

CHAPTER

1

Homeostasis and Cellular Signaling

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CHAPTER OUTLINE

- THE BASIS OF PHYSIOLOGICAL REGULATION
- MODES OF COMMUNICATION AND SIGNALING
- THE MOLECULAR BASIS OF CELLULAR SIGNALING
- SIGNAL TRANSDUCTION BY PLASMA MEMBRANE RECEPTORS
- SECOND MESSENGER SYSTEMS AND INTRACELLULAR SIGNALING PATHWAYS
- INTRACELLULAR RECEPTORS AND HORMONE SIGNALING

KEY CONCEPTS

1. Physiology is the study of the functions of living organisms and how they are regulated and integrated.
2. A stable internal environment is necessary for normal cell function and survival of the organism.
3. Homeostasis is the maintenance of steady states in the body by coordinated physiological mechanisms.
4. Negative and positive feedback are used to modulate the body's responses to changes in the environment.
5. Steady state and equilibrium are distinct conditions. Steady state is a condition that does not change over time, while equilibrium represents a balance between opposing forces.
6. Cellular communication is essential to integrate and coordinate the systems of the body so they can participate in different functions.
7. Different modes of cell communication differ in terms of distance and speed.
8. Chemical signaling molecules (first messengers) provide the major means of intercellular communication; they include ions, gases, small peptides, protein hormones, metabolites, and steroids.
9. Receptors are the receivers and transmitters of signaling molecules; they are located either on the plasma membrane or within the cell.
10. Second messengers are important for amplification of the signal received by plasma membrane receptors.
11. Steroid and thyroid hormone receptors are intracellular receptors that participate in the regulation of gene expression.

Physiology is the study of processes and functions in living organisms. It is a broad field that encompasses many disciplines and has strong roots in physics, chemistry, and mathematics. Physiologists assume that the same chemical and physical laws that apply to the inanimate world govern processes in the body. They attempt to describe functions in chemical, physical, or engineering terms. For example, the

distribution of ions across cell membranes is described in thermodynamic terms, muscle contraction is analyzed in terms of forces and velocities, and regulation in the body is described in terms of control systems theory. Because the functions of living systems are carried out by their constituent structures, knowledge of structure from gross anatomy to the molecular level is germane to an understanding of physiology.

The scope of physiology ranges from the activities or functions of individual molecules and cells to the interaction of our bodies with the external world. In recent years, we have seen many advances in our understanding of physiological processes at the molecular and cellular levels. In higher organisms, changes in cell function always occur in the context of a whole organism, and different tissues and organs obviously affect one another. The independent activity of an organism requires the coordination of function at all levels, from molecular and cellular to the organism as a whole. An important part of physiology is understanding how different parts of the body are controlled, how they interact, and how they adapt to changing conditions.

For a person to remain healthy, physiological conditions in the body must be kept at optimal levels and closely regulated. Regulation requires effective communication between cells and tissues. This chapter discusses several topics related to regulation and communication: the internal environment, homeostasis of extracellular fluid, intracellular homeostasis, negative and positive feedback, feedforward control, compartments, steady state and equilibrium, intercellular and intracellular communication, nervous and endocrine systems control, cell membrane transduction, and important signal transduction cascades.

THE BASIS OF PHYSIOLOGICAL REGULATION

Our bodies are made up of incredibly complex and delicate materials, and we are constantly subjected to all kinds of disturbances, yet we keep going for a lifetime. It is clear that conditions and processes in the body must be closely controlled and regulated, i.e., kept at appropriate values. Below we consider, in broad terms, physiological regulation in the body.

A Stable Internal Environment Is Essential for Normal Cell Function

The nineteenth-century French physiologist Claude Bernard was the first to formulate the concept of the internal environment (*milieu intérieur*). He pointed out that an external environment surrounds multicellular organisms (air or water), but the cells live in a liquid internal environment (extracellular fluid). Most body cells are not directly exposed to the external world but, rather, interact with it through the internal environment, which is continuously renewed by the circulating blood (Fig. 1.1).

For optimal cell, tissue, and organ function in animals, several conditions in the internal environment must be maintained within narrow limits. These include but are not limited to (1) oxygen and carbon dioxide tensions, (2) concentrations of glucose and other metabolites, (3) osmotic pressure, (4) concentrations of hydrogen, potassium, calcium, and magnesium ions, and (5) temperature. Departures from optimal conditions may result in disordered functions, disease, or death.

Bernard stated that “stability of the internal environment is the primary condition for a free and independent existence.” He recognized that an animal’s independence from changing external conditions is related to its capacity to

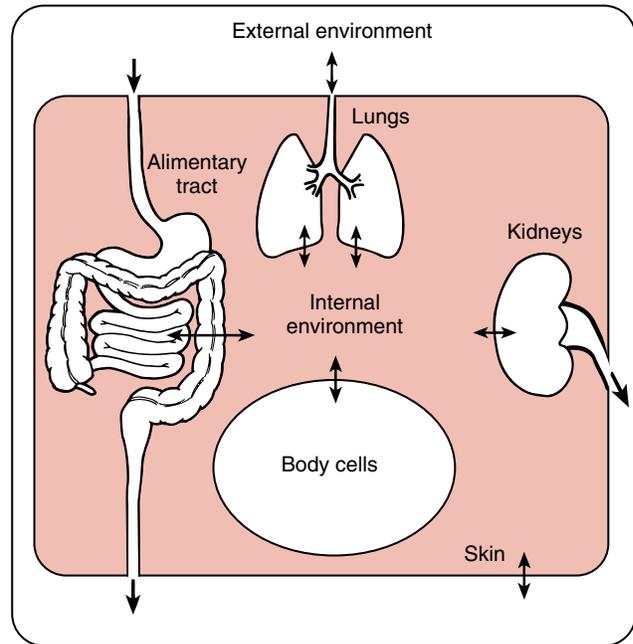


FIGURE 1.1 The living cells of our body, surrounded by an internal environment (extracellular fluid), communicate with the external world through this medium. Exchanges of matter and energy between the body and the external environment (indicated by arrows) occur via the gastrointestinal tract, kidneys, lungs, and skin (including the specialized sensory organs).

maintain a relatively constant internal environment. A good example is the ability of warm-blooded animals to live in different climates. Over a wide range of external temperatures, core temperature in mammals is maintained constant by both physiological and behavioral mechanisms. This stability has a clear survival value.

Homeostasis Is the Maintenance of Steady States in the Body by Coordinated Physiological Mechanisms

The key to maintaining stability of the internal environment is the presence of regulatory mechanisms in the body. In the first half of the twentieth century, the American physiologist Walter B. Cannon introduced a concept describing this capacity for self-regulation: **homeostasis**, the maintenance of steady states in the body by coordinated physiological mechanisms.

The concept of homeostasis is helpful in understanding and analyzing conditions in the body. The existence of steady conditions is evidence of regulatory mechanisms in the body that maintain stability. To function optimally under a variety of conditions, the body must sense departures from normal and must engage mechanisms for restoring conditions to normal. Departures from normal may be in the direction of too little or too much, so mechanisms exist for opposing changes in either direction. For example, if blood glucose concentration is too low, the hormone glucagon, from alpha cells of the pancreas, and epinephrine, from the adrenal medulla, will increase it. If blood glucose concentra-

tion is too high, insulin from the beta cells of the pancreas will lower it by enhancing the cellular uptake, storage, and metabolism of glucose. Behavioral responses also contribute to the maintenance of homeostasis. For example, a low blood glucose concentration stimulates feeding centers in the brain, driving the animal to seek food.

Homeostatic regulation of a physiological variable often involves several cooperating mechanisms activated at the same time or in succession. The more important a variable, the more numerous and complicated are the mechanisms that keep it at the desired value. Disease or death is often the result of dysfunction of homeostatic mechanisms.

The effectiveness of homeostatic mechanisms varies over a person's lifetime. Some homeostatic mechanisms are not fully developed at the time of birth. For example, a newborn infant cannot concentrate urine as well as an adult and is, therefore, less able to tolerate water deprivation. Homeostatic mechanisms gradually become less efficient as people age. For example, older adults are less able to tolerate stresses, such as exercise or changing weather, than are younger adults.

Intracellular Homeostasis Is Essential for Normal Cell Function

The term *homeostasis* has traditionally been applied to the internal environment—the extracellular fluid that bathes our tissues—but it can also be applied to conditions within cells. In fact, the ultimate goal of maintaining a constant internal environment is to promote intracellular homeostasis, and toward this end, conditions in the cytosol are closely regulated.

The many biochemical reactions within a cell must be tightly regulated to provide metabolic energy and proper rates of synthesis and breakdown of cellular constituents. Metabolic reactions within cells are catalyzed by enzymes and are therefore subject to several factors that regulate or influence enzyme activity.

- First, the final product of the reactions may inhibit the catalytic activity of enzymes, **end-product inhibition**. End-product inhibition is an example of negative-feedback control (see below).
- Second, intracellular regulatory proteins, such as the calcium-binding protein calmodulin, may control enzyme activity.
- Third, enzymes may be controlled by covalent modification, such as phosphorylation or dephosphorylation.
- Fourth, the ionic environment within cells, including hydrogen ion concentration ($[H^+]$), ionic strength, and calcium ion concentration, influences the structure and activity of enzymes.

Hydrogen ion concentration or pH affects the electrical charge of protein molecules and, hence, their configuration and binding properties. pH affects chemical reactions in cells and the organization of structural proteins. Cells regulate their pH via mechanisms for buffering intracellular hydrogen ions and by extruding H^+ into the extracellular fluid (see Chapter 25).

The structure and activity of cellular proteins are also affected by ionic strength. Cytosolic ionic strength depends on the total number and charge of ions per unit volume of

water within cells. Cells can regulate their ionic strength by maintaining the proper mixture of ions and un-ionized molecules (e.g., organic osmolytes, such as sorbitol).

Many cells use calcium as an intracellular signal or “messenger” for enzyme activation, and, therefore, must possess mechanisms for regulating cytosolic $[Ca^{2+}]$. Such fundamental activities as muscle contraction, the secretion of neurotransmitters, hormones, and digestive enzymes, and the opening or closing of ion channels are mediated via transient changes in cytosolic $[Ca^{2+}]$. Cytosolic $[Ca^{2+}]$ in resting cells is low, about 10^{-7} M, and far below extracellular fluid $[Ca^{2+}]$ (about 2.5 mM). Cytosolic $[Ca^{2+}]$ is regulated by the binding of calcium to intracellular proteins, transport is regulated by adenosine triphosphate (ATP)-dependent calcium pumps in mitochondria and other organelles (e.g., sarcoplasmic reticulum in muscle), and the extrusion of calcium is regulated via cell membrane Na^+/Ca^{2+} exchangers and calcium pumps. Toxins or diminished ATP production can lead to an abnormally elevated cytosolic $[Ca^{2+}]$. A high cytosolic $[Ca^{2+}]$ activates many enzyme pathways, some of which have detrimental effects and may cause cell death.

Negative Feedback Promotes Stability; Feedforward Control Anticipates Change

Engineers have long recognized that stable conditions can be achieved by negative-feedback control systems (Fig. 1.2). Feedback is a flow of information along a closed loop. The components of a simple negative-feedback control system include a regulated variable, sensor (or detector), controller (or comparator), and effector. Each component controls the next component. Various disturbances may arise within or

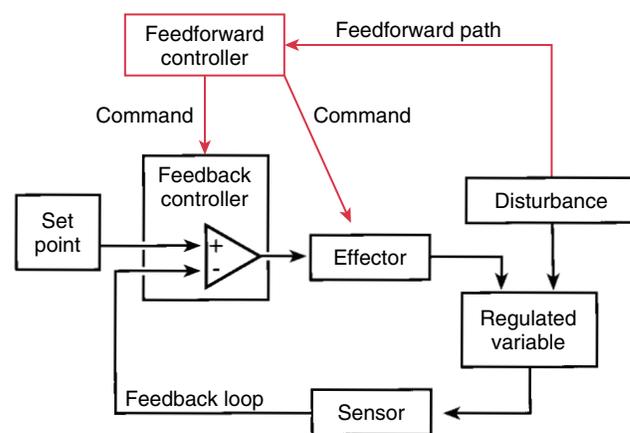


FIGURE 1.2 Elements of negative feedback and feedforward control systems (red). In a negative-

feedback control system, information flows along a closed loop. The regulated variable is sensed, and information about its level is fed back to a feedback controller, which compares it to a desired value (set point). If there is a difference, an error signal is generated, which drives the effector to bring the regulated variable closer to the desired value. A feedforward controller generates commands without directly sensing the regulated variable, although it may sense a disturbance. Feedforward controllers often operate through feedback controllers.

outside the system and cause undesired changes in the regulated variable. With **negative feedback**, a regulated variable is sensed, information is fed back to the controller, and the effector acts to oppose change (hence, the term *negative*).

