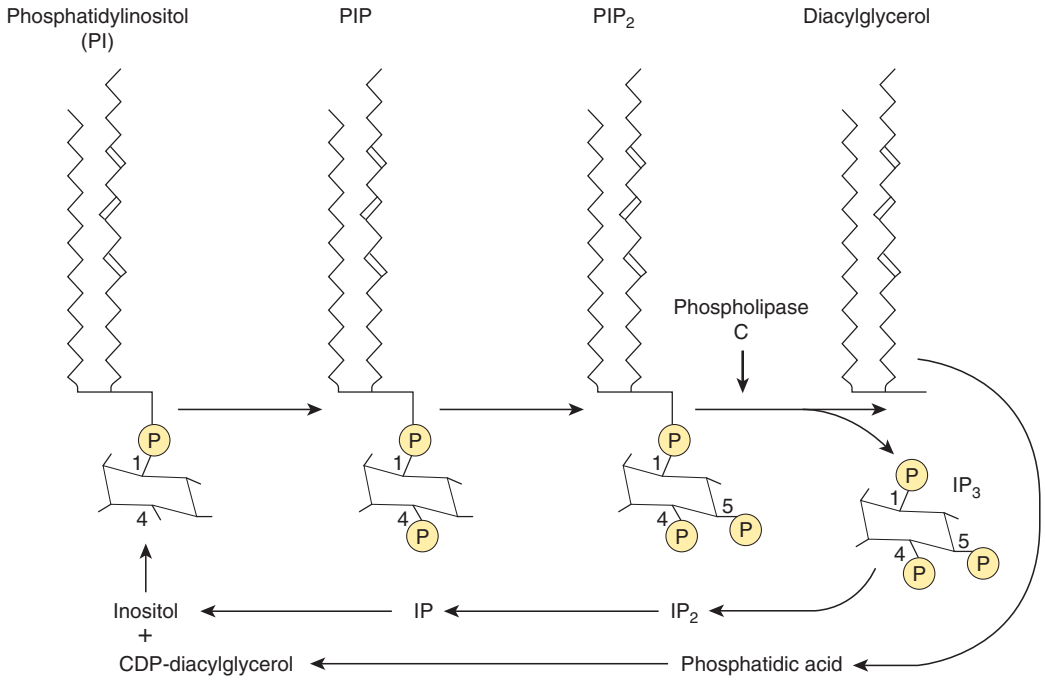


FIGURE 2–25 Metabolism of phosphatidylinositol in cell membranes. Phosphatidylinositol is successively phosphorylated to form phosphatidylinositol 4-phosphate (PIP), then phosphatidylinositol 4,5-bisphosphate (PIP_2). Phospholipase C_β and phospholipase Cγ catalyze the breakdown of PIP_2 to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Other inositol phosphates and phosphatidylinositol derivatives can also be formed. IP_3 is dephosphorylated to inositol, and diacylglycerol is metabolized to cytosine diphosphate (CDP)-diacylglycerol. CDP-diacylglycerol and inositol then combine to form phosphatidylinositol, completing the cycle. (Modified from Berridge MJ: Inositol triphosphate and diacylglycerol as second messengers. Biochem J 1984;220:345.)

phosphodiesterase isoforms that break down cAMP are inhibited by methylxanthines such as caffeine and theophylline. Consequently, these compounds can augment hormonal and transmitter effects mediated via cAMP. Cyclic AMP activates one of the cyclic nucleotide-dependent protein kinases **(protein kinase A, PKA)** that, like protein kinase C, catalyzes the phosphorylation of proteins, changing their conformation and altering their activity. In addition, the active catalytic subunit of PKA moves to the nucleus and phosphorylates the **cAMP-responsive element-binding protein (CREB).** This transcription factor then binds to DNA and alters transcription of a number of genes.

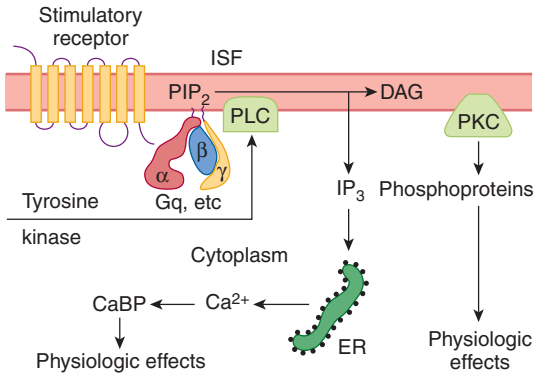


FIGURE 2–26 Diagrammatic representation of release of inositol triphosphate (IP_3) and diacylglycerol (DAG) as second messengers. Binding of ligand to G protein-coupled receptor activates phospholipase C $(PLC)_\beta$. Alternatively, activation of receptors with intracellular tyrosine kinase domains can activate PLCγ. The resulting hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP_2) produces IP_3, which releases Ca^{2+} from the endoplasmic reticulum (ER), and DAG, which activates protein kinase C (PKC). CaBP, Ca^{2+}-binding proteins; ISF, interstitial fluid.

PRODUCTION OF cAMP BY ADENYLYL CYCLASE

Adenylyl cyclase is a membrane bound protein with 12 transmembrane regions. Ten isoforms of this enzyme have been described and each can have distinct regulatory properties, permitting the cAMP pathway to be customized to specific tissue needs. Notably, stimulatory heterotrimeric G proteins (G_s) activate, while inhibitory heterotrimeric G proteins (G_i) inactivate adenylyl cyclase (Figure 2–28). When the appropriate ligand binds to a stimulatory receptor, a G_s α subunit activates one of the adenylyl cyclases. Conversely, when the appropriate ligand binds to an inhibitory receptor, a G_i α sub-unit inhibits adenylyl cyclase. The receptors are specific, responding at low threshold to only one or a select group of related ligands. However, heterotrimeric G proteins mediate the stimulatory and inhibitory effects produced by many different ligands. In addition, cross-talk occurs between the

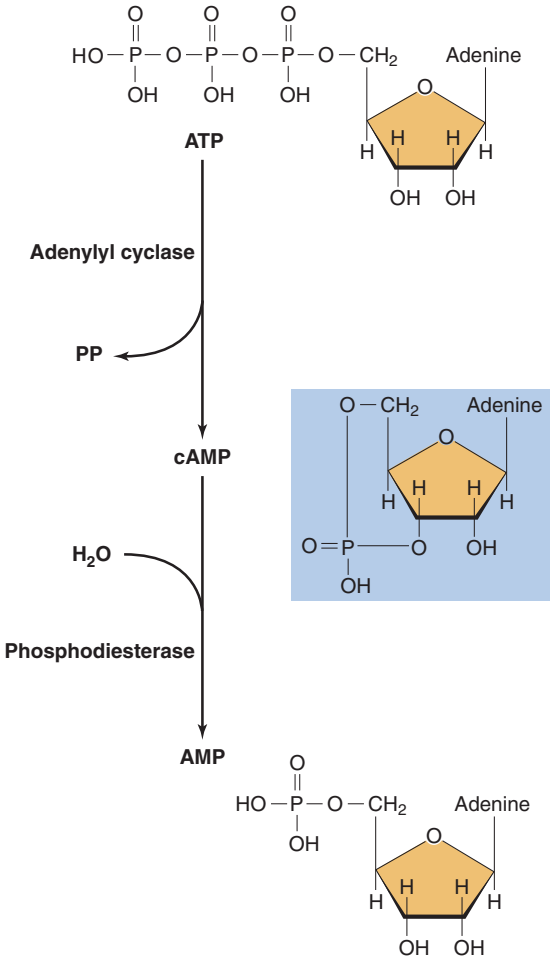


FIGURE 2–27 Formation and metabolism of cAMP. The second messenger cAMP is made from ATP by adenylyl cyclase and broken down into AMP by phosphodiesterase.

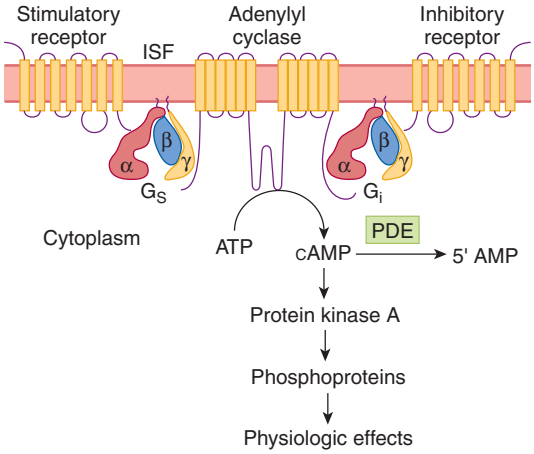


FIGURE 2–28 The cAMP system. Activation of adenylyl cyclase catalyzes the conversion of ATP to cAMP. Cyclic AMP activates protein kinase A, which phosphorylates proteins, producing physiologic effects. Stimulatory ligands bind to stimulatory receptors and activate adenylyl cyclase via G_s. Inhibitory ligands inhibit adenylyl cyclase via inhibitory receptors and G_i. ISF, interstitial fluid.

phospholipase C system and the adenylyl cyclase system, as several of the isoforms of adenylyl cyclase are stimulated by calmodulin. Finally, the effects of protein kinase A and protein kinase C are very widespread and can also affect directly, or indirectly, the activity at adenylyl cyclase. The close relationship between activation of G proteins and adenylyl cyclases also allows for spatial regulation of cAMP production. All of these events, and others, allow for fine-tuning the cAMP response for a particular physiological outcome in the cell.

Two bacterial toxins have important effects on adenylyl cyclase that are mediated by G proteins. The A subunit of **cholera toxin** catalyzes the transfer of ADP ribose to an arginine residue in the middle of the α subunit of G_s. This inhibits its GTPase activity, producing prolonged stimulation of adenylyl cyclase. **Pertussis toxin** catalyzes ADP-ribosylation of a cysteine residue near the carboxyl terminal of the α subunit of G_i. This inhibits the function of G_i. In addition to the implications of these alterations in disease, both toxins are used for fundamental research on G protein function. The drug forskolin also stimulates adenylyl cyclase activity by a direct action on the enzyme.

GUANYLYL CYCLASE

Another cyclic nucleotide of physiologic importance is **cyclic guanosine monophosphate (cyclic GMP** or **cGMP).** Cyclic GMP is important in vision in both rod and cone cells. In addition, there are cGMP-regulated ion channels, and cGMP activates cGMP-dependent kinase, producing a number of physiologic effects.

Guanylyl cyclases are a family of enzymes that catalyze the formation of cGMP. They exist in two forms (**Figure 2–29**). One form has an extracellular amino terminal domain that is a receptor, a single transmembrane domain, and a cytoplasmic portion with guanylyl cyclase catalytic activity. Several such guanylyl cyclases have been characterized. Two are receptors for atrial natriuretic peptide (ANP; also known as atrial natriuretic factor), and a third binds an *Escherichia coli* enterotoxin and the gastrointestinal polypeptide guanylin. The other form of guanylyl cyclase is soluble, contains heme, and is not bound to the membrane. There appear to be several isoforms of the intracellular enzyme. They are activated by nitric oxide (NO) and NO-containing compounds.

GROWTH FACTORS

Growth factors have become increasingly important in many different aspects of physiology. They are polypeptides and proteins that are conveniently divided into three groups. One group is made up of agents that foster the multiplication or development of various types of cells; NGF, insulin-like growth factor I (IGF-I), activins and inhibins, and epidermal growth

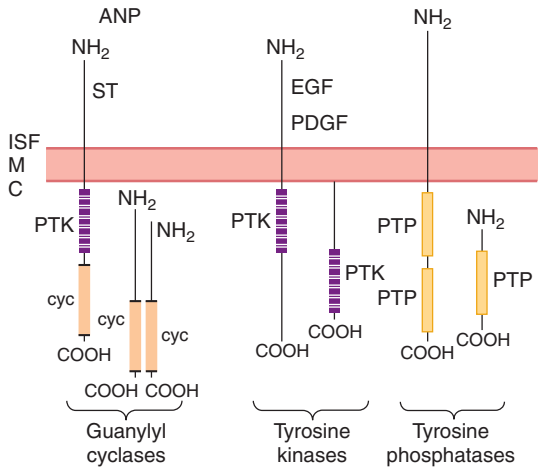


FIGURE 2–29 Diagrammatic representation of guanylyl cyclases, tyrosine kinases, and tyrosine phosphatases. ANP, atrial natriuretic peptide; C, cytoplasm; cyc, guanylyl cyclase domain; EGF, epidermal growth factor; ISF, interstitial fluid; M, cell membrane; PDGF, platelet-derived growth factor; PTK, tyrosine kinase domain; PTP, tyrosine phosphatase domain; ST, *E. coli* enterotoxin. (Modified from Koesling D, Böhme E, Schultz G: Guanylyl cyclases, a growing family of signal transducing enzymes. FASEB J 1991;5:2785.)

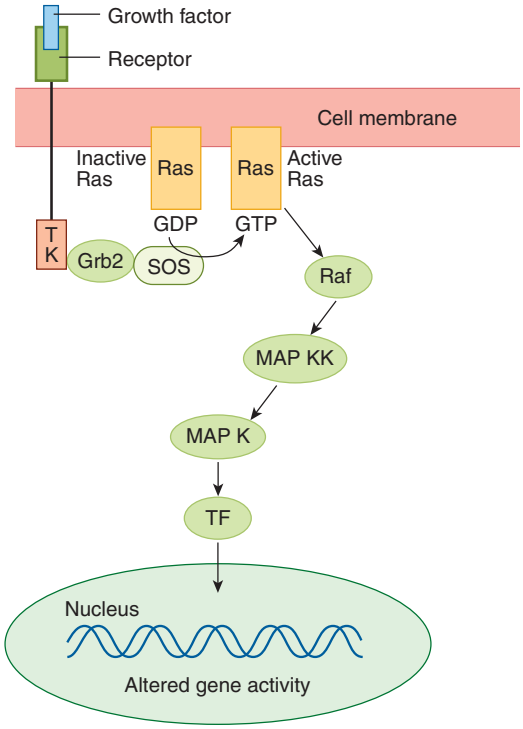


FIGURE 2–30 One of the direct pathways by which growth factors alter gene activity. TK, tyrosine kinase domain; Grb2, Ras activator controller; Sos, Ras activator; Ras, product of the ras gene; MAP K, mitogen-activated protein kinase; MAP KK, MAP kinase kinase; TF, transcription factors. There is a cross-talk between this pathway and the cAMP pathway, as well as a cross-talk with the IP_3–DAG pathway.

factor (EGF) are examples. More than 20 have been described. The cytokines are a second group. These factors are produced by macrophages and lymphocytes, as well as other cells, and are important in regulation of the immune system (see Chapter 3). Again, more than 20 have been described. The third group is made up of the colony-stimulating factors that regulate proliferation and maturation of red and white blood cells.

Receptors for EGF, platelet-derived growth factor (PDGF), and many of the other factors that foster cell multiplication and growth have a single membrane-spanning domain with an intracellular tyrosine kinase domain (Figure 2–29). When ligand binds to a tyrosine kinase receptor, it first causes a dimerization of two similar receptors. The dimerization results in partial activation of the intracellular tyrosine kinase domains and a cross-phosphorylation to fully activate each other. One of the pathways activated by phosphorylation leads, through the small G protein Ras, to MAP kinases, and eventually to the production of transcription factors in the nucleus that alter gene expression (**Figure 2–30**).

Receptors for cytokines and colony-stimulating factors differ from the other growth factors in that most of them do not have tyrosine kinase domains in their cytoplasmic portions and some have little or no cytoplasmic tail. However, they initiate tyrosine kinase activity in the cytoplasm. In particular, they activate the so-called Janus tyrosine kinases **(JAKs)** in the cytoplasm (**Figure 2–31**). These in turn phosphorylate **STAT** proteins. The phosphorylated STATs form homo- and heterodimers and move to the nucleus, where they act as transcription factors. There are four known mammalian JAKs and seven known STATs. Interestingly, the JAK–STAT pathway can also be activated by growth hormone and is another important direct path from the cell surface to the nucleus. However, it should be emphasized that both the Ras and the JAK–STAT pathways are complex and there is cross-talk between them and other signaling pathways discussed previously.

Finally, note that the whole subject of second messengers and intracellular signaling has become immensely complex, with multiple pathways and interactions. It is only possible in a book such as this to list highlights and present general themes that will aid the reader in understanding the rest of physiology (**see Clinical Box 2–9**).

HOMEOSTASIS

The actual environment of the cells of the body is the interstitial component of the ECF. Because normal cell function depends on the constancy of this fluid, it is not surprising that in multicellular animals, an immense number of regulatory mechanisms have evolved to maintain it. To describe "the various physiologic arrangements which serve to restore the normal state, once it has been disturbed," W.B. Cannon coined the term **homeostasis.** The buffering properties of the body fluids and the renal and respiratory adjustments to the presence of excess acid or alkali are examples of homeostatic mechanisms. There are countless other examples, and a large

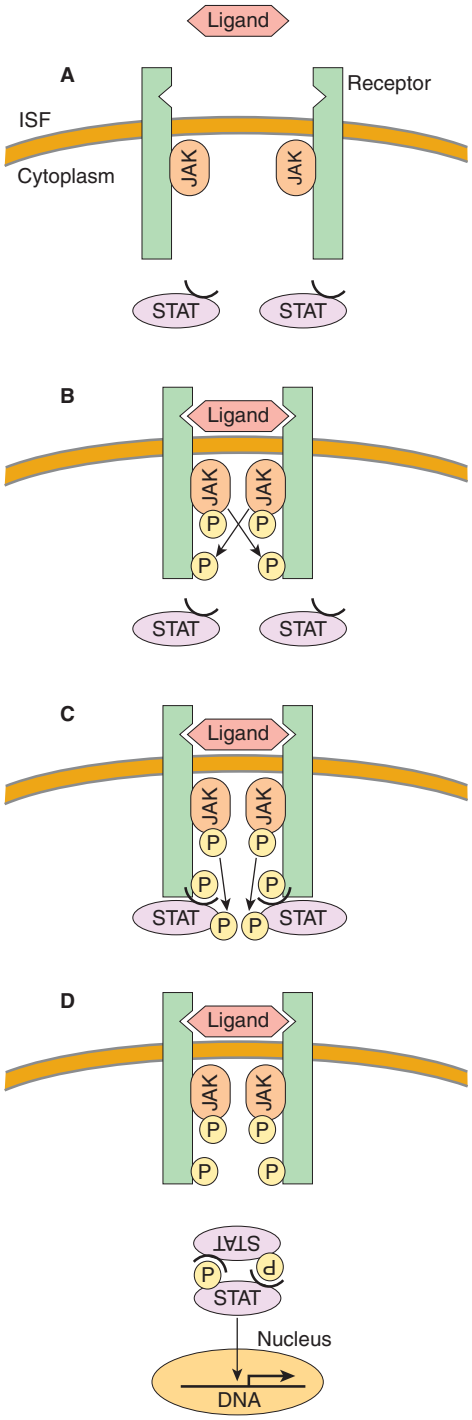


FIGURE 2–31 Signal transduction via the JAK–STAT pathway. A) Ligand binding leads to dimerization of receptor. **B)** Activation and tyrosine phosphorylation of JAKs. **C)** JAKs phosphorylate STATs. **D)** STATs dimerize and move to nucleus, where they bind to response elements on DNA. (Modified from Takeda K, Kishimoto T, Akira S: STAT6: Its role in interleukin 4-mediated biological functions. J Mol Med 1997;75:317.)

CLINICAL BOX 2–9

Receptor & G Protein Diseases

Many diseases are being traced to mutations in the genes for receptors. For example, loss-of-function receptor mutations that cause disease have been reported for the 1,25-dihydroxycholecalciferol receptor and the insulin receptor. Certain other diseases are caused by production of antibodies against receptors. Thus, antibodies against thyroid-stimulating hormone (TSH) receptors cause Graves' disease, and antibodies against nicotinic acetylcholine receptors cause myasthenia gravis.

