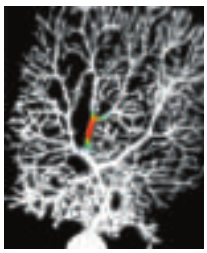
Chapter 7



Molecular Signaling within Neurons

Overview

As is apparent in the preceding chapters, electrical and chemical signaling mechanisms allow one nerve cell to receive and transmit information to another. This chapter focuses on the related events within neurons and other cells that are triggered by the interaction of a chemical signal with its receptor. This intracellular processing typically begins when extracellular chemical signals, such as neurotransmitters, hormones, and trophic factors, bind to specific receptors located either on the surface or within the cytoplasm or nucleus of the target cells. Such binding activates the receptors and in so doing stimulates cascades of intracellular reactions involving GTP-binding proteins, second messenger molecules, protein kinases, ion channels, and many other effector proteins whose modulation temporarily changes the physiological state of the target cell. These same intracellular signal transduction pathways can also cause longer-lasting changes by altering the transcription of genes, thus affecting the protein composition of the target cells on a more permanent basis. The large number of components involved in intracellular signaling pathways allows precise temporal and spatial control over the function of individual neurons, thereby allowing the coordination of electrical and chemical activity in the related populations of neurons that comprise neural circuits and systems.

Strategies of Molecular Signaling

Chemical communication coordinates the behavior of individual nerve and glial cells in physiological processes that range from neural differentiation to learning and memory. Indeed, molecular signaling ultimately mediates and modulates all brain functions. To carry out such communication, a series of extraordinarily diverse and complex chemical signaling pathways has evolved. The preceding chapters have described in some detail the electrical signaling mechanisms that allow neurons to generate action potentials for conduction of information. These chapters also described synaptic transmission, a special form of chemical signaling that transfers information from one neuron to another. Chemical signaling is not, however, limited to synapses (Figure 7.1A). Other well-characterized forms of chemical communication include **paracrine** signaling, which acts over a longer range than synaptic transmission and involves the secretion of chemical signals onto a group of nearby target cells, and **endocrine** signaling, which refers to the secretion of hormones into the bloodstream where they can affect targets throughout the body.

Chemical signaling of any sort requires three components: a molecular *signal* that transmits information from one cell to another, a *receptor* molecule

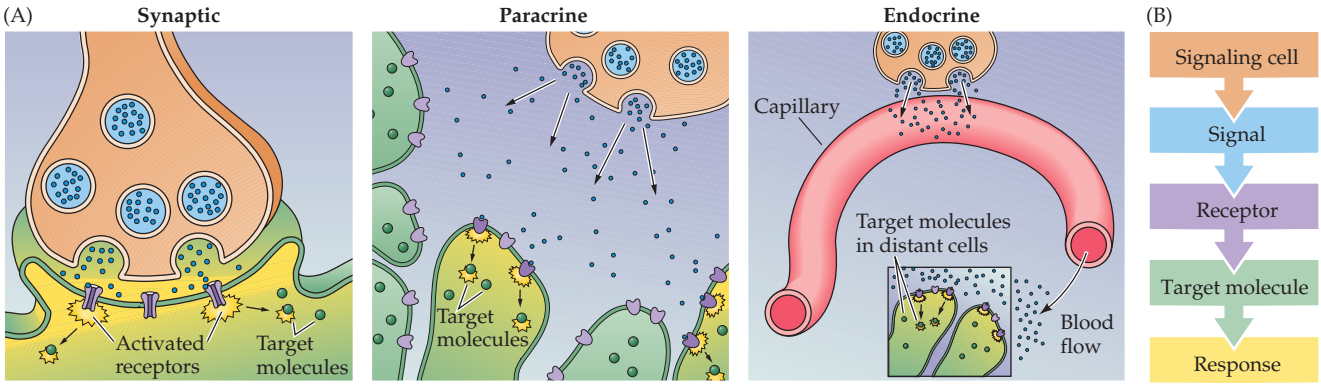


Figure 7.1 Chemical signaling mechanisms. (A) Forms of chemical communication include synaptic transmission, paracrine signaling, and endocrine signaling. (B) The essential components of chemical signaling are: cells that initiate the process by releasing signaling molecules; specific receptors on target cells; second messenger target molecules; and subsequent cellular responses.

that transduces the information provided by the signal, and a *target* molecule that mediates the cellular response (Figure 7.1B). The part of this process that take place within the confines of the target cell is called **intracellular signal transduction**. A good example of transduction in the context of *intercellular* communication is the sequence of events triggered by chemical synaptic transmission (see Chapter 5): Neurotransmitters serve as the signal, neurotransmitter receptors serve as the transducing receptor, and the target molecule is an ion channel that is altered to cause the electrical response of the postsynaptic cell. In many cases, however, synaptic transmission activates additional *intracellular* pathways that have a variety of functional consequences. For example, the binding of the neurotransmitter norepinephrine to its receptor activates GTP-binding proteins, which produces second messengers within the postsynaptic target, activates enzyme cascades, and eventually changes the chemical properties of numerous target molecules within the affected cell.