A familiar example of a negative-feedback control system is the thermostatic control of room temperature. Room temperature (regulated variable) is subjected to disturbances. For example, on a cold day, room temperature falls. A thermometer (sensor) in the thermostat (controller) detects the room temperature. The thermostat is set for a certain temperature (set point). The controller compares the actual temperature (feedback signal) to the set point temperature, and an error signal is generated if the room temperature falls below the set temperature. The error signal activates the furnace (effector). The resulting change in room temperature is monitored, and when the temperature rises sufficiently, the furnace is turned off. Such a negative-feedback system allows some fluctuation in room temperature, but the components act together to maintain the set temperature. Effective communication between the sensor and effector is important in keeping these oscillations to a minimum.

Similar negative-feedback systems maintain homeostasis in the body. One example is the system that regulates arterial blood pressure (see Chapter 18). This system's sensors (arterial baroreceptors) are located in the carotid sinuses and aortic arch. Changes in stretch of the walls of the carotid sinus and aorta, which follow from changes in blood pressure, stimulate these sensors. Afferent nerve fibers transmit impulses to control centers in the medulla oblongata. Efferent nerve fibers send impulses from the medullary centers to the system's effectors, the heart and blood vessels. The output of blood by the heart and the resistance to blood flow are altered in an appropriate direction to maintain blood pressure, as measured at the sensors, within a given range of values. This negative-feedback control system compensates for any disturbance that affects blood pressure, such as changing body position, exercise, anxiety, or hemorrhage. Nerves accomplish continuous rapid communication between the feedback elements. Various hormones are also involved in regulating blood pressure, but their effects are generally slower and last longer.

Feedforward control is another strategy for regulating systems in the body, particularly when a change with time is desired. In this case, a command signal is generated, which specifies the target or goal. The moment-to-moment operation of the controller is "open loop"; that is, the regulated variable itself is not sensed. Feedforward control mechanisms often sense a disturbance and can, therefore, take corrective action that anticipates change. For example, heart rate and breathing increase even before a person has begun to exercise.

Feedforward control usually acts in combination with negative-feedback systems. One example is picking up a pencil. The movements of the arm, hand, and fingers are directed by the cerebral cortex (feedforward controller); the movements are smooth, and forces are appropriate only in part because of the feedback of visual information and sensory information from receptors in the joints and muscles. Another example of this combination occurs during exercise. Respiratory and cardiovascular adjustments closely match muscular activity, so that arterial blood oxygen and carbon

dioxide tensions hardly change during all but exhausting exercise. One explanation for this remarkable behavior is that exercise simultaneously produces a centrally generated feedforward signal to the active muscles and the respiratory and cardiovascular systems; feedforward control, together with feedback information generated as a consequence of increased movement and muscle activity, adjusts the heart, blood vessels, and respiratory muscles. In addition, control system function can adapt over a period of time. Past experience and learning can change the control system's output so that it behaves more efficiently or appropriately.

Although homeostatic control mechanisms usually act for the good of the body, they are sometimes deficient, inappropriate, or excessive. Many diseases, such as cancer, diabetes, and hypertension, develop because of a defective control mechanism. Homeostatic mechanisms may also result in inappropriate actions, such as autoimmune diseases, in which the immune system attacks the body's own tissue. Scar formation is one of the most effective homeostatic mechanisms of healing, but it is excessive in many chronic diseases, such as pulmonary fibrosis, hepatic cirrhosis, and renal interstitial disease.

Positive Feedback Promotes a Change in One Direction

With **positive feedback**, a variable is sensed and action is taken to reinforce a change of the variable. Positive feedback does not lead to stability or regulation, but to the opposite—a progressive change in one direction. One example of positive feedback in a physiological process is the upstroke of the action potential in nerve and muscle (Fig. 1.3). Depolarization of the cell membrane to a value greater than threshold leads to an increase in sodium (Na^+) permeability. Positively charged Na^+ ions rush into the cell through membrane Na^+ channels and cause further membrane depolarization; this leads to a further increase in Na^+ permeability and more Na^+ entry. This snowballing event, which occurs in a fraction of a mil-

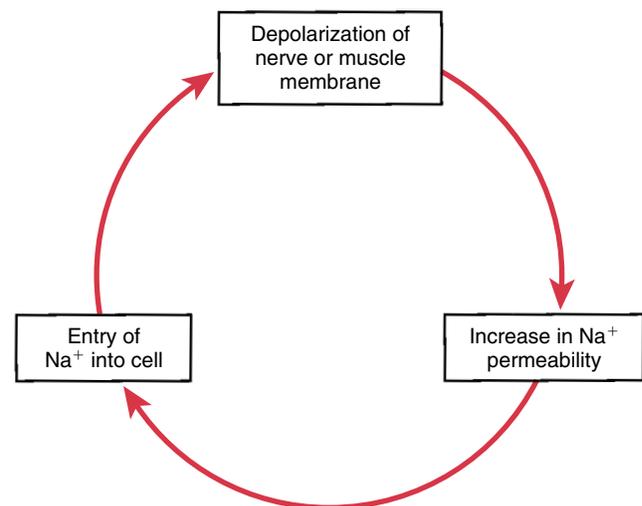


FIGURE 1.3 A positive-feedback cycle involved in the upstroke of an action potential.

lisecond, leads to an actual reversal of membrane potential and an electrical signal (action potential) conducted along the nerve or muscle fiber membrane. The process is stopped by inactivation (closure) of the Na^+ channels.

Another example of positive feedback occurs during the follicular phase of the menstrual cycle. The female sex hormone estrogen stimulates the release of luteinizing hormone, which in turn causes further estrogen synthesis by the ovaries. This positive feedback culminates in ovulation.

A third example is calcium-induced calcium release, which occurs with each heartbeat. Depolarization of the cardiac muscle plasma membrane leads to a small influx of calcium through membrane calcium channels. This leads to an explosive release of calcium from the muscle's sarcoplasmic reticulum, which rapidly increases the cytosolic calcium level and activates the contractile machinery. Many other examples are described in this textbook.

Positive feedback, if unchecked, can lead to a vicious cycle and dangerous situations. For example, a heart may be so weakened by disease that it cannot provide adequate blood flow to the muscle tissue of the heart. This leads to a further reduction in cardiac pumping ability, even less coronary blood flow, and further deterioration of cardiac function. The physician's task is sometimes to interrupt or "open" such a positive-feedback loop.

Steady State and Equilibrium Are Separate Ideas

Physiology often involves the study of exchanges of matter or energy between different defined spaces or **compartments**, separated by some type of limiting structure or **membrane**. The whole body can be divided into two major compartments: extracellular fluid and intracellular fluid. These two compartments are separated by cell plasma membranes. The extracellular fluid consists of all the body fluids outside of cells and includes the interstitial fluid, lymph, blood plasma, and specialized fluids, such as cerebrospinal fluid. It constitutes the internal environment of the body. Ordinary extracellular fluid is subdivided into interstitial fluid—lymph and plasma; these fluid compartments are separated by the endothelium, which lines the blood vessels. Materials are exchanged between these two compartments

at the blood capillary level. Even within cells there is compartmentalization. The interiors of organelles are separated from the cytosol by membranes, which restrict enzymes and substrates to structures such as mitochondria and lysosomes and allow for the fine regulation of enzymatic reactions and a greater variety of metabolic processes.

When two compartments are in **equilibrium**, opposing forces are balanced, and there is no net transfer of a particular substance or energy from one compartment to the other. Equilibrium occurs if sufficient time for exchange has been allowed and if no physical or chemical driving force would favor net movement in one direction or the other. For example, in the lung, oxygen in alveolar spaces diffuses into pulmonary capillary blood until the same oxygen tension is attained in both compartments. Osmotic equilibrium between cells and extracellular fluid is normally present in the body because of the high water permeability of most cell membranes. An equilibrium condition, if undisturbed, remains stable. No energy expenditure is required to maintain an equilibrium state.

Equilibrium and steady state are sometimes confused with each other. A **steady state** is simply a condition that does not change with time. It indicates that the amount or concentration of a substance in a compartment is constant. In a steady state, there is no net gain or net loss of a substance in a compartment. Steady state and equilibrium both suggest stable conditions, but a steady state does not necessarily indicate an equilibrium condition, and energy expenditure may be required to maintain a steady state. For example, in most body cells, there is a steady state for Na^+ ions; the amounts of Na^+ entering and leaving cells per unit time are equal. But intracellular and extracellular Na^+ ion concentrations are far from equilibrium. Extracellular $[\text{Na}^+]$ is much higher than intracellular $[\text{Na}^+]$, and Na^+ tends to move into cells down concentration and electrical gradients. The cell continuously uses metabolic energy to pump Na^+ out of the cell to maintain the cell in a steady state with respect to Na^+ ions. In living systems, conditions are often displaced from equilibrium by the constant expenditure of metabolic energy.

Figure 1.4 illustrates the distinctions between steady state and equilibrium. In Figure 1.4A, the fluid level in the

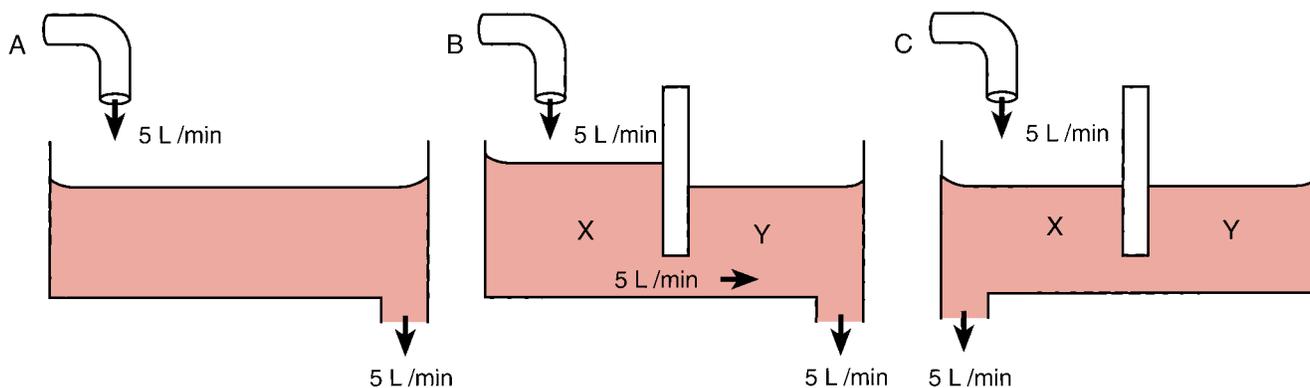


FIGURE 1.4 Models of the concepts of steady state and equilibrium. A, B, and C, Depiction of a steady state. In C, compartments X and Y are in equilibrium.

(Modified from Riggs DS. *The Mathematical Approach to Physiological Problems*. Cambridge, MA: MIT Press, 1970;169.)

sink is constant (a steady state) because the rates of inflow and outflow are equal. If we were to increase the rate of inflow (open the tap), the fluid level would rise, and with time, a new steady state might be established at a higher level. In Figure 1.4B, the fluids in compartments X and Y are not in equilibrium (the fluid levels are different), but the system as a whole and each compartment are in a steady state, since inputs and outputs are equal. In Figure 1.4C, the system is in a steady state and compartments X and Y are in equilibrium. Note that the term *steady state* can apply to a single or several compartments; the term *equilibrium* describes the relation between at least two adjacent compartments that can exchange matter or energy with each other.

