3

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

LEARNING OBJECTIVES

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

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

Cell-to-Cell Communication

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

IN THE CLINIC

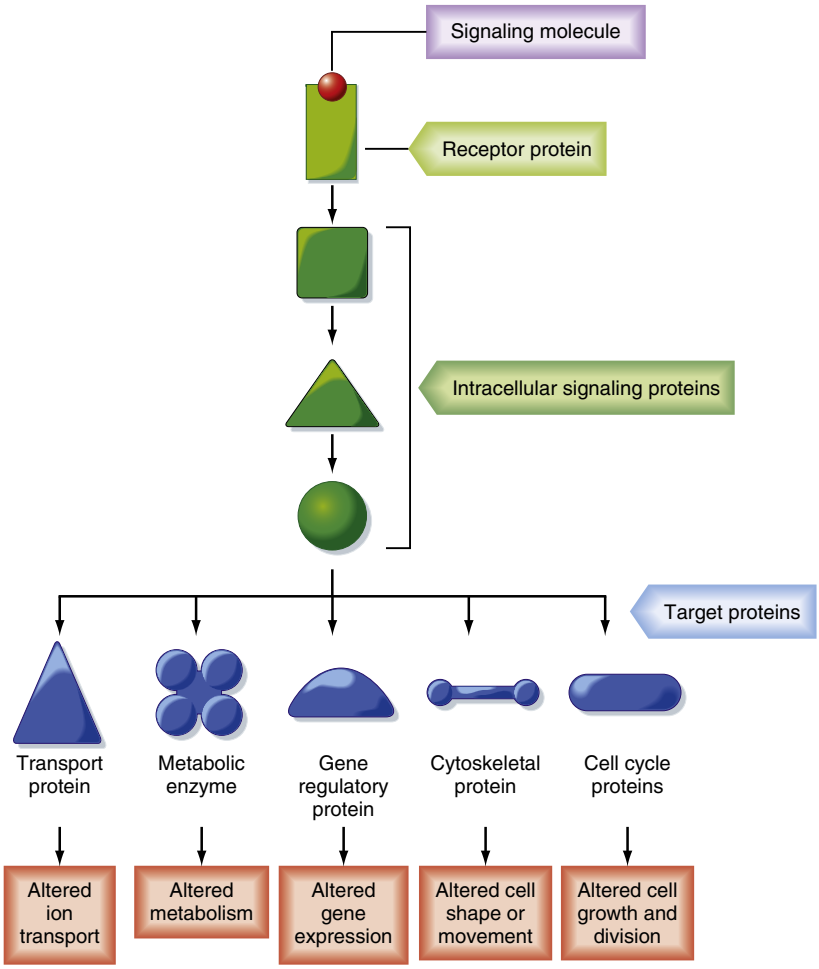
The significance of signaling pathways in medicine is illustrated by the following short list of popular drugs that act by regulating signaling pathways. Details on these pathways are presented later in this and other chapters.

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

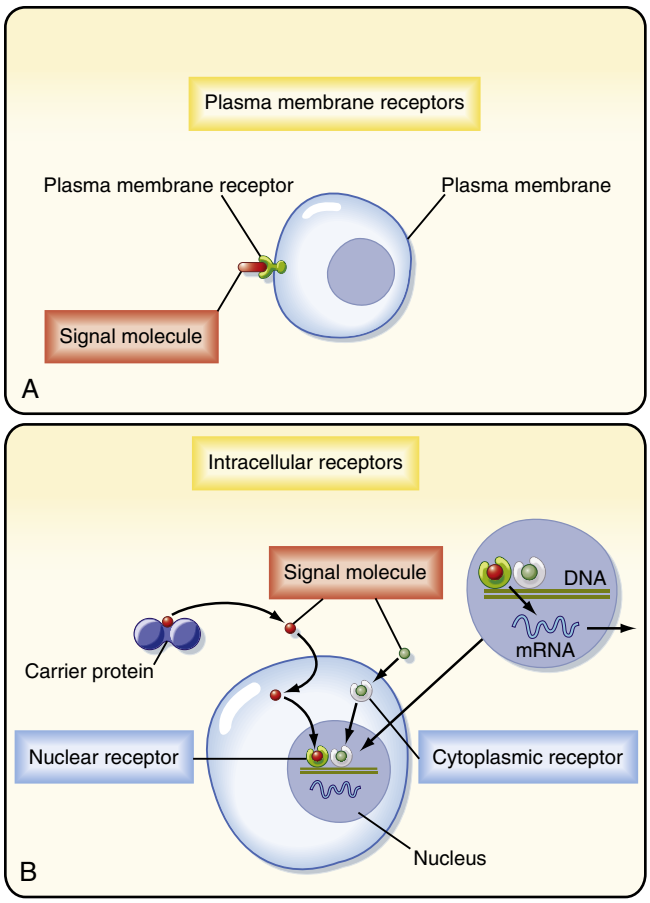
of how cells communicate follows. Readers who desire a more in-depth presentation of this material are encouraged to consult one of the many cellular and molecular biology textbooks currently available.

Cells in higher animals release into the extracellular space hundreds of chemicals, including (1) **peptides and proteins** (e.g., insulin); **(2) amines** (e.g., epinephrine and norepinephrine); (3) **steroid hormones** (e.g., aldosterone, estrogen); and (4) **small molecules,** including amino acids, nucleotides, ions (e.g., Ca^{++}), and gases, such as nitric oxide and carbon dioxide. Secretion of signaling molecules is cell-type specific. For example, beta cells in the pancreas release insulin, which stimulates glucose uptake into cells. The ability of a cell to respond to a specific signaling molecule depends on the expression of receptors that bind the signaling molecule with high affinity and specificity. Receptors are located in the plasma membrane, the cytosol, and the nucleus (Fig. 3.2).

Signaling molecules can act over long or short distances and can require cell-to-cell contact or very close cellular proximity (Fig. 3.3). **Contact-dependent signaling**, in which a membrane-bound signaling molecule of one cell binds directly to a plasma membrane receptor of another cell, is important during development, in immune responses, and in cancer (see Fig. 3.3*A*). Molecules that are released and act locally are called **paracrine** (see Fig. 3.3*B*) or **autocrine** (see Fig. 3.3*C*) **hormones**. Paracrine signals are released by one type of cell and act on another type; they are usually taken up by target cells or rapidly degraded (within minutes) by enzymes. For example, enterochromaffin-like cells in the stomach secrete histamine, which stimulates the production of acid by neighboring parietal cells (see Chapter 27 for details). Autocrine signaling involves the release of a molecule that affects the same cell or other cells of the same type (e.g., cancer cells). In **synaptic signaling**



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



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

(see Fig. 3.3*D*), neurons transmit electrical signals along their axons and release neurotransmitters at synapses that affect the function of other neurons or cells that are distant from the neuron cell body. The close physical relationship between the nerve terminal and the target cell ensures that the neurotransmitter is delivered to a specific cell. Details on synaptic signaling are discussed in Chapter 6. **Endocrine** signals are hormones that are secreted into the blood and are widely dispersed in the body (see Fig. 3.3*E*). Details on endocrine signaling are discussed in Chapter 38.