A general advantage of chemical signaling in both intercellular and intracellular contexts is **signal amplification**. Amplification occurs because individual signaling reactions can generate a much larger number of molecular products than the number of molecules that initiate the reaction. In the case of norepinephrine signaling, for example, a single norepinephrine molecule binding to its receptor can generate many thousands of second messenger molecules (such as cyclic AMP), yielding an amplification of tens of thousands of phosphates transferred to target proteins (Figure 7.2). Similar amplification occurs in all signal transduction pathways. Because the transduction processes often are mediated by a sequential set of enzymatic reactions, each with its own amplification factor, a small number of signal molecules ultimately can activate a very large number of target molecules. Such amplification guarantees that a physiological response is evoked in the face of other, potentially countervailing, influences.

Another rationale for these complex signal transduction schemes is to permit precise control of cell behavior over a wide range of times. Some molecular interactions allow information to be transferred rapidly, while others are slower and longer lasting. For example, the signaling cascades associated with synaptic transmission at neuromuscular junctions allow a person to respond to rapidly changing cues, such as the trajectory of a pitched ball, while the slower responses triggered by adrenal medullary hormones (epinephrine and norepinephrine) secreted during a challenging game produce slower (and longer lasting) effects on muscle metabolism (see Chapter 20) and emotional state (see Chapter 29). To encode information that varies so