Coordinated Body Activity Requires Integration of Many Systems

Body functions can be analyzed in terms of several systems, such as the nervous, muscular, cardiovascular, respiratory, renal, gastrointestinal, and endocrine systems. These divisions are rather arbitrary, however, and all systems interact and depend on each other. For example, walking involves the activity of many systems. The nervous system coordinates the movements of the limbs and body, stimulates the muscles to contract, and senses muscle tension and limb position. The cardiovascular system supplies blood to the muscles, providing for nourishment and the removal of metabolic wastes and heat. The respiratory system supplies oxygen and removes carbon dioxide. The renal system maintains an optimal blood composition. The gastrointestinal system supplies energy-yielding metabolites. The endocrine system helps adjust blood flow and the supply of various metabolic substrates to the working muscles. Coordinated body activity demands the integration of many systems.

Recent research demonstrates that many diseases can be explained on the basis of abnormal function at the molecular level. This reductionist approach has led to incredible advances in our knowledge of both normal and abnormal function. Diseases occur within the context of a whole organism, however, and it is important to understand how all cells, tissues, organs, and organ systems respond to a disturbance (disease process) and interact. The saying, "The whole is more than the sum of its parts," certainly applies to what happens in living organisms. The science of physiology has the unique challenge of trying to make sense of the complex interactions that occur in the body. Understanding the body's processes and functions is clearly fundamental to the intelligent practice of medicine.

MODES OF COMMUNICATION AND SIGNALING

The human body has several means of transmitting information between cells. These mechanisms include direct communication between adjacent cells through gap junctions, autocrine and paracrine signaling, and the release of neurotransmitters and hormones produced by endocrine and nerve cells (Fig. 1.5).

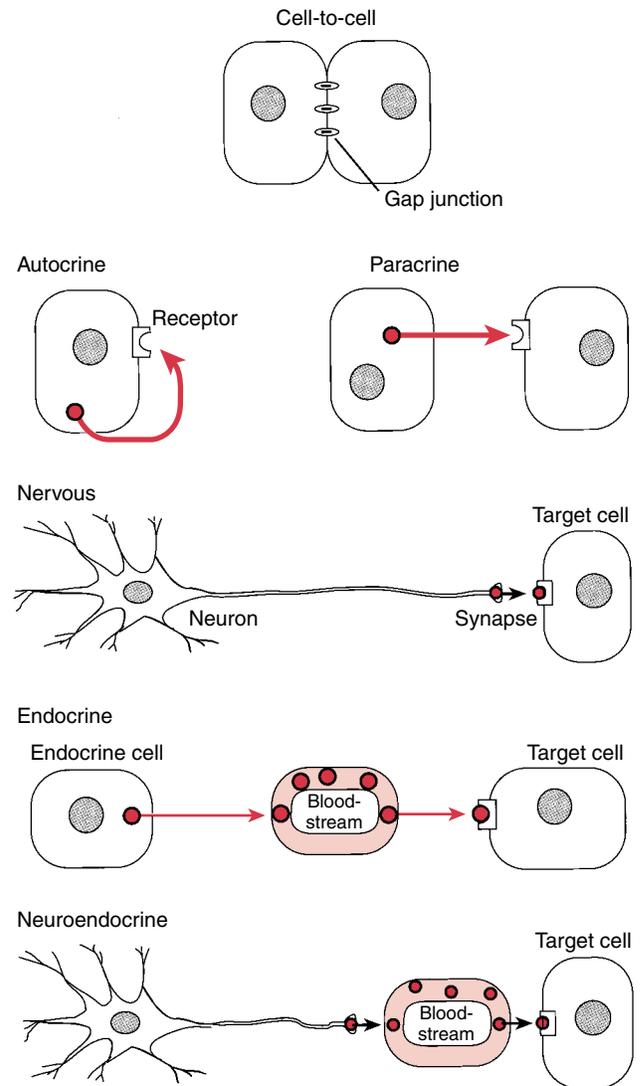


FIGURE 1.5 Modes of intercellular signaling. Cells may communicate with each other directly via gap junctions or chemical messengers. With autocrine and paracrine signaling, a chemical messenger diffuses a short distance through the extracellular fluid and binds to a receptor on the same cell or a nearby cell. Nervous signaling involves the rapid transmission of action potentials, often over long distances, and the release of a neurotransmitter at a synapse. Endocrine signaling involves the release of a hormone into the bloodstream and the binding of the hormone to specific target cell receptors. Neuroendocrine signaling involves the release of a hormone from a nerve cell and the transport of the hormone by the blood to a distant target cell.

Gap Junctions Provide a Pathway for Direct Communication Between Adjacent Cells

Adjacent cells sometimes communicate directly with each other via **gap junctions**, specialized protein channels made of the protein **connexin** (Fig. 1.6). Six connexins form a half-channel called a **connexon**. Two connexons join end to end to form an intercellular channel between adjacent cells. Gap junctions allow the flow of ions (hence, electrical current) and small molecules between the cytosol of

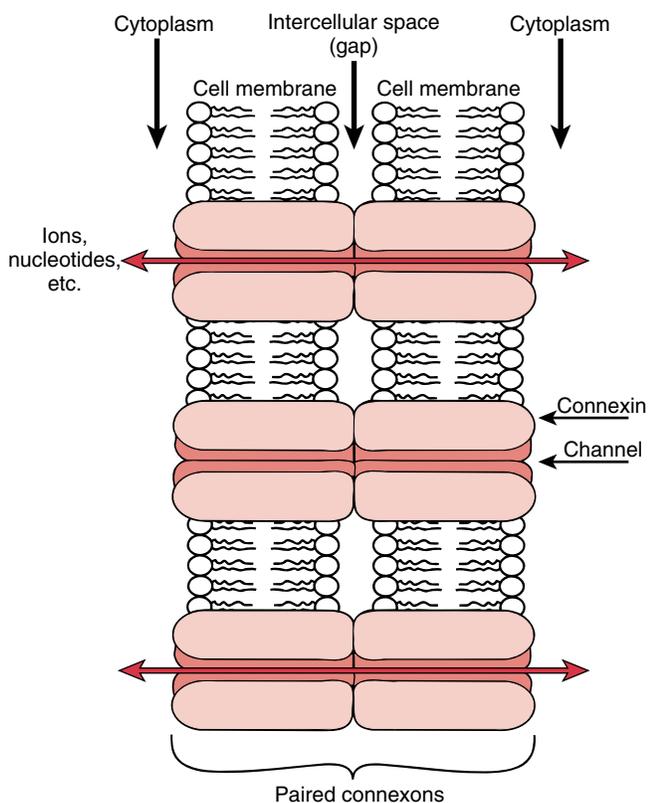


FIGURE 1.6 The structure of gap junctions. The channel connects the cytosol of adjacent cells. Six molecules of the protein connexin form a half-channel called a connexon. Ions and small molecules, such as nucleotides, can flow through the pore formed by the joining of connexons from adjacent cells.

neighboring cells (see Fig. 1.5). Gap junctions appear to be important in the transmission of electrical signals between neighboring cardiac muscle cells, smooth muscle cells, and some nerve cells. They may also functionally couple adjacent epithelial cells. Gap junctions are thought to play a role in the control of cell growth and differentiation by allowing adjacent cells to share a common intracellular environment. Often when a cell is injured, gap junctions close, isolating a damaged cell from its neighbors. This isolation process may result from a rise in calcium and a fall in pH in the cytosol of the damaged cell.

Cells May Communicate Locally by Paracrine and Autocrine Signaling

Cells may signal to each other via the local release of chemical substances. This means of communication, present in primitive living forms, does not depend on a vascular system. With **paracrine signaling**, a chemical is liberated from a cell, diffuses a short distance through interstitial fluid, and acts on nearby cells. Paracrine signaling factors affect only the immediate environment and bind with high specificity to cell receptors. They are also rapidly destroyed by extracellular enzymes or bound to extracellular matrix, thus preventing their widespread diffusion. **Nitric oxide (NO)**, originally called "endothelium-derived relaxing factor

(EDRF)," is an example of a paracrine signaling molecule. Although most cells can produce NO, it has major roles in mediating vascular smooth muscle tone, facilitating central nervous system neurotransmission activities, and modulating immune responses (see Chapters 16 and 26).

The production of NO results from the activation of **nitric oxide synthase (NOS)**, which deaminates arginine to citrulline (Fig. 1.7). NO, produced by endothelial cells, regulates vascular tone by diffusing from the endothelial cell to the underlying vascular smooth muscle cell, where it activates its effector target, a cytoplasmic enzyme **guanylyl cyclase**. The activation of cytoplasmic guanylyl cyclase results in increased intracellular **cyclic guanosine monophosphate (cGMP)** levels and the activation of **cGMP-dependent protein kinase**. This enzyme phosphorylates potential target substrates, such as calcium pumps in the sarcoplasmic reticulum or sarcolemma, leading to reduced cytoplasmic levels of calcium. In turn, this deactivates the contractile machinery in the vascular smooth muscle cell and produces relaxation or a decrease of tone (see Chapter 16).

In contrast, during **autocrine signaling**, the cell releases a chemical into the interstitial fluid that affects its own activity by binding to a receptor on its own surface (see Fig. 1.5). Eicosanoids (e.g., prostaglandins), are examples of signaling molecules that often act in an autocrine manner. These molecules act as local hormones to influence a variety of physiological processes, such as uterine smooth muscle contraction during pregnancy.

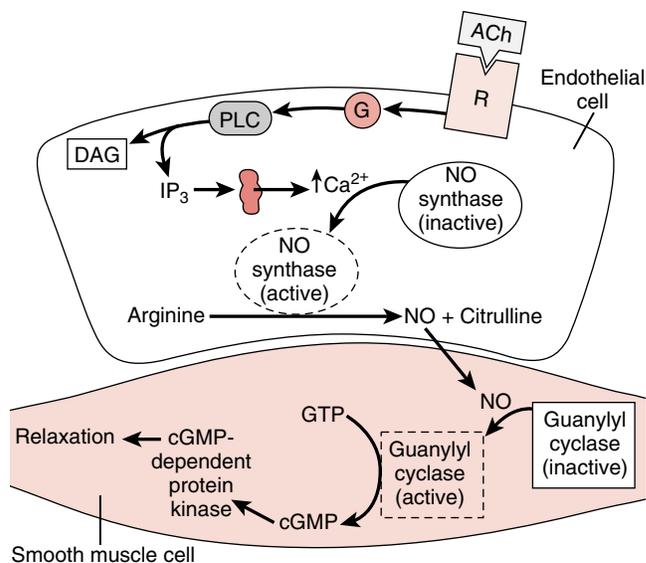


FIGURE 1.7 Paracrine signaling by nitric oxide (NO) after stimulation of endothelial cells with acetylcholine (ACh). The NO produced diffuses to the underlying vascular smooth muscle cell and activates its effector, cytoplasmic guanylyl cyclase, leading to the production of cGMP. Increased cGMP leads to the activation of cGMP-dependent protein kinase, which phosphorylates target substrates, leading to a decrease in cytoplasmic calcium and relaxation. Relaxation can also be mediated by nitroglycerin, a pharmacological agent that is converted to NO in smooth muscle cells, which can then activate guanylyl cyclase. G, G protein; PLC, phospholipase C; DAG, diacylglycerol; IP₃, inositol trisphosphate.

The Nervous System Provides for Rapid and Targeted Communication

The nervous system provides for rapid communication between body parts, with conduction times measured in milliseconds. This system is also organized for discrete activities; it has an enormous number of “private lines” for sending messages from one distinct locus to another. The conduction of information along nerves occurs via action potentials, and signal transmission between nerves or between nerves and effector structures takes place at a **synapse**. Synaptic transmission is almost always mediated by the release of specific chemicals or **neurotransmitters** from the nerve terminals (see Fig. 1.5). Innervated cells have specialized protein molecules (receptors) in their cell membranes that selectively bind neurotransmitters. Chapter 3 discusses the actions of various neurotransmitters and how they are synthesized and degraded. Chapters 4 to 6 discuss the role of the nervous system in coordinating and controlling body functions.