An example of loss of function of a receptor is the type of nephrogenic diabetes insipidus that is due to loss of the ability of mutated V2 vasopressin receptors to mediate concentration of the urine. Mutant receptors can gain as well as lose function. A gain-of-function mutation of the Ca^{2+} receptor causes excess inhibition of parathyroid hormone secretion and familial hypercalciuric hypocalcemia. G proteins can also undergo loss-of-function or gain-of-function mutations that cause disease (**Table 2–6**). In one form of pseudohypoparathyroidism, a mutated $G_s\alpha$ fails to respond to parathyroid hormone, producing the symptoms of hypoparathyroidism without any decline in circulating parathyroid hormone. Testotoxicosis is an interesting disease that combines gain and loss of function. In this condition, an activating mutation of $G_s\alpha$ causes excess testosterone secretion and prepubertal sexual maturation. However, this mutation is temperature-sensitive and is active only at the relatively low temperature of the testes (33°C). At 37°C, the normal temperature of the rest of the body, it is replaced by loss of function, with the production of hypoparathyroidism and decreased responsiveness to TSH. A different activating mutation in $G_s\alpha$ is associated with the rough-bordered areas of skin pigmentation and hypercortisolism of the McCune–Albright syndrome. This mutation occurs during fetal development, creating a mosaic of normal and abnormal cells. A third mutation in $G_s\alpha$ reduces its intrinsic GTPase activity. As a result, it is much more active than normal, and excess cAMP is produced. This causes hyperplasia and eventually neoplasia in somatotrope cells of the anterior pituitary. Forty per cent of somatotrope tumors causing acromegaly have cells containing a somatic mutation of this type.

part of physiology is concerned with regulatory mechanisms that act to maintain the constancy of the internal environment. Many of these regulatory mechanisms operate on the principle of negative feedback; deviations from a given normal set point are detected by a sensor, and signals from the sensor trigger compensatory changes that continue until the set point is again reached.

TABLE 2–6 Examples of abnormalities caused by loss- or gain-of-function mutations of heterotrimeric G protein-coupled receptors and G proteins.

Site	Type of Mutation	Disease
Receptor		
Cone opsins	Loss	Color blindness
Rhodopsin	Loss	Congenital night blindness; two forms of retinitis pigmentosa
V_2 vasopressin	Loss	X-linked nephrogenic diabetes insipidus
ACTH	Loss	Familial glucocorticoid deficiency
LH	Gain	Familial male precocious puberty
TSH	Gain	Familial nonautoimmune hyperthyroidism
TSH	Loss	Familial hypothyroidism
Ca^{2+}	Gain	Familial hypercalciuric hypocalcemia
Thromboxane A_2	Loss	Congenital bleeding
Endothelin B	Loss	Hirschsprung disease
G protein		
G_s α	Loss	Pseudohypothyroidism type 1a
G_s α	Gain/loss	Testotoxicosis
G_s α	Gain (mosaic)	McCune–Albright syndrome
G_s α	Gain	Somatotroph adenomas with acromegaly
G_i α	Gain	Ovarian and adrenocortical tumors

CHAPTER SUMMARY

- The cell and the intracellular organelles are surrounded by semipermeable membranes. Biological membranes have a lipid bilayer core that is populated by structural and functional proteins. These proteins contribute greatly to the semipermeable properties of biological membrane.
- Cells contain a variety of organelles that perform specialized cell functions. The nucleus is an organelle that contains the cellular DNA and is the site of transcription. The endoplasmic reticulum and the Golgi apparatus are important in protein processing and the targeting of proteins to correct compartments within the cell. Lysosomes and peroxisomes are membrane-bound organelles that contribute to protein and lipid processing. Mitochondria are organelles that allow for oxidative phosphorylation in eukaryotic cells and also are important in specialized cellular signaling.
- The cytoskeleton is a network of three types of filaments that provide structural integrity to the cell as well as a means for trafficking of organelles and other structures around the cell. Actin filaments are important in cellular contraction, migration, and signaling. Actin filaments also provide the backbone for muscle contraction. Intermediate filaments are primarily structural. Microtubules provide a dynamic structure in cells that allows for the movement of cellular components around the cell.
- There are three superfamilies of molecular motor proteins in the cell that use the energy of ATP to generate force, movement, or both. Myosin is the force generator for muscle cell contraction. Cellular myosins can also interact with the cytoskeleton (primarily thin filaments) to participate in contraction as well as movement of cell contents. Kinesins and cellular dyneins are motor proteins that primarily interact with microtubules to move cargo around the cells.
- Cellular adhesion molecules aid in tethering cells to each other or to the extracellular matrix as well as providing for initiation of cellular signaling. There are four main families of these proteins: integrins, immunoglobulins, cadherins, and selectins.
- Cells contain distinct protein complexes that serve as cellular connections to other cells or the extracellular matrix. Tight junctions provide intercellular connections that link cells into a regulated tissue barrier and also provide a barrier to movement of proteins in the cell membrane. Gap junctions provide contacts between cells that allow for direct passage of small molecules between two cells. Desmosomes and adherens junctions are specialized structures that hold cells together. Hemidesmosomes and focal adhesions attach cells to their basal lamina.
- Exocytosis and endocytosis are vesicular fusion events that allow for movement of proteins and lipids between the cell interior, the plasma membrane, and the cell exterior. Exocytosis can be constitutive or nonconstitutive; both are regulated processes that require specialized proteins for vesicular fusion. Endocytosis is the formation of vesicles at the plasma membrane to take material from the extracellular space into the cell interior.
- Cells can communicate with one another via chemical messengers. Individual messengers (or ligands) typically bind to a plasma membrane receptor to initiate intracellular changes that lead to physiologic changes. Plasma membrane receptor families include ion channels, G protein-coupled receptors, or a variety of enzyme-linked receptors (eg, tyrosine kinase receptors). There are additional cytosolic receptors (eg, steroid receptors) that can bind membrane-permeant compounds. Activation of receptors leads to cellular changes that include changes in membrane potential, activation of heterotrimeric G proteins, increase in second messenger molecules, or initiation of transcription.
- Second messengers are molecules that undergo a rapid concentration changes in the cell following primary messenger recognition. Common second messenger molecules include Ca^{2+}, cyclic adenosine monophosphate (cAMP), cyclic guanine monophosphate (cGMP), inositol trisphosphate (IP_3), and nitric oxide (NO).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. The electrogenic Na, K ATPase plays a critical role in cellular physiology by
   A. using the energy in ATP to extrude 3 Na^+ out of the cell in exchange for taking two K^+ into the cell.
   B. using the energy in ATP to extrude 3 K^+ out of the cell in exchange for taking two Na^+ into the cell.
   C. using the energy in moving Na^+ into the cell or K^+ outside the cell to make ATP.
   D. using the energy in moving Na^+ outside of the cell or K^+ inside the cell to make ATP.
2. Cell membranes
   A. contain relatively few protein molecules.
   B. contain many carbohydrate molecules.
   C. are freely permeable to electrolytes but not to proteins.
   D. have variable protein and lipid contents depending on their location in the cell.
   E. have a stable composition throughout the life of the cell.
3. Second messengers
   A. are substances that interact with first messengers outside cells.
   B. are substances that bind to first messengers in the cell membrane.
   C. are hormones secreted by cells in response to stimulation by another hormone.
   D. mediate the intracellular responses to many different hormones and neurotransmitters.
   E. are not formed in the brain.
4. The Golgi complex
   A. is an organelle that participates in the breakdown of proteins and lipids.
   B. is an organelle that participates in posttranslational processing of proteins.
   C. is an organelle that participates in energy production.
   D. is an organelle that participates in transcription and translation.
   E. is a subcellular compartment that stores proteins for trafficking to the nucleus.
5. Endocytosis
   A. includes phagocytosis and pinocytosis, but not clathrin-mediated or caveolae-dependent uptake of extracellular contents.
   B. refers to the merging of an intracellular vesicle with the plasma membrane to deliver intracellular contents to the extracellular milieu.
   C. refers to the invagination of the plasma membrane to uptake extracellular contents into the cell.
   D. refers to vesicular trafficking between Golgi stacks.
6. G protein-coupled receptors
   A. are intracellular membrane proteins that help to regulate movement within the cell.
   B. are plasma membrane proteins that couple the extracellular binding of primary signaling molecules to exocytosis.
   C. are plasma membrane proteins that couple the extracellular binding of primary signaling molecules to the activation of heterotrimeric G proteins.
   D. are intracellular proteins that couple the binding of primary messenger molecules with transcription.
7. Gap junctions are intercellular connections that
   A. primarily serve to keep cells separated and allow for transport across a tissue barrier.
   B. serve as a regulated cytoplasmic bridge for sharing of small molecules between cells.
   C. serve as a barrier to prevent protein movement within the cellular membrane.
   D. are cellular components for constitutive exocytosis that occurs between adjacent cells.
8. F-actin is a component of the cellular cytoskeleton that
   A. provides a structural component for cell movement.
   B. is defined as the "functional" form of actin in the cell.
   C. refers to the actin subunits that provide the molecular building blocks of the extended actin molecules found in the cell.
   D. provide the molecular architecture for cell to cell communication.

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CHAPTER 3

Immunity, Infection, & Inflammation

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the significance of immunity, particularly with respect to defending the body against microbial invaders.
- Define the circulating and tissue cell types that contribute to immune and inflammatory responses.
- Describe how phagocytes are able to kill internalized bacteria.
- Identify the functions of hematopoietic growth factors, cytokines, and chemokines.
- Delineate the roles and mechanisms of innate, acquired, humoral, and cellular immunity.
- Understand the basis of inflammatory responses and wound healing.

INTRODUCTION

As an open system, the body is continuously called upon to defend itself from potentially harmful invaders such as bacteria, viruses, and other microbes. This is accomplished by the immune system, which is subdivided into innate and adaptive (or acquired) branches. The immune system is composed of specialized effector cells that sense and respond to foreign antigens and other molecular patterns not found in human tissues. Likewise, the immune system clears the body's own cells that have become senescent or abnormal, such as cancer cells. Finally, normal host tissues occasionally become the subject of inappropriate immune attack, such as in autoimmune diseases or in settings where normal cells are harmed as innocent bystanders when the immune system mounts an inflammatory response to an invader. It is beyond the scope of this volume to provide a full treatment of all aspects of modern immunology. Nevertheless, the student of physiology should have a working knowledge of immune functions and their regulation, due to a growing appreciation for the ways in which the immune system can contribute to normal physiological regulation in a variety of tissues, as well as contributions of immune effectors to pathophysiology.

IMMUNE EFFECTOR CELLS

Many immune effector cells circulate in the blood as the white blood cells. In addition, the blood is the conduit for the precursor cells that eventually develop into the immune cells of the tissues. The circulating immunologic cells include **granulocytes (polymorphonuclear leukocytes, PMNs),** comprising **neutrophils, eosinophils,** and **basophils; lymphocytes;** and **monocytes.** Immune responses in the tissues are further amplified by these cells following their extravascular migration, as well as tissue **macrophages** (derived from monocytes) and **mast cells** (related to basophils). Acting together, these cells provide the body with powerful defenses against tumors and viral, bacterial, and parasitic infections.

GRANULOCYTES

All granulocytes have cytoplasmic granules that contain biologically active substances involved in inflammatory and allergic reactions.

The average half-life of a neutrophil in the circulation is 6 h. To maintain the normal circulating blood level, it is therefore necessary to produce over 100 billion neutrophils per day. Many neutrophils enter the tissues, particularly if triggered to

do so by an infection or by inflammatory cytokines. They are attracted to the endothelial surface by cell adhesion molecules known as selectins, and they roll along it. They then bind firmly to neutrophil adhesion molecules of the integrin family. They next insinuate themselves through the walls of the capillaries between endothelial cells by a process called **diapedesis.** Many of those that leave the circulation enter the gastrointestinal tract and are eventually lost from the body.

Invasion of the body by bacteria triggers the **inflammatory response.** The bone marrow is stimulated to produce and release large numbers of neutrophils. Bacterial products interact with plasma factors and cells to produce agents that attract neutrophils to the infected area **(chemotaxis).** The chemotactic agents, which are part of a large and expanding family of **chemokines** (see following text), include a component of the complement system (C5a); leukotrienes; and polypeptides from lymphocytes, mast cells, and basophils. Other plasma factors act on the bacteria to make them "tasty" to the phagocytes **(opsonization).** The principal opsonins that coat the bacteria are immunoglobulins of a particular class (IgG) and complement proteins (see following text). The coated bacteria then bind to G protein-coupled receptors on the neutrophil cell membrane. This triggers increased motor activity of the cell, exocytosis, and the so-called respiratory burst. The increased motor activity leads to prompt ingestion of the bacteria by endocytosis **(phagocytosis).** By **exocytosis,** neutrophil granules discharge their contents into the phagocytic vacuoles containing the bacteria and also into the interstitial space **(degranulation).** The granules contain various proteases plus antimicrobial proteins called **defensins.** In addition, the cell membrane-bound enzyme **NADPH oxidase** is activated, with the production of toxic oxygen metabolites. The combination of the toxic oxygen metabolites and the proteolytic enzymes from the granules makes the neutrophil a very effective killing machine.

Activation of NADPH oxidase is associated with a sharp increase in O_2 uptake and metabolism in the neutrophil (the **respiratory burst**) and generation of O_2^- by the following reaction:

$$NADPH + H^+ + 2O_2 + \rightarrow NADP^+ + 2H^+ + 2O_2^-$$

O_2^- is a **free radical** formed by the addition of one electron to O_2. Two O_2^- react with two H^+ to form H_2O_2 in a reaction catalyzed by the cytoplasmic form of superoxide dismutase (SOD-1):

$$O_2^- + O_2^- + H^+ \xrightarrow{\text{SOD-1}} \rightarrow H_2O_2 + O_2$$

O_2^- and H_2O_2 are both oxidants that are effective bactericidal agents, but H_2O_2 is converted to H_2O and O_2 by the enzyme **catalase.** The cytoplasmic form of SOD contains both Zn and Cu. It is found in many parts of the body. It is defective as a result of genetic mutation in a familial form of **amyotrophic lateral sclerosis** (ALS; see Chapter 15). Therefore, it may be that O_2^- accumulates in motor neurons and kills them in at least one form of this progressive, fatal disease. Two other forms of SOD encoded by at least one different gene are also found in humans.

Neutrophils also discharge the enzyme **myeloperoxidase,** which catalyzes the conversion of Cl^-, Br^-, I^-, and SCN^- to the corresponding acids (HOCl, HOBr, etc). These acids are also potent oxidants. Because Cl^- is present in greatest abundance in body fluids, the principal product is HOCl.

In addition to myeloperoxidase and defensins, neutrophil granules contain elastase, metalloproteinases that attack collagen, and a variety of other proteases that help destroy invading organisms. These enzymes act in a cooperative fashion with O_2^-, H_2O_2, and HOCl to produce a killing zone around the activated neutrophil. This zone is effective in killing invading organisms, but in certain diseases (eg, rheumatoid arthritis) the neutrophils may also cause local destruction of host tissue.

Like neutrophils, **eosinophils** have a short half-life in the circulation, are attracted to the surface of endothelial cells by selectins, bind to integrins that attach them to the vessel wall, and enter the tissues by diapedesis. Like neutrophils, they release proteins, cytokines, and chemokines that produce inflammation but are capable of killing invading organisms. However, eosinophils have some selectivity in the way in which they respond and in the killing molecules they secrete. Their maturation and activation in tissues is particularly stimulated by IL-3, IL-5, and GM-CSF (see below). They are especially abundant in the mucosa of the gastrointestinal tract, where they defend against parasites, and in the mucosa of the respiratory and urinary tracts. Circulating eosinophils are increased in allergic diseases such as asthma and in various other respiratory and gastrointestinal diseases.

Basophils also enter tissues and release proteins and cytokines. They resemble but are not identical to mast cells, and like mast cells they contain histamine (see below). They release histamine and other inflammatory mediators when activated by binding of specific antigens to cell-fixed IgE molecules, and participate in immediate-type hypersensitivity (allergic) reactions. These range from mild urticaria and rhinitis to severe anaphylactic shock. The antigens that trigger IgE formation and basophil (and mast cell) activation are innocuous to most individuals, and are referred to as allergens.

MAST CELLS

Mast cells are heavily granulated cells of the connective tissue that are abundant in tissues that come into contact with the external environment, such as beneath epithelial surfaces. Their granules contain proteoglycans, histamine, and many proteases. Like basophils, they degranulate when allergens bind to cell-bound IgE molecules directed against them. They are involved in inflammatory responses initiated by immunoglobulins IgE and IgG (see below). The inflammation combats invading parasites. In addition to this involvement in acquired immunity, they release TNF-α in response to bacterial products by an antibody-independent mechanism, thus participating in the nonspecific **innate immunity** that combats infections prior to the development of an adaptive immune response (see following text). Marked mast cell degranulation produces clinical manifestations of allergy up to and including anaphylaxis.

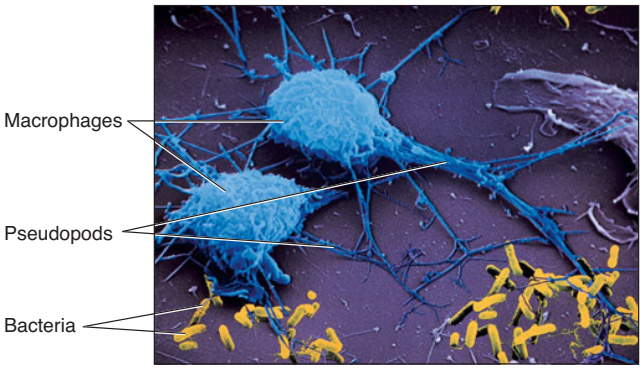

FIGURE 3–1 Macrophages contacting bacteria and preparing to engulf them. Figure is a colorized version of a scanning electronmicrograph.

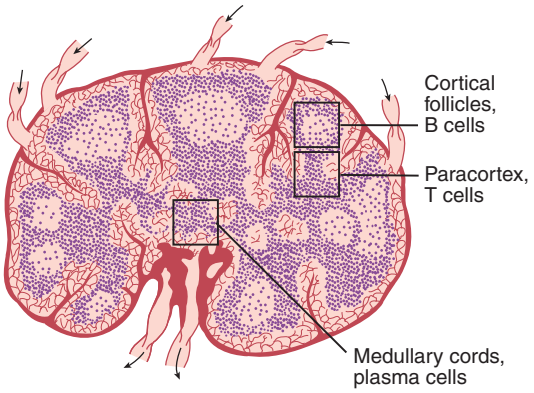

FIGURE 3–2 Anatomy of a normal lymph node. (After Chandrasoma. Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 4th ed. McGraw-Hill, 2003.)

MONOCYTES

Monocytes enter the blood from the bone marrow and circulate for about 72 h. They then enter the tissues and become **tissue macrophages** (Figure 3–1). Their life span in the tissues is unknown, but bone marrow transplantation data in humans suggest that they persist for about 3 months. It appears that they do not reenter the circulation. Some may end up as the multinucleated giant cells seen in chronic inflammatory diseases such as tuberculosis. The tissue macrophages include the Kupffer cells of the liver, pulmonary alveolar macrophages (see Chapter 34), and microglia in the brain, all of which come originally from the circulation. In the past, they have been called the **reticuloendothelial system,** but the general term **tissue macrophage system** seems more appropriate.

Macrophages are activated by cytokines released from T lymphocytes, among others. Activated macrophages migrate in response to chemotactic stimuli and engulf and kill bacteria by processes generally similar to those occurring in neutrophils. They play a key role in innate immunity (see below). They also secrete up to 100 different substances, including factors that affect lymphocytes and other cells, prostaglandins of the E series, and clot-promoting factors.

LYMPHOCYTES

Lymphocytes are key elements in the production of acquired immunity (see below). After birth, some lymphocytes are formed in the bone marrow. However, most are formed in the lymph nodes (Figure 3–2), thymus, and spleen from precursor cells that originally came from the bone marrow and were processed in the thymus (T cells) or bursal equivalent (B cells, see below). Lymphocytes enter the bloodstream for the most part via the lymphatics. At any given time, only about 2% of the body lymphocytes are in the peripheral blood. Most of the rest are in the lymphoid organs. It has been calculated that in humans, 3.5×10^{10} lymphocytes per day enter the circulation via the thoracic duct alone; however, this count includes cells that reenter the lymphatics and thus traverse the thoracic duct more than once. The effects of adrenocortical hormones on the lymphoid organs, the circulating lymphocytes, and the granulocytes are discussed in Chapter 20.