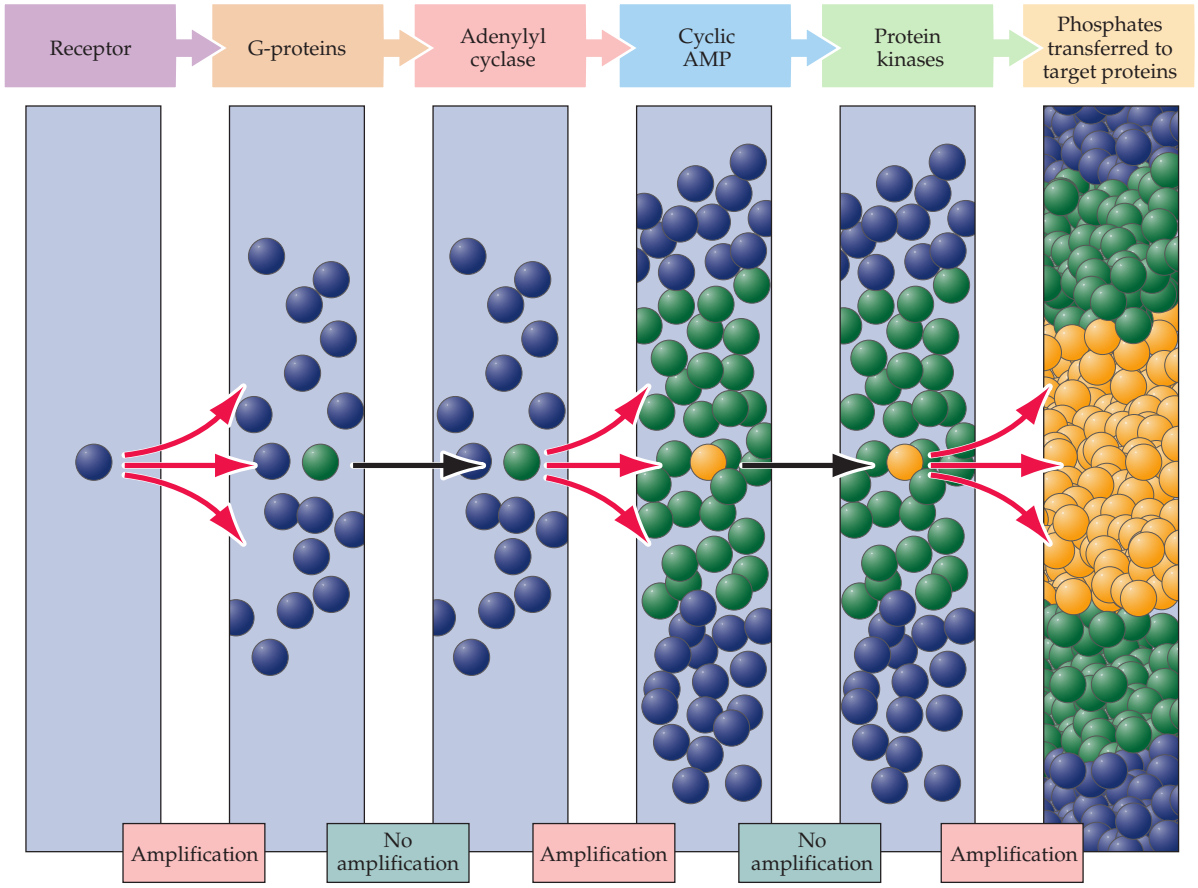


Figure 7.2 Amplification in signal transduction pathways. The activation of a single receptor by a signaling molecule, such as the neurotransmitter norepinephrine, can lead to the activation of numerous G-proteins inside cells. These activated proteins can bind to other signaling molecules, such as the enzyme adenylyl cyclase. Each activated enzyme molecule generates a large number of cAMP molecules. cAMP binds to and activates another family of enzymes, protein kinases. These enzymes can then phosphorylate many target proteins. While not every step in this signaling pathway involves amplification, overall the cascade results in a tremendous increase in the potency of the initial signal.

widely over time, the concentration of the relevant signaling molecules must be carefully controlled. On one hand, the concentration of every signaling molecule within the signaling cascade must return to subthreshold values before the arrival of another stimulus. On the other hand, keeping the intermediates in a signaling pathway activated is critical for a sustained response. Having multiple levels of molecular interactions facilitates the intricate timing of these events.

The Activation of Signaling Pathways

The molecular components of these signal transduction pathways are always activated by a chemical signaling molecule. Such signaling molecules can be grouped into three classes: **cell-impermeant**, **cell-permeant**, and **cell-associated signaling molecules** (Figure 7.3). The first two classes are secreted molecules and thus can act on target cells removed from the site of signal synthesis or release. Cell-impermeant signaling molecules typically bind to receptors associated with cell membranes. Hundreds of secreted molecules have now been identified, including the neurotransmitters discussed in Chapter 6, as well as proteins such as neurotrophic factors (see Chapter 22), and peptide hormones such as glucagon, insulin, and various reproductive hormones. These signaling molecules are typically short-lived, either because they are rapidly metabolized or because they are internalized by endocytosis once bound to their receptors.

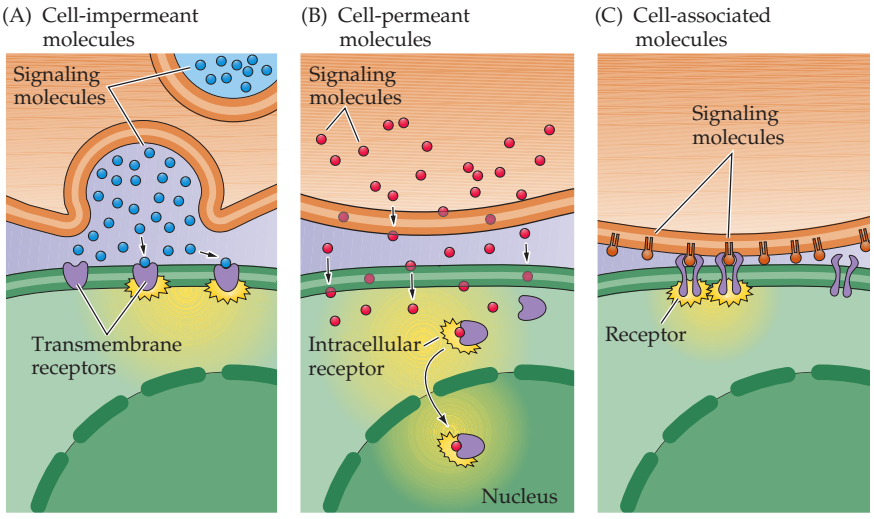


Figure 7.3 Three classes of cell signaling molecules. (A) Cell-impermeant molecules, such as neurotransmitters, cannot readily traverse the plasma membrane of the target cell and must bind to the extracellular portion of transmembrane receptor proteins. (B) Cell-permeant molecules are able to cross the plasma membrane and bind to receptors in the cytoplasm or nucleus of target cells. (C) Cell-associated molecules are presented on the extracellular surface of the plasma membrane. These signals activate receptors on target cells only if they are directly adjacent to the signaling cell.