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

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

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

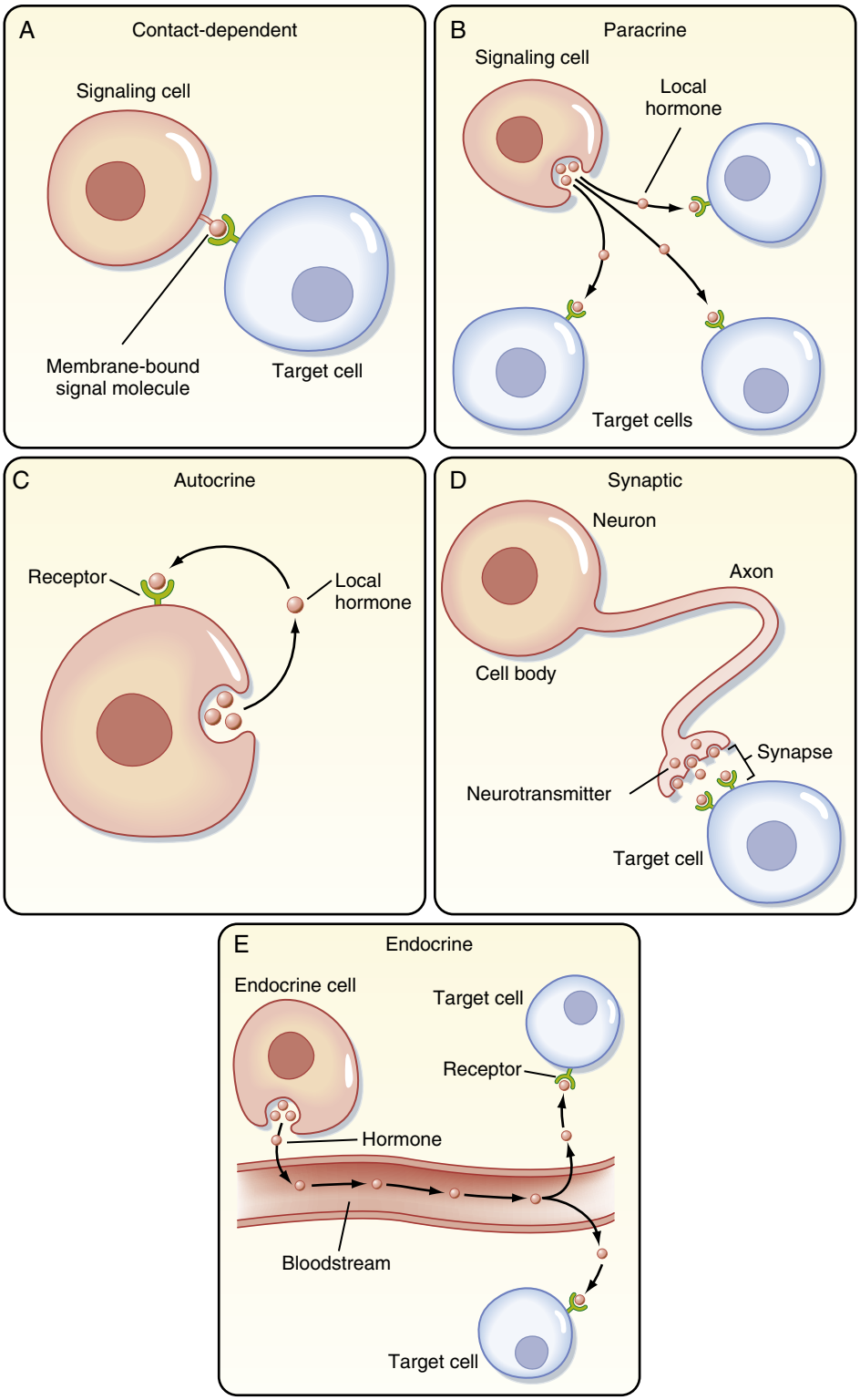
Receptors

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

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

GPCRs regulate the activity of other proteins, such as enzymes and ion channels (see Fig. 3.4*B*). In the example in Fig. 3.4*B*, the interaction between the receptor and the target protein is mediated by heterotrimeric G proteins,

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



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

TABLE 3.1 Classes of Membrane Receptors

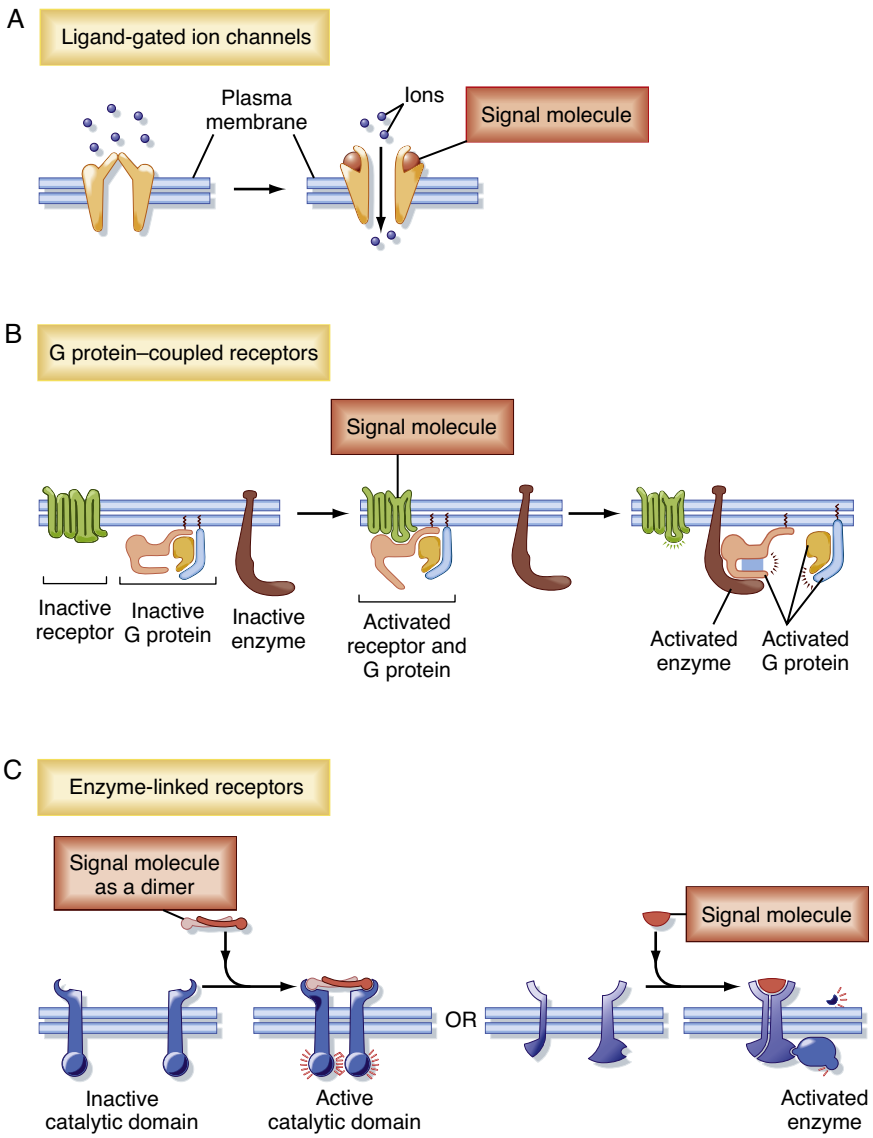
Receptor Class	Ligand	Signal Transduction Pathway/Target
Ligand-gated ion channels	**Extracellular ligand:**	**Membrane currents:**
	GABA	**Cl^-**
	ACh (muscle)	**Na^+, K^+, Ca^{++}**
	ATP	**Ca^{++}, Na^+, K^+**
	Glutamate: NMDA	**Na^+, K^+, Ca^{++}**
	Intracellular ligand:	
	cAMP (olfaction)	**K^+**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

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

which are composed of α, β, and γ subunits. Stimulation of G proteins by ligand-bound receptors activates or inhibits downstream target proteins that regulate signaling pathways if the target protein is an enzyme or changes membrane ion permeability if the target protein is an ion channel.