During fetal development, and to a much lesser extent during adult life, lymphocyte precursors come from the bone marrow. Those that populate the thymus (Figure 3–3) become transformed by the environment in this organ into T lymphocytes. In birds, the precursors that populate the bursa of Fabricius, a lymphoid structure near the cloaca, become transformed into B lymphocytes. There is no bursa in mammals, and the transformation to B lymphocytes occurs in **bursal equivalents,** that is, the fetal liver and, after birth, the bone marrow. After residence in the thymus or liver, many of the T and B lymphocytes migrate to the lymph nodes.

T and B lymphocytes are morphologically indistinguishable but can be identified by markers on their cell membranes. B cells differentiate into **plasma cells** and **memory B cells.** There are three major types of T cells: **cytotoxic T cells, helper T cells,** and **memory T cells.** There are two subtypes of helper T cells: T helper 1 (TH1) cells secrete IL-2 and γ-interferon and are concerned primarily with cellular immunity; T helper 2 (TH2) cells secrete IL-4 and IL-5 and interact primarily with B cells in relation to humoral immunity. Cytotoxic T cells destroy transplanted and other foreign cells, with their development aided and directed by helper T cells. Markers on the surface of lymphocytes are assigned CD (clusters of differentiation) numbers on the basis of their reactions to a panel of monoclonal antibodies. Most cytotoxic T cells display the glycoprotein CD8, and helper T cells display the glycoprotein CD4. These proteins are closely associated with the T cell receptors and may function as coreceptors. On the basis of differences in their receptors and functions, cytotoxic T cells are divided into αβ and γδ types (see below). Natural killer (NK) cells (see above) are also cytotoxic lymphocytes, though they are not T cells. Thus, there are three main types of cytotoxic lymphocytes in the body: αβ T cells, γδ T cells, and NK cells.

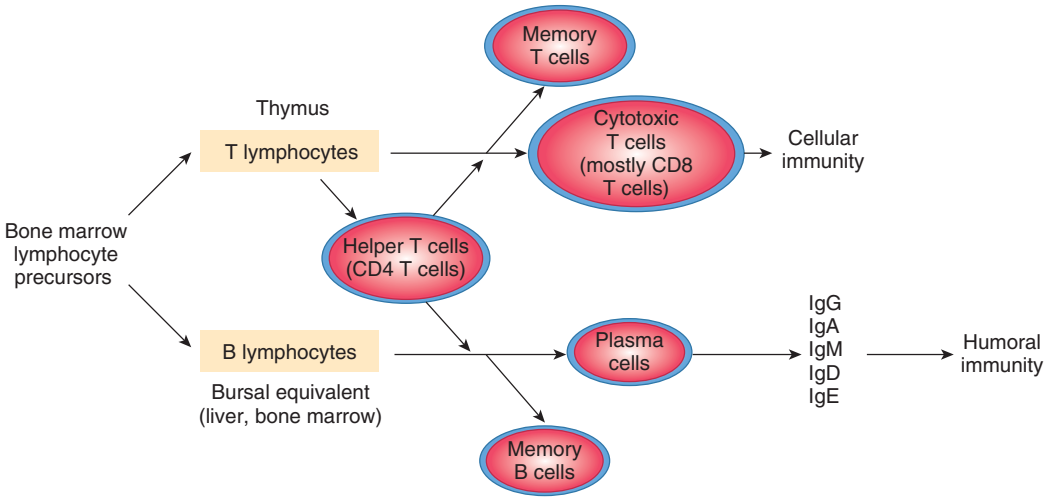

FIGURE 3–3 **Development of the system mediating acquired immunity.**

MEMORY B CELLS & T CELLS

After exposure to a given antigen, a small number of activated B and T cells persist as memory B and T cells. These cells are readily converted to effector cells by a later encounter with the same antigen. This ability to produce an accelerated response to a second exposure to an antigen is a key characteristic of acquired immunity. The ability persists for long periods of time, and in some instances (eg, immunity to measles) it can be life-long.

After activation in lymph nodes, lymphocytes disperse widely throughout the body and are especially plentiful in areas where invading organisms enter the body, for example, the mucosa of the respiratory and gastrointestinal tracts. This puts memory cells close to sites of reinfection and may account in part for the rapidity and strength of their response. Chemokines are involved in guiding activated lymphocytes to these locations.

GRANULOCYTE & MACROPHAGE COLONY-STIMULATING FACTORS

The production of white blood cells is regulated with great precision in healthy individuals, and the production of granulocytes is rapidly and dramatically increased in infections. The proliferation and self-renewal of hematopoietic stem cells (HSCs) depends on **stem cell factor (SCF).** Other factors specify particular lineages. The proliferation and maturation of the cells that enter the blood from the marrow are regulated by growth factors that cause cells in one or more of the committed cell lines to proliferate and mature (Table 3–1). The regulation of erythrocyte production by **erythropoietin** is discussed in Chapter 38. Three additional factors are called **colony-stimulating factors (CSFs),** because they cause appropriate single stem cells to proliferate in soft agar, forming colonies. The factors stimulating the production of committed stem cells include **granulocyte–macrophage CSF (GM-CSF), granulocyte CSF (G-CSF),** and **macrophage CSF (M-CSF).** Interleukins **IL-1** and **IL-6** followed by **IL-3** (Table 3–1) act in sequence to convert pluripotential uncommitted stem cells to committed progenitor cells. IL-3 is also known as **multi-CSF.** Each of the CSFs has a predominant action, but all the CSFs and interleukins also have other overlapping actions. In addition, they activate and sustain mature blood cells. It is interesting in this regard that the genes for many of these factors are located together on the long arm of chromosome 5 and may have originated by duplication of an ancestral gene. It is also interesting that basal hematopoiesis is normal in mice in which the GM-CSF gene is knocked out indicating that loss of one factor can be compensated for by others. On the other hand, the absence of GM-CSF causes accumulation of surfactant in the lungs (see Chapter 34).

As noted in Chapter 38, erythropoietin is produced in part by kidney cells and is a circulating hormone. The other factors are produced by macrophages, activated T cells, fibroblasts, and endothelial cells. For the most part, the factors act locally in the bone marrow (Clinical Box 3–1).

IMMUNITY

OVERVIEW

Insects and other invertebrates have only **innate immunity.** This system is triggered by receptors that bind sequences of sugars, lipids, amino acids, or nucleic acids that are common on bacteria and other microorganisms, but are not found in eukaryotic cells. These receptors, in turn, activate various defense mechanisms. The receptors are coded in the germ line, and their fundamental structure is not modified by exposure to antigen. The activated defenses include, in various species, release of interferons, phagocytosis, production of antibacterial peptides, activation of the complement system, and several proteolytic cascades. Even plants release antibacterial peptides

TABLE 3–1 Hematopoietic growth factors.

Cytokine	Cell Lines Stimulated	Cytokine Source
IL-1	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
IL-3	Erythrocyte Granulocyte Megakaryocyte Monocyte	T lymphocytes
IL-4	Basophil	T lymphocytes
IL-5	Eosinophil	T lymphocytes
IL-6	Erythrocyte Granulocyte Megakaryocyte Monocyte	Endothelial cells Fibroblasts Macrophages
IL-11	Erythrocyte Granulocyte Megakaryocyte	Fibroblasts Osteoblasts
Erythropoietin	Erythrocyte	Kidney Kupffer cells of liver
SCF	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
G-CSF	Granulocyte	Endothelial cells Fibroblasts Monocytes
GM-CSF	Erythrocyte Granulocyte Megakaryocyte	Endothelial cells Fibroblasts Monocytes T lymphocytes
M-CSF	Monocyte	Endothelial cells Fibroblasts Monocytes
Thrombopoietin	Megakaryocyte	Liver, kidney

Key: CSF, colony stimulating factor; G, granulocyte; IL, interleukin; M, macrophage; SCF, stem cell factor.

Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF (editors): *Pathophysiology of Disease*, 6th ed. McGraw-Hill, 2010.

CLINICAL BOX 3–1

Disorders of Phagocytic Function

More than 15 primary defects in neutrophil function have been described, along with at least 30 other conditions in which there is a secondary depression of the function of neutrophils. Patients with these diseases are prone to infections that are relatively mild when only the neutrophil system is involved, but which can be severe when the monocyte-tissue macrophage system is also involved. In one syndrome (neutrophil hypomotility), actin in the neutrophils does not polymerize normally, and the neutrophils move slowly. In another, there is a congenital deficiency of leukocyte integrins. In a more serious disease (chronic granulomatous disease), there is a failure to generate O_2^- in both neutrophils and monocytes and consequent inability to kill many phagocytosed bacteria. In severe congenital glucose 6-phosphate dehydrogenase deficiency, there are multiple infections because of failure to generate the NADPH necessary for O_2^- production. In congenital myeloperoxidase deficiency, microbial killing power is reduced because hypochlorous acid is not formed.

THERAPEUTIC HIGHLIGHTS

The cornerstones of treatment in disorders of phagocytic function include scrupulous efforts to avoid exposure to infectious agents, and antibiotic and antifungal prophylaxis. Antimicrobial therapies must also be implemented aggressively if infections occur. Sometimes, surgery is needed to excise and/or drain abscesses and relieve obstructions. Bone marrow transplantation may offer the hope of a definitive cure for severe conditions, such as chronic granulomatous disease. Sufferers of this condition have a significantly reduced life expectancy due to recurrent infections and their complications, and so the risks of bone marrow transplantation may be deemed acceptable. Gene therapy, on the other hand, remains a distant goal.

in response to infection. This primitive immune system is also important in vertebrates, particularly in the early response to infection. However, in vertebrates, innate immunity is also complemented by **adaptive** or **acquired immunity,** a system in which T and B lymphocytes are activated by specific antigens. T cells bear receptors related to antibody molecules, but which remain cell-bound. When these receptors encounter their cognate antigen, the T cell is stimulated to proliferate and produce cytokines that orchestrate the immune response, including that of B cells. Activated B lymphocytes form clones that produce secreted antibodies, which attack foreign proteins. After the invasion is repelled, small numbers of lymphocytes persist as memory cells so that a second exposure to the same antigen provokes a prompt and magnified immune attack. The genetic event that led to acquired immunity occurred 450 million years ago in the ancestors of jawed vertebrates and was probably insertion of a transposon into the genome in a way that made possible the generation of the immense repertoire of T cell receptors and antibodies that can be produced by the body.

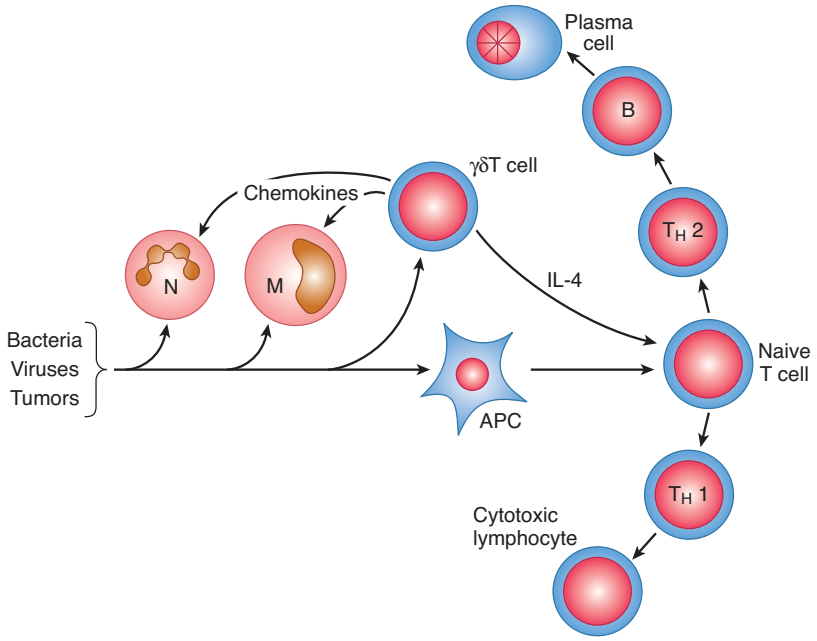

FIGURE 3–4 How bacteria, viruses, and tumors trigger innate immunity and initiate the acquired immune response. Arrows indicate mediators/cytokines that act on the target cell shown and/or pathways of differentiation. APC, antigen-presenting cell; M, monocyte; N, neutrophil; TH1 and TH2, helper T cells type 1 and type 2, respectively.

In vertebrates, including humans, innate immunity provides the first line of defense against infections, but it also triggers the slower but more specific acquired immune response (Figure 3–4). In vertebrates, natural and acquired immune mechanisms also attack tumors and tissue transplanted from other animals.

Once activated, immune cells communicate by means of cytokines and chemokines. They kill viruses, bacteria, and other foreign cells by secreting other cytokines and activating the complement system.

CYTOKINES

Cytokines are hormone-like molecules that act—generally in a paracrine fashion—to regulate immune responses. They are secreted not only by lymphocytes and macrophages but by endothelial cells, neurons, glial cells, and other types of cells. Most of the cytokines were initially named for their actions, for example, B cell-differentiating factor, or B cell-stimulating factor 2. However, the nomenclature has since been rationalized by international agreement to that of the **interleukins.** For example, the name of B cell-differentiating factor was changed to interleukin-4. A number of cytokines selected for their biological and clinical relevance are listed in Table 3–2, but it would be beyond the scope of this text to list all cytokines, which now number more than 100.

Many of the receptors for cytokines and hematopoietic growth factors (see above), as well as the receptors for prolactin (see Chapter 22), and growth hormone (see Chapter 18) are members of a cytokine-receptor superfamily that has three subfamilies (Figure 3–5). The members of subfamily 1, which includes the receptors for IL-4 and IL-7, are homodimers. The members of subfamily 2, which includes the receptors for IL-3, IL-5, and IL-6, are heterodimers. The receptor for IL-2 (and several other cytokines) consists of a heterodimer plus an unrelated protein, the so-called Tac antigen. The other members of subfamily 3 have the same γ chain as IL-2R. The extracellular domain of the homodimer and heterodimer subunits all contain four conserved cysteine residues plus a conserved Trp-Ser-X-Trp-Ser domain, and although the intracellular portions do not contain tyrosine kinase catalytic domains, they activate cytoplasmic tyrosine kinases when ligand binds to the receptors.

The effects of the principal cytokines are listed in Table 3–2. Some of them have systemic as well as local paracrine effects. For example, IL-1, IL-6, and tumor necrosis factor α cause fever, and IL-1 increases slow-wave sleep and reduces appetite.

Another superfamily of cytokines is the **chemokine** family. Chemokines are substances that attract neutrophils (see previous text) and other white blood cells to areas of inflammation or immune response. Over 40 have now been identified, and it is clear that they also play a role in the regulation of cell growth and angiogenesis. The chemokine receptors are G protein-coupled receptors that cause, among other things, extension of pseudopodia with migration of the cell toward the source of the chemokine.

THE COMPLEMENT SYSTEM

The cell-killing effects of innate and acquired immunity are mediated in part by a system of more than 30 plasma proteins originally named the **complement system** because they "complemented" the effects of antibodies. Three different pathways or enzyme

TABLE 3–2 Examples of cytokines and their clinical relevance.

Cytokine	Cellular Sources	Major Activities	Clinical Relevance
Interleukin-1	Macrophages	Activation of T cells and macrophages; promotion of inflammation	Implicated in the pathogenesis of septic shock, rheumatoid arthritis, and atherosclerosis
Interleukin-2	Type 1 (TH1) helper T cells	Activation of lymphocytes, natural killer cells, and macrophages	Used to induce lymphokine-activated killer cells; used in the treatment of metastatic renal-cell carcinoma, melanoma, and various other tumors
Interleukin-4	Type 2 (TH2) helper T cells, mast cells, basophils, and eosinophils	Activation of lymphocytes, monocytes, and IgE class switching	As a result of its ability to stimulate IgE production, plays a part in mast-cell sensitization and thus in allergy and in defense against nematode infections
Interleukin-5	Type 2 (TH2) helper T cells, mast cells, and eosinophils	Differentiation of eosinophils	Monoclonal antibody against interleukin-5 used to inhibit the antigen-induced late-phase eosinophilia in animal models of allergy
Interleukin-6	Type 2 (TH2) helper T cells and macrophages	Activation of lymphocytes; differentiation of B cells; stimulation of the production of acute-phase proteins	Overproduced in Castleman's disease; acts as an autocrine growth factor in myeloma and in mesangial proliferative glomerulonephritis
Interleukin-8	T cells and macrophages	Chemotaxis of neutrophils, basophils, and T cells	Levels are increased in diseases accompanied by neutrophilia, making it a potentially useful marker of disease activity
Interleukin-11	Bone marrow stromal cells	Stimulation of the production of acute-phase proteins	Used to reduce chemotherapy-induced thrombocytopenia in patients with cancer
Interleukin-12	Macrophages and B cells	Stimulation of the production of interferon γ by type 1 (TH1) helper T cells and by natural killer cells; induction of type 1 (TH1) helper T cells	May be useful as an adjuvant for vaccines
Tumor necrosis factor α	Macrophages, natural killer cells, T cells, B cells, and mast cells	Promotion of inflammation	Treatment with antibodies against tumor necrosis factor α beneficial in rheumatoid arthritis and Crohn's disease
Lymphotoxin (tumor necrosis factor β)	Type 1 (TH1) helper T cells and B cells	Promotion of inflammation	Implicated in the pathogenesis of multiple sclerosis and insulin-dependent diabetes mellitus
Transforming growth factor β	T cells, macrophages, B cells, and mast cells	Immunosuppression	May be useful therapeutic agent in multiple sclerosis and myasthenia gravis
Granulocyte-macrophage colony-stimulating factor	T cells, macrophages, natural killer cells, and B cells	Promotion of the growth of granulocytes and monocytes	Used to reduce neutropenia after chemotherapy for tumors and in ganciclovir-treated patients with AIDS; used to stimulate cell production after bone marrow transplantation
Interferon-α	Virally infected cells	Induction of resistance of cells to viral infection	Used to treat AIDS-related Kaposi sarcoma, melanoma, chronic hepatitis B infection, and chronic hepatitis C infection
Interferon-β	Virally infected cells	Induction of resistance of cells to viral infection	Used to reduce the frequency and severity of relapses in multiple sclerosis
Interferon-γ	Type 1 (TH1) helper T cells and natural killer cells	Activation of macrophages; inhibition of type 2 (TH2) helper T cells	Used to enhance the killing of phagocytosed bacteria in chronic granulomatous disease

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cascades activate the system: the **classic pathway,** triggered by immune complexes; the **mannose-binding lectin pathway,** triggered when this lectin binds mannose groups in bacteria; and the **alternative** or **properdin pathway,** triggered by contact with various viruses, bacteria, fungi, and tumor cells. The proteins that are produced have three functions: they help kill invading organisms by opsonization, chemotaxis, and eventual lysis of the cells; they serve in part as a bridge from innate to acquired immunity by activating B cells and aiding immune memory; and they help dispose of waste products after apoptosis. Cell lysis, one of the principal ways the complement system kills cells, is brought about by inserting proteins called **perforins** into their cell membranes. These create holes, which permit free flow of ions and thus disruption of membrane polarity.

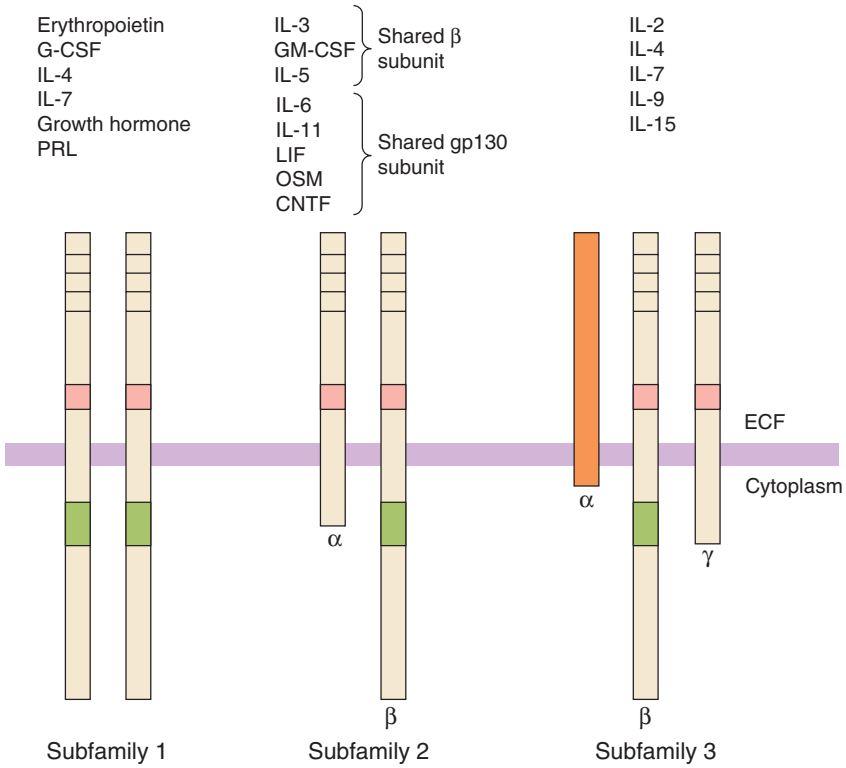

FIGURE 3–5 Members of one of the cytokine receptor superfamilies, showing shared structural elements. Note that all the subunits except the α subunit in subfamily 3 have four conserved cysteine residues (open boxes at top) and a Trp-Ser-X-Trp-Ser motif (pink). Many subunits also contain a critical regulatory domain in their cytoplasmic portions (green). CNTF, ciliary neurotrophic factor; LIF, leukemia inhibitory factor; OSM, oncostatin M; PRL, prolactin. (Modified from D'Andrea AD: Cytokine receptors in congenital hematopoietic disease. *N Engl J Med* 1994;330:839.)