Cell-permeant signaling molecules can cross the plasma membrane to act directly on receptors that are inside the cell. Examples include numerous steroid (glucocorticoids, estradiol, and testosterone) and thyroid (thyroxin) hormones, and retinoids. These signaling molecules are relatively insoluble in aqueous solutions and are often transported in blood and other extracellular fluids by binding to specific carrier proteins. In this form, they may persist in the bloodstream for hours or even days.

The third group of chemical signaling molecules, cell-associated signaling molecules, are arrayed on the extracellular surface of the plasma membrane. As a result, these molecules act only on other cells that are physically in contact with the cell that carries such signals. Examples include proteins such as the integrins and neural cell adhesion molecules (NCAMs) that influence axonal growth (see Chapter 22). Membrane-bound signaling molecules are more difficult to study, but are clearly important in neuronal development and other circumstances where physical contact between cells provides information about cellular identities.

Receptor Types

Regardless of the nature of the initiating signal, cellular responses are determined by the presence of receptors that specifically bind the signaling molecules. Binding of signal molecules causes a conformational change in the receptor, which then triggers the subsequent signaling cascade within the affected cell. Given that chemical signals can act either at the plasma membrane or within the cytoplasm (or nucleus) of the target cell, it is not surprising that receptors are actually found on both sides of the plasma membrane. The receptors for impermeant signal molecules are membrane-spanning proteins. The extracellular domain of such receptors includes the binding site for the signal, while the intracellular domain activates intracellular signaling cascades after the signal binds. A large number of these receptors have been identified and are grouped into families defined by the mechanism used to transduce signal binding into a cellular response (Figure 7.4).

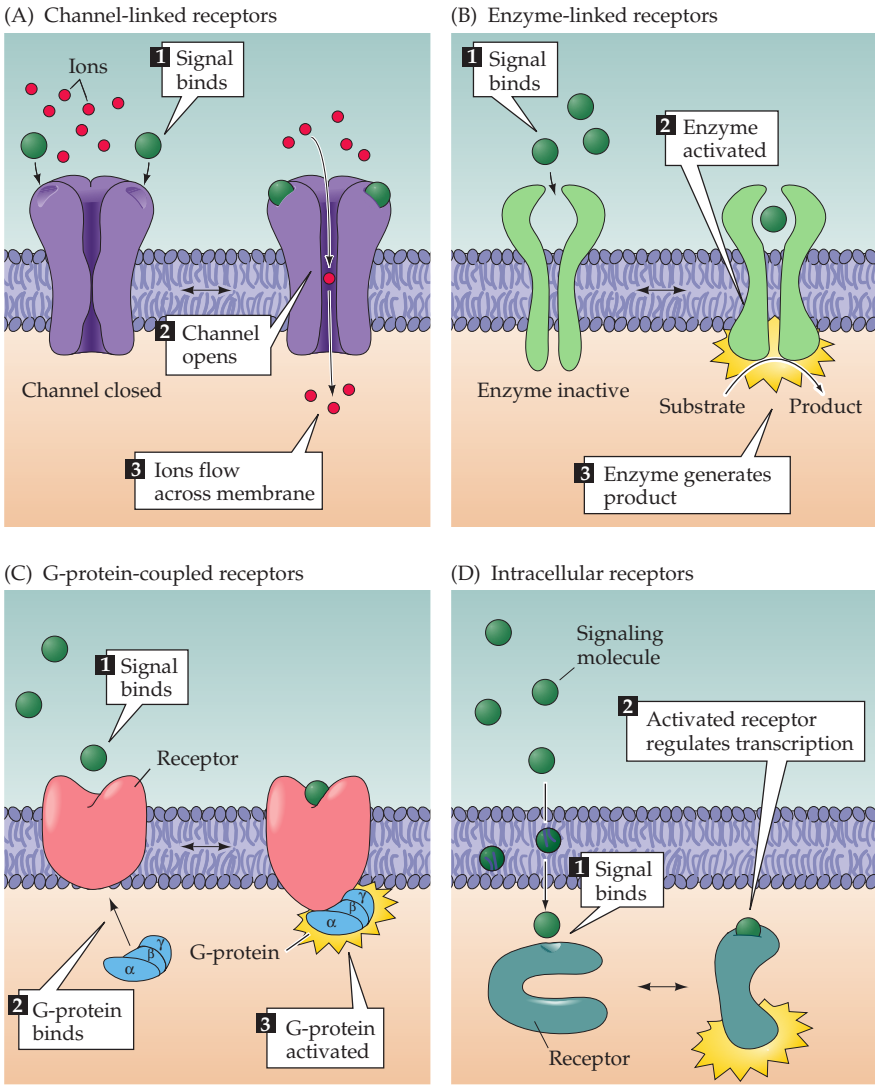


Figure 7.4 Categories of cellular receptors. Membrane-impermeant signaling molecules can bind to and activate either channel-linked receptors (A), enzyme-linked receptors (B), or G-protein-coupled receptors (C). Membrane permeant signaling molecules activate intracellular receptors (D).