The Endocrine System Provides for Slower and More Diffuse Communication

The endocrine system produces **hormones** in response to a variety of stimuli. In contrast to the effects of nervous system stimulation, responses to hormones are much slower (seconds to hours) in onset, and the effects often last longer. Hormones are carried to all parts of the body by the bloodstream (see Fig. 1.5). A particular cell can respond to a hormone only if it possesses the specific receptor (“receiver”) for the hormone. Hormone effects may be discrete. For example, arginine vasopressin increases the water permeability of kidney collecting duct cells but does not change the water permeability of other cells. They may also be diffuse, influencing practically every cell in the body. For example, thyroxine has a general stimulatory effect on metabolism. Hormones play a critical role in controlling such body functions as growth, metabolism, and reproduction.

Cells that are not traditional endocrine cells produce a special category of chemical messengers called **tissue growth factors**. These growth factors are protein molecules that influence cell division, differentiation, and cell survival. They may exert effects in an autocrine, paracrine, or endocrine fashion. Many growth factors have been identified, and probably many more will be recognized in years to come. **Nerve growth factor** enhances nerve cell development and stimulates the growth of axons. **Epidermal growth factor** stimulates the growth of epithelial cells in the skin and other organs. **Platelet-derived growth factor** stimulates the proliferation of vascular smooth muscle and endothelial cells. **Insulin-like growth factors** stimulate the proliferation of a wide variety of cells and mediate many of the effects of growth hormone. Growth factors appear to be important in the development of multicellular organisms and in the regeneration and repair of damaged tissues.

The Nervous and Endocrine Control Systems Overlap

The distinction between nervous and endocrine control systems is not always clear. First, the nervous system ex-

erts important controls over endocrine gland function. For example, the hypothalamus controls the secretion of hormones from the pituitary gland. Second, specialized nerve cells, called **neuroendocrine cells**, secrete hormones. Examples are the hypothalamic neurons, which liberate releasing factors that control secretion by the anterior pituitary gland, and the hypothalamic neurons, which secrete arginine vasopressin and oxytocin into the circulation. Third, many proven or potential neurotransmitters found in nerve terminals are also well-known hormones, including arginine vasopressin, cholecystokinin, enkephalins, norepinephrine, secretin, and vasoactive intestinal peptide. Therefore, it is sometimes difficult to classify a particular molecule as either a hormone or a neurotransmitter.

THE MOLECULAR BASIS OF CELLULAR SIGNALING

Cells communicate with one another by many complex mechanisms. Even unicellular organisms, such as yeast cells, utilize small peptides called **pheromones** to coordinate mating events that eventually result in haploid cells with new assortments of genes. The study of intercellular communication has led to the identification of many complex signaling systems that are used by the body to network and coordinate functions. These studies have also shown that these signaling pathways must be tightly regulated to maintain cellular homeostasis. Dysregulation of these signaling pathways can transform normal cellular growth into uncontrolled cellular proliferation or cancer (see Clinical Focus Box 1.1).

Signaling systems consist of **receptors** that reside either in the plasma membrane or within cells and are activated by a variety of extracellular signals or **first messengers**, including peptides, protein hormones and growth factors, steroids, ions, metabolic products, gases, and various chemical or physical agents (e.g., light). Signaling systems also include **transducers** and **effectors** that are involved in conversion of the signal into a physiological response. The pathway may include additional intracellular messengers, called **second messengers** (Fig. 1.8). Examples of second messengers are cyclic nucleotides such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG), and calcium.

A general outline for a signaling system is as follows: The signaling cascade is initiated by binding of a hormone to its appropriate ligand-binding site on the outer surface domain of its cognate membrane receptor. This results in activation of the receptor; the receptor may adopt a new conformation, form aggregates (multimerize), or become phosphorylated. This change usually results in an association of adapter signaling molecules that transduce and amplify the signal through the cell by activating specific effector molecules and generating a second messenger. The outcome of the signal transduction cascade is a physiological response, such as secretion, movement, growth, division, or death.

CLINICAL FOCUS BOX 1.1

From Signaling Molecules to Oncoproteins and Cancer

Cancer may result from defects in critical signaling molecules that regulate many cell properties, including cell proliferation, differentiation, and survival. Normal cellular regulatory proteins or **proto-oncogenes** may become altered by mutation or abnormally expressed during cancer development. **Oncoproteins**, the altered proteins that arise from proto-oncogenes, in many cases are signal transduction proteins that normally function in the regulation of cellular proliferation. Examples of signaling molecules that can become oncogenic span the entire signal transduction pathway and include ligands (e.g., growth factors), receptors, adapter and effector molecules, and transcription factors.

There are many examples of how normal cellular proteins can be converted into oncoproteins. One occurs in chronic myeloid leukemia (CML). This disease usually results from an acquired chromosomal abnormality that involves translocation between chromosomes 9 and 22. This translocation results in the fusion of the *bcr* gene, whose function is unknown, with part of the *cellular abl* (*c-abl*) gene. The *c-abl* gene encodes a protein tyrosine kinase

whose normal substrates are unknown. The chimeric (composed of fused parts of *bcr* and *c-abl*) Bcr-Abl fusion protein has unregulated tyrosine kinase activity and, through the Abl SH2 and SH3 domains, binds to and phosphorylates many signal transduction proteins that the wild-type Abl tyrosine kinase would not normally activate. Increased expression of the unregulated Bcr-Abl protein activates many growth regulatory genes in the absence of normal growth factor signaling.

The chromosomal translocation that results in the formation of the Bcr-Abl oncoprotein occurs during the development of hematopoietic stem cells and is observed as the diagnostic, shorter, Philadelphia chromosome 22. It results in chronic myeloid leukemia that is characterized by a progressive leukocytosis (increase in number of circulating white blood cells) and the presence of circulating immature blast cells. Other secondary mutations may spontaneously occur within the mutant stem cell and can lead to acute leukemia, a rapidly progressing disease that is often fatal. Understanding of the molecules and signaling pathways that regulate normal cell physiology can help us understand what happens in some types of cancer.

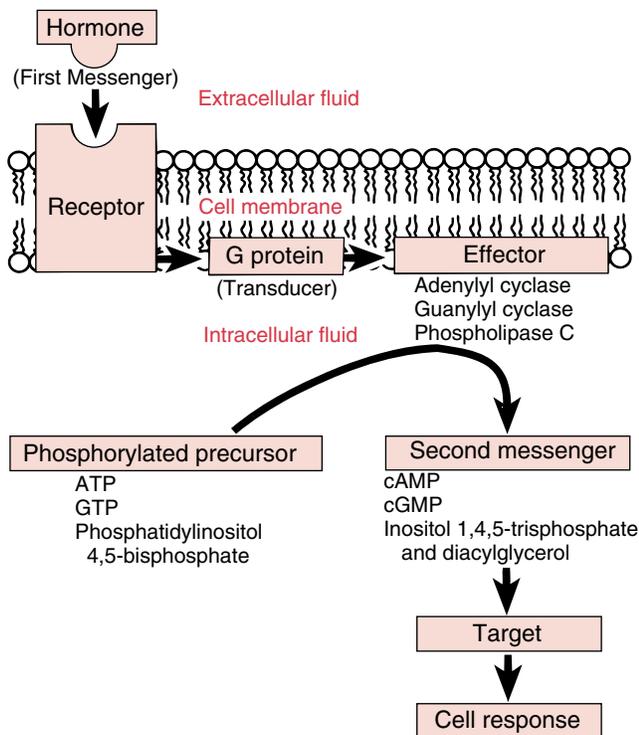


FIGURE 1.8 Signal transduction patterns common to second messenger systems. A protein or peptide hormone binds to a plasma membrane receptor, which stimulates or inhibits a membrane-bound effector enzyme via a G protein. The effector catalyzes the production of many second messenger molecules from a phosphorylated precursor (e.g., cAMP from ATP, cGMP from GTP, or inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate). The second messengers, in turn, activate protein kinases (targets) or cause other intracellular changes that ultimately lead to the cell response.

SIGNAL TRANSDUCTION BY PLASMA MEMBRANE RECEPTORS

As mentioned above, the molecules that are produced by one cell to act on itself (autocrine signaling) or other cells (paracrine, neural, or endocrine signaling) are ligands or first messengers. Many of these ligands bind directly to receptor proteins that reside in the plasma membrane, and others cross the plasma membrane and interact with cellular receptors that reside in either the cytoplasm or the nucleus. Thus, cellular receptors are divided into two general types, **cell-surface receptors** and **intracellular receptors**. Three general classes of cell-surface receptors have been identified: G-protein-coupled receptors, ion channel-linked receptors, and enzyme-linked receptors. Intracellular receptors include steroid and thyroid hormone receptors and are discussed in a later section in this chapter.

G-Protein-Coupled Receptors Transmit Signals Through the Trimeric G Proteins

G-protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors, with more than 1,000 members. These receptors indirectly regulate their effector targets, which can be ion channels or plasma membrane-bound effector enzymes, through the intermediary activity of a separate membrane-bound adapter protein complex called the **trimeric GTP-binding regulatory protein** or **G protein** (see Clinical Focus Box 1.2). GPCRs mediate cellular responses to numerous types of first messenger signaling molecules, including proteins, small peptides, amino acids, and fatty acid derivatives. Many first messenger ligands can activate several different GPCRs. For example, serotonin can activate at least 15 different GPCRs.

G-protein-coupled receptors are structurally and functionally similar molecules. They have a ligand-binding ex-

CLINICAL FOCUS BOX 1.2

G Proteins and Disease

G proteins function as key transducers of information across cell membranes by coupling receptors to effectors such as adenylyl cyclase (AC) or phospholipase C (see Fig. 1.9). They are part of a large family of proteins that bind and hydrolyze guanosine triphosphate (GTP) as part of an “on” and “off” switching mechanism. G proteins are heterotrimers, consisting of G_α , G_β , and G_γ subunits, each of which is encoded by a different gene.

Some strains of bacteria have developed toxins that can modify the activity of the α subunit of G proteins, resulting in disease. For example, **cholera toxin**, produced by the microorganism that causes cholera, *Vibrio cholerae*, causes ADP ribosylation of the stimulatory (G_{α_s}) subunit of G proteins. This modification abolishes the GTPase activity of G_{α_s} and results in an α_s subunit that is always in the “on” or active state. Thus, cholera toxin results in continuous stimulation of AC. The main cells affected by this bacterial toxin are the epithelial cells of the intestinal tract, and the excessive production of cAMP causes them to secrete chloride ions and water. This causes severe diarrhea and dehydration and may result in death.

Another toxin, **pertussis toxin**, is produced by *Bordetella pertussis* bacteria and causes whooping cough. The pertussis toxin alters the activity of G_{α_i} by ADP ribosylation. This modification inhibits the function of the α_i subunit by preventing association with an activated receptor. Thus, the α_i subunit remains GDP-bound and in an “off” state, unable to inhibit the activity of AC. The molecular mechanism by which pertussis toxin causes whooping cough is not understood.

The understanding of the actions of cholera and pertussis toxins highlights the importance of normal G-protein function and illustrates that dysfunction of this signaling pathway can cause acute disease. In the years since the discovery of these proteins, there has been an explosion of information on G proteins and several chronic human diseases have been linked to genetic mutations that cause ab-

normal function or expression of G proteins. These mutations can occur either in the G proteins themselves or in the receptors to which they are coupled.

Mutations in G-protein-coupled receptors (GPCRs) can result in the receptor being in an active conformation in the absence of ligand binding. This would result in sustained stimulation of G proteins. Mutations of G-protein subunits can result in either constitutive activation (e.g., continuous stimulation of effectors such as AC) or loss of activity (e.g., loss of cAMP production).

Many factors influence the observed manifestations resulting from defective G-protein signaling. These include the specific GPCRs and the G proteins that associate with them, their complex patterns of expression in different tissues, and whether the mutation is germ-line or somatic. Mutation of a ubiquitously expressed GPCR or G protein results in widespread manifestations, while mutation of a GPCR or G protein with restricted expression will result in more focused manifestations.