INNATE IMMUNITY

The cells that mediate innate immunity include neutrophils, macrophages, and **natural killer cells,** large cytotoxic lymphocytes distinct from both T and B cells . All these cells respond to molecular patterns produced by bacteria and to other substances characteristic of viruses, tumor, and transplant cells. Many cells that are not professional immunocytes may nevertheless also contribute to innate immune responses, such as endothelial and epithelial cells. The activated cells produce their effects via the release of cytokines, as well as, in some cases, complement and other systems.

Innate immunity in *Drosophila* centers around a receptor protein named **toll,** which binds fungal antigens and triggers activation of genes coding for antifungal proteins. An expanding list of toll-like receptors (TLRs) have now been identified in humans and other vertebrates. One of these, TLR4, binds bacterial lipopolysaccharide and a protein called CD14, and this initiates intracellular events that activate transcription of genes for a variety of proteins involved in innate immune responses. This is important because bacterial lipopolysaccharide produced by gram-negative organisms is the cause of septic shock. TLR2 mediates the response to microbial lipoproteins, TLR6 cooperates with TLR2 in recognizing certain peptidoglycans, TLR5 recognizes a molecule known as flagellin in bacterial flagellae, and TLR9 recognizes bacterial DNA. TLRs are referred to as **pattern recognition receptors (PRRs),** because they recognize and respond to the molecular patterns expressed by pathogens. Other PRRs may be intracellular, such as the so-called NOD proteins. One NOD protein, NOD2, has received attention as a candidate gene leading to the intestinal inflammatory condition, Crohn's disease (Clinical Box 3–2).

ACQUIRED IMMUNITY

As noted previously, the key to acquired immunity is the ability of lymphocytes to produce antibodies (in the case of B cells) or cell-surface receptors (in the case of T cells) that are specific for one of the many millions of foreign agents that may invade the body. The antigens stimulating production of T cell receptors or antibodies are usually proteins and polypeptides, but antibodies can also be formed against nucleic acids and lipids if these are presented as nucleoproteins and lipoproteins. Antibodies to small molecules can also be produced experimentally if the molecules are bound to protein. Acquired immunity has two components: humoral immunity and cellular immunity. **Humoral immunity** is mediated by circulating immunoglobulin antibodies in the

γ-globulin fraction of the plasma proteins. Immunoglobulins are produced by differentiated forms of B lymphocytes known as **plasma cells,** and they activate the complement system and attack and neutralize antigens. Humoral immunity is a major defense against bacterial infections. **Cellular immunity** is mediated by T lymphocytes. It is responsible for delayed allergic reactions and rejection of transplants of foreign tissue. Cytotoxic T cells attack and destroy cells bearing the antigen that activated them. They kill by inserting perforins (see above) and by initiating apoptosis. Cellular immunity constitutes a major defense against infections due to viruses, fungi, and a few bacteria such as the tubercle bacillus. It also helps defend against tumors.

CLINICAL BOX 3–2

Crohn's Disease

Crohn's disease is a chronic, relapsing, and remitting disease that involves transmural inflammation of the intestine that can occur at any point along the length of the gastrointestinal tract, but most commonly is confined to the distal small intestine and colon. Patients with this condition suffer from changes in bowel habits, bloody diarrhea, severe abdominal pain, weight loss, and malnutrition. Evidence is accumulating that the disease reflects a failure to down-regulate inflammatory responses to the normal gut commensal microbiota. In genetically-susceptible individuals, mutations in genes controlling innate immune responses (eg, NOD2) or regulators of acquired immunity appear to predispose to disease when individuals are exposed to appropriate environmental factors, which can include a change in the microbiota or stress

THERAPEUTIC HIGHLIGHTS

During flares of Crohn's disease, the mainstay of treatment remains high-dose corticosteroids to suppress inflammation nonspecifically. Surgery is often required to treat complications such as strictures, fistulas, and abscesses. Some patients with severe disease also benefit from ongoing treatment with immunosuppressive drugs, or from treatment with antibodies targeted against tumor necrosis factor-α. Probiotics, therapeutic microorganisms designed to restore a "healthy" microbiota, may have some role in prophylaxis. The pathogenesis of Crohn's disease, as well as the related inflammatory bowel disease, ulcerative colitis, remains the subject of intense investigation, and therapies that target specific facets of the inflammatory cascade that may be selectively implicated in individual patients with differing genetic backgrounds are under development.

ANTIGEN RECOGNITION

The number of different antigens recognized by lymphocytes in the body is extremely large. The repertoire develops initially without exposure to the antigen. Stem cells differentiate into many million different T and B lymphocytes, each with the ability to respond to a particular antigen. When the antigen first enters the body, it can bind directly to the appropriate receptors on B cells. However, a full antibody response requires that the B cells contact helper T cells. In the case of T cells, the antigen is taken up by an antigen-presenting cell (APC) and partially digested. A peptide fragment of it is presented to the appropriate receptors on T cells. In either case, the cells are stimulated to divide, forming **clones** of cells that respond to this antigen **(clonal selection).** Effector cells are also subject to **negative selection,** during which lymphocyte precursors that are reactive with self-antigens are normally deleted. This results in immune **tolerance.** It is this latter process that presumably goes awry in autoimmune diseases, where the body reacts to and destroys cells expressing normal proteins, with accompanying inflammation that may lead to tissue destruction.

ANTIGEN PRESENTATION

APCs include specialized cells called **dendritic cells** in the lymph nodes and spleen and the Langerhans dendritic cells in the skin. Macrophages and B cells themselves, and likely many other cell types, can also function as APCs. For example, in the intestine, the epithelial cells that line the tract are likely important in presenting antigens derived from commensal bacteria. In APCs, polypeptide products of antigen digestion are coupled to protein products of the **major histocompatibility complex (MHC)** genes and presented on the surface of the cell. The products of the MHC genes are called human leukocyte antigens (HLA).

The genes of the MHC, which are located on the short arm of human chromosome 6, encode glycoproteins and are divided into two classes on the basis of structure and function. Class I antigens are composed of a 45-kDa heavy chain associated noncovalently with β_2-microglobulin encoded by a gene outside the MHC (Figure 3–6). They are found on all nucleated cells. Class II antigens are heterodimers made up of a 29–34-kDa α chain associated noncovalently with a 25–28-kDa β chain. They are present in "professional" APCs, including B cells, and in activated T cells.

The class I MHC proteins (MHC-I proteins) are coupled primarily to peptide fragments generated from proteins synthesized within cells. Peptides to which the host is not tolerant (eg, those from mutant or viral proteins) are recognized by T cells. The digestion of these proteins occurs in complexes of proteolytic enzymes known as **proteasomes,** and the peptide fragments bind to MHC proteins in the endoplasmic reticulum. The class II MHC proteins (MHC-II proteins) are concerned primarily with peptide products of extracellular antigens, such as bacteria, that enter the cell by endocytosis and are digested in the late endosomes.

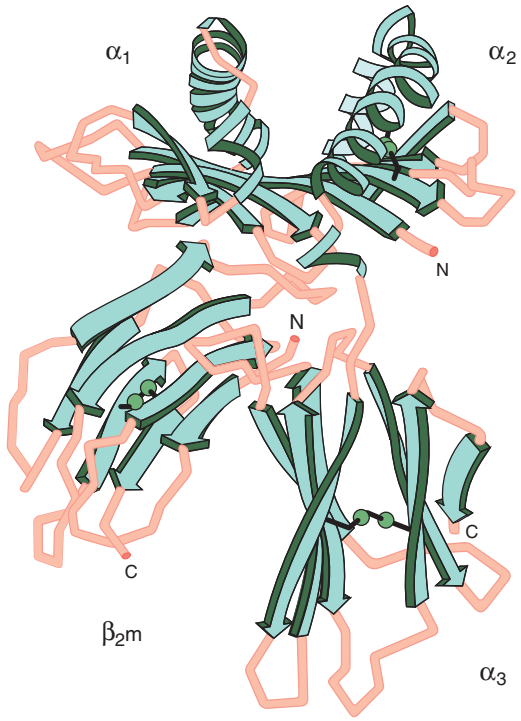

FIGURE 3–6 Structure of human histocompatibility antigen HLA-A2. The antigen-binding pocket is at the top and is formed by the α_1 and α_2 parts of the molecule. The α_3 portion and the associated β_2-microglobulin (β_2m) are close to the membrane. The extension of the C terminal from α 3 that provides the transmembrane domain and the small cytoplasmic portion of the molecule have been omitted. (Reproduced with permission from Bjorkman PJ, et al: Structure of the human histocompatibility antigen HLA-A2. Nature 1987;329:506.)

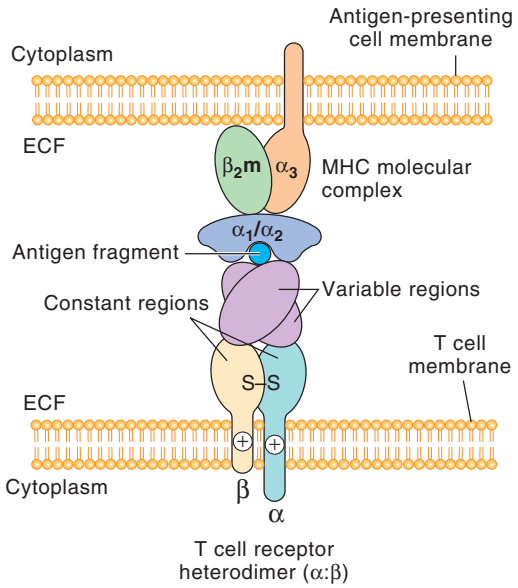

FIGURE 3–7 Interaction between antigen-presenting cell (top) and αβ T lymphocyte (bottom). The MHC proteins (in this case, MHC-I) and their peptide antigen fragment bind to the α and β units that combine to form the T cell receptor.

T CELL RECEPTORS

The MHC protein–peptide complexes on the surface of the APCs bind to appropriate T cells. Therefore, receptors on the T cells must recognize a very wide variety of complexes. Most of the receptors on circulating T cells are made up of two polypeptide units designated α and β. They form heterodimers that recognize the MHC proteins and the antigen fragments with which they are combined (Figure 3–7). These cells are called αβ T cells. On the other hand, about 10% of circulating T cells have two different polypeptides designated γ and δ in their receptors, and they are called γδ T cells. These T cells are prominent in the mucosa of the gastrointestinal tract, and there is evidence that they form a link between the innate and acquired immune systems by way of the cytokines they secrete (Figure 3–3).

CD8 occurs on the surface of cytotoxic T cells that bind MHC-I proteins, and CD4 occurs on the surface of helper T cells that bind MHC-II proteins (Figure 3–8). The CD8 and CD4 proteins facilitate the binding of the MHC proteins to the T cell receptors, and they also foster lymphocyte development. The activated CD8 cytotoxic T cells kill their targets directly, whereas the activated CD4 helper T cells secrete cytokines that activate other lymphocytes.

The T cell receptors are surrounded by adhesion molecules and proteins that bind to complementary proteins in the APC when the two cells transiently join to form the "immunologic synapse" that permits T cell activation to occur (Figure 3–7). It is now generally accepted that two signals are necessary to produce activation. One is produced by the binding of the digested antigen to the T cell receptor. The other is produced by the joining of the surrounding proteins in the "synapse."

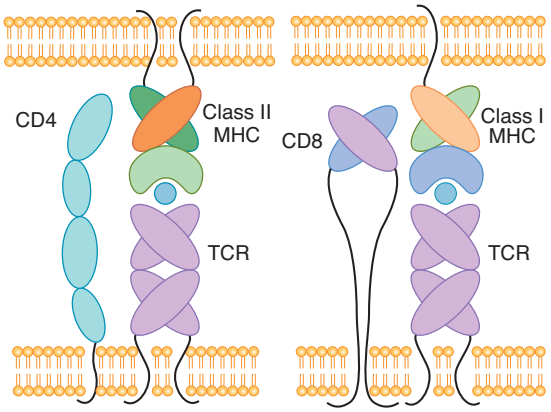

FIGURE 3–8 Diagrammatic summary of the structure of CD4 and CD8, and their relation to MHC-I and MHC-II proteins. Note that CD4 is a single protein, whereas CD8 is a heterodimer.

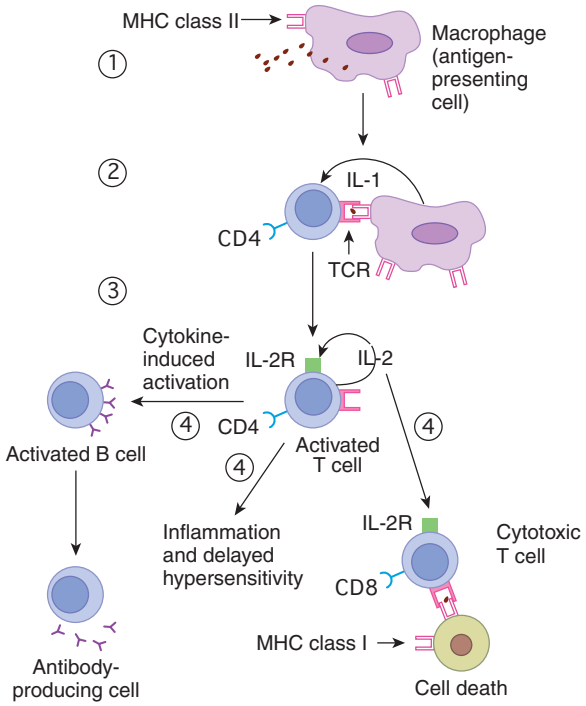

FIGURE 3–9 Summary of acquired immunity. (1) An antigen-presenting cell ingests and partially digests an antigen, then presents part of the antigen along with MHC peptides (in this case, MHC II peptides on the cell surface). **(2)** An "immune synapse" forms with a naive CD4 T cell, which is activated to produce IL-2. **(3)** IL-2 acts in an auto-crine fashion to cause the cell to multiply, forming a clone. **(4)** The activated CD4 cell may promote B cell activation and production of plasma cells or it may activate a cytotoxic CD8 cell. The CD8 cell can also be activated by forming a synapse with an MCH I antigen-presenting cell. (Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease,* 6th ed. McGraw-Hill, 2010.)

If the first signal occurs but the second does not, the T cell is inactivated and becomes unresponsive.

B CELLS

As noted above, B cells can bind antigens directly, but they must contact helper T cells to produce full activation and antibody formation. It is the TH2 subtype that is mainly involved. Helper T cells develop along the TH2 lineage in response to IL-4 (see below). On the other hand, IL-12 promotes the TH1 phenotype. IL-2 acts in an autocrine fashion to cause activated T cells to proliferate. The role of various cytokines in B cell and T cell activation is summarized in Figure 3–9.

The activated B cells proliferate and transform into **memory B cells** (see above) and **plasma cells.** The plasma cells secrete large quantities of antibodies into the general circulation. The antibodies circulate in the globulin fraction of the plasma and, like antibodies elsewhere, are called **immunoglobulins.** The immunoglobulins are actually the secreted form of antigen-binding receptors on the B cell membrane.

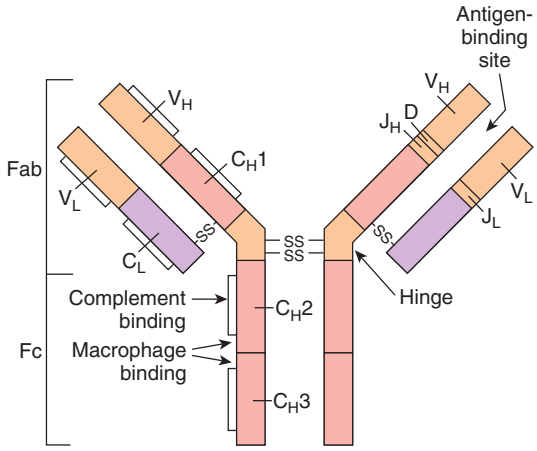

FIGURE 3–10 Typical immunoglobulin G molecule. Fab, portion of the molecule that is concerned with antigen binding; Fc, effector portion of the molecule. The constant regions are pink and purple, and the variable regions are orange. The constant segment of the heavy chain is subdivided into CH1, CH2, and CH3. SS lines indicate intersegmental disulfide bonds. On the right side, the C labels are omitted to show regions J_H, D, and J_L.

IMMUNOGLOBULINS

Circulating antibodies protect their host by binding to and neutralizing some protein toxins, by blocking the attachment of some viruses and bacteria to cells, by opsonizing bacteria (see above), and by activating complement. Five general types of immunoglobulin antibodies are produced by plasma cells. The basic component of each is a symmetric unit containing four polypeptide chains (Figure 3–10). The two long chains are called **heavy chains,** whereas the two short chains are called **light chains.** There are two types of light chains, k and λ, and eight types of heavy chains. The chains are joined by disulfide bridges that permit mobility, and there are intrachain disulfide bridges as well. In addition, the heavy chains are flexible in a region called the hinge. Each heavy chain has a variable (V) segment in which the amino acid sequence is highly variable, a diversity (D) segment in which the amino acid segment is also highly variable, a joining (J) segment in which the sequence is moderately variable, and a constant (C) segment in which the sequence is constant. Each light chain has a V, J, and C segment. The V segments form part of the antigen-binding sites (Fab portion of the molecule [Figure 3–10]). The Fc portion of the molecule is the effector portion, which mediates the reactions initiated by antibodies.

Two of the classes of immunoglobulins contain additional polypeptide components (Table 3–3). In IgM, five of the basic immunoglobulin units join around a polypeptide called the J chain to form a pentamer. In IgA, the **secretory immunoglobulin,** the immunoglobulin units form dimers and trimers around a J chain and a polypeptide that comes from epithelial cells, the secretory component (SC).

TABLE 3–3 Human immunoglobulins.[a]

Immunoglobulin	Function	Heavy Chain	Additional Chain	Structure	Plasma Concentration (mg/dL)
IgG	Complement activation	γ_1, γ_2, γ_3, γ_4		Monomer	1000
IgA	Localized protection in external secretions (tears, intestinal secretions, etc)	α_1, α_2	J, SC	Monomer; dimer with J or SC chain; trimer with J chain	200
IgM	Complement activation	μ	J	Pentamer with J chain	120
IgD	Antigen recognition by B cells	Δ	Monomer	3	
IgE	Reagin activity; releases histamine from basophils and mast cells	ε	Monomer	0.05	

[a]In all instances, the light chains are k or γ.

In the intestine, bacterial and viral antigens are taken up by M cells (see Chapter 26) and passed on to underlying aggregates of lymphoid tissue **(Peyer's patches),** where they activate naive T cells. These lymphocytes then form B cells that infiltrate mucosa of the gastrointestinal, respiratory, genitourinary, and female reproductive tracts and the breast. There they secrete large amounts of IgA when exposed again to the antigen that initially stimulated them. The epithelial cells produce the SC, which acts as a receptor for, and binds to, IgA. The resulting secretory immunoglobulin passes through the epithelial cell and is secreted by exocytosis. This system of **secretory immunity** is an important and effective defense mechanism at all mucosal surfaces. It also accounts for the immune protection that is conferred by breast feeding of infants whose immune systems are otherwise immature, because IgA is secreted into the breast milk.