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

Nuclear receptors are small hydrophobic molecules, including steroid hormones, thyroid hormones, retinoids, and vitamin D, that have a long biological half-life (hours to days), diffuse across the plasma membrane, and bind to nuclear receptors or to cytoplasmic receptors that, once bound to their ligand, translocate to the nucleus (see Fig. 3.5). Some nuclear receptors, such as those that bind cortisol and aldosterone, are located in the cytosol and enter the nucleus after binding to hormone, whereas other receptors, including the thyroid hormone receptor, are located in the nucleus. In both cases, inactive receptors are bound to inhibitory proteins, and binding of hormone results in dissociation of the inhibitory complex. Hormone binding causes the receptor to bind coactivator proteins that activate gene transcription. Once activated, the hormone-receptor complex regulates the transcription of specific genes. Activation of specific genes usually occurs in two steps: an early primary response (≈30 minutes), which activates genes that stimulate other genes to produce a delayed (hours to days) secondary response (see Fig. 3.5). Each hormone elicits a specific response that is based on cellular expression of the cognate receptor, as well as on cell type–specific expression of gene regulatory proteins that interact with the activated receptor to regulate the transcription of a specific set of



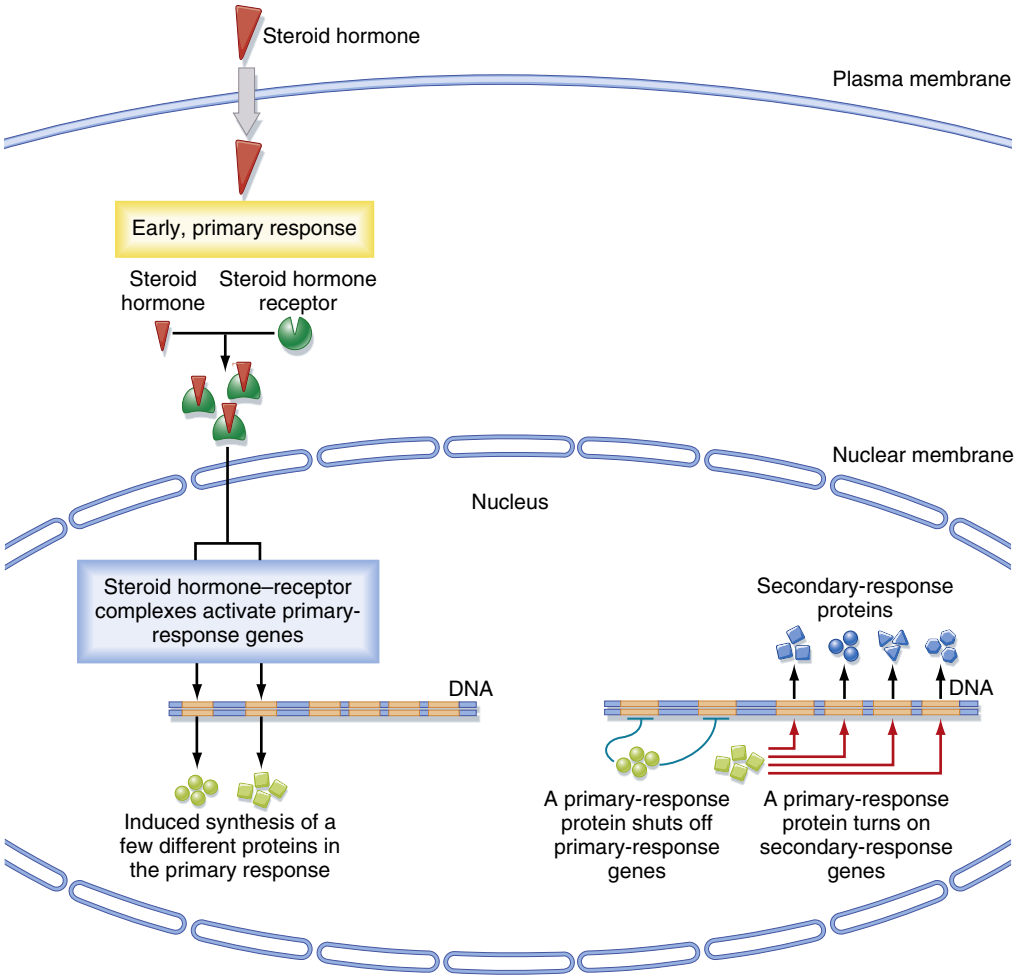
• **Fig. 3.4** Three of the four classes of plasma membrane receptors. See text for details. (Redrawn from Alberts B, et al. *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

genes (see Chapter 38 for more details). In addition to steroid receptors that regulate gene expression, evidence also suggests the existence of membrane and juxtamembrane steroid receptors that mediate the rapid, nongenomic effects of steroid hormones.

Some membrane proteins do not fit the classic definition of receptors, but they subserve a receptor-like function in that they recognize extracellular signals and transduce the signals into an intracellular second messenger that has a biological effect. For example, on activation by a ligand, some membrane proteins undergo **regulated intramembrane proteolysis (RIP)**, which elaborates a cytosolic peptide fragment that enters the nucleus and regulates gene expression (Fig. 3.6). In this signaling pathway, binding of ligand to a plasma membrane receptor leads to ectodomain shedding, facilitated by members of the metalloproteinase-disintegrin family, and produces a carboxy-terminal fragment that is the substrate for γ-secretase. γ-Secretase induces RIP, thereby causing the release of an intracellular domain of the protein that enters the nucleus and regulates transcription (see Fig. 3.6). The best characterized example of RIP is the sterol regulatory element–binding protein (SREBP), a transmembrane protein expressed in the membrane of the endoplasmic reticulum. When cellular cholesterol levels are low, SREBP undergoes RIP, and the proteolytically cleaved fragment is translocated into the nucleus, where it transcriptionally activates genes that promote cholesterol biosynthesis.

Receptors and Signal Transduction Pathways

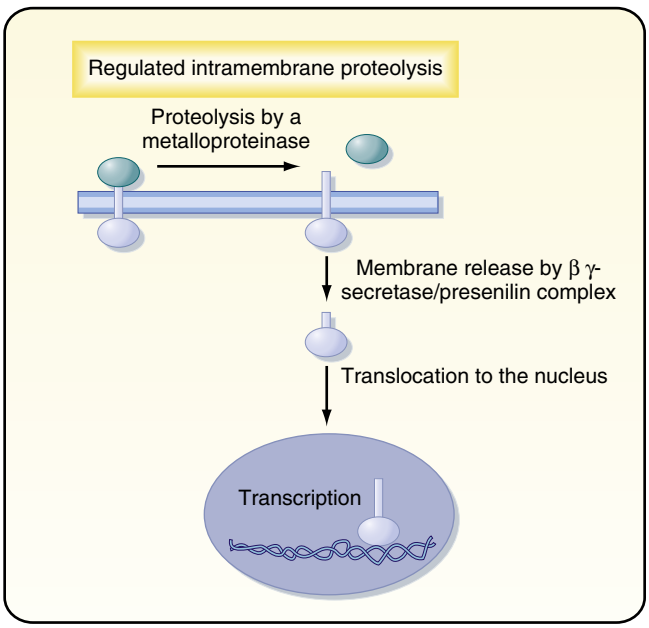
When hormones bind to plasma membrane receptors, signals are relayed to effector proteins via intracellular signaling pathways. When hormones bind to nuclear or cytosolic



• **Fig. 3.5** Steroid hormones stimulate the transcription of early-response genes and late-response genes. See text for details. (Redrawn from Alberts B, et al. *Molecular Biology of Cell.* 6th ed. New York: Garland Science; 2015.)

IN THE CLINIC

Alzheimer's disease, a progressive neurodegenerative brain disease characterized by the formation of amyloid plaques, affects approximately 44 million people worldwide. In Alzheimer's disease, regulated intramembrane proteolysis of amyloid β-protein precursor (APP) causes the accumulation of amyloid β-protein (Aβ), which forms amyloid plaques that are thought to contribute to the pathogenesis of Alzheimer's disease. APP is a type I transmembrane protein (i.e., its spans the membrane only once). After ectodomain shedding, its sequential proteolysis by β-secretase and γ-secretase produces the Aβ40 and Aβ42 peptides that are normally produced throughout life but accumulate in individuals with Alzheimer's disease. Missense mutations in presenilins, proteins that regulate γ-secretase protease activity, enhance the production of Aβ42, which is more hydrophobic and prone to aggregation into amyloid fibrils than is the more abundant Aβ40 protein.



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

receptors, they relay signals primarily through regulation of gene expression. Signaling pathways can amplify and integrate signals but can also downregulate and desensitize

signals, reducing or terminating the response, even in the continued presence of hormone.