Somatic mutation of G_{α_s} during embryogenesis can result in the dysregulated activation of this G protein and is the source of several diseases that have multiple pleiotropic or local manifestations, depending on when the mutation occurs. For example, early somatic mutation of G_{α_s} and its overactivity can lead to McCune-Albright syndrome (MAS). The consequences of the mutant G_{α_s} in MAS are manifested in many ways, with the most common being a triad of features that includes polyostotic (affecting many bones) fibrous dysplasia, *café-au-lait* skin hyperpigmentation, and precocious puberty. A later mutation of G_{α_s} can result in a more restricted focal syndrome, such as monostotic (affecting a single bone) fibrous dysplasia.

The complexity of the involvement of GPCR or G proteins in the pathogenesis of many human diseases is beginning to be appreciated, but already this information underscores the critical importance of understanding the molecular events involved in hormone signaling so that rational therapeutic interventions can be designed.

tracellular domain on one end of the molecule, separated by a seven-pass transmembrane-spanning region from the cytosolic regulatory domain at the other end, where the receptor interacts with the membrane-bound G protein. Binding of ligand or hormone to the extracellular domain results in a conformational change in the receptor that is transmitted to the cytosolic regulatory domain. This conformational change allows an association of the ligand-bound, activated receptor with a trimeric G protein associated with the inner leaflet of the plasma membrane. The interaction between the ligand-bound, activated receptor and the G protein, in turn, activates the G protein, which dissociates from the receptor and transmits the signal to its effector enzyme or ion channel (Fig. 1.9).

The trimeric G proteins are named for their requirement for GTP binding and hydrolysis and have been shown to have a broad role in linking various seven-pass transmembrane receptors to membrane-bound effector systems that generate intracellular messengers. G proteins are tethered to the membrane through lipid linkage and are het-

erotrimeric, that is, composed of three distinct subunits. The subunits of a G protein are an α subunit, which binds and hydrolyzes GTP, and β and γ subunits, which form a stable, tight noncovalent-linked dimer. When the α subunit binds GDP, it associates with the $\beta\gamma$ subunits to form a trimeric complex that can interact with the cytoplasmic domain of the GPCR (Fig. 1.10). The conformational change that occurs upon ligand binding causes the GDP-bound trimeric (α , β , γ complex) G protein to associate with the ligand-bound receptor. The association of the GDP-bound trimeric complex with the GPCR activates the exchange of GDP for GTP. Displacement of GDP by GTP is favored in cells because GTP is in higher concentration.

The displacement of GDP by GTP causes the α subunit to dissociate from the receptor and from the $\beta\gamma$ subunits of the G protein. This exposes an effector binding site on the α subunit, which then associates with an effector molecule (e.g., adenylyl cyclase or phospholipase C) to result in the generation of second messengers (e.g., cAMP or IP_3 and DAG). The hydrolysis of GTP to GDP by the α subunit re-

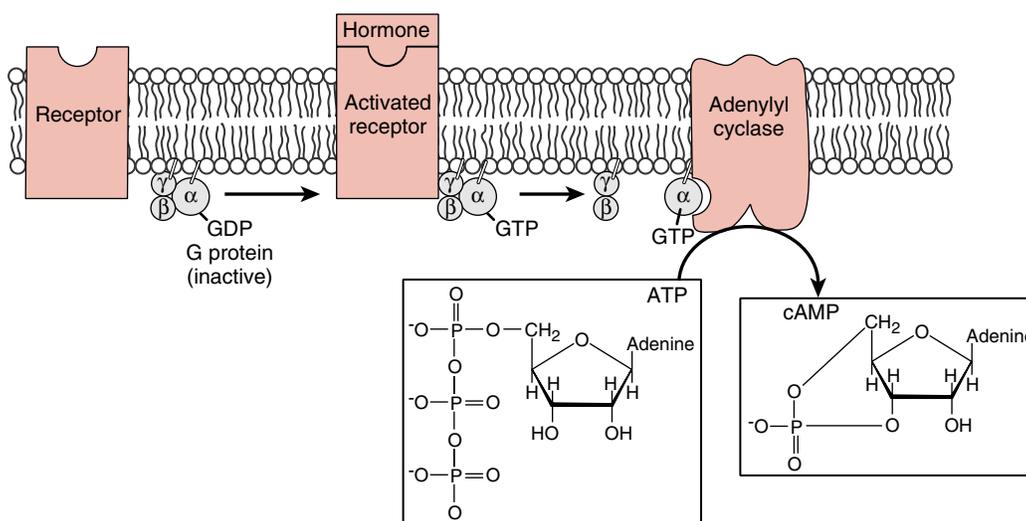


FIGURE 1.9 Activation of a G-protein-coupled receptor and the production of cAMP. Binding of a hormone causes the interaction of the activated receptor with the inactive, GDP-bound G protein. This interaction results in activa-

tion of the G protein through GDP to GTP exchange and dissociation of the α and $\beta\gamma$ subunits. The activated α subunit of the G protein can then interact with and activate the membrane protein adenylyl cyclase to catalyze the conversion of ATP to cAMP.

sults in the reassociation of the α and $\beta\gamma$ subunits, which are then ready to repeat the cycle.

The cycling between inactive (GDP-bound) and active forms (GTP-bound) places the G proteins in the family of **molecular switches**, which regulate many biochemical events. When the switch is "off," the bound nucleotide is GDP. When the switch is "on," the hydrolytic enzyme (G protein) is bound to GTP, and the cleavage of GTP to GDP will reverse the switch to an "off" state. While most of the signal transduction produced by G proteins is due to the ac-

tivities of the α subunit, a role for $\beta\gamma$ subunits in activating effectors during signal transduction is beginning to be appreciated. For example, $\beta\gamma$ subunits can activate K^+ channels. Therefore, both α and $\beta\gamma$ subunits are involved in regulating physiological responses.

The catalytic activity of a G protein, which is the hydrolysis of GTP to GDP, resides in its $G\alpha$ subunit. Each $G\alpha$ subunit within this large protein family has an intrinsic rate of GTP hydrolysis. The intrinsic catalytic activity rate of G proteins is an important factor contributing to the ampli-

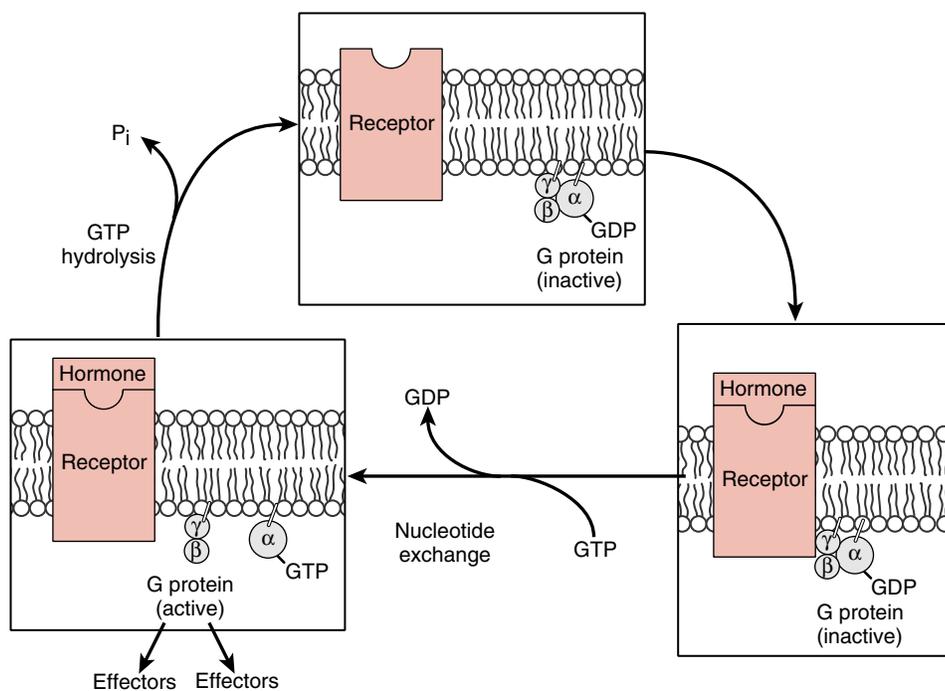


FIGURE 1.10 Activation and inactivation of G proteins.

When bound to GDP, G proteins are in an inactive state and are not associated with a receptor. Binding of a hormone to a G-protein-coupled receptor results in an association of the inactive, GDP-bound G protein with the receptor. The interaction of the GDP-bound G protein with the activated receptor results in activation of the G protein via the exchange of GDP for GTP by the α subunit. The α and $\beta\gamma$ subunits of the activated GTP-bound G protein dissociate and can then interact with their effector proteins. The intrinsic GTPase activity in the α subunit of the G protein hydrolyzes the bound GTP to GDP. The GDP-bound α subunit reassociates with the $\beta\gamma$ subunit to form an inactive, membrane-bound G-protein complex.

cation of the signal produced by a single molecule of ligand binding to a G-protein-coupled receptor. For example, a $G\alpha$ subunit that remains active longer (slower rate of GTP hydrolysis) will continue to activate its effector for a longer period and result in greater production of second messenger.

The G proteins functionally couple receptors to several different effector molecules. Two major effector molecules that are regulated by G-protein subunits are **adenylyl cyclase (AC)** and phospholipase C (PLC). The association of an activated $G\alpha$ subunit with AC can result in either the stimulation or the inhibition of the production of cAMP. This disparity is due to the two types of α subunit that can couple AC to cell-surface receptors. Association of an α_s subunit (*s* for stimulatory) promotes the activation of AC and production of cAMP. The association of an α_i (*i* for inhibitory) subunit promotes the inhibition of AC and a decrease in cAMP. Thus, bidirectional regulation of adenylyl cyclase is achieved by coupling different classes of cell-surface receptors to the enzyme by either G_s or G_i (Fig. 1.11).

In addition to α_s and α_i subunits, other isoforms of G-protein subunits have been described. For example, α_q activates PLC, resulting in the production of the second messengers diacylglycerol and inositol trisphosphate. Another

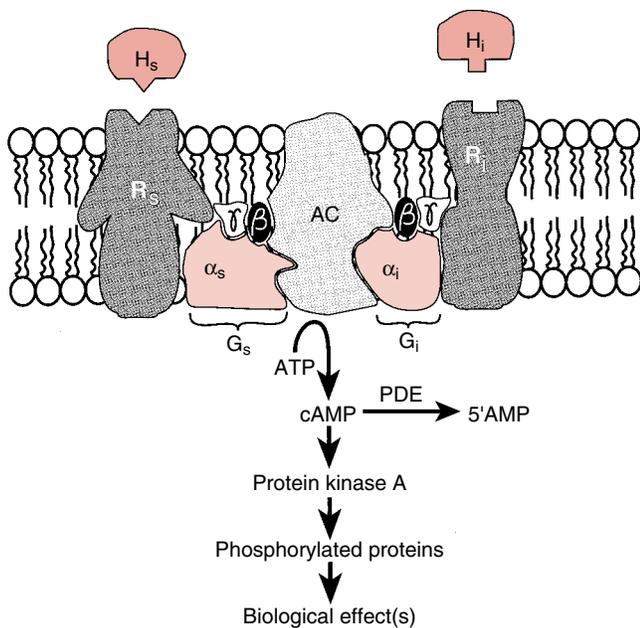


FIGURE 1.11 Stimulatory and inhibitory coupling of G proteins to adenylyl cyclase (AC). Stimulatory (G_s) and inhibitory (G_i) G proteins couple hormone binding to the receptor with either activation or inhibition of AC. Each G protein is a trimer consisting of $G\alpha$, $G\beta$, and $G\gamma$ subunits. The $G\alpha$ subunits in G_s and G_i are distinct in each and provide the specificity for either AC activation or AC inhibition. Hormones (H_s) that stimulate AC interact with “stimulatory” receptors (R_s) and are coupled to AC through stimulatory G proteins (G_s). Conversely, hormones (H_i) that inhibit AC interact with “inhibitory” receptors (R_i) that are coupled to AC through inhibitory G proteins (G_i). Intracellular levels of cAMP are modulated by the activity of phosphodiesterase (PDE), which converts cAMP to 5'AMP and turns off the signaling pathway by reducing the level of cAMP.