GENETIC BASIS OF DIVERSITY IN THE IMMUNE SYSTEM

The genetic mechanism for the production of the immensely large number of different configurations of immunoglobulins produced by human B cells, as well as T cell receptors, is a fascinating biologic problem. Diversity is brought about in part by the fact that in immunoglobulin molecules there are two kinds of light chains and eight kinds of heavy chains. As noted previously, there are areas of great variability **(hypervariable regions)** in each chain. The variable portion of the heavy chains consists of the V, D, and J segments. In the gene family responsible for this region, there are several hundred different coding regions for the V segment, about 20 for the D segment, and four for the J segment. During B cell development, one V coding region, one D coding region, and one J coding region are selected at random and recombined to form the gene that produces that particular variable portion. A similar variable recombination takes place in the coding regions responsible for the two variable segments (V and J) in the light chain. In addition, the J segments are variable because the gene segments join in an imprecise and variable fashion (junctional site diversity) and nucleotides are sometimes added (junctional insertion diversity). It has been calculated that these mechanisms permit the production of about 10^{15} different immunoglobulin molecules. Additional variability is added by somatic mutation.

Similar gene rearrangement and joining mechanisms operate to produce the diversity in T cell receptors. In humans, the α subunit has a V region encoded by 1 of about 50 different genes and a J region encoded by 1 of another 50 different genes. The β subunits have a V region encoded by 1 of about 50 genes, a D region encoded by 1 of 2 genes, and a J region encoded by 1 of 13 genes. These variable regions permit the generation of up to an estimated 10^{15} different T cell receptors (Clinical Box 3–3 and Clinical Box 3–4).

A variety of immunodeficiency states can arise from defects in these various stages of B and T lymphocyte maturation. These are summarized in Figure 3–11.

PLATELETS

Platelets are circulating cells that are important mediators of hemostasis. While not immune cells, per se, they often participate in the response to tissue injury in cooperation with inflammatory cell types (see below). They have a ring of microtubules around their periphery and an extensively invaginated membrane with an intricate canalicular system in contact with the ECF. Their membranes contain receptors for collagen, ADP, vessel wall von Willebrand factor (see below), and fibrinogen. Their cytoplasm contains actin, myosin, glycogen, lysosomes, and two types of granules: (1) dense granules, which contain the nonprotein substances that are secreted in response to platelet activation, including serotonin, ADP, and other adenine nucleotides; and (2) α-granules, which contain secreted proteins. These proteins include clotting factors and **platelet-derived growth factor (PDGF).** PDGF is also produced by macrophages and endothelial cells. It is a dimer made up of A and B subunit polypeptides. Homodimers (AA and BB), as well as the heterodimer (AB), are produced.

CLINICAL BOX 3–3

Autoimmunity

Sometimes the processes that eliminate antibodies against self-antigens fail and a variety of different **autoimmune diseases** are produced. These can be B cell- or T cell-mediated and can be organ-specific or systemic. They include type 1 diabetes mellitus (antibodies against pancreatic islet B cells), myasthenia gravis (antibodies against nicotinic cholinergic receptors), and multiple sclerosis (antibodies against myelin basic protein and several other components of myelin). In some instances, the antibodies are against receptors and are capable of activating those receptors; for example, antibodies against TSH receptors increase thyroid activity and cause Graves' disease (see Chapter 19). Other conditions are due to the production of antibodies against invading organisms that cross-react with normal body constituents **(molecular mimicry).** An example is rheumatic fever following a streptococcal infection; a portion of cardiac myosin resembles a portion of the streptococcal M protein, and antibodies induced by the latter attack the former and damage the heart. Some conditions may be due to **bystander effects,** in which inflammation sensitizes T cells in the neighborhood, causing them to become activated when otherwise they would not respond.

THERAPEUTIC HIGHLIGHTS

The therapy of autoimmune disorders rests on efforts to replace or restore the damaged function (eg, provision of exogenous insulin in type 1 diabetes) as well as nonspecific efforts to reduce inflammation (using corticosteroids) or to suppress immunity. Recently, agents that deplete or dampen the function of B cells have been shown to have some efficacy in a range of autoimmune disorders, including rheumatoid arthritis, most likely by interrupting the production of autoantibodies that contribute to disease pathogenesis.

CLINICAL BOX 3–4

Tissue Transplantation

The T lymphocyte system is responsible for the rejection of transplanted tissue. When tissues such as skin and kidneys are transplanted from a donor to a recipient of the same species, the transplants "take" and function for a while but then become necrotic and are "rejected" because the recipient develops an immune response to the transplanted tissue. This is generally true even if the donor and recipient are close relatives, and the only transplants that are never rejected are those from an identical twin. Nevertheless, organ transplantation remains the only viable option in a number of end stage diseases.

THERAPEUTIC HIGHLIGHTS

A number of treatments have been developed to overcome the rejection of transplanted organs in humans. The goal of treatment is to stop rejection without leaving the patient vulnerable to massive infections. One approach is to kill T lymphocytes by killing all rapidly dividing cells with drugs such as azathioprine, a purine antimetabolite, but this makes patients susceptible to infections and cancer. Another is to administer glucocorticoids, which inhibit cytotoxic T cell proliferation by inhibiting production of IL-2, but these cause osteoporosis, mental changes, and the other facets of Cushing syndrome (see Chapter 20). More recently, immunosuppressive drugs such as **cyclosporine** or **tacrolimus (FK-506)** have found favor. Activation of the T cell receptor normally increases intracellular Ca^{2+}, which acts via calmodulin to activate calcineurin. Calcineurin dephosphorylates the transcription factor NF-AT, which moves to the nucleus and increases the activity of genes coding for IL-2 and related stimulatory cytokines. Cyclosporine and tacrolimus prevent the dephosphorylation of NF-AT. However, these drugs inhibit all T cell-mediated immune responses, and cyclosporine causes kidney damage and cancer. A new and promising approach to transplant rejection is the production of T cell unresponsiveness by using drugs that block the costimulation that is required for normal activation (see text). Clinically effective drugs that act in this fashion could be of great value to transplant surgeons.

PDGF stimulates wound healing and is a potent mitogen for vascular smooth muscle. Blood vessel walls as well as platelets contain von Willebrand factor, which, in addition to its role in adhesion, regulates circulating levels of factor VIII (see below).

When a blood vessel wall is injured, platelets adhere to the exposed collagen and **von Willebrand factor** in the wall via receptors on the platelet membrane. Von Willebrand factor is a very large circulating molecule that is produced by endothelial cells. Binding produces platelet activations, which release the contents of their granules. The released ADP acts on the ADP receptors in the platelet membranes to produce further accumulation of more platelets **(platelet aggregation).** Humans have at least three different types of platelet ADP receptors: $P2Y_1$, $P2Y_2$, and $P2X_1$. These are obviously attractive targets for drug development, and several new inhibitors have shown

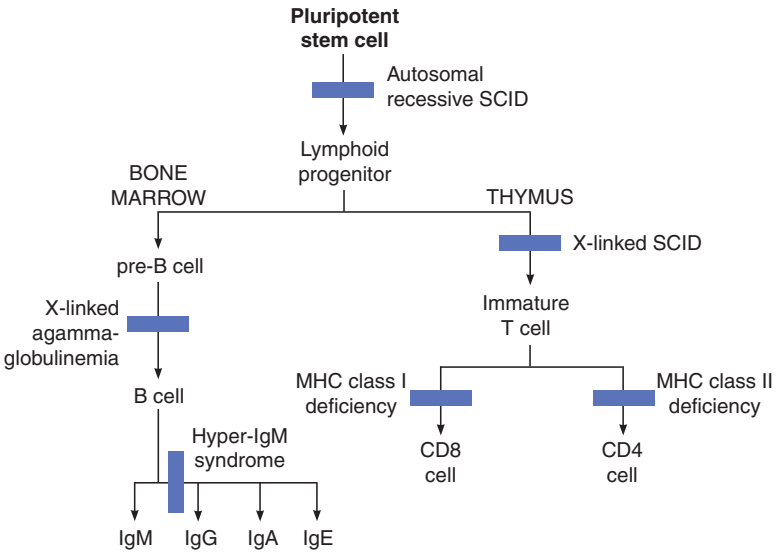

FIGURE 3–11 Sites of congenital blockade of B and T lymphocyte maturation in various immunodeficiency states. SCID, severe combined immune deficiency. (Modified from Rosen FS, Cooper MD, Wedgwood RJP: The primary immunodeficiencies. *N Engl J Med* 1995;333:431.)

promise in the prevention of heart attacks and strokes. Aggregation is also fostered by **platelet-activating factor (PAF),** a cytokine secreted by neutrophils and monocytes as well as platelets. This compound also has inflammatory activity. It is an ether phospholipid, 1-alkyl-2-acetylglyceryl-3-phosphorylcholine, which is produced from membrane lipids. It acts via a G protein-coupled receptor to increase the production of arachidonic acid derivatives, including thromboxane A_2. The role of this compound in the balance between clotting and anticlotting activity at the site of vascular injury is discussed in Chapter 31.

Platelet production is regulated by the CSFs that control the production of the platelet precursors in the bone marrow, known as megakaryocytes, plus **thrombopoietin,** a circulating protein factor. This factor, which facilitates megakaryocyte maturation, is produced constitutively by the liver and kidneys, and there are thrombopoietin receptors on platelets. Consequently, when the number of platelets is low, less is bound and more is available to stimulate production of platelets. Conversely, when the number of platelets is high, more is bound and less is available, producing a form of feedback control of platelet production. The amino terminal portion of the thrombopoietin molecule has the platelet-stimulating activity, whereas the carboxyl terminal portion contains many carbohydrate residues and is concerned with the bioavailability of the molecule.

When the platelet count is low, clot retraction is deficient and there is poor constriction of ruptured vessels. The resulting clinical syndrome **(thrombocytopenic purpura)** is characterized by easy bruisability and multiple subcutaneous hemorrhages. Purpura may also occur when the platelet count is normal, and in some of these cases, the circulating platelets are abnormal **(thrombasthenic purpura).** Individuals with thrombocytosis are predisposed to thrombotic events.

INFLAMMATION & WOUND HEALING

LOCAL INJURY

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances. It includes a sequence of reactions initially involving cytokines, neutrophils, adhesion molecules, complement, and IgG. PAF, an agent with potent inflammatory effects, also plays a role. Later, monocytes and lymphocytes are involved. Arterioles in the inflamed area dilate, and capillary permeability is increased (see Chapters 32 and 33). When the inflammation occurs in or just under the skin (Figure 3–12), it is characterized by redness, swelling, tenderness, and pain. Elsewhere, it is a key component of asthma, ulcerative colitis, Crohn's disease, rheumatoid arthritis, and many other diseases (Clinical Box 3–2).

Evidence is accumulating that a transcription factor, **nuclear factor-κB,** plays a key role in the inflammatory response. NF-κB is a heterodimer that normally exists in the cytoplasm of cells bound to IκBα, which renders it inactive. Stimuli such as cytokines, viruses, and oxidants induce signals that allow NF-κB to dissociate from IκBα, which is then degraded. NF-κB moves to the nucleus, where it binds to the DNA of the genes for numerous inflammatory mediators, resulting in their increased production and secretion. Glucocorticoids inhibit the activation of NF-κB by increasing the production of IκBα, and this is probably the main basis of their anti-inflammatory action (see Chapter 20).

SYSTEMIC RESPONSE TO INJURY

Cytokines produced in response to inflammation and other injuries, as well as disseminated infection, also produce systemic responses. These include alterations in plasma **acute**

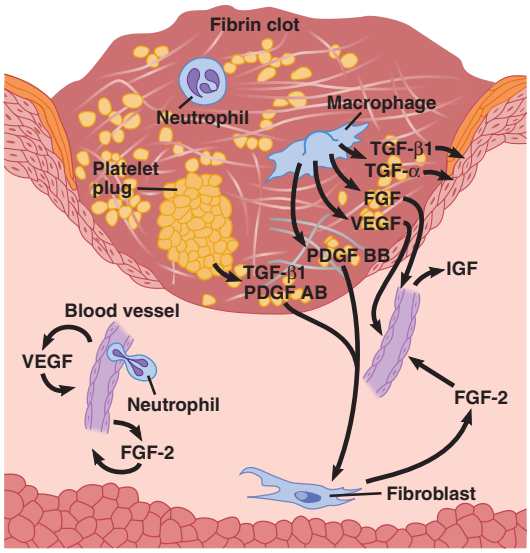

FIGURE 3–12 Cutaneous wound 3 days after injury, showing the multiple cytokines and growth factors affecting the repair process. VEGF, vascular endothelial growth factor. For other abbreviations, see Appendix. Note the epidermis growing down under the fibrin clot, restoring skin continuity. (Modified from Singer AJ, Clark RAF: Cutaneous wound healing. *N Engl J Med* 1999;341:738.)

phase proteins, defined as proteins whose concentration is increased or decreased by at least 25% following injury. Many of the proteins are of hepatic origin. A number of them are shown in Figure 3–13. The causes of the changes in concentration are incompletely understood, but it can be said that many of the changes make homeostatic sense. Thus, for example, an increase in C-reactive protein activates monocytes and causes further production of cytokines. Other changes that occur in response to injury include somnolence, negative nitrogen balance, and fever.

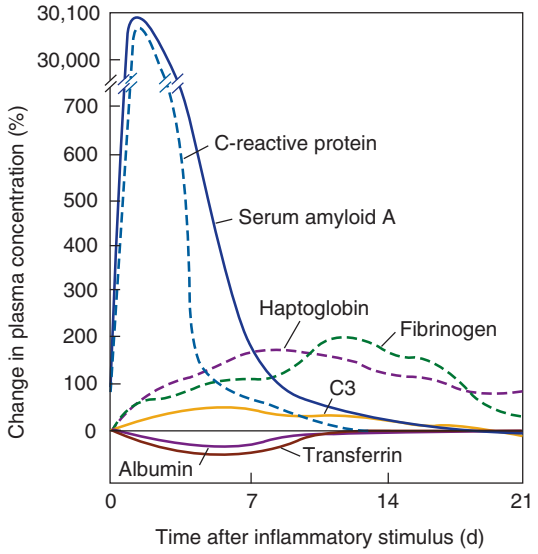

FIGURE 3–13 Time course of changes in some major acute phase proteins. C3, C3 component of complement. (Modified and reproduced with permission from McAdam KP, Elin RJ, Sipe JD, Wolff SM: Changes in human serum amyloid A and C-reactive protein after etiocholanolone-induced inflammation. J Clin Invest, 1978 Feb;61(2):390–394.)

WOUND HEALING

When tissue is damaged, platelets adhere to exposed matrix via integrins that bind to collagen and laminin (Figure 3–12). Blood coagulation produces thrombin, which promotes platelet aggregation and granule release. The platelet granules generate an inflammatory response. White blood cells are attracted by selectins and bind to integrins on endothelial cells, leading to their extravasation through the blood vessel walls. Cytokines released by the white blood cells and platelets up-regulate integrins on macrophages, which migrate to the area of injury, and on fibroblasts and epithelial cells, which mediate wound healing and scar formation. Plasmin aids healing by removing excess fibrin. This aids the migration of keratinocytes into the wound to restore the epithelium under the scab. Collagensynthesis is upregulated, producing the scar. Wounds gain 20% of their ultimate strength in 3 weeks and later gain more strength, but they never reach more than about 70% of the strength of normal skin.

CHAPTER SUMMARY

- Immune and inflammatory responses are mediated by several different cell types—granulocytes, lymphocytes, monocytes, mast cells, tissue macrophages, and antigen-presenting cells—that arise predominantly from the bone marrow and may circulate or reside in connective tissues.
- Granulocytes mount phagocytic responses that engulf and destroy bacteria. These are accompanied by the release of reactive oxygen species and other mediators into adjacent tissues that may cause tissue injury.
- Mast cells and basophils underpin allergic reactions to substances that would be treated as innocuous by nonallergic individuals.
- A variety of soluble mediators orchestrate the development of immunologic effector cells and their subsequent immune and inflammatory reactions.
- Innate immunity represents an evolutionarily conserved, primitive response to stereotypical microbial components.
- Acquired immunity is slower to develop than innate immunity, but long-lasting and more effective.
- Genetic rearrangements endow B and T lymphocytes with a vast array of receptors capable of recognizing billions of foreign antigens.
- Self-reactive lymphocytes are normally deleted; a failure of this process leads to autoimmune disease. Disease can also result from abnormal function or development of granulocytes and lymphocytes. In these latter cases, deficient immune responses to microbial threats usually result.

- Inflammatory responses occur in response to infection or injury, and serve to resolve the threat, although they may cause damage to otherwise healthy tissue. A number of chronic diseases reflect excessive inflammatory responses that persist even once the threat is controlled, or are triggered by stimuli that healthy individuals would not respond to.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. In an experiment, a scientist treats a group of mice with an antiserum that substantially depletes the number of circulating neutrophils. Compared with untreated control animals, the mice with reduced numbers of neutrophils were found to be significantly more susceptible to death induced by bacterial inoculation. The increased mortality can be ascribed to a relative deficit in which of the following?
   A. Acquired immunity
   B. Oxidants
   C. Platelets
   D. Granulocyte/macrophage colony stimulating factor (GM-CSF)
   E. Integrins
2. A 20-year-old college student comes to the student health center in April complaining of runny nose and congestion, itchy eyes, and wheezing. She reports that similar symptoms have occurred at the same time each year, and that she obtains some relief from over-the-counter antihistamine drugs, although they make her too drowsy to study. Her symptoms can most likely be attributed to inappropriate synthesis of which of the following antibodies specific for tree pollen?
   A. IgA
   B. IgD
   C. IgE
   D. IgG
   E. IgM
3. If a nasal biopsy were performed on the patient described in Question 2 while symptomatic, histologic examination of the tissue would most likely reveal degranulation of which of the following cell types?
   A. Dendritic cells
   B. Lymphocytes
   C. Neutrophils
   D. Monocytes
   E. Mast cells
4. A biotechnology company is working to design a new therapeutic strategy for cancer that involves triggering an enhanced immune response to cellular proteins that are mutated in the disease. Which of the following immune cells or processes will most likely **not** be required for a successful therapy?
   A. Cytotoxic T cells
   B. Antigen presentation in the context of MHC-II
   C. Proteosomal degradation
   D. Gene rearrangements producing T cell receptors
   E. The immune synapse
5. The ability of the blood to phagocytose pathogens and mount a respiratory burst is increased by
   A. interleukin-2 (IL-2)
   B. granulocyte colony-stimulating factor (G-CSF)
   C. erythropoietin
   D. interleukin-4 (IL-4)
   E. interleukin-5 (IL-5)
6. Cells responsible for innate immunity are activated most commonly by
   A. glucocorticoids
   B. pollen
   C. carbohydrate sequences in bacterial cell walls
   D. eosinophils
   E. thrombopoietin
7. A patient suffering from an acute flare in his rheumatoid arthritis undergoes a procedure where fluid is removed from his swollen and inflamed knee joint. Biochemical analysis of the inflammatory cells recovered from the removed fluid would most likely reveal a decrease in which of the following proteins?
   A. Interleukin 1
   B. Tumor necrosis factor-α
   C. Nuclear factor-κB
   D. IκBα
   E. von Willbrand factor

CHAPTER RESOURCES

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CHAPTER 4

Excitable Tissue: Nerve

OBJECTIVES

After studying this chapter, you should be able to:

- Name the various types of glia and their functions.
- Name the parts of a neuron and their functions.
- Describe the chemical nature of myelin, and summarize the differences in the ways in which unmyelinated and myelinated neurons conduct impulses.
- Describe orthograde and retrograde axonal transport.
- Describe the changes in ionic channels that underlie the action potential.
- List the various nerve fiber types found in the mammalian nervous system.
- Describe the function of neurotrophins.

INTRODUCTION

The human central nervous system (CNS) contains about 10^{11} (100 billion) **neurons.** It also contains 10–50 times this number of **glial cells.** The CNS is a complex organ; it has been calculated that 40% of the human genes participate, at least to a degree, in its formation. The neurons, the basic building blocks of the nervous system, have evolved from primitive neuroeffector cells that respond to various stimuli by contracting. In more complex animals, contraction has become the specialized function of muscle cells, whereas integration and transmission of nerve impulses have become the specialized functions of neurons. Neurons and glial cells along with brain capillaries form a functional unit that is required for normal brain function, including synaptic activity, extracellular fluid homeostasis, energy metabolism, and neural protection. Disturbances in the interaction of these elements are the pathophysiological basis for many neurological disorders (eg, cerebral ischemia, seizures, neurodegenerative diseases, and cerebral edema). This chapter describes the cellular components of the CNS and the excitability of neurons, which involves the genesis of electrical signals that enable neurons to integrate and transmit impulses (eg, action potentials, receptor potentials, and synaptic potentials).