Intracellular signaling molecules—so-called second messengers (the first messenger of the signal is the ligand that binds to the receptor)—include small molecules such as cAMP, cGMP, Ca^{++}, and diacylglycerol. Signaling pathways often include dozens of small molecules that form complicated networks within the cell (Fig. 3.7). Some proteins in the intracellular signaling pathways relay the signal by passing the message directly to another protein (e.g., by phosphorylating a target, or by binding and causing an allosteric change). Such intracellular signaling proteins act as **reversible molecular switches**: When a signal is received, they switch from an inactive to an active form or vice versa, until another signaling molecule reverses the process. This principle of reversibility is central to many signaling pathways. In many cases, activation is achieved by reversing inhibition: For example, the thyroid hormone receptor is bound to an inhibitory protein in the absence of signal.

Signaling complexes, composed of multiple proteins that interact physically, enhance the speed, efficiency, and specificity of signaling. Many proteins, usually enzymes or ion channels, transduce the signal into a different chemical form and simultaneously amplify the signal either by producing large amounts of additional signaling molecules or by activating a large number of downstream signaling proteins. For example, adenylyl cyclase, the enzyme that makes cAMP, transduces a signal (receptor activation of G proteins) and amplifies the signal by generating large amounts of cAMP. Other types of signaling proteins include those that integrate multiple signals. Other proteins carry the signal from one region of the cell to another: for example, by translocating from the cytosol to the nucleus.

Cells can respond quickly and in a graded manner to increasing concentrations of hormone, and the effect of a signaling molecule can be either long- or short-lived. Cells can also adjust their sensitivity to a signal by **desensitization,** whereby prolonged exposure to a hormone decreases the cell's response over time. Desensitization is a reversible process that can involve a reduction in the number of receptors expressed in the plasma membrane, inactivation of receptors, or changes in signaling proteins that mediate the downstream effect of the receptors. Homologous desensitization involves a reduction in the response only to the signaling molecule that caused the response (e.g., opioid dependence and tolerance), whereas heterologous desensitization is when one ligand desensitizes the response to another ligand.

Table 3.1 summarizes the four general classes of receptors and provides a few examples of the signal transduction pathways associated with each class of receptors.

Ligand-Gated Ion Channel Signal Transduction Pathways

This class of receptors transduces a chemical signal into an electrical signal, which elicits a response. For example, the ryanodine receptor, located in the membrane of the sarcoplasmic reticulum of skeletal muscle, is activated by Ca^{++}, caffeine, adenosine triphosphate (ATP), or metabolites of arachidonic acid to release Ca^{++} into the cytosol, which facilitates muscle contraction (see Chapter 12 for details). In glutamatergic synapses in which high levels of prior synaptic activity have led to partial membrane depolarization, activation of the *N*-methyl-D-aspartate receptor by glutamate stimulates Ca^{++} influx important for synaptic plasticity.

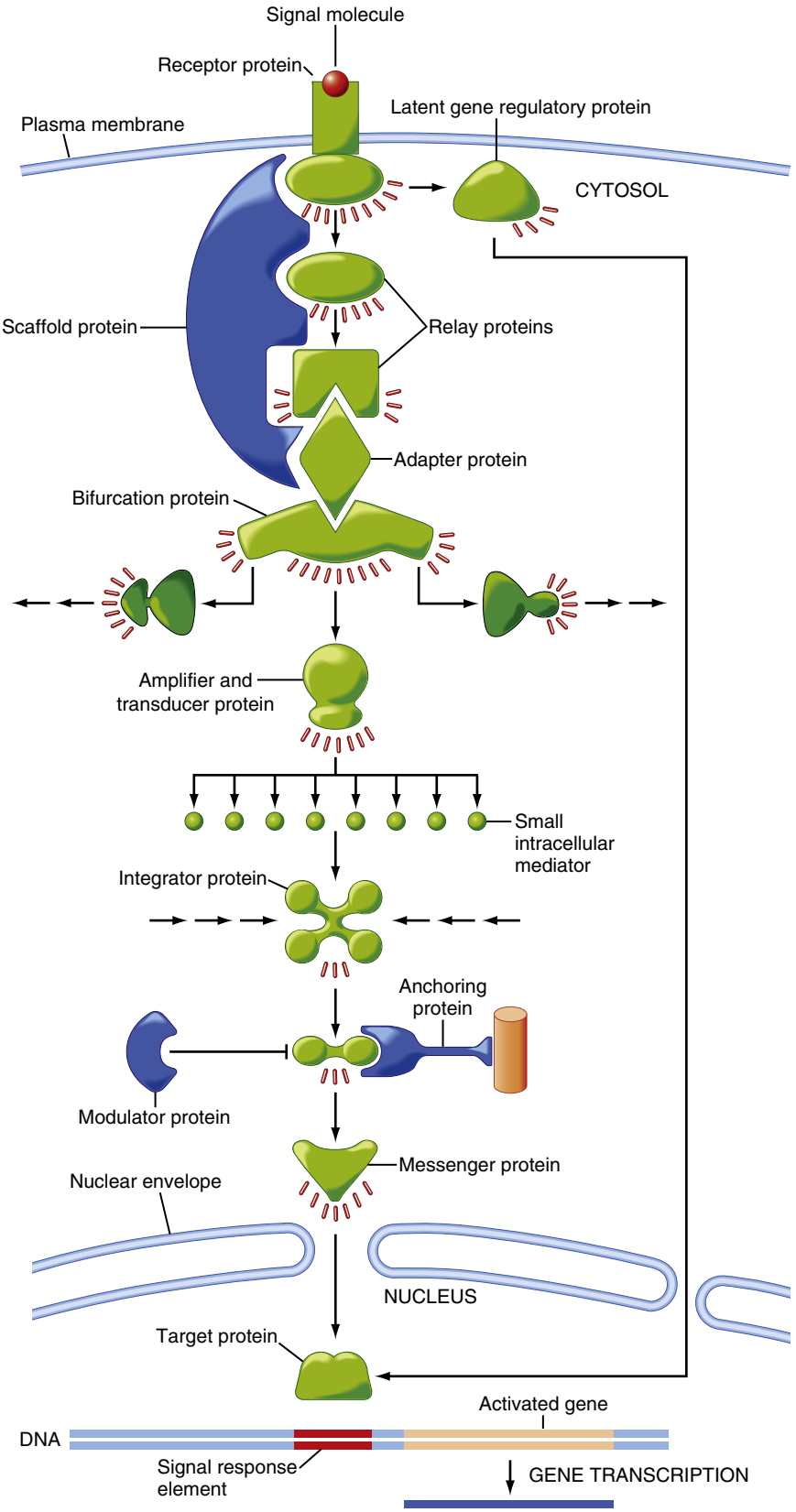
G Protein–Coupled Signal Transduction Pathways

There are two classes of **GTP-binding proteins** (i.e., GTPases, which are named for their ability to hydrolyze GTP to guanosine diphosphate [GDP] and an inorganic phosphate): low-molecular-weight, **monomeric G proteins** and **heterotrimeric G proteins** composed of α, β, and γ subunits. GTP binding activates, whereas hydrolysis of GTP to GDP inactivates, GTP-binding proteins (Fig. 3.8*A*). All GTPases are controlled by regulatory proteins, including **GTPase-activating proteins,** which induce the hydrolysis of GTP to GDP and thus inactivate the GTPase, and **guanine nucleotide exchange factors (GEFs)** that cause the GTPase to release GDP, which is rapidly replaced by GTP, thereby activating the GTPase (see Fig. 3.8*B*).