$G\alpha$ subunit, α_T or **transducin**, is expressed in photoreceptor tissues, and has an important role in signaling in rod cells by activation of the effector **cGMP phosphodiesterase**, which degrades cGMP to 5'GMP (see Chapter 4). All three subunits of G proteins belong to large families that are expressed in different combinations in different tissues. This tissue distribution contributes to both the specificity of the transduced signal and the second messenger produced.

The Ion Channel-Linked Receptors Help Regulate the Intracellular Concentration of Specific Ions

Ion channels, found in all cells, are transmembrane proteins that cross the plasma membrane and are involved in regulating the passage of specific ions into and out of cells.

Ion channels may be opened or closed by changing the membrane potential or by the binding of ligands, such as neurotransmitters or hormones, to membrane receptors. In some cases, the receptor and ion channel are one and the same molecule. For example, at the neuromuscular junction, the neurotransmitter acetylcholine binds to a muscle membrane nicotinic cholinergic receptor that is also an ion channel. In other cases, the receptor and an ion channel are linked via a G protein, second messengers, and other downstream effector molecules, as in the muscarinic cholinergic receptor on cells innervated by parasympathetic postganglionic nerve fibers. Another possibility is that the ion channel is directly activated by a cyclic nucleotide, such as cGMP or cAMP, produced as a consequence of receptor activation. This mode of ion channel control is predominantly found in the sensory tissues for sight, smell, and hearing. The opening or closing of ion channels plays a key role in signaling between electrically excitable cells.

The Tyrosine Kinase Receptors Signal Through Adapter Proteins to the Mitogen-Activated Protein Kinase Pathway

Many hormones, growth factors, and cytokines signal their target cells by binding to a class of receptors that have tyrosine kinase activity and result in the phosphorylation of tyrosine residues in the receptor and other target proteins. Many of the receptors in this class of plasma membrane receptors have an intrinsic tyrosine kinase domain that is part of the cytoplasmic region of the receptor (Fig. 1.12). Another group of related receptors lacks an intrinsic tyrosine kinase but, when activated, becomes associated with a cytoplasmic tyrosine kinase (see Fig. 1.12). This family of tyrosine kinase receptors utilizes similar signal transduction pathways, and we discuss them together.

The **tyrosine kinase receptors** consist of a hormone-binding region that is exposed to the extracellular fluid. Typical agonists for these receptors include hormones (e.g., insulin), growth factors (e.g., epidermal, fibroblast, and platelet-derived growth factors), or cytokines. The cytokine receptors include receptors for interferons, interleukins (e.g., IL-1 to IL-17), tumor necrosis factor, and colony-stimulating factors (e.g., granulocyte and monocyte colony-stimulating factors).

The signaling cascades generated by the activation of tyrosine kinase receptors can result in the amplification of

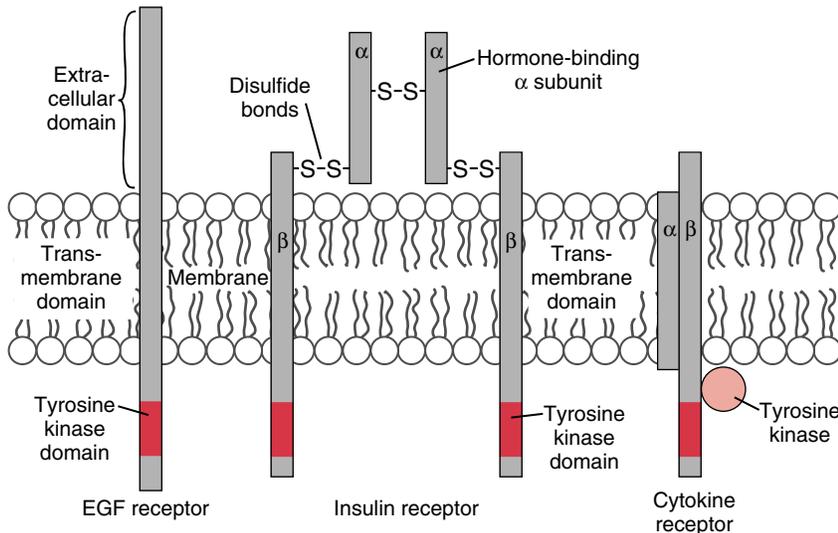


FIGURE 1.12 General structures of the tyrosine kinase receptor family.

Tyrosine kinase receptors have an intrinsic protein tyrosine kinase activity that resides in the cytoplasmic domain of the molecule. Examples are the epidermal growth factor (EGF) and insulin receptors. The EGF receptor is a single-chain transmembrane protein consisting of an extracellular region containing the hormone-binding domain, a trans-membrane domain, and an intracellular region that contains the tyrosine kinase domain. The insulin receptor is a heterotetramer consisting of two α and two β subunits held together by disulfide bonds. The α subunits are entirely extracellular and involved in insulin binding. The β subunits are transmembrane proteins and contain the tyrosine kinase activity within the cytoplasmic domain of the subunit. Some receptors become associated with cytoplasmic tyrosine kinases following their activation. Examples can be found in the family of cytokine receptors, which generally consist of an agonist-binding subunit and a signal-transducing subunit that become associated with a cytoplasmic tyrosine kinase.

gene transcription and *de novo* transcription of genes involved in growth, cellular differentiation, and movements such as crawling or shape changes. The general scheme for this signaling pathway begins with the agonist binding to the extracellular portion of the receptor (Fig. 1.13). The binding of agonists causes two of the agonist-bound receptors to associate or dimerize, and the associated or intrinsic tyrosine kinases become activated. The tyrosine kinases then phosphorylate tyrosine residues in the other subunit

of the dimer. The phosphorylated tyrosine residues in the cytoplasmic domains of the dimerized receptor serve as "docking sites" for additional signaling molecules or adapter proteins that have a specific sequence called an SH2 domain. The SH2-containing adapter proteins may be serine/threonine protein kinases, phosphatases, or other proteins that help in the assembly of signaling complexes that transmit the signal from an activated receptor to many signaling pathways, resulting in a cellular response.

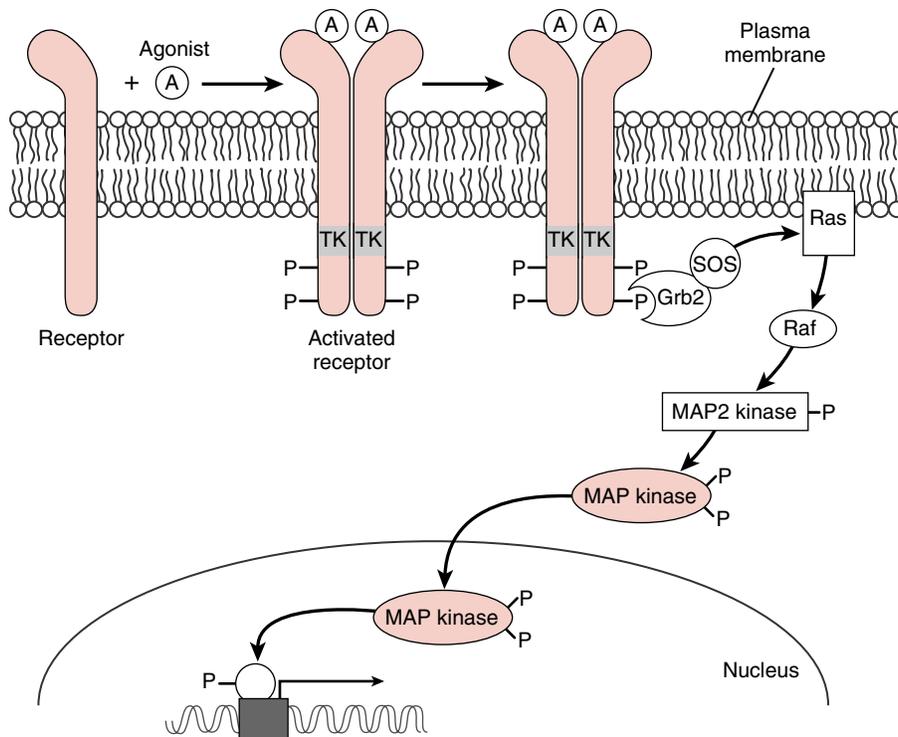


FIGURE 1.13 A signaling pathway for tyrosine kinase receptors.

Binding of agonist to the tyrosine kinase receptor (TK) causes dimerization, activation of the intrinsic tyrosine kinase activity, and phosphorylation of the receptor subunits. The phosphotyrosine residues serve as docking sites for intracellular proteins, such as Grb2 and SOS, which have SH2 domains. Ras is activated by the exchange of GDP for GTP. Ras-GTP (active form) activates the serine/threonine kinase Raf, initiating a phosphorylation cascade that results in the activation of MAP kinase. MAP kinase translocates to the nucleus and phosphorylates transcription factors to modulate gene transcription.

One of these signaling pathways includes the activation of another GTPase that is related to the trimeric G proteins. Members of the *ras* family of **monomeric G proteins** are activated by many tyrosine kinase receptor growth factor agonists and, in turn, activate an intracellular signaling cascade that involves the phosphorylation and activation of several protein kinases called **mitogen-activated protein kinases** (MAP kinases). Activated MAP kinase translocates to the nucleus, where it activates the transcription of genes involved in the transcription of other genes, the **immediate early genes**.

SECOND MESSENGER SYSTEMS AND INTRACELLULAR SIGNALING PATHWAYS

Second messengers transmit and amplify the first messenger signal to signaling pathways inside the cell. Only a few second messengers are responsible for relaying these signals within target cells, and because each target cell has a different complement of intracellular signaling pathways, the physiological responses can vary. Thus, it is useful to keep in mind that every cell in our body is programmed to respond to specific combinations of messengers and that the same messenger can elicit a distinct physiological response in different cell types. For example, the neurotransmitter acetylcholine can cause heart muscle to relax, skeletal muscle to contract, and secretory cells to secrete.

cAMP Is an Important Second Messenger in All Cells

As a result of binding to specific G-protein-coupled receptors, many peptide hormones and catecholamines produce an almost immediate increase in the intracellular concentration of cAMP. For these ligands, the receptor is coupled to a stimulatory G protein (G_{α_s}), which upon activation and exchange of GDP for GTP can diffuse in the membrane to interact with and activate adenylyl cyclase (AC), a large transmembrane protein that converts intracellular ATP to the second messenger, cAMP.

In addition to those hormones that stimulate the production of cAMP through a receptor coupled to G_{α_s} , some hormones act to decrease cAMP formation and, therefore, have opposing intracellular effects. These hormones bind to receptors that are coupled to an inhibitory (G_{α_i}) rather than a stimulatory (G_{α_s}) G protein. cAMP is perhaps the most widely distributed second messenger and has been shown to mediate various cellular responses to both hormonal and nonhormonal stimuli, not only in higher organisms but also in various primitive life forms, including slime molds and yeasts. The intracellular signal provided by cAMP is rapidly terminated by its hydrolysis to 5'AMP by a group of enzymes known as **phosphodiesterases**, which are also regulated by hormones in some instances.