CELLULAR ELEMENTS IN THE CNS

GLIAL CELLS

For many years following their discovery, glial cells (or glia) were viewed as CNS connective tissue. In fact, the word *glia* is Greek for *glue.* However, today theses cells are recognized for their role in communication within the CNS in partnership with neurons. Unlike neurons, glial cells continue to undergo cell division in adulthood and their ability to proliferate is particularly noticeable after brain injury (eg, stroke).

There are two major types of glial cells in the vertebrate nervous system: **microglia** and **macroglia.** Microglia are scavenger cells that resemble tissue macrophages and remove debris resulting from injury, infection, and disease (eg, multiple sclerosis, AIDS-related dementia, Parkinson disease, and Alzheimer disease). Microglia arise from macrophages outside of the nervous system and are physiologically and embryologically unrelated to other neural cell types.

There are three types of macroglia: oligodendrocytes, Schwann cells, and astrocytes (Figure 4–1). **Oligodendrocytes** and **Schwann cells** are involved in myelin formation around axons in the CNS and peripheral nervous system, respectively. **Astrocytes,** which are found throughout the brain, are of two subtypes. **Fibrous astrocytes,** which contain many intermediate filaments, are found primarily in white matter.

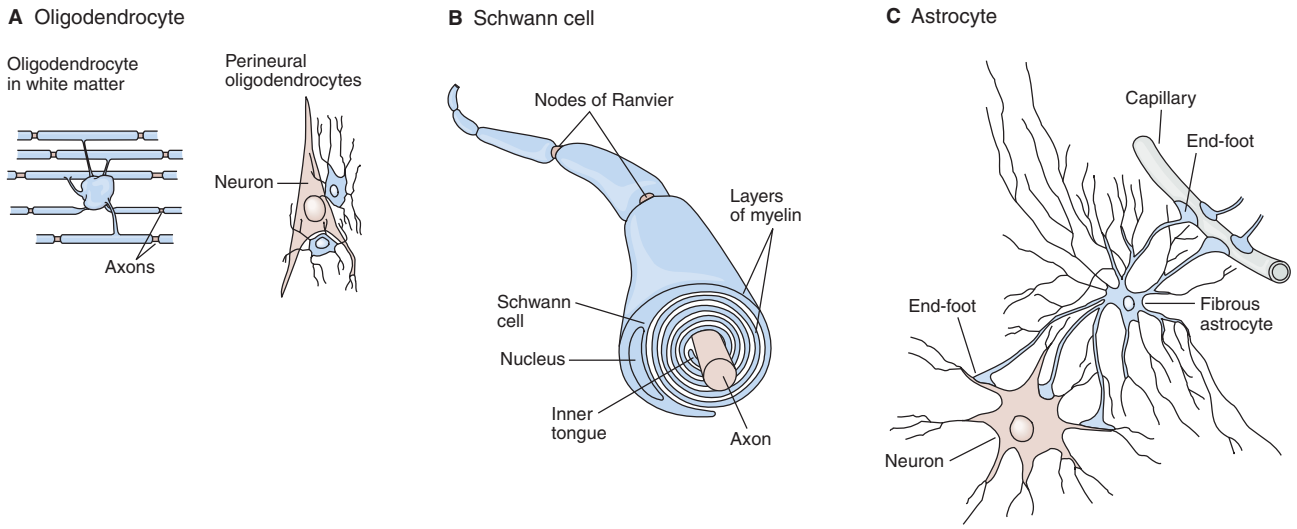

FIGURE 4–1 The principal types of macroglia in the nervous system. A) Oligodendrocytes are small with relatively few processes. Those in the white matter provide myelin, and those in the gray matter support neurons. **B)** Schwann cells provide myelin to the peripheral nervous system. Each cell forms a segment of myelin sheath about 1 mm long; the sheath assumes its form as the inner tongue of the Schwann cell turns around the axon several times, wrapping in concentric layers. Intervals between segments of myelin are the nodes of Ranvier. **C)** Astrocytes are the most common glia in the CNS and are characterized by their starlike shape. They contact both capillaries and neurons and are thought to have a nutritive function. They are also involved in forming the blood–brain barrier. (From Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science,* 4th ed. McGraw-Hill, 2000.)

Protoplasmic astrocytes are found in gray matter and have a granular cytoplasm. Both types send processes to blood vessels, where they induce capillaries to form the tight junctions making up the **blood–brain barrier.** They also send processes that envelop synapses and the surface of nerve cells. Protoplasmic astrocytes have a membrane potential that varies with the external K^+ concentration but do not generate propagated potentials. They produce substances that are tropic to neurons, and they help maintain the appropriate concentration of ions and neurotransmitters by taking up K^+ and the neurotransmitters glutamate and γ-aminobutyrate (GABA).

NEURONS

Neurons in the mammalian CNS come in many different shapes and sizes. Most have the same parts as the typical spinal motor neuron illustrated in Figure 4–2. The cell body **(soma)** contains the nucleus and is the metabolic center of the neuron. Neurons have several processes called **dendrites** that extend outward from the cell body and arborize extensively. Particularly in the cerebral and cerebellar cortex, the dendrites have small knobby projections called **dendritic spines.** A typical neuron also has a long fibrous **axon** that originates from a somewhat thickened area of the cell body, the **axon hillock.** The first portion

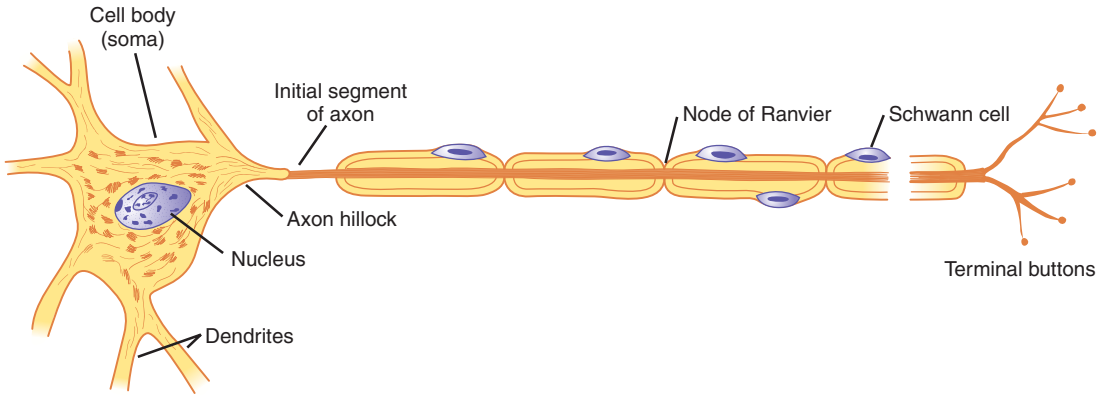

FIGURE 4–2 Motor neuron with a myelinated axon. A motor neuron is comprised of a cell body (soma) with a nucleus, several processes called dendrites, and a long fibrous axon that originates from the axon hillock. The first portion of the axon is called the initial segment. A myelin sheath forms from Schwann cells and surrounds the axon except at its ending and at the nodes of Ranvier. Terminal buttons (boutons) are located at the terminal endings.

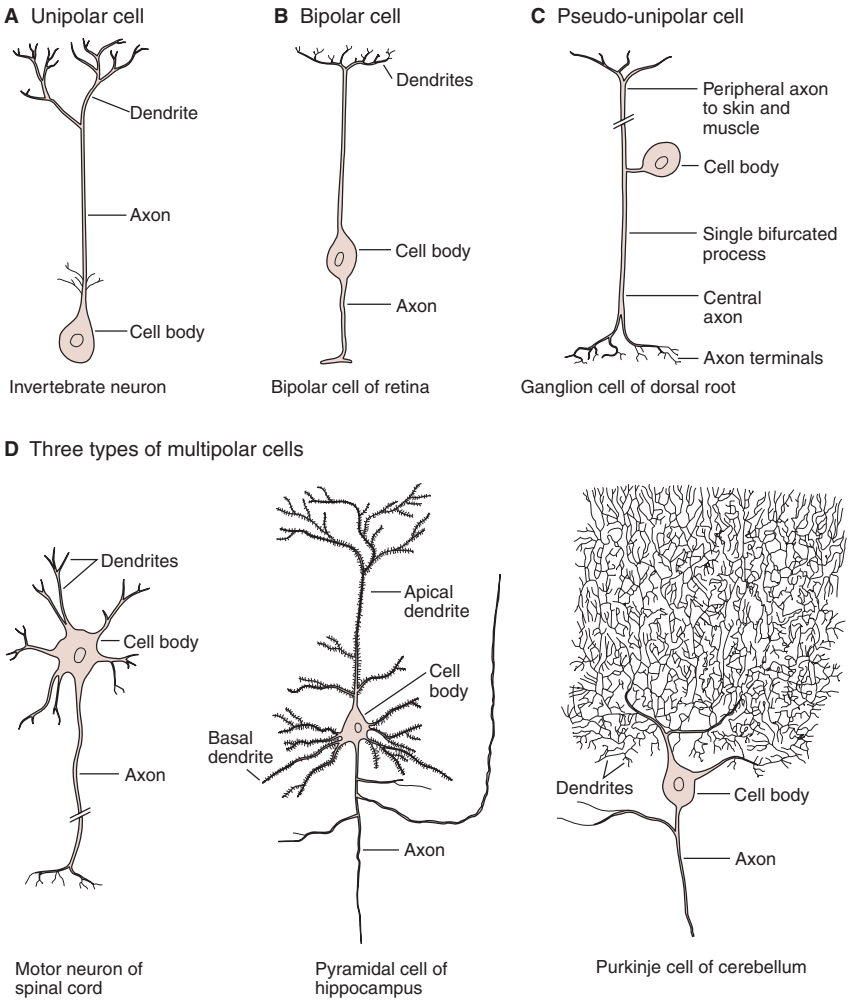

FIGURE 4–3 Some of the types of neurons in the mammalian nervous system. A) Unipolar neurons have one process, with different segments serving as receptive surfaces and releasing terminals. **B)** Bipolar neurons have two specialized processes: a dendrite that carries information to the cell and an axon that transmits information from the cell. **C)** Some sensory neurons are in a subclass of bipolar cells called pseudo-unipolar cells. As the cell develops, a single process splits into two, both of which function as axons—one going to skin or muscle and another to the spinal cord. **D)** Multipolar cells have one axon and many dendrites. Examples include motor neurons, hippocampal pyramidal cells with dendrites in the apex and base, and cerebellar Purkinje cells with an extensive dendritic tree in a single plane. (From Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science,* 4th ed. McGraw-Hill, 2000.)

of the axon is called the **initial segment.** The axon divides into **presynaptic terminals,** each ending in a number of **synaptic knobs** which are also called **terminal buttons** or **boutons.** They contain granules or vesicles in which the synaptic transmitters secreted by the nerves are stored. Based on the number of processes that emanate from their cell body, neurons can be classified as **unipolar, bipolar,** and **multipolar** (Figure 4–3).

The conventional terminology used for the parts of a neuron works well enough for spinal motor neurons and interneurons, but there are problems in terms of "dendrites" and "axons" when it is applied to other types of neurons found in the nervous system. From a functional point of view, neurons generally have four important zones: (1) a receptor, or dendritic zone, where multiple local potential changes generated by synaptic connections are integrated; (2) a site where propagated action potentials are generated (the initial segment in spinal motor neurons, the initial node of Ranvier in cutaneous sensory neurons); (3) an axonal process that transmits propagated impulses to the nerve endings; and (4) the nerve endings, where action potentials cause the release of synaptic transmitters. The cell body is often located at the dendritic zone end of the axon, but it can be within the axon (eg, auditory neurons) or attached to the side of the axon (eg, cutaneous neurons). Its location makes no difference as far as the receptor function of the dendritic zone and the transmission function of the axon are concerned.

The axons of many neurons are myelinated, that is, they acquire a sheath of **myelin,** a protein–lipid complex that is wrapped around the axon (Figure 4–1B). In the peripheral nervous system, myelin forms when a Schwann cell wraps its membrane around an axon up to 100 times. The myelin is then

CLINICAL BOX 4–1

Demyelinating Diseases

Normal conduction of action potentials relies on the insulating properties of **myelin.** Thus, defects in myelin can have major adverse neurological consequences. One example is **multiple sclerosis (MS),** an autoimmune disease that affects over 3 million people worldwide, usually striking between the ages of 20 and 50 and affecting women about twice as often as men. The cause of MS appears to include both genetic and environmental factors. It is most common among Caucasians living in countries with temperate climates including Europe, southern Canada, northern United States, and southeastern Australia. Environmental triggers include early exposure to viruses such as Epstein-Barr virus and those that cause measles, herpes, chicken pox, or influenza. In MS, antibodies and white blood cells in the immune system attack myelin, causing inflammation and injury to the sheath and eventually the nerves that it surrounds. Loss of myelin leads to leakage of K^+ through voltage-gated channels, hyperpolarization, and failure to conduct action potentials. Initial presentation commonly includes reports of **paraparesis** (weakness in lower extremities) that may be accompanied by mild spasticity and hyperreflexia; **paresthesia;** numbness; urinary incontinence; and heat intolerance. Clinical assessment often reports **optic neuritis,** characterized by blurred vision, a change in color perception, visual field defect (**central scotoma**), and pain with eye movements; **dysarthria;** and **dysphagia.** Symptoms are often exacerbated by increased body temperature or ambient temperature. Progression of the disease is quite variable. In the most common form called **relapsing-remitting MS,** transient episodes appear suddenly, last a few weeks or months, and then gradually disappear. Subsequent episodes can appear years later, and eventually full recovery does not occur. Many of these individuals later develop a steadily worsening course with only minor periods of remission (**secondary-progressive MS**). Others have a progressive form of the disease in which there are no periods of remission (**primary-progressive MS**). Diagnosing MS is very difficult and generally is delayed until multiple episodes occur with deficits separated in time and space. **Nerve conduction tests** can detect slowed conduction in motor and sensory pathways. Cerebral spinal fluid analysis can detect the presence of **oligoclonal** bands indicative of an abnormal immune reaction against myelin. The most definitive assessment is **magnetic resonance imaging (MRI)** to visualize multiple scarred (sclerotic) areas or plaques in the brain. These plaques often appear in the periventricular regions of the cerebral hemispheres.

THERAPEUTIC HIGHLIGHTS

Although there is no cure for MS, **corticosteroids** (eg, **prednisone**) are the most common treatment used to reduce the inflammation that is accentuated during a relapse. Some drug treatments are designed to modify the course of the disease. For example, daily injections of **β-interferons** suppress the immune response to reduce the severity and slow the progression of the disease. **Glatiramer acetate** may block the immune system's attack on the myelin. **Natalizumab** interferes with the ability of potentially damaging immune cells to move from the bloodstream to the CNS. A recent clinical trial using B cell–depleting therapy with **rituximab,** an anti-CD20 monoclonal antibody, showed that the progression of the disease was slowed in patients under the age of 51 who were diagnosed with the primary-progressive form of MS. Another recent clinical trial has shown that oral administration of **fingolimod** slowed the progression of the relapsing-remitting form of MS. This immunosuppressive drug acts by sequestering lymphocytes in the lymph nodes, thereby limiting their access to the CNS.

compacted when the extracellular portions of a membrane protein called protein zero (P_0) lock to the extracellular portions of P_0 in the apposing membrane. Various mutations in the gene for P_0 cause peripheral neuropathies; 29 different mutations have been described that cause symptoms ranging from mild to severe. The myelin sheath envelops the axon except at its ending and at the **nodes of Ranvier,** periodic 1-μm constrictions that are about 1 mm apart (Figure 4–2). The insulating function of myelin is discussed later in this chapter. Not all neurons are myelinated; some are **unmyelinated,** that is, simply surrounded by Schwann cells without the wrapping of the Schwann cell membrane that produces myelin around the axon.

In the CNS of mammals, most neurons are myelinated, but the cells that form the myelin are oligodendrocytes rather than Schwann cells (Figure 4–1). Unlike the Schwann cell, which forms the myelin between two nodes of Ranvier on a single neuron, oligodendrocytes emit multiple processes that form myelin on many neighboring axons. In multiple sclerosis, a crippling autoimmune disease, patchy destruction of myelin occurs in the CNS (see Clinical Box 4–1). The loss of myelin is associated with delayed or blocked conduction in the demyelinated axons.

AXONAL TRANSPORT

Neurons are secretory cells, but they differ from other secretory cells in that the secretory zone is generally at the end of the axon, far removed from the cell body. The apparatus for protein synthesis is located for the most part in the cell body, with transport of proteins and polypeptides to the axonal ending by

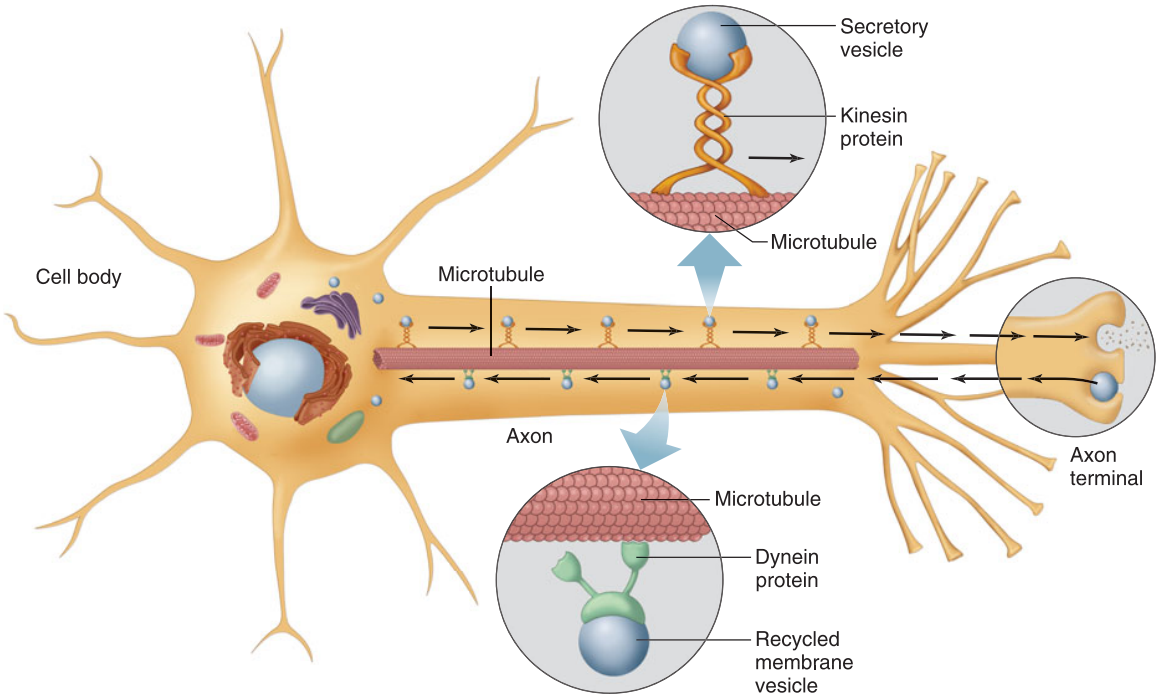

FIGURE 4–4 Axonal transport along microtubules by dynein and kinesin. Fast (400 mm/day) and slow (0.5–10 mm/day) axonal orthograde transport occurs along microtubules that run along the length of the axon from the cell body to the terminal. Retrograde transport (200 mm/day) occurs from the terminal to the cell body. (From Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology.* McGraw-Hill, 2008.)

axoplasmic flow. Thus, the cell body maintains the functional and anatomic integrity of the axon; if the axon is cut, the part distal to the cut degenerates **(wallerian degeneration).**

Orthograde transport occurs along microtubules that run along the length of the axon and requires two molecular motors, dynein and kinesin (Figure 4–4). Orthograde transport moves from the cell body toward the axon terminals. It has both fast and slow components; **fast axonal transport** occurs at about 400 mm/day, and **slow axonal transport** occurs at 0.5 to 10 mm/day. **Retrograde transport,** which is in the opposite direction (from the nerve ending to the cell body), occurs along microtubules at about 200 mm/day. Synaptic vesicles recycle in the membrane, but some used vesicles are carried back to the cell body and deposited in lysosomes. Some materials taken up at the ending by endocytosis, including **nerve growth factor (NGF)** and some viruses, are also transported back to the cell body. A potentially important exception to these principles seems to occur in some dendrites. In them, single strands of mRNA transported from the cell body make contact with appropriate ribosomes, and protein synthesis appears to create local protein domains.