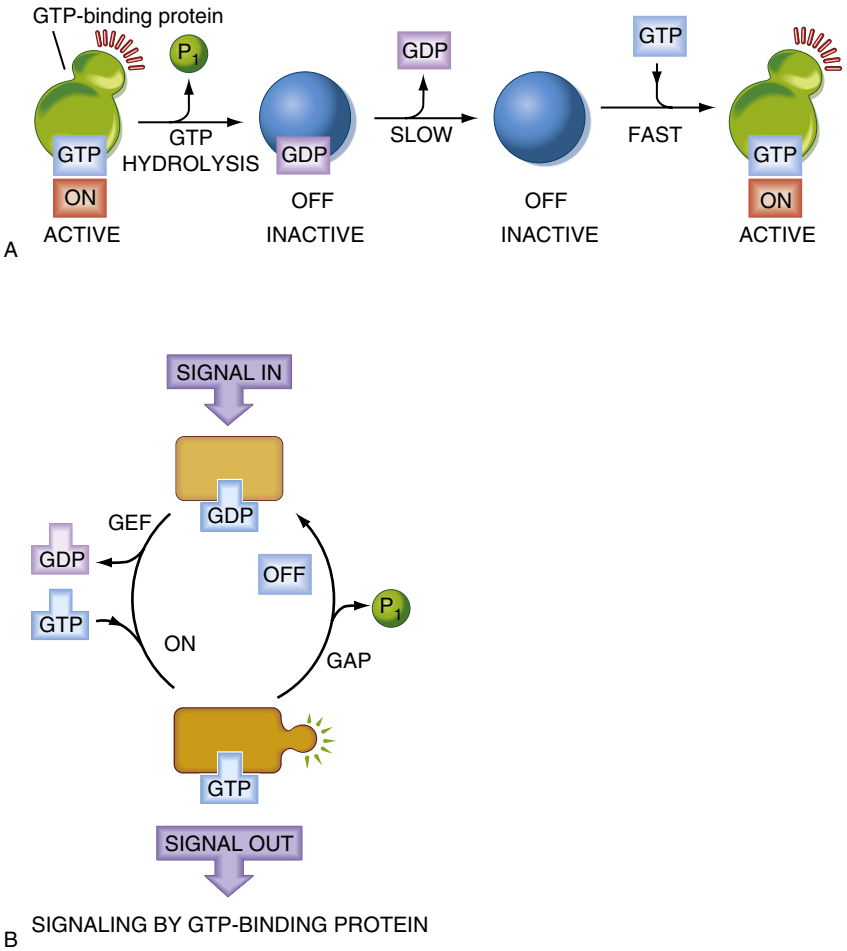
Monomeric G proteins are composed of a single 20- to 40-kDa protein and can be membrane bound because of the addition of lipids post-translationally. Monomeric G proteins have been classified into five families (Ras, Rho, Rab, Ran, and Arf), play a central role in many enzyme-linked receptor pathways, and regulate gene expression and cell proliferation, differentiation, and survival. Rho GTPases regulate actin cytoskeletal organization, cell cycle progression, and gene expression. The Rab GTPases regulate intravesicular transport and trafficking of proteins between organelles in the secretory and endocytic pathways. Ran GTPases regulate nucleocytoplasmic transport of RNA and proteins. Ras GTPases are involved in many signaling pathways that control cell division, proliferation, and death. Arf GTPases, like Rab GTPases, regulate vesicular transport.

Heterotrimeric G proteins couple to more than 1000 different receptors and thereby mediate the cellular response to an incredibly diverse set of signaling molecules, including hormones, neurotransmitters, peptides, and odorants. Like monomeric G proteins, they can be membrane bound because of the addition of lipids post-translationally. Heterotrimeric complexes are composed of three subunits: α, β, and γ. There exist 16 α subunits, 5 β subunits, and 12 γ subunits, which can assemble into hundreds of different combinations and thereby interact with a diverse number of receptors and effectors. The assembly of subunits and the association with receptors and effectors depend on the cell type.

An overview of heterotrimeric G protein activation is illustrated in Fig. 3.9. In the absence of ligand, these



• **Fig. 3.7** Illustration of how intracellular signals are amplified and integrated. Signaling pathways often include dozens of proteins and small molecules that form complicated networks within the cell. Some signaling proteins relay the signal by passing the message to another protein. Many proteins amplify the signal either by producing large amounts of additional signaling molecules or by activating a large number of downstream signaling proteins. Other proteins carry the signal from one region of the cell to another. See text for more details. (Redrawn from Alberts B, et al. *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

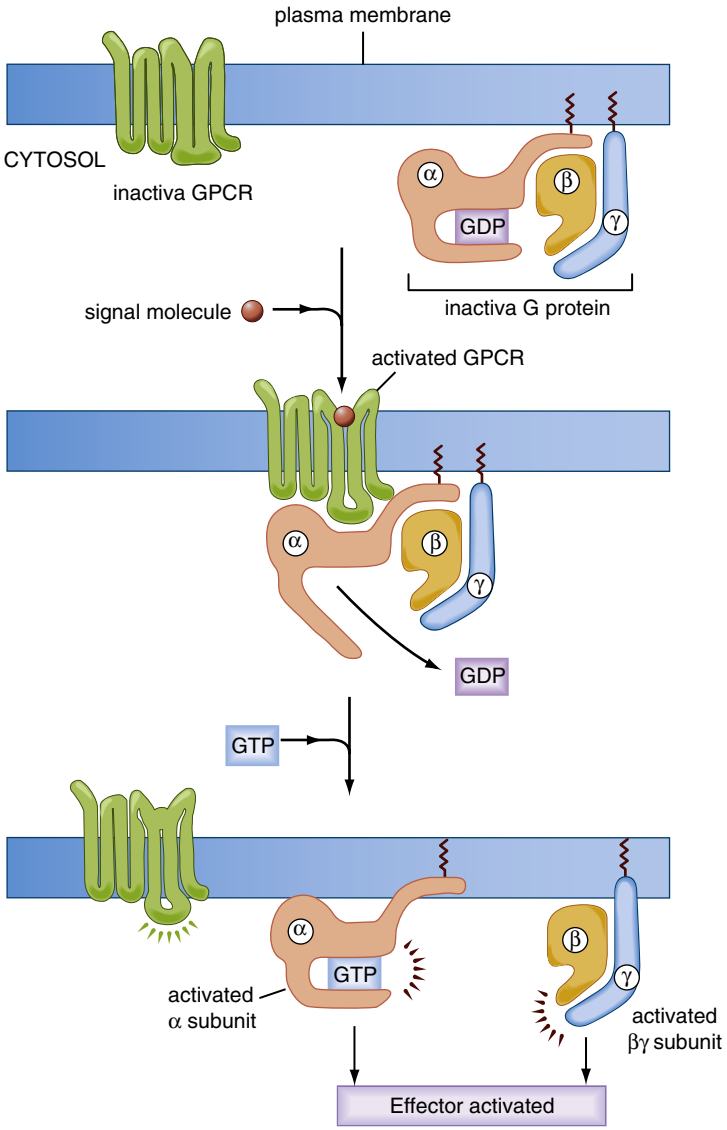


• **Fig. 3.8** GTP-binding proteins. GTP binding activates whereas hydrolysis of GTP to GDP inactivates GTP-binding proteins **(A)**. All GTPases are controlled by regulatory proteins, including GTPase-activating proteins (GAP), which induce the hydrolysis of GTP to GDP, thus inactivating the GTPase, and guanine nucleotide exchange factors (GEF), which cause the GTPase to release GDP, which is rapidly replaced by GTP, thereby activating the GTPase **(B)**. (Redrawn from Kantrowitx ER, Lipscomb WN. Escherichia coli aspartate transcarbamoylase: the molecular basis for a concerted allosteric transition. *Trends Biochem Sci.* 1990;15:53-59.)

G proteins are inactive and form a heterotrimeric complex in which GDP binds to the α subunit. Binding of a signal molecule to an inactive GPCR induces a conformational change in the G protein that promotes the release of GDP and the subsequent binding of GTP to the α subunit. Binding of GTP to the α subunit stimulates dissociation of the α subunit from the heterotrimeric complex and results in release of the α subunit from the βγ dimer, each of which can interact with and regulate downstream effectors such as adenylyl cyclase and phospholipases (see Fig. 3.9). Activation of downstream effectors by the α subunit and βγ dimer is terminated when the α subunit hydrolyzes the bound GTP to GDP and inorganic phosphate (P_i). The α subunit bound to GDP associates with the βγ dimer and terminates the activation of effectors.