Protein Kinase A Is the Major Mediator of the Signaling Effects of cAMP

cAMP activates an enzyme, **protein kinase A** (or **cAMP-dependent protein kinase**), which in turn catalyzes the phosphorylation of various cellular proteins, ion channels, and

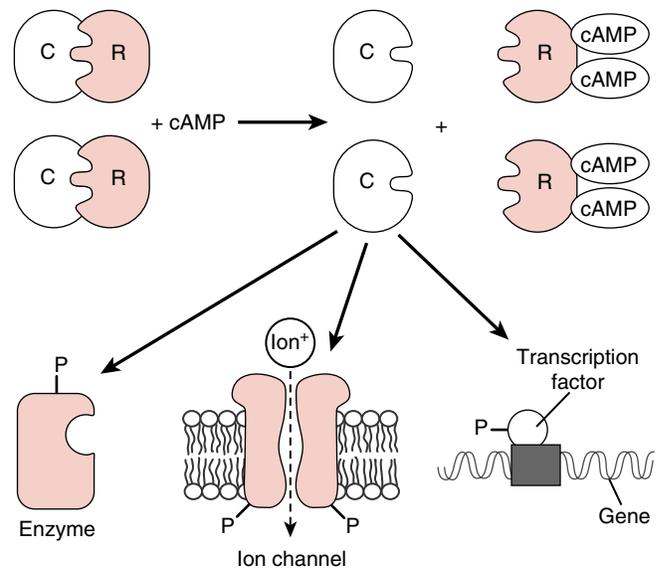


FIGURE 1.14 Activation and targets of protein kinase A. Inactive protein kinase A consists of two regulatory subunits complexed with two catalytic subunits. Activation of adenylyl cyclase results in increased cytosolic levels of cAMP. Two molecules of cAMP bind to each of the regulatory subunits, leading to the release of the active catalytic subunits. These subunits can then phosphorylate target enzymes, ion channels, or transcription factors, resulting in a cellular response. R, regulatory subunit; C, catalytic subunit; P, phosphate group.

transcription factors. This phosphorylation alters the activity or function of the target proteins and ultimately leads to a desired cellular response. However, in addition to activating protein kinase A and phosphorylating target proteins, in some cell types, cAMP directly binds to and affects the activity of ion channels.

Protein kinase A consists of catalytic and regulatory subunits, with the kinase activity residing in the catalytic subunit. When cAMP concentrations in the cell are low, two regulatory subunits bind to and inactivate two catalytic subunits, forming an inactive tetramer (Fig. 1.14). When cAMP is formed in response to hormonal stimulation, two molecules of cAMP bind to each of the regulatory subunits, causing them to dissociate from the catalytic subunits. This relieves the inhibition of catalytic subunits and allows them to catalyze the phosphorylation of target substrates and produce the resultant biological response to the hormone (see Fig. 1.14).

cGMP Is an Important Second Messenger in Smooth Muscle and Sensory Cells

cGMP, a second messenger similar and parallel to cAMP, is formed, much like cAMP, by the enzyme **guanylyl cyclase**. Although the full role of cGMP as a second messenger is not as well understood, its importance is finally being appreciated with respect to signal transduction in sensory tissues (see Chapter 4) and smooth muscle tissues (see Chapters 9 and 16).

One reason for its less apparent role is that few substrates for **cGMP-dependent protein kinase**, the main target of

cGMP production, are known. The production of cGMP is mainly regulated by the activation of a cytoplasmic form of guanylyl cyclase, a target of the paracrine mediator nitric oxide (NO) that is produced by endothelial as well as other cell types and can mediate smooth muscle relaxation (see Chapter 16). Atrial natriuretic peptide and guanylin (an intestinal hormone) also use cGMP as a second messenger, and in these cases, the plasma membrane receptors for these hormones express guanylyl cyclase activity.

Second Messengers 1,2-Diacylglycerol (DAG) and Inositol Trisphosphate (IP₃) Are Generated by the Hydrolysis of Phosphatidylinositol 4,5-Bisphosphate (PIP₂)

Some G-protein-coupled receptors are coupled to a different effector enzyme, **phospholipase C (PLC)**, which is localized to the inner leaflet of the plasma membrane. Similar to other GPCRs, binding of ligand or agonist to the receptor results in activation of the associated G protein, usually G α_q (or G q). Depending on the isoform of the G protein associated with the receptor, either the α or the $\beta\gamma$ subunit may stimulate PLC. Stimulation of PLC results in the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP₂), into 1,2-diacylglycerol (DAG) and inositol trisphosphate (IP₃). Both DAG and IP₃ serve as second messengers in the cell (Fig. 1.15).

In its second messenger role, DAG accumulates in the plasma membrane and activates the membrane-bound calcium- and lipid-sensitive enzyme **protein kinase C** (see Fig. 1.15). When activated, this enzyme catalyzes the phosphorylation of specific proteins, including other enzymes and transcription factors, in the cell to produce appropriate physiological effects, such as cell proliferation. Several tumor-promoting phorbol esters that mimic the structure of DAG have been shown to activate protein kinase C. They can, therefore, bypass the receptor by passing through the plasma membrane and directly activating protein kinase C, causing the phosphorylation of downstream targets to result in cellular proliferation.

IP₃ promotes the release of calcium ions into the cytoplasm by activation of endoplasmic or sarcoplasmic reticulum IP₃-gated calcium release channels (see Chapter 9). The concentration of free calcium ions in the cytoplasm of most cells is in the range of 10⁻⁷M. With appropriate stimulation, the concentration may abruptly increase 1,000 times or more. The resulting increase in free cytoplasmic calcium synergizes with the action of DAG in the activation of some forms of protein kinase C and may also activate many other calcium-dependent processes.

Mechanisms exist to reverse the effects of DAG and IP₃ by rapidly removing them from the cytoplasm. The IP₃ is dephosphorylated to inositol, which can be reused for phosphoinositide synthesis. The DAG is converted to phosphatidic acid by the addition of a phosphate group to carbon number 3. Like inositol, phosphatidic acid can be used for the resynthesis of membrane inositol phospholipids (see Fig. 1.15). On removal of the IP₃ signal, calcium is quickly pumped back into its storage sites, restoring cytoplasmic calcium concentrations to their low prestimulus levels.

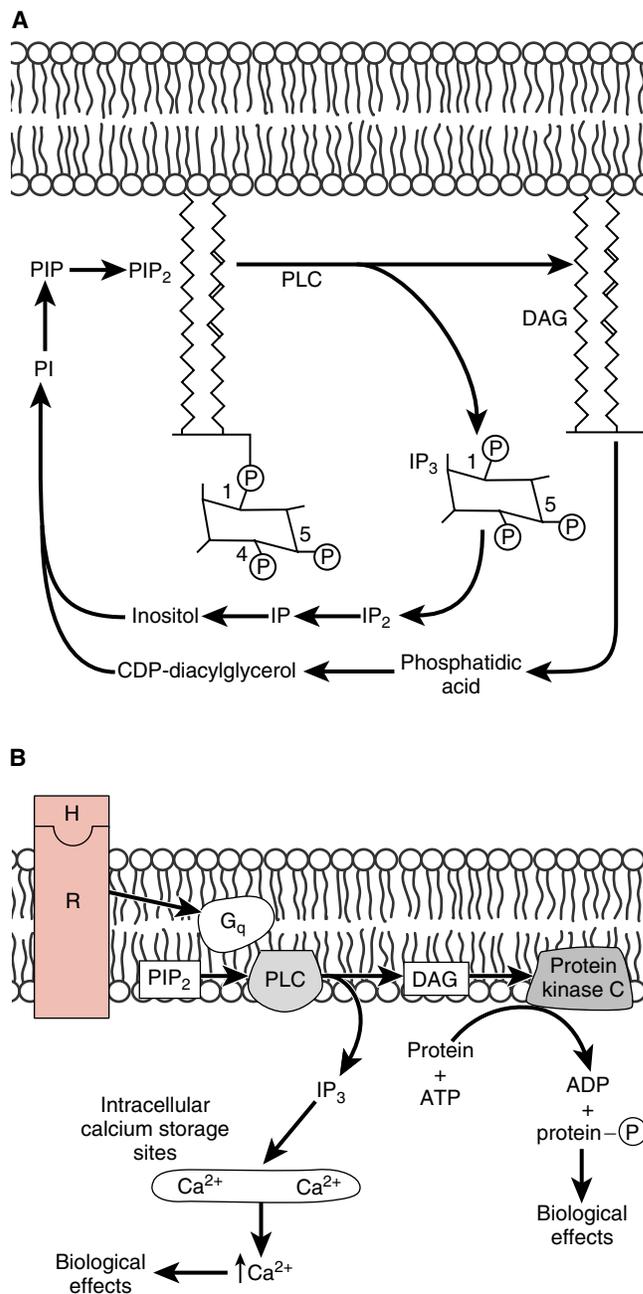


FIGURE 1.15 The phosphatidylinositol second messenger system. **A**, The pathway leading to the generation of inositol trisphosphate and diacylglycerol. The successive phosphorylation of phosphatidylinositol (PI) leads to the generation of phosphatidylinositol 4,5-bisphosphate (PIP₂). Phospholipase C (PLC) catalyzes the breakdown of PIP₂ to inositol trisphosphate (IP₃) and diacylglycerol (DAG), which are used for signaling and can be recycled to generate phosphatidylinositol. **B**, The generation of IP₃ and DAG and their intracellular signaling roles. The binding of hormone (H) to a G-protein-coupled receptor (R) can lead to the activation of PLC. In this case, the G α subunit is G q , a G protein that couples receptors to PLC. The activation of PLC results in the cleavage of PIP₂ to IP₃ and DAG. IP₃ interacts with calcium release channels in the endoplasmic reticulum, causing the release of calcium to the cytoplasm. Increased intracellular calcium can lead to the activation of calcium-dependent enzymes. An accumulation of DAG in the plasma membrane leads to the activation of the calcium- and phospholipid-dependent enzyme protein kinase C and phosphorylation of its downstream targets.

In addition to IP_3 , other, perhaps more potent phosphoinositols, such as IP_4 or IP_5 , may also be produced in response to stimulation. These are formed by the hydrolysis of appropriate phosphatidylinositol phosphate precursors found in the cell membrane. The precise role of these phosphoinositols is unknown. Evidence suggests that the hydrolysis of other phospholipids, such as phosphatidylcholine, may play an analogous role in hormone-signaling processes.

Cells Use Calcium as a Second Messenger by Keeping Resting Intracellular Calcium Levels Low

The levels of cytosolic calcium in an unstimulated cell are about 10,000 times less (10^{-7} M versus 10^{-3} M) than in the extracellular fluid. This large gradient of calcium is maintained by the limited permeability of the plasma membrane to calcium, by calcium transporters in the plasma membrane that extrude calcium, by calcium pumps in intracellular organelles, and by cytoplasmic and organellar proteins that bind calcium to buffer its free cytoplasmic concentration. Several plasma membrane ion channels serve to increase cytosolic calcium levels. Either these ion channels are voltage-gated and open when the plasma membrane depolarizes, or they may be controlled by phosphorylation by protein kinase A or protein kinase C.

In addition to the plasma membrane ion channels, the endoplasmic reticulum has two other main types of ion channels that, when activated, release calcium into the cytoplasm, causing an increase in cytoplasmic calcium. The small water-soluble molecule IP_3 activates the IP_3 -gated calcium release channel in the endoplasmic reticulum. The activated channel opens to allow calcium to flow down a concentration gradient into the cytoplasm. The IP_3 -gated channels are structurally similar to the second type of calcium release channel, the **ryanodine receptor**, found in the sarcoplasmic reticulum of muscle cells. Ryanodine receptors release calcium to trigger muscle contraction when an action potential invades the transverse tubule system of skeletal or cardiac muscle fibers (see Chapter 8). Both types of channels are regulated by positive feedback, in which the released cytosolic calcium can bind to the receptor to enhance further calcium release. This causes the calcium to be released suddenly in a spike, followed by a wave-like flow of the ion throughout the cytoplasm.

Increasing cytosolic free calcium activates many different signaling pathways and leads to numerous physiological events, such as muscle contraction, neurotransmitter secretion, and cytoskeletal polymerization. Calcium acts as a second messenger in two ways:

- It binds directly to an effector molecule, such as protein kinase C, to participate in its activation.
- It binds to an intermediary cytosolic calcium-binding protein, such as calmodulin.