EXCITATION & CONDUCTION

A hallmark of nerve cells is their excitable membrane. Nerve cells respond to electrical, chemical, or mechanical stimuli. Two types of physicochemical disturbances are produced: local, nonpropagated potentials called, depending on their location, **synaptic, generator,** or **electrotonic potentials;** and propagated potentials, the **action potentials** (or **nerve impulses**). Action potentials are the primary electrical responses of neurons and other excitable tissues, and they are the main form of communication within the nervous system. They are due to changes in the conduction of ions across the cell membrane. The electrical events in neurons are rapid, being measured in **milliseconds (ms);** and the potential changes are small, being measured in **millivolts (mV).**

The impulse is normally transmitted **(conducted)** along the axon to its termination. Nerves are not "telephone wires" that transmit impulses passively; conduction of nerve impulses, although rapid, is much slower than that of electricity. Nerve tissue is in fact a relatively poor passive conductor, and it would take a potential of many volts to produce a signal of a fraction of a volt at the other end of a meter-long axon in the absence of active processes in the nerve. Instead, conduction is an active, self-propagating process, and the impulse moves along the nerve at a constant amplitude and velocity. The process is often compared to what happens when a match is applied to one end of a trail of gunpowder; by igniting the powder particles immediately in front of it, the flame moves steadily down the trail to its end as it is extinguished in its wake.

RESTING MEMBRANE POTENTIAL

When two electrodes are connected through a suitable amplifier and placed on the surface of a single axon, no potential difference is observed. However, if one electrode is inserted

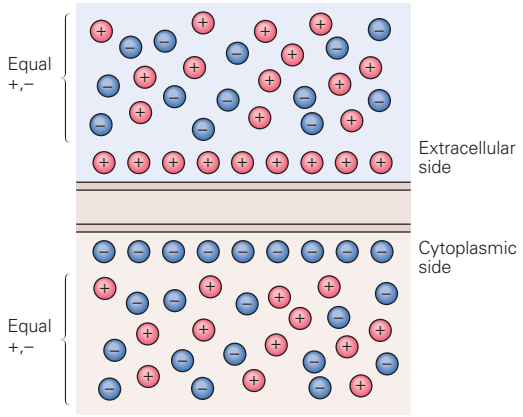

FIGURE 4–5 A membrane potential results from separation of positive and negative charges across the cell membrane. The excess of positive charges (red circles) outside the cell and negative charges (blue circles) inside the cell at rest represents a small fraction of the total number of ions present. (From Kandel ER, Schwartz JH, Jessell TM (editors): *Principles of Neural Science,* 4th ed. McGraw-Hill, 2000.).

into the interior of the cell, a constant **potential difference** is observed, with the inside negative relative to the outside of the cell at rest. A membrane potential results from separation of positive and negative charges across the cell membrane (Figure 4–5).

In order for a potential difference to be present across a membrane lipid bilayer, two conditions must be met. First, there must be an unequal distribution of ions of one or more species across the membrane (ie, a concentration gradient). Second, the membrane must be permeable to one or more of these ion species. The permeability is provided by the existence of channels or pores in the bilayer; these channels are usually permeable to a single species of ions. The resting membrane potential represents an equilibrium situation at which the driving force for the membrane-permeant ions down their concentration gradients across the membrane is equal and opposite to the driving force for these ions down their electrical gradients.

In neurons, the concentration of K^+ is much higher inside than outside the cell, while the reverse is the case for Na^+. This concentration difference is established by Na, K ATPase. The outward K^+ concentration gradient results in passive movement of K^+ out of the cell when K^+-selective channels are open. Similarly, the inward Na^+ concentration gradient results in passive movement of Na^+ into the cell when Na^+-selective channels are open.

In neurons, the **resting membrane potential** is usually about –70 mV, which is close to the equilibrium potential for K^+ (step 1 in Figure 4–6). Because there are more open K^+ channels than Na^+ channels at rest, the membrane permeability to K^+ is greater. Consequently, the intracellular and extracellular K^+ concentrations are the prime determinants of the resting membrane potential, which is therefore close to the equilibrium potential for K^+. Steady ion leaks cannot continue forever without eventually dissipating the ion gradients. This is prevented by the Na, K ATPase, which actively moves Na^+ and K^+ against their electrochemical gradients.

IONIC FLUXES DURING THE ACTION POTENTIAL

The cell membranes of nerves, like those of other cells, contain many different types of ion channels. Some of these are voltage-gated and others are ligand-gated. It is the behavior of these channels, and particularly Na^+ and K^+ channels, which explains the electrical events in neurons.

The changes in membrane conductance of Na^+ and K^+ that occur during the action potentials are shown by steps 1 through 7 in Figure 4–6. The conductance of an ion is the reciprocal of its electrical resistance in the membrane and is a measure of the membrane permeability to that ion. In response to a depolarizing stimulus, some of the voltage-gated Na^+ channels open and Na^+ enters the cell and the membrane is brought to its **threshold potential** (step 2) and the voltage-gated Na^+ channels overwhelm the K^+ and other channels. The entry of Na^+ causes the opening of more voltage-gated Na^+ channels and further depolarization, setting up a **positive feedback loop.** The rapid upstroke in the membrane potential ensues (step 3). The membrane potential moves toward the equilibrium potential for Na^+ (+60 mV) but does not reach it during the action potential (step 4), primarily because the increase in Na^+ conductance is short-lived. The Na^+ channels rapidly enter a closed state called the **inactivated state** and remain in this state for a few milliseconds before returning to the resting state, when they again can be activated. In addition, the direction of the electrical gradient for Na^+ is reversed during the **overshoot** because the membrane potential is reversed, and this limits Na^+ influx; also the voltage-gated K^+ channels open. These factors contribute to **repolarization.** The opening of voltage-gated K^+ channels is slower and more prolonged than the opening of the Na^+ channels, and consequently, much of the increase in K^+ conductance comes after the increase in Na^+ conductance (step 5). The net movement of positive charge out of the cell due to K^+ efflux at this time helps complete the process of repolarization. The slow return of the K^+ channels to the closed state also explains the **after-hyperpolarization** (step 6), followed by a return to the resting membrane potential (step 7). Thus, voltage-gated K^+ channels bring the action potential to an end and cause closure of their gates through a **negative feedback process.** Figure 4–7 shows the sequential feedback control in voltage-gated K^+ and Na^+ channels during the action potential.

Decreasing the external Na^+ concentration reduces the size of the action potential but has little effect on the resting membrane potential. The lack of much effect on the resting membrane potential would be predicted, since the permeability of the membrane to Na^+ at rest is relatively low. In contrast, since the resting membrane potential is close to the equilibrium potential for K^+, changes in the changes in the external concentration of this ion can have major effects on the resting membrane potential. If the extracellular level of K^+ is

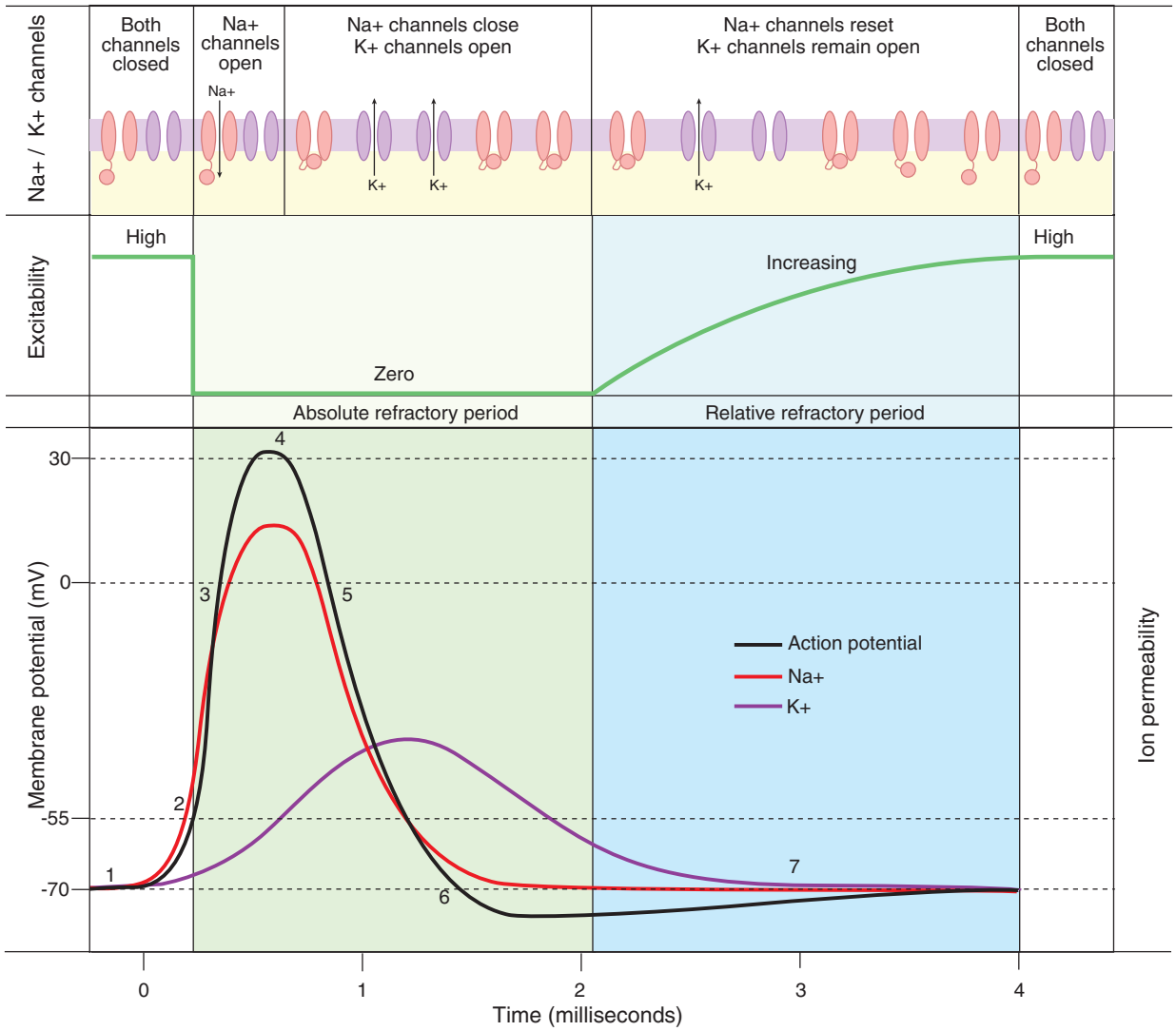

FIGURE 4–6 Changes in membrane potential and relative membrane permeability to Na^+ and K^+ during an action potential. Steps 1 through 7 are detailed in the text. These changes in threshold for activation (excitability) are correlated with the phases of the action potential. (Modified from Silverthorn DU: *Human Physiology: An Integrated Approach,* 5th ed. Pearson, 2010.)

increased **(hyperkalemia),** the resting potential moves closer to the threshold for eliciting an action potential, thus the neuron becomes more excitable. If the extracellular level of K^+ is decreased **(hypokalemia),** the membrane potential is reduced and the neuron is hyperpolarized.

Although Na^+ enters the nerve cell and K^+ leaves it during the action potential, very few ions actually move across the membrane. It has been estimated that only 1 in 100,000 K^+ ions cross the membrane to change the membrane potential from +30 mV (peak of the action potential) to –70 mV (resting potential). Significant differences in ion concentrations can be measured only after prolonged, repeated stimulation.

Other ions, notably Ca^{2+}, can affect the membrane potential through both channel movement and membrane interactions. A decrease in extracellular Ca^{2+} concentration increases the excitability of nerve and muscle cells by decreasing the amount of depolarization necessary to initiate the changes in the Na^+ and K^+ conductance that produce the action potential. Conversely, an increase in extracellular Ca^{2+} concentration can stabilize the membrane by decreasing excitability.

ALL-OR-NONE ACTION POTENTIALS

It is possible to determine the minimal intensity of stimulating current **(threshold intensity)** that, acting for a given duration, will just produce an action potential. The threshold intensity varies with the duration; with weak stimuli it is long, and with strong stimuli it is short. The relation between the strength and the duration of a threshold stimulus is called the **strength–duration curve.** Slowly rising currents fail to fire the nerve because the nerve adapts to the applied stimulus, a process called **adaptation.**

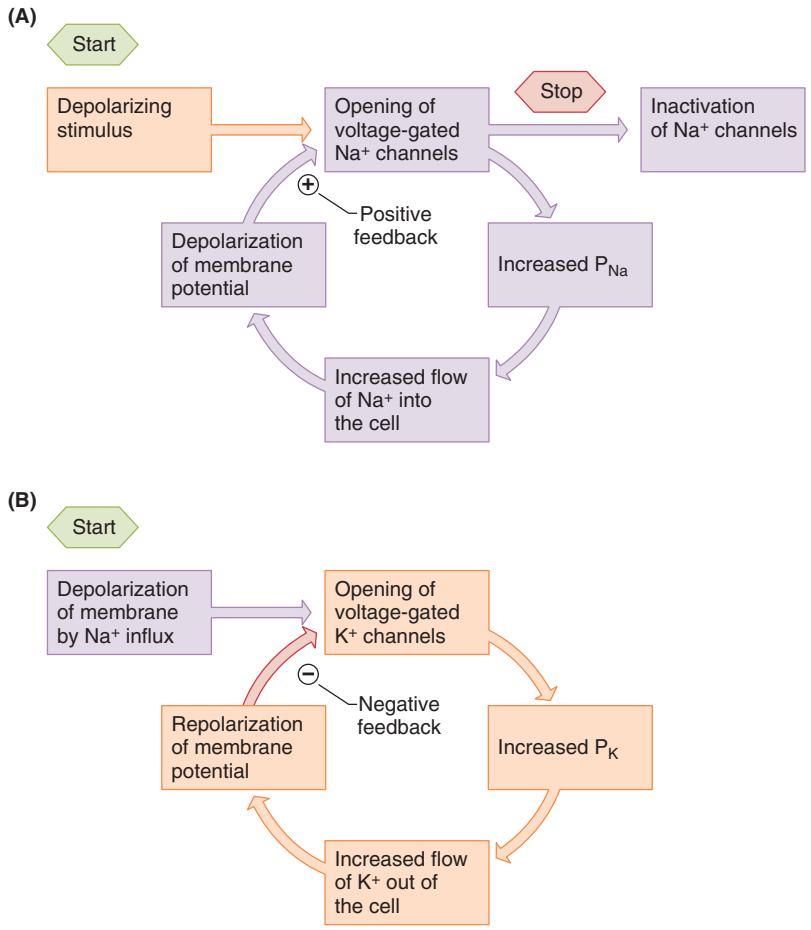

FIGURE 4–7 Feedback control in voltage-gated ion channels in the membrane. A) Na^+ channels exert positive feedback. **B)** K^+ channels exert negative feedback. P_{Na}, P_K is permeability to Na^+ and K^+, respectively. (From Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology.* McGraw-Hill, 2008.)

Once threshold intensity is reached, a full-fledged action potential is produced. Further increases in the intensity of a stimulus produce no increment or other change in the action potential as long as the other experimental conditions remain constant. The action potential fails to occur if the stimulus is subthreshold in magnitude, and it occurs with constant amplitude and form regardless of the strength of the stimulus if the stimulus is at or above threshold intensity. The action potential is therefore **all-or-none** in character.

ELECTROTONIC POTENTIALS, LOCAL RESPONSE, & FIRING LEVEL

Although subthreshold stimuli do not produce an action potential, they do have an effect on the membrane potential. This can be demonstrated by placing recording electrodes within a few millimeters of a stimulating electrode and applying subthreshold stimuli of fixed duration. Application of such currents leads to a localized depolarizing potential change that rises sharply and decays exponentially with time. The magnitude of this response drops off rapidly as the distance between the stimulating and recording electrodes is increased. Conversely, an anodal current produces a hyperpolarizing potential change of similar duration. These potential changes are called **electrotonic potentials.** As the strength of the current is increased, the response is greater due to the increasing addition of a **local response** of the membrane (Figure 4–8). Finally, at 7–15 mV of depolarization (potential of –55 mV), the **firing level** (threshold potential) is reached and an action potential occurs.

CHANGES IN EXCITABILITY DURING ELECTROTONIC POTENTIALS & THE ACTION POTENTIAL

During the action potential, as well as during electrotonic potentials and the local response, the threshold of the neuron to stimulation changes (Figure 4–6). Hyperpolarizing responses elevate the threshold, and depolarizing potentials

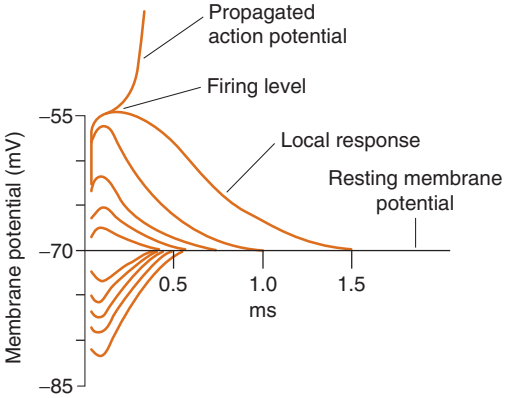

FIGURE 4–8 Electrotonic potentials and local response. The changes in the membrane potential of a neuron following application of stimuli of 0.2, 0.4, 0.6, 0.8, and 1.0 times threshold intensity are shown superimposed on the same time scale. The responses below the horizontal line are those recorded near the anode, and the responses above the line are those recorded near the cathode. The stimulus of threshold intensity was repeated twice. Once it caused a propagated action potential (top line), and once it did not.

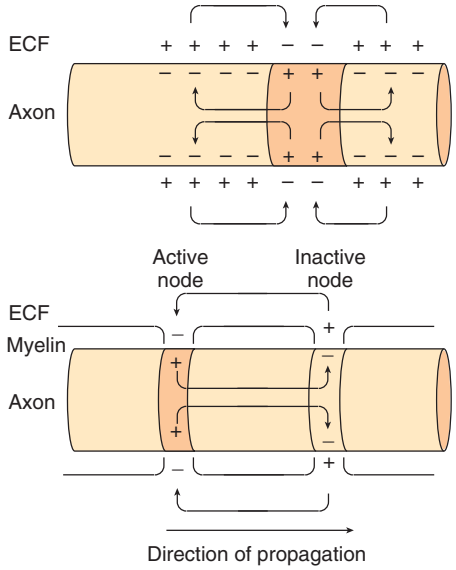

FIGURE 4–9 Local current flow (movement of positive charges) around an impulse in an axon. Top: Unmyelinated axon. **Bottom:** Myelinated axon. Positive charges from the membrane ahead of and behind the action potential flow into the area of negativity represented by the action potential ("current sink"). In myelinated axons, depolarization appears to "jump" from one node of Ranvier to the next (saltatory conduction).

lower it as they move the membrane potential closer to the firing level. During the local response, the threshold is lowered, but during the rising and much of the falling phases of the spike potential, the neuron is refractory to stimulation. This **refractory period** is divided into an **absolute refractory period,** corresponding to the period from the time the firing level is reached until repolarization is about one-third complete, and a **relative refractory period,** lasting from this point to the start of after-depolarization. During the absolute refractory period, no stimulus, no matter how strong, will excite the nerve, but during the relative refractory period, stronger than normal stimuli can cause excitation. These changes in threshold are correlated with the phases of the action potential in Figure 4–6.

CONDUCTION OF THE ACTION POTENTIAL

The nerve cell membrane is polarized at rest, with positive charges lined up along the outside of the membrane and negative charges along the inside. During the action potential, this polarity is abolished and for a brief period is actually reversed (Figure 4–9). Positive charges from the membrane ahead of and behind the action potential flow into the area of negativity represented by the action potential ("current sink"). By drawing off positive charges, this flow decreases the polarity of the membrane ahead of the action potential. Such electrotonic depolarization initiates a local response, and when the firing level is reached, a propagated response occurs that in turn electrotonically depolarizes the membrane in front of it.