Another way to attenuate or terminate signaling through a GPCR involves desensitization and endocytic removal of receptors from the plasma membrane. Binding of hormone to a GPCR increases the ability of **GPCR kinases** to phosphorylate the intracellular domain of GPCRs, which recruits proteins called **β-arrestins** to bind to the GPCR. β-Arrestins inactivate the receptor and promote endocytic removal of the GPCR from the plasma membrane. GPCR kinase/β-arrestin inactivation with endocytosis of GPCRs is an important mechanism whereby cells downregulate (desensitize) a response during prolonged exposure to elevated hormone levels. One of the major benefits of β blockers, given for congestive heart failure, is that they reverse chronic desensitization and the associated recovery of adrenergic responsiveness.

Activated G protein α subunits couple to a variety of effector proteins, including adenylyl cyclase, **phosphodiesterases,** and **phospholipases** (A_2, C, and D). A very common downstream effector of heterotrimeric G proteins is adenylyl cyclase, which facilitates the conversion of ATP to cAMP (Fig. 3.10). When a signal molecule binds to a GPCR that interacts with a G protein composed of an α subunit of the α_s class, adenylyl cyclase is activated, which

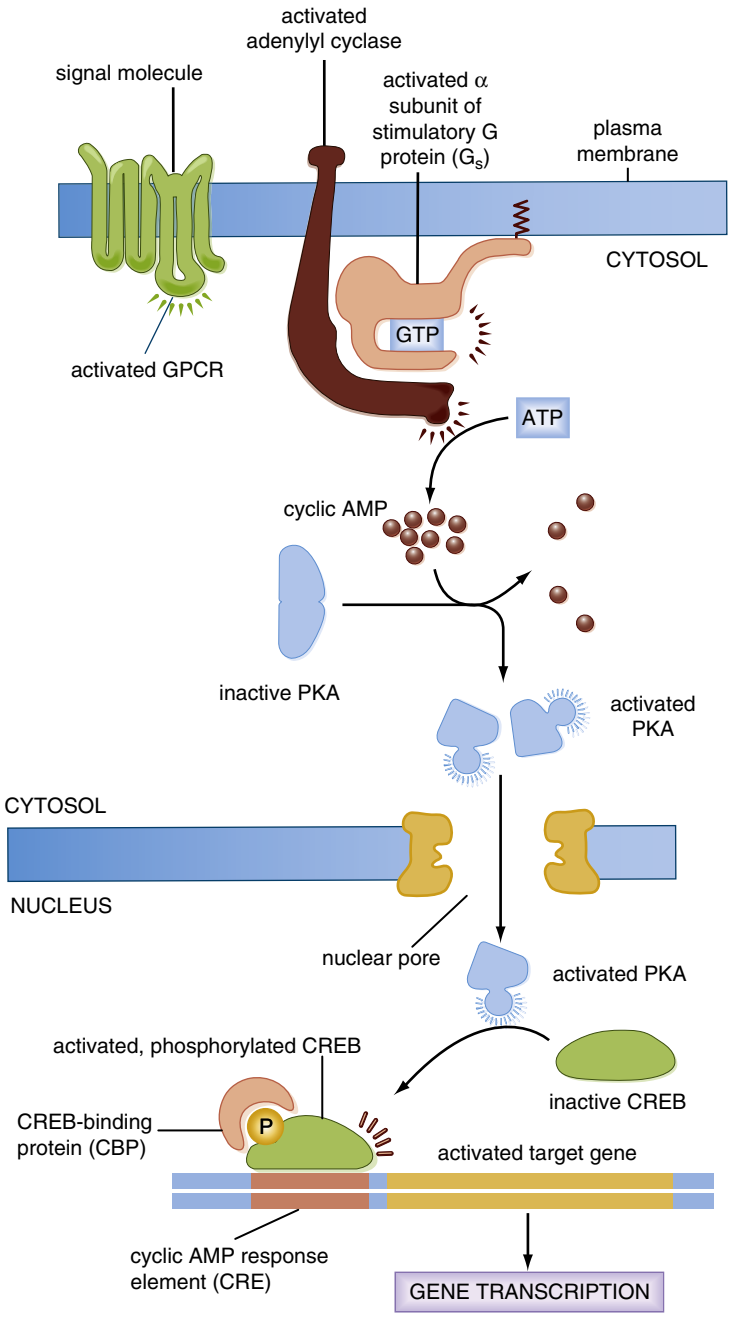


• **Fig. 3.9** Activation of a G protein–coupled receptor (GPCR) and effector activation. In the absence of ligand, heterotrimeric G proteins are in an inactive state because GDP binds to the α subunit. Binding of a signal molecule to an inactive GPCR induces a conformational change in the G protein that promotes the release of GDP and the subsequent binding of GTP to the α subunit. Binding of GTP to the α subunit stimulates dissociation of the α subunit from the heterotrimeric complex and results in release of the α subunit from the βγ dimer, each of which can interact with and regulate downstream effectors. (Redrawn from Alberts B, et al. *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

causes an increase in cAMP levels and, as a result, activation of **protein kinase A (PKA).** By phosphorylating specific serine and threonine residues on downstream effector proteins, PKA regulates effector protein activity. In contrast, when a ligand binds to a receptor that interacts with a G protein composed of an α subunit of the α_i class, adenylyl cyclase is inhibited, which causes reductions in cAMP levels and, consequently, in PKA activity.

Some effector proteins, such as ion-gated channels, are also regulated directly by cAMP. cAMP is degraded to AMP by cAMP phosphodiesterases, which are inhibited by caffeine and other methylxanthines. Thus by interfering with a constitutive "off" signal, caffeine can prolong a cellular response mediated by cAMP and PKA. Because these effects target existing proteins, they can be extremely rapid (e.g., adrenaline response). In addition to cytoplasmic signaling, the catalytic subunit of PKA can enter the nucleus of cells and phosphorylate and activate the transcription factor **cAMP response element–binding (CREB) protein** (see Fig. 3.10). Phospho-CREB protein increases the transcription of many genes, which can in turn produce a distinct set of responses with much slower kinetics. Hence, cAMP has many cellular effects, including direct and indirect effects mediated by PKA.

Heterotrimeric G proteins also regulate phototransduction. In rod cells in the eye, absorption of light by



• **Fig. 3.10** GPCR stimulation of adenylyl cyclase, cAMP, and protein kinase A (PKA). Binding of a signal molecule to a GPCR mediates Gs stimulation of adenylyl cyclase, which increases cytosolic cAMP, which in turn activates PKA. Activated PKA phosphorylates a number of target proteins to elicit many effects. PKA also enters the nucleus where it phosphorylates CREB (cyclic adenosine monophosphate [cyclic AMP] response element–binding protein). Phosphorylated CREB recruits coactivator CBP, which stimulates gene transcription. *GPCR*, G Protein–Coupled Receptor. (Redrawn from Alberts B, et al. *Molecular Biology of the Cell.* 6th ed. New York: Garland Science; 2015.)

rhodopsin activates the G protein transducin, which via the α_t subunit activates cGMP phosphodiesterase. Activation of this phosphodiesterase lowers the concentration of cGMP and thereby closes a cGMP-activated cation channel. The ensuing change in cation channel activity alters the membrane voltage. The exquisite sensitivity of rods to light—rods can detect a single photon of light—is due to the abundance of rhodopsin in rods and amplification of the signal (a photon of light) by the G protein–cGMP phosphodiesterase–cGMP channel signaling pathway (see Chapter 8 for more details).