Calmodulin is a small protein (16 kDa) with four binding sites for calcium. The binding of calcium to calmodulin causes calmodulin to undergo a dramatic conformational change and increases the affinity of this intracellular calcium "receptor" for its effectors (Fig. 1.16). Calcium-calmodulin complexes bind to and activate a variety of cellular proteins, including protein kinases that are important in many physiological processes, such as smooth muscle

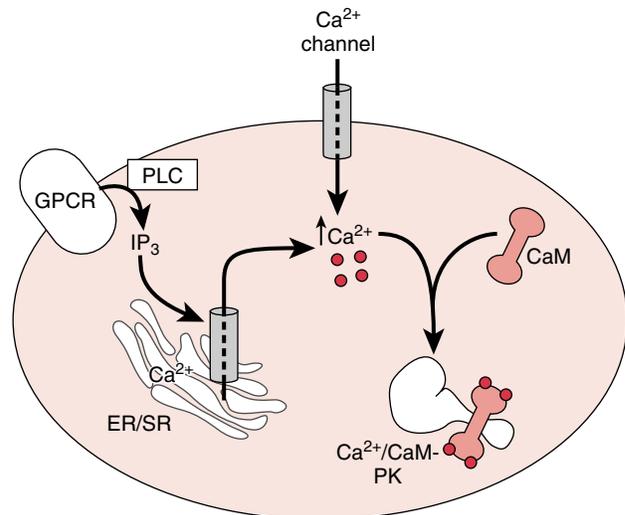


FIGURE 1.16 The role of calcium in intracellular signaling and activation of calcium-calmodulin-dependent protein kinases.

Levels of intracellular calcium are regulated by membrane-bound ion channels that allow the entry of calcium from the extracellular space or release calcium from internal stores (e.g., endoplasmic reticulum, sarcoplasmic reticulum in muscle cells, and mitochondria). Calcium can also be released from intracellular stores via the G-protein-mediated activation of PLC and the generation of IP_3 . IP_3 causes the release of calcium from the endoplasmic or sarcoplasmic reticulum in muscle cells by interaction with calcium ion channels. When intracellular calcium rises, four calcium ions complex with the dumbbell-shaped calmodulin protein (CaM) to induce a conformational change. Ca^{2+} /CaM can then bind to a spectrum of target proteins including Ca^{2+} /CaM-PKs, which then phosphorylate other substrates, leading to a response. IP_3 , inositol trisphosphate; PLC, phospholipase C; CaM, calmodulin; Ca^{2+} /CaM-PK, calcium-calmodulin-dependent protein kinases; ER/SR, endoplasmic/sarcoplasmic reticulum.

contraction (myosin light-chain kinase; see Chapter 9) and hormone synthesis (aldosterone synthesis; see Chapter 34), and ultimately result in altered cellular function.

Two mechanisms operate to terminate calcium action. The IP_3 generated by the activation of PLC can be dephosphorylated and, thus, inactivated by cellular phosphatases. In addition, the calcium that enters the cytosol can be rapidly removed. The plasma membrane, endoplasmic reticulum, sarcoplasmic reticulum, and mitochondrial membranes all have ATP-driven calcium pumps that drive the free calcium out of the cytosol to the extracellular space or into an intracellular organelle. Lowering cytosolic calcium concentrations shifts the equilibrium in favor of the release of calcium from calmodulin. Calmodulin then dissociates from the various proteins that were activated, and the cell returns to its basal state.

INTRACELLULAR RECEPTORS AND HORMONE SIGNALING

The intracellular receptors, in contrast to the plasma membrane-bound receptors, can be located in either the cytosol or the nucleus and are distinguished by their mode of acti-

vation and function. The ligands for these receptors must be lipid soluble because of the plasma membranes that must be traversed for the ligand to reach its receptor. The main result of activation of the intracellular receptors is altered gene expression.

Steroid and Thyroid Hormone Receptors Are Intracellular Receptors Located in the Cytoplasm or Nucleus

For the activation of intracellular receptors to occur, ligands must cross the plasma membrane. The hormone ligands that belong to this group include the steroids (e.g., estradiol, testosterone, progesterone, cortisone, and aldosterone), 1,25-dihydroxy vitamin D₃, thyroid hormone, and retinoids. These hormones are typically delivered to their target cells bound to specific carrier proteins. Because of their lipid solubility, these hormones freely diffuse through both plasma and nuclear membranes. These hormones bind to specific receptors that reside either in the cytoplasm or the nucleus. Steroid hormone receptors are located in the cytoplasm and are usually found complexed with other proteins that maintain the receptor in an inactive conformation. In contrast, the thyroid hormones and retinoic acid bind to receptors that are already bound to response elements in the DNA of specific genes. The unoccupied receptors are inactive until the hormone binds, and they serve as repressors in the absence of hormone. These receptors are discussed in Chapters 31 and 33. The model of steroid hormone action shown in Figure 1.17 is generally applicable to all steroid and thyroid hormones.

All **steroid hormone receptors** have similar structures, with three main domains. The N-terminal regulatory domain regulates the transcriptional activity of the receptor and may have sites for phosphorylation by protein kinases that may also be involved in modifying the transcriptional activity of the receptor. There is a centrally located DNA-binding domain and a carboxyl-terminal hormone-binding and dimerization domain. When hormones bind, the hormone-receptor complex moves to the nucleus, where it binds to specific DNA sequences in the gene regulatory (promoter) region of specific hormone-responsive genes. The targeted DNA sequence in the promoter is called a **hormone response element (HRE)**. Binding of the hormone-receptor complex to the HRE can either activate or repress transcription. The end result of stimulation by steroid hormones is a change in the readout or transcription of the genome. While most effects involve increased production of specific proteins, repressed production of certain proteins by steroid hormones can also occur. These newly synthesized proteins and/or enzymes will affect cellular metabolism with responses attributable to that particular steroid hormone.

Hormones Bound to Their Receptors Regulate Gene Expression

The interaction of hormone and receptor leads to the activation (or transformation) of receptors into forms with

an increased affinity for binding to specific HRE or acceptor sites on the chromosomes. The molecular basis of activation *in vivo* is unknown but appears to involve a decrease in apparent molecular weight or in the aggregation state of receptors, as determined by density gradient centrifugation. The binding of hormone-receptor complexes to chromatin results in alterations in RNA polymerase activity that lead to either increased or decreased transcription of specific portions of the genome. As a result, mRNA is produced, leading to the production of new cellular proteins or changes in the rates of synthesis of pre-existing proteins (see Fig. 1.17).

The molecular mechanism of steroid hormone-receptor activation and/or transformation, how the hormone-receptor complex activates transcription, and how the hormone-receptor complex recognizes specific response elements of the genome are not well understood but are under active investigation. Steroid hormone receptors are also known to undergo phosphorylation/dephosphorylation reactions. The effect of this covalent modification is also an area of active research.

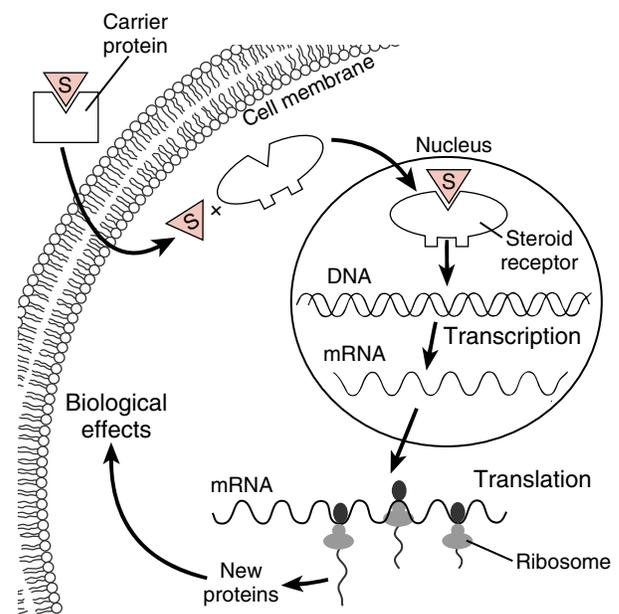


FIGURE 1.17 The general mechanism of action of steroid hormones.

Steroid hormones (S) are lipid soluble and pass through the plasma membrane, where they bind to a cognate receptor in the cytoplasm. The steroid hormone-receptor complex then moves to the nucleus and binds to a hormone response element in the promoter-regulatory region of specific hormone-responsive genes. Binding of the steroid hormone-receptor complex to the response element initiates transcription of the gene, to form messenger RNA (mRNA). The mRNA moves to the cytoplasm, where it is translated into a protein that participates in a cellular response. Thyroid hormones are thought to act by a similar mechanism, although their receptors are already bound to a hormone response element, repressing gene expression. The thyroid hormone-receptor complex forms directly in the nucleus and results in the activation of transcription from the thyroid hormone-responsive gene.

REVIEW QUESTIONS

DIRECTIONS: Each of the numbered items or incomplete statements in this section is followed by answers or by completions of the statement. Select the ONE lettered answer or completion that is BEST in each case.

- If a region or compartment is in a steady state with respect to a particular substance, then
 - The amount of the substance in the compartment is increasing
 - The amount of the substance in the compartment is decreasing
 - The amount of the substance in the compartment does not change with respect to time
 - There is no movement into or out of the compartment
 - The compartment must be in equilibrium with its surroundings
 - A 62-year-old woman eats a high carbohydrate meal. Her plasma glucose concentration rises, and this results in increased insulin secretion from the pancreatic islet cells. The insulin response is an example of
 - Chemical equilibrium
 - End-product inhibition
 - Feedforward control
 - Negative feedback
 - Positive feedback
 - In animal models of autosomal recessive polycystic kidney disease, epidermal growth factor (EGF) receptors may be abnormally expressed on the urine side of kidney epithelial cells and may be stimulated by EGF in the urine, causing excessive cell proliferation and formation of numerous kidney cysts. What type of drug might be useful in treating this condition?
 - Adenylyl cyclase stimulator
 - EGF agonist
 - Phosphatase inhibitor
 - Phosphodiesterase inhibitor
 - Tyrosine kinase inhibitor
 - Second messengers
 - Are extracellular ligands
 - Are always available for signal transduction
 - Always produce the same cellular response
 - Include nucleotides, ions, and gases
 - Are produced only by tyrosine kinase receptors
 - The second messengers cyclic AMP and cyclic GMP
 - Activate the same signal transduction pathways
 - Are generated by the activation of cyclases
 - Activate the same protein kinase
 - Are important only in sensory transduction
 - Can activate phospholipase C
 - Binding of estrogen to its steroid hormone receptor
 - Stimulates the GTPase activity of the trimeric G protein coupled to the estrogen receptor
 - Stimulates the activation of the IP₃ receptor in the sarcoplasmic reticulum to increase intracellular calcium
 - Stimulates phosphorylation of tyrosine residues in the cytoplasmic domain of the receptor
 - Stimulates the movement of the hormone-receptor complex to the nucleus to cause gene activation
 - Stimulates the activation of the MAP kinase pathway and results in the regulation of several transcription factors
 - A single cell within a culture of freshly isolated cardiac muscle cells is injected with a fluorescent dye that cannot cross cell membranes. Within minutes, several adjacent cells become fluorescent. The most likely explanation for this observation is the presence of
 - Ryanodine receptors
 - IP₃ receptors
 - Transverse tubules
 - Desmosomes
 - Gap junctions
 - Many signaling pathways involve the generation of inositol trisphosphate (IP₃) and diacylglycerol (DAG). These molecules
 - Are first messengers
 - Activate phospholipase C
 - Can activate tyrosine kinase receptors
 - Can activate calcium calmodulin-dependent protein kinases
 - Are derived from PIP₂
 - Tyrosine kinase receptors
 - Have constitutively active tyrosine kinase domains
 - Phosphorylate and activate ras directly
 - Mediate cellular processes involved in growth and differentiation
 - Are not phosphorylated upon activation
 - Are monomeric receptors upon activation
10. A pituitary tumor is removed from a 40-year-old man with acromegaly resulting from excessive secretion of growth hormone. It is known that G proteins and adenylyl cyclase normally mediate the stimulation of growth hormone secretion produced by growth hormone-releasing hormone (GHRH). Which of the following problems is most likely to be present in the patient's tumor cells?
- Adenylyl cyclase activity is abnormally low
 - The G α_s subunit is unable to hydrolyze GTP
 - The G α_s subunit is inactivated
 - The G α_s subunit is activated
 - The cells lack GHRH receptors

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