The spatial distribution of ion channels along the axon plays a key role in the initiation and regulation of the action potential. Voltage-gated Na^+ channels are highly concentrated in the nodes of Ranvier and the initial segment in myelinated neurons. The number of Na^+ channels per square micrometer of membrane in myelinated mammalian neurons has been estimated to be 50–75 in the cell body, 350–500 in the initial segment, less than 25 on the surface of the myelin, 2000–12,000 at the nodes of Ranvier, and 20–75 at the axon terminals. Along the axons of unmyelinated neurons, the number is about 110. In many myelinated neurons, the Na^+ channels are flanked by K^+ channels that are involved in repolarization.

Conduction in myelinated axons depends on a similar pattern of circular current flow as described above. However, myelin is an effective insulator, and current flow through it is negligible. Instead, depolarization in myelinated axons travels from one node of Ranvier to the next, with the current sink at the active node serving to electrotonically depolarize the node ahead of the action potential to the firing level (Figure 4–9). This "jumping" of depolarization from node to node is called **saltatory conduction.** It is a rapid process that allows myelinated axons to conduct up to 50 times faster than the fastest unmyelinated fibers.

ORTHODROMIC & ANTIDROMIC CONDUCTION

An axon can conduct in either direction. When an action potential is initiated in the middle of the axon, two impulses traveling in opposite directions are set up by electrotonic

TABLE 4–1 Types of mammalian nerve fibers.

Fiber Type	Function	Fiber Diameter (μm)	Conduction Velocity (m/s)	Spike Duration (ms)	Absolute Refractory Period (ms)
Aα	Proprioception; somatic motor	12–20	70–120		
Aβ	Touch, pressure	5–12	30–70	0.4–0.5	0.4–1
Aγ	Motor to muscle spindles	3–6	15–30		
Aδ	Pain, temperature	2–5	12–30		
B	Preganglionic autonomic	<3	3–15	1.2	1.2
C, Dorsal root	Pain, temperature	0.4–1.2	0.5–2	2	2
C, Sympathetic	Postganglionic sympathetic	0.3–1.3	0.7–2.3	2	2

depolarization on either side of the initial current sink. In the natural situation, impulses pass in one direction only, ie, from synaptic junctions or receptors along axons to their termination. Such conduction is called **orthodromic.** Conduction in the opposite direction is called **antidromic.** Because synapses, unlike axons, permit conduction in one direction only, an antidromic impulse will fail to pass the first synapse they encounter and die out at that point.

PROPERTIES OF MIXED NERVES

Peripheral nerves in mammals are made up of many axons bound together in a fibrous envelope called the **epineurium.** Potential changes recorded extracellularly from such nerves therefore represent an algebraic summation of the all-or-none action potentials of many axons. The thresholds of the individual axons in the nerve and their distance from the stimulating electrodes vary. With subthreshold stimuli, none of the axons are stimulated and no response occurs. When the stimuli are of threshold intensity, axons with low thresholds fire and a small potential change is observed. As the intensity of the stimulating current is increased, the axons with higher thresholds are also discharged. The electrical response increases proportionately until the stimulus is strong enough to excite all of the axons in the nerve. The stimulus that produces excitation of all the axons is the **maximal stimulus,** and application of greater, supramaximal stimuli produces no further increase in the size of the observed potential.

After a stimulus is applied to a nerve, there is a **latent period** before the start of the action potential. This interval corresponds to the time it takes the impulse to travel along the axon from the site of stimulation to the recording electrodes. Its duration is proportionate to the distance between the stimulating and recording electrodes and inversely proportionate to the speed of conduction. If the duration of the latent period and the distance between the stimulating and recording electrodes are known, **axonal conduction velocity** can be calculated.

NERVE FIBER TYPES & FUNCTION

Erlanger and Gasser divided mammalian nerve fibers into A, B, and C groups, further subdividing the A group into α, β, γ, and δ fibers. In Table 4–1, the various fiber types are listed with their diameters, electrical characteristics, and functions. By comparing the neurologic deficits produced by careful dorsal root section and other nerve-cutting experiments with the histologic changes in the nerves, the functions and histologic characteristics of each of the families of axons responsible for the various peaks of the compound action potential have been established. In general, the greater the diameter of a given nerve fiber, the greater is its speed of conduction. The large axons are concerned primarily with proprioceptive sensation, somatic motor function, conscious touch, and pressure, while the smaller axons subserve pain and temperature sensations and autonomic function.

Further research has shown that not all the classically described lettered components are homogeneous, and a numerical system (Ia, Ib, II, III, and IV) has been used by some physiologists to classify sensory fibers. Unfortunately, this has led to confusion. A comparison of the number system and the letter system is shown in Table 4–2.

TABLE 4–2 Numerical classification of sensory nerve fibers.

Number	Origin	Fiber Type
Ia	Muscle spindle, annulo-spiral ending	Aα
Ib	Golgi tendon organ	Aα
II	Muscle spindle, flower-spray ending; touch, pressure	Aβ
III	Pain and cold receptors; some touch receptors	Aδ
IV	Pain, temperature, and other receptors	Dorsal root C

TABLE 4–3 Relative susceptibility of mammalian A, B, and C nerve fibers to conduction block produced by various agents.

Susceptibility To:	Most Susceptible	Intermediate	Least Susceptible
Hypoxia	B	A	C
Pressure	A	B	C
Local anesthetics	C	B	A

TABLE 4–4 Neurotrophins.

Neurotrophin	Receptor
Nerve growth factor (NGF)	Trk A
Brain-derived neurotrophic factor (BDNF)	Trk B
Neurotrophin 3 (NT-3)	Trk C, less on Trk A and Trk B
Neurotrophin 4/5 (NT-4/5)	Trk B

In addition to variations in speed of conduction and fiber diameter, the various classes of fibers in peripheral nerves differ in their sensitivity to hypoxia and anesthetics (Table 4–3). This fact has clinical as well as physiologic significance. Local anesthetics depress transmission in the group C fibers before they affect group A touch fibers (see Clinical Box 4–2). Conversely, pressure on a nerve can cause loss of conduction in large-diameter motor, touch, and pressure fibers while pain sensation remains relatively intact. Patterns of this type are sometimes seen in individuals who sleep with their arms under their heads for long periods, causing compression of the nerves in the arms. Because of the association of deep sleep with alcoholic intoxication, the syndrome is most common on weekends and has acquired the interesting name Saturday night or Sunday morning paralysis.

NEUROTROPHINS

A number of proteins necessary for survival and growth of neurons have been isolated and studied. Some of these **neurotrophins** are products of the muscles or other structures that the neurons innervate, but many in the CNS are produced by astrocytes. These proteins bind to receptors at the endings of a neuron. They are internalized and then transported by retrograde transport to the neuronal cell body, where they foster the production of proteins associated with neuronal development, growth, and survival. Other neurotrophins are produced in neurons and transported in an anterograde fashion to the nerve ending, where they maintain the integrity of the postsynaptic neuron.

RECEPTORS

Four established neurotrophins and their three high-affinity **tyrosine kinase associated (Trk) receptors** are listed in Table 4–4. Each of these Trk receptors dimerizes, and this initiates autophosphorylation in the cytoplasmic tyrosine kinase domains of the receptors. An additional low-affinity NGF receptor that is a 75-kDa protein is called **$p75^{NTR}$.** This receptor binds all four of the listed neurotrophins with equal affinity. There is some evidence that it can form a heterodimer with Trk A monomer and that the dimer has increased affinity and specificity

CLINICAL BOX 4–2

Local Anesthesia

Local or regional anesthesia is used to block the conduction of action potentials in sensory and motor nerve fibers. This usually occurs as a result of blockade of voltage-gated Na^+ channels on the nerve cell membrane. This causes a gradual increase in the threshold for electrical excitability of the nerve, a reduction in the rate of rise of the action potential, and a slowing of axonal conduction velocity. There are two major categories of local anesthetics: **ester-linked** (eg, **cocaine, procaine, tetracaine**) or **amide-linked** (eg, **lidocaine, bupivacaine**). In addition to either the ester or amide, all local anesthetics contain an aromatic and an amine group. The structure of the aromatic group determines the drug's hydrophobic characteristics, and the amine group determines its latency to onset of action and its potency. Application of these drugs into the vicinity of a central **(**eg, **epidural, spinal anesthesia)** or peripheral nerve can lead to rapid, temporary, and near complete interruption of neural traffic to allow a surgical or other potentially noxious procedure to be done without eliciting pain. Cocaine (from the coca shrub, *Erythroxylan coca*) was the first chemical to be identified as having local anesthetic properties and remains the only naturally occurring local anesthetic. In 1860, Albert Niemann isolated the chemical, tasted it, and reported a numbing effect on his tongue. The first clinical use of cocaine as a local anesthetic was in 1886 when Carl Koller used it as a topical ophthalmic anesthetic. Its addictive and toxic properties prompted the development of other local anesthetics. In 1905, procaine was synthesized as the first suitable substitute for cocaine. Nociceptive fibers (unmyelinated C fibers) are the most sensitive to the blocking effect of local anesthetics. This is followed by sequential loss of sensitivity to temperature, touch, and deep pressure. Motor nerve fibers are the most resistant to the actions of local anesthetics.

CLINICAL BOX 4–3

Axonal Regeneration

Peripheral nerve damage is often reversible. Although the axon will degenerate distal to the damage, connective elements of the so-called **distal stump** often survive. **Axonal sprouting** occurs from the proximal stump, growing toward the nerve ending. This results from **growth-promoting factors** secreted by **Schwann cells** that attract axons toward the distal stump. Adhesion molecules of the immunoglobulin superfamily (eg, NgCAM/L1) promote axon growth along cell membranes and extracellular matrices. Inhibitory molecules in the perineurium assure that the regenerating axons grow in a correct trajectory. Denervated distal stumps are able to upregulate production of **neurotrophins** that promote growth. Once the regenerated axon reaches its target, a new functional connection (eg, neuromuscular junction) is formed. Regeneration allows for considerable, although not full, recovery. For example, fine motor control may be permanently impaired because some motor neurons are guided to an inappropriate motor fiber. Nonetheless, recovery of peripheral nerves from damage far surpasses that of central nerve pathways. The proximal stump of a damaged axon in the CNS will form short sprouts, but distant stump recovery is rare, and the damaged axons are unlikely to form new synapses. This is in part because CNS neurons do not have the growth-promoting chemicals needed for regeneration. In fact, CNS myelin is a potent inhibitor of axonal growth. In addition, following CNS injury several events—**astrocytic proliferation, activation of microglia, scar formation, inflammation,** and **invasion of immune cells**—provide an inappropriate environment for regeneration. Thus, treatment of brain and spinal cord injuries frequently focuses on rehabilitation rather than reversing the nerve damage. New research is aiming to identify ways to initiate and maintain axonal growth, to direct regenerating axons to reconnect with their target neurons, and to reconstitute original neuronal circuitry.

THERAPEUTIC HIGHLIGHTS

There is evidence showing that the use of **nonsteroidal anti-inflammatory drugs** (NSAIDs) like ibuprofen can overcome the factors that inhibit axonal growth following injury. This effect is thought to be mediated by the ability of NSAIDs to inhibit RhoA, a small GTPase protein that normally prevents repair of neural pathways and axons. Growth cone collapse in response to myelin-associated inhibitors after nerve injury is prevented by drugs such as **pertussis toxin,** which interfere with signal transduction via trimeric G protein. Experimental drugs that inhibit the **phosphoinositide 3-kinase (PI3) pathway** or the **inositol triphosphate (IP_3) receptor** have also been shown to promote regeneration after nerve injury.

for NGF. However, it now appears that $p75^{NTR}$ receptors can form homodimers that in the absence of Trk receptors cause apoptosis, an effect opposite to the usual growth-promoting and nurturing effects of neurotrophins. Research is ongoing to characterize the distinct roles of $p75^{NTR}$ and Trk receptors and factors that influence their expression in neurons.

FUNCTION OF NEUROTROPHINS

The first neurotrophin to be characterized was NGF, a protein growth factor that is necessary for the growth and maintenance of sympathetic neurons and some sensory neurons. It is present in a broad spectrum of animal species, including humans, and is found in many different tissues. In male mice, there is a particularly high concentration in the submandibular salivary glands, and the level is reduced by castration to that seen in females. The factor is made up of two α, two β, and two γ subunits. The β subunits, each of which has a molecular mass of 13,200 Da, have all the nerve growth-promoting activity, the α subunits have trypsin-like activity, and the γ subunits are serine proteases. The function of the proteases is unknown. The structure of the β subunit of NGF resembles that of insulin.

NGF is picked up by neurons and is transported in retrograde fashion from the endings of the neurons to their cell bodies. It is also present in the brain and appears to be responsible for the growth and maintenance of cholinergic neurons in the basal forebrain and the striatum. Injection of antiserum against NGF in newborn animals leads to almost total destruction of the sympathetic ganglia; it thus produces an **immunosympathectomy.** There is evidence that the maintenance of neurons by NGF is due to a reduction in apoptosis.

Brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), NT-4/5, and NGF each maintain a different pattern of neurons, although there is some overlap. Disruption of NT-3 by gene knockout causes a marked loss of cutaneous mechanoreceptors, even in heterozygotes. BDNF acts rapidly and can actually depolarize neurons. BDNF-deficient mice lose peripheral sensory neurons and have severe degenerative changes in their vestibular ganglia and blunted long-term potentiation.

OTHER FACTORS AFFECTING NEURONAL GROWTH

The regulation of neuronal growth is a complex process. Schwann cells and astrocytes produce **ciliary neurotrophic factor (CNTF).** This factor promotes the survival of damaged and embryonic spinal cord neurons and may prove to be of value in treating human diseases in which motor neurons degenerate. **Glial cell line-derived neurotrophic factor (GDNF)**

maintains midbrain dopaminergic neurons in vitro. However, GDNF knockouts have dopaminergic neurons that appear normal, but they have no kidneys and fail to develop an enteric nervous system. Another factor that enhances the growth of neurons is **leukemia inhibitory factor (LIF).** In addition, neurons as well as other cells respond to **insulin-like growth factor I (IGF-I)** and the various forms of **transforming growth factor (TGF), fibroblast growth factor (FGF),** and **platelet-derived growth factor (PDGF).**

Clinical Box 4–3 compares the ability to regenerate neurons after central and peripheral nerve injury.

CHAPTER SUMMARY

- There are two main types of glia: microglia and macroglia. Microglia are scavenger cells. Macroglia include oligodendrocytes, Schwann cells, and astrocytes. The first two are involved in myelin formation; astrocytes produce substances that are tropic to neurons, and they help maintain the appropriate concentration of ions and neurotransmitters.
- Neurons are composed of a cell body (soma) that is the metabolic center of the neuron, dendrites that extend outward from the cell body and arborize extensively, and a long fibrous axon that originates from a somewhat thickened area of the cell body, the axon hillock.
- The axons of many neurons acquire a sheath of myelin, a protein–lipid complex that is wrapped around the axon. Myelin is an effective insulator, and depolarization in myelinated axons travels from one node of Ranvier to the next, with the current sink at the active node serving to electrotonically depolarize to the firing level the node ahead of the action potential.
- Orthograde transport occurs along microtubules that run the length of the axon and requires two molecular motors: dynein and kinesin. It moves from the cell body toward the axon terminals and has both fast (400 mm/day) and slow (0.5–10 mm/day) components. Retrograde transport, which is in the opposite direction (from the nerve ending to the cell body), occurs along microtubules at about 200 mm/day.
- In response to a depolarizing stimulus, voltage-gated Na^+ channels become active, and when the threshold potential is reached, an action potential results. The membrane potential moves toward the equilibrium potential for Na^+. The Na^+ channels rapidly enter a closed state (inactivated state) before returning to the resting state. The direction of the electrical gradient for Na^+ is reversed during the overshoot because the membrane potential is reversed, and this limits Na^+ influx. Voltage-gated K^+ channels open and the net movement of positive charge out of the cell helps complete the process of repolarization. The slow return of the K^+ channels to the closed state explains after-hyperpolarization, followed by a return to the resting membrane potential.
- Nerve fibers are divided into different categories (A, B, and C) based on axonal diameter, conduction velocity, and function. A numerical classification (Ia, Ib, II, III, and IV) is also used for sensory afferent fibers.
- Neurotrophins such as NGF are carried by retrograde transport to the neuronal cell body, where they foster the production of proteins associated with neuronal development, growth, and survival.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following statements about glia is true?
   A. Microglia arise from macrophages outside of the nervous system and are physiologically and embryologically similar to other neural cell types.
   B. Glia do not undergo proliferation.
   C. Protoplasmic astrocytes produce substances that are tropic to neurons to help maintain the appropriate concentration of ions and neurotransmitters by taking up K^+ and the neurotransmitters glutamate and GABA.
   D. Oligodendrocytes and Schwann cells are involved in myelin formation around axons in the peripheral and central nervous systems, respectively.
   E. Macroglia are scavenger cells that resemble tissue macrophages and remove debris resulting from injury, infection, and disease.
2. A 13-year-old girl was being seen by her physician because of experiencing frequent episodes of red, painful, warm extremities. She was diagnosed with primary erythromelalgia, which may be due to a peripheral nerve sodium channelopathy. Which part of a neuron has the highest concentration of Na^+ channels per square micrometer of cell membrane?
   A. dendrites
   B. cell body near dendrites
   C. initial segment
   D. axonal membrane under myelin
   E. none of Ranvier
3. A 45-year-old female office worker had been experiencing tingling in her index and middle fingers and thumb of her right hand. Recently, her wrist and hand had become weak. Her physician ordered a nerve conduction test to evaluate her for carpal tunnel syndrome. Which one of the following nerves has the slowest conduction velocity?
   A. Aα fibers
   B. Aβ fibers
   C. Aγ fibers
   D. B fibers
   E. C fibers
4. Which of the following is *not* correctly paired?
   A. Synaptic transmission: Antidromic conduction
   B. Molecular motors: Dynein and kinesin
   C. Fast axonal transport: ~400 mm/day
   D. Slow axonal transport: 0.5–10 mm/day
   E. Nerve growth factor: Retrograde transport
5. A 32-year-old female received an injection of a local anesthetic for a tooth extraction. Within 2 h, she noted palpitations, diaphoresis, and dizziness. Which of the following ionic changes is correctly matched with a component of the action potential?
   A. Opening of voltage-gated K^+ channels: After-hyperpolarization
   B. A decrease in extracellular Ca^{2+}: Repolarization
   C. Opening of voltage-gated Na^+ channels: Depolarization
   D. Rapid closure of voltage-gated Na^+ channels: Resting membrane potential
   E. Rapid closure of voltage-gated K^+ channels: Relative refractory period

6. A man falls into a deep sleep with one arm under his head. This arm is paralyzed when he awakens, but it tingles, and pain sensation in it is still intact. The reason for the loss of motor function without loss of pain sensation is that in the nerves to his arm,
   A. A fibers are more susceptible to hypoxia than B fibers.
   B. A fibers are more sensitive to pressure than C fibers.
   C. C fibers are more sensitive to pressure than A fibers.
   D. Motor nerves are more affected by sleep than sensory nerves.
   E. Sensory nerves are nearer the bone than motor nerves and hence are less affected by pressure.
7. Which of the following statements about nerve growth factor is *not* true?
   A. It is made up of three polypeptide subunits.
   B. It is responsible for the growth and maintenance of adrenergic neurons in the basal forebrain and the striatum.
   C. It is necessary for the growth and development of the sympathetic nervous system.
   D. It is picked up by nerves from the organs they innervate.
   E. It can express both $p75^{NTR}$ and Trk A receptors.
8. A 20-year old female student awakens one morning with severe pain and blurry vision in her left eye; the symptoms abate over several days. About 6 months later, on a morning after playing volleyball with friends, she notices weakness but not pain in her right leg; the symptoms intensify while taking a hot shower. Which of the following is most likely to be the case?
   A. The two episodes described are not likely to be related.
   B. She may have primary-progressive multiple sclerosis.
   C. She may have relapsing-remitting multiple sclerosis.
   D. She may have a lumbar disk rupture.
   E. She may have Guillain–Barre syndrome.

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