Heterotrimeric G proteins also regulate **phospholipases**, a family of enzymes that modulate a variety of signaling pathways. Ligands that activate receptors that are coupled to the α_q subunit stimulate phospholipase C, an enzyme that converts phosphatidylinositol 4,5-biphosphate to inositol 1,4,5-triphosphate (InsP3) and diacylglycerol. InsP3 is a second messenger that diffuses to the endoplasmic reticulum, where it

IN THE CLINIC

Cholera toxin, secreted by ***Vibrio cholera***, catalyzes the ADP ribosylation of the G-protein α_s subunit, which inhibits the GTPase activity of α_s. Thus α_s remains in its activated, GTP-bound state, which in turn causes activation of adenylyl cyclase and an increase in cAMP/PKA levels. In the intestines, elevated levels of PKA increase cystic fibrosis transmembrane conductance regulator (CFTR)–mediated chloride secretion, which leads to secretory diarrhea and extensive loss of fluids, characteristic of cholera. ***Bordetella pertussis***, the bacterium that causes whooping cough, secretes pertussis toxin, which ADP ribosylates the α_i subunit. In this case, the ribosylation inactivates α_i, reducing the inhibition of adenylyl cyclase and thus also leading to increased levels of cAMP/PKA.

activates a ligand-activated Ca^{++} channel to release Ca^{++} into the cytosol, whereas diacylglycerol activates protein kinase C, which phosphorylates effector proteins. As noted earlier, both Ca^{++} and protein kinase C influence effector proteins, as well as other signaling pathways, to elicit responses.

Ligand binding to GPCRs can also activate **phospholipase A_2**, an enzyme that releases arachidonic acid from membrane phospholipids. **Arachidonic acid**, which can also be released from diacylglycerol via an indirect pathway, can be released from cells and thereby regulate neighboring cells or stimulate inflammation. It can also be retained within cells, where it is incorporated into the plasma membrane or is metabolized in the cytosol to form intracellular second messengers that affect the activity of enzymes and ion channels. In one pathway, cytosolic **cyclooxygenases** facilitate the metabolism of arachidonic acid to prostaglandins, thromboxanes, and prostacyclins. Prostaglandins mediate aggregation of platelets, cause constriction of the airways, and induce inflammation. Thromboxanes also induce platelet aggregation and constrict blood vessels, whereas prostacyclin inhibits platelet aggregation and causes dilation of blood vessels. In a second pathway of arachidonic acid metabolism, the enzyme 5-lipoxygenase initiates the conversion of arachidonic acid to **leukotrienes**, which participate in allergic and inflammatory responses, including those causing asthma, rheumatoid arthritis, and inflammatory bowel disease. The third pathway of arachidonic acid metabolism is initiated by epoxygenase, an enzyme that facilitates the generation of hydroxyeicosatetraenoic acid (HETE) and *cis*-epoxyeicosatrienoic acid (*cis*-EET). HETE and *cis*-EET and their metabolites increase release of Ca^{++} from the endoplasmic reticulum, stimulate cell proliferation, and regulate inflammatory responses.

Ca^{++} is also an intracellular messenger that elicits cellular effects via Ca^{++}-binding proteins, most notably **calmodulin** (CaM). When Ca^{++} binds to CaM, its conformation is altered, and the structural change in CaM allows it to bind to and regulate other signaling proteins, including cAMP phosphodiesterase, an enzyme that degrades cAMP to AMP, which is inactive and unable to activate PKA. By binding to **CaM-dependent kinases**, CaM also phosphorylates specific serine and threonine residues in many proteins, including myosin light-chain kinase, which facilitates smooth muscle contraction (see Chapter 14).

Protein Phosphatases and Phosphodiesterases Counteract the Activation of Cyclic Nucleotide Kinases

There are two ways to terminate a signal initiated by cAMP and cGMP: enhancing degradation of these cyclic nucleotides by phosphodiesterases and dephosphorylation of effectors by protein **phosphatases**. Phosphodiesterases facilitate the breakdown of cAMP and cGMP to AMP and GMP, respectively, and are activated by ligand activation of GPCRs. Phosphatases dephosphorylate effector proteins that were phosphorylated by kinases such as PKA. The balance between kinase-mediated phosphorylation and phosphatase-mediated dephosphorylation allows rapid and exquisite regulation of the phosphorylated state and thus the activity of signaling proteins.

Enzyme Receptor–Linked Signal Transduction Pathways

There are several classes of receptors that have enzymatic activity or are intimately associated with proteins that have enzymatic activity. Four of these classes are discussed next, including receptors that mediate the cellular responses to atrial natriuretic peptide (ANP) and nitric oxide **(guanylyl cyclase receptors)**; transforming growth factor-β (TGF-β; **threonine/serine kinase receptors**); EGF, platelet-derived growth factor (PDGF), and insulin **(tyrosine kinase receptors)**; and interleukins **(tyrosine kinase–associated receptors)**.

Guanylyl Cyclase Receptors

ANP binds to the extracellular domain of the plasma membrane receptor guanylyl cyclase and induces a conformational change in the receptor that causes receptor dimerization and activation of guanylyl cyclase, which metabolizes GTP to cGMP. cGMP activates **cGMP-dependent protein kinase,** which phosphorylates proteins on specific serine and threonine residues. In the kidney, ANP inhibits reabsorption of sodium and water by the collecting duct.

Nitric oxide activates a soluble receptor guanylyl cyclase that converts GTP to cGMP, which relaxes smooth muscle. Because nitroglycerin increases blood concentrations of nitric oxide, which increases cGMP and thereby relaxes smooth muscle in coronary arteries, it has long been used to treat **angina pectoris** (i.e., chest pain caused by inadequate blood flow to heart muscle; see Chapter 17).

Threonine/Serine Kinase Receptors

The TGF-β receptor is a threonine/serine kinase that has two subunits. Binding of TGF-β to the type II subunit

IN THE CLINIC

There are two isoforms of cyclooxygenase: COX1 and COX2. When activated in endothelial cells, COX1 facilitates the production of prostacyclins, which inhibit blood clots. In vascular smooth muscle cells and platelets, COX1 facilitates the production of thromboxane A_2, which is prothrombotic (i.e., promotes blood clots). Thus cardiovascular health depends in part on the balance between prostacyclins and thromboxane A_2, generated by distinct cell types. Low doses of aspirin, a nonsteroidal anti-inflammatory drug (NSAID), reduce thromboxane A_2 production by platelets with little effect on endothelial prostacyclin production. Thus low-dose aspirin is antithrombotic (i.e., reduces blood clots). COX2 is activated by inflammatory stimuli. Thus the ability of NSAIDs (e.g., aspirin, ibuprofen, naproxen, acetaminophen, indomethacin) to suppress the inflammatory response is due to inhibition of COX2. Both COX1 and COX2 facilitate the production of prostanoids that protect the stomach. Several lines of evidence suggest that both COX1 and COX2 must be inhibited to elicit damage to the gastrointestinal tract. Consequently, the negative effects of NSAIDs on the gastric mucosa (e.g., increased incidence of gastrointestinal bleeding) are most likely due to inhibition of COX1 and COX2 by these nonselective COX inhibitors.

Selective COX2 inhibitors (e.g., Celecoxib, Rofecoxib) are very effective in selectively inhibiting COX2 and are used extensively to reduce the inflammatory response. Because COX2 inhibitors are thought to lack the negative effects elicited by NSAIDs on the gastrointestinal tract, their use has increased dramatically. However, in 2005, the U.S. Food and Drug Administration (FDA) announced that selective COX2 inhibitors, as well as nonselective NSAIDs, increase the risk for heart attacks and strokes and required that COX2-selective and nonselective NSAIDs carry a warning label on product packaging that highlighted the potential for the increased risk for adverse cardiovascular events and stroke. In addition, although much evidence suggests that COX2-selective inhibitors do not cause gastrointestinal bleeding, in 2005 the FDA also required the pharmaceutical industry to add to the warning label on COX2-selective drugs a caution about the potential for increased risk for gastrointestinal bleeding. In 2015, the FDA strengthened warnings that both COX2-selective and COX2-nonselective NSAIDs increase the risk of heart attacks and strokes.[b]

[b]See U.S. Food and Drug Administration. FDA Drug Safety Communication: FDA Strengthens Warning That Non-aspirin Nonsteroidal Anti-inflammatory Drugs (NSAIDs) Can Cause Heart Attacks or Strokes 2015. Accessed July 9, 2022. http://www.fda.gov/Drugs/DrugSafety/ucm451800.htm

induces it to phosphorylate the type I subunit on specific serine and threonine residues, which in turn phosphorylates other downstream effector proteins on serine and threonine residues and thereby elicits cellular responses, including cell growth, cell differentiation, and apoptosis.

Tyrosine Kinase Receptors

There are two classes of tyrosine kinase receptors. Nerve growth factor (NGF) receptors are typical examples of one class. Ligand binding to two NGF receptors facilitates their dimerization and thus enables the cytoplasmic tyrosine kinase domain of each monomer to phosphorylate and

AT THE CELLULAR LEVEL

Ras GTPases, monomeric G proteins, are involved in many signaling pathways that control cell division, proliferation, and death. Many mutations of proteins in the Ras signaling pathway are oncogenic (cancer causing) or inactivate tumor suppressors. Mutations in *Ras* genes that inhibit GTPase activity, as well as overexpression of Ras proteins as a result of transcriptional activation, lead to continuous cell proliferation, a major step in the development of cancer in many organs, including the pancreas, colon, and lungs. In addition, mutations in and overexpression of GEFs, which facilitate exchange of GTP for GDP, and GTPase-activating proteins, which accelerate GTP hydrolysis, may also be oncogenic (see Fig. 3.8*B*).

activate the other monomer. Once the other monomer is phosphorylated, the cytoplasmic domains can recruit GEFs such as growth factor receptor–bound protein 2 to the plasma membrane, which in turn activates Ras and downstream kinases that regulate gene transcription programs important for cell survival and proliferation.

Activation of the insulin receptor (which is tetrameric and composed of two α and two β subunits) by insulin is an example of the other type of tyrosine kinase receptor. Binding of insulin to the α subunits produces a conformational change that facilitates interaction between the two α and β pairs. Binding of insulin to its receptor causes autophosphorylation of tyrosine residues in the catalytic domains of the β subunits, and the activated receptor then phosphorylates cytoplasmic proteins to initiate its cellular effects, including stimulating the absorption of glucose from the blood into skeletal muscle and fat tissue.

Tyrosine Kinase–Associated Receptors

The tyrosine kinase–associated receptors have no intrinsic kinase activity but associate with proteins that do have tyrosine kinase activity, including tyrosine kinases of the Src family and Janus family. Receptors in this class bind several cytokines, including interleukin-6, a proinflammatory cytokine that is necessary for resistance to bacterial infections, and erythropoietin, which stimulates the production of red blood cells. Tyrosine kinase–associated receptor subunits assemble into homodimers (αα), heterodimers (αβ), or heterotrimers (αβγ) when ligands bind. Subunit assembly enhances the binding of tyrosine kinases, which induces kinase activity and thereby phosphorylates tyrosine residues on the kinases, as well as on the receptor. Most polypeptide growth factors bind to tyrosine kinase–associated receptors.

Regulation of Gene Expression by Signal Transduction Pathways

Steroid and thyroid hormones, cAMP, and receptor tyrosine kinases are transcription factors that regulate gene expression and thereby participate in signal transduction pathways.

This section discusses the regulation of gene expression by steroid and thyroid hormones, cAMP, and receptor tyrosine kinases.

Nuclear Receptor Signal Transduction Pathways

The family of nuclear receptors includes more than 30 genes and has been divided into two subfamilies on the basis of structure and mechanism of action: (1) steroid hormone receptors and (2) receptors that bind retinoic acid, thyroid hormones (iodothyronines), and vitamin D. When ligands bind to these receptors, the ligand-receptor complex activates transcription factors that bind to DNA and regulate the expression of genes (see Figs. 3.2*B*, 3.5, and 3.7).

The location of nuclear receptors varies. Glucocorticoid and mineralocorticoid receptors are located in the cytoplasm, where they interact with chaperones (i.e., heat shock proteins; see Fig. 3.2*B*). Binding of hormone to these receptors results in a conformational change that causes chaperones to dissociate from the receptor, thereby revealing a nuclear localization motif that facilitates translocation of the hormone-bound receptor complex to the nucleus. Estrogen and progesterone receptors are located primarily in the nucleus, and thyroid hormone and retinoic acid receptors are located in the nucleus bound to DNA.

When activated by hormone binding, nuclear receptors bind to specific DNA sequences in the regulatory regions of responsive genes called **hormone response elements.** Ligand-receptor binding to DNA causes a conformational change in DNA that initiates transcription. Nuclear receptors also regulate gene expression by acting as transcriptional repressors. For example, glucocorticoids suppress the **transcription activator protein-1 (AP-1)** and **nuclear factor κB**, which stimulate the expression of genes that cause inflammation. By this mechanism glucocorticoids reduce inflammation.

Cell-Surface Signal Transduction Pathways Control Gene Expression

As noted previously, cAMP is an important second messenger. In addition to its importance in activating PKA, which phosphorylates specific serine and threonine residues on proteins, cAMP stimulates the transcription of many genes, including those that code for hormones, including somatostatin, glucagon, and vasoactive intestinal polypeptide (see Fig. 3.10). Many genes activated by cAMP have a **cAMP response element (CRE)** in their DNA. Increases in cAMP stimulate PKA, which not only acts in the cytoplasm but also can translocate to the nucleus, where it phosphorylates **CREB** and thereby increases its affinity for **CREB-binding protein (CBP)**. The CREB-CBP complex activates transcription. The response is terminated when PKA phosphorylates a phosphatase that dephosphorylates CREB (see Fig. 3.10).

Many growth factors, including EGF, PDGF, NGF, and insulin, bind to and activate enzyme-linked receptors that have tyrosine kinase activity. Activation of tyrosine kinases initiates a cascade of events that enhance the activity of the small GTP-binding protein Ras, which in a series of steps and intermediary proteins phosphorylates the **mitogen-activated protein kinase**, which then translocates to the nucleus and stimulates transcription of genes that stimulate cell growth.

Tyrosine kinase–associated receptors, as noted earlier, are activated by a variety of hormones, including cytokines, growth hormone, and interferon. Although these receptors do not have tyrosine kinase activity, they are associated with **Janus family proteins**, which do have tyrosine kinase activity. Once activated, hormone tyrosine kinase–associated receptors activate Janus family protein, which phosphorylates latent transcription factors called **signal transducers and activators of transcription (STATs)**. When phosphorylated on tyrosine residues, STATs dimerize and then enter the nucleus and regulate transcription.

Key Points

1. The function of cells is tightly coordinated and integrated by external chemical signals, including hormones, neurotransmitters, growth factors, odorants, and products of cellular metabolism that serve as chemical messengers and provide cell-to-cell communication. Chemical and physical signals interact with receptors located in the plasma membrane, cytoplasm, and nucleus. Interaction of these signals with receptors initiates a cascade of events that mediate the response to each stimulus. These pathways ensure that the cellular response to external signals is specific, amplified, tightly regulated, and coordinated.
2. There are two classes of GTP-binding proteins: monomeric G proteins and heterotrimeric G proteins composed of α, β, and γ subunits. Monomeric G proteins regulate actin cytoskeleton organization, cell cycle progression, intracellular vesicular transport, and gene expression. Heterotrimeric G proteins regulate ion channels, adenylyl cyclase and the cAMP-PKA signaling pathway, phosphodiesterases (which also regulate cAMP and cGMP signaling pathways), and phospholipases, which regulate the production of prostaglandins, prostacyclins, and thromboxanes.
3. There are four subtypes of enzyme-linked receptors that mediate the cellular response to a wide variety of signals, including ANP, nitric oxide, TGF-β, PDGF, insulin, and interleukins.
4. There are two types of nuclear receptors: (1) one type that in the absence of ligand is located in the cytoplasm and when bound to ligand translocates to the nucleus and (2) another type that permanently resides in the nucleus. Both classes of receptors regulate gene transcription.