Channel-linked receptors (also called ligand-gated ion channels) have the receptor and transducing functions as part of the same protein molecule. Interaction of the chemical signal with the binding site of the receptor causes the opening or closing of an ion channel pore in another part of the same molecule. The resulting ion flux changes the membrane potential of the target cell and, in some cases, can also lead to entry of Ca^{2+} ions that serve as a second messenger signal within the cell. Good examples of such receptors are the ionotropic neurotransmitter receptors described in Chapters 5 and 6.

Enzyme-linked receptors also have an extracellular binding site for chemical signals. The intracellular domain of such receptors is an enzyme whose catalytic activity is regulated by the binding of an extracellular signal. The great majority of these receptors are **protein kinases**, often tyrosine kinases, that phosphorylate intracellular target proteins, thereby changing the physiological function of the target cells. Noteworthy members of this

group of receptors are the Trk family of neurotrophin receptors (see Chapter 22) and other receptors for growth factors.

G-protein-coupled receptors regulate intracellular reactions by an indirect mechanism involving an intermediate transducing molecule, called the **GTP-binding proteins** (or **G-proteins**). Because these receptors all share the structural feature of crossing the plasma membrane seven times, they are also referred to as 7-transmembrane receptors (or metabotropic receptors; see Chapter 5). Hundreds of different G-protein-linked receptors have been identified. Well-known examples include the β-adrenergic receptor, the muscarinic type of acetylcholine receptor, metabotropic glutamate receptors, receptors for odorants in the olfactory system, and many types of receptors for peptide hormones. Rhodopsin, a light-sensitive, 7-transmembrane protein in retinal photoreceptors, is another form of G-protein-linked receptor (see Chapter 10).

Intracellular receptors are activated by cell-permeant or lipophilic signaling molecules (Figure 7.4D). Many of these receptors lead to the activation of signaling cascades that produce new mRNA and protein within the target cell. Often such receptors comprise a receptor protein bound to an inhibitory protein complex. When the signaling molecule binds to the receptor, the inhibitory complex dissociates to expose a DNA-binding domain on the receptor. This activated form of the receptor can then move into the nucleus and directly interact with nuclear DNA, resulting in altered transcription. Some intracellular receptors are located primarily in the cytoplasm, while others are in the nucleus. In either case, once these receptors are activated they can affect gene expression by altering DNA transcription.

G-Proteins and Their Molecular Targets

Both G-protein-linked receptors and enzyme-linked receptors can activate biochemical reaction cascades that ultimately modify the function of target proteins. For both these receptor types, the coupling between receptor activation and their subsequent effects are the GTP-binding proteins. There are two general classes of GTP-binding protein (Figure 7.5). **Heterotrimeric G-proteins** are composed of three distinct subunits (α, β, and γ). There are many different α, β, and γ subunits, allowing a bewildering number of G-protein permutations. Regardless of the specific composition of the heterotrimeric G-protein, its α subunit binds to guanine nucleotides, either GTP or GDP. Binding of GDP then allows the α subunit to bind to the β and γ subunits to form an inactive trimer. Binding of an extracellular signal to a G-protein-coupled receptor in turn allows the G-protein to bind to the receptor and causes GDP to be replaced with GTP (Figure 7.5A). When GTP is bound to the G-protein, the α subunit dissociates from the βγ complex and activates the G-protein. Following activation, both the GTP-bound α subunit and the free βγ complex can bind to downstream effector molecules that mediate a variety of responses in the target cell.

The second class of GTP-binding proteins are **monomeric G-proteins** (also called **small G-proteins**). These monomeric GTPases also relay signals from activated cell surface receptors to intracellular targets such as the cytoskeleton and the vesicle trafficking apparatus of the cell. The first small G-protein was discovered in a virus that causes *ra*t *s*arcoma tumors and was therefore called **ras**. Ras is a molecule that helps regulate cell differentiation and proliferation by relaying signals from receptor kinases to the nucleus; the viral form of ras is defective, which accounts for the ability of the virus to cause the uncontrolled cell proliferation that leads to tumors. Since then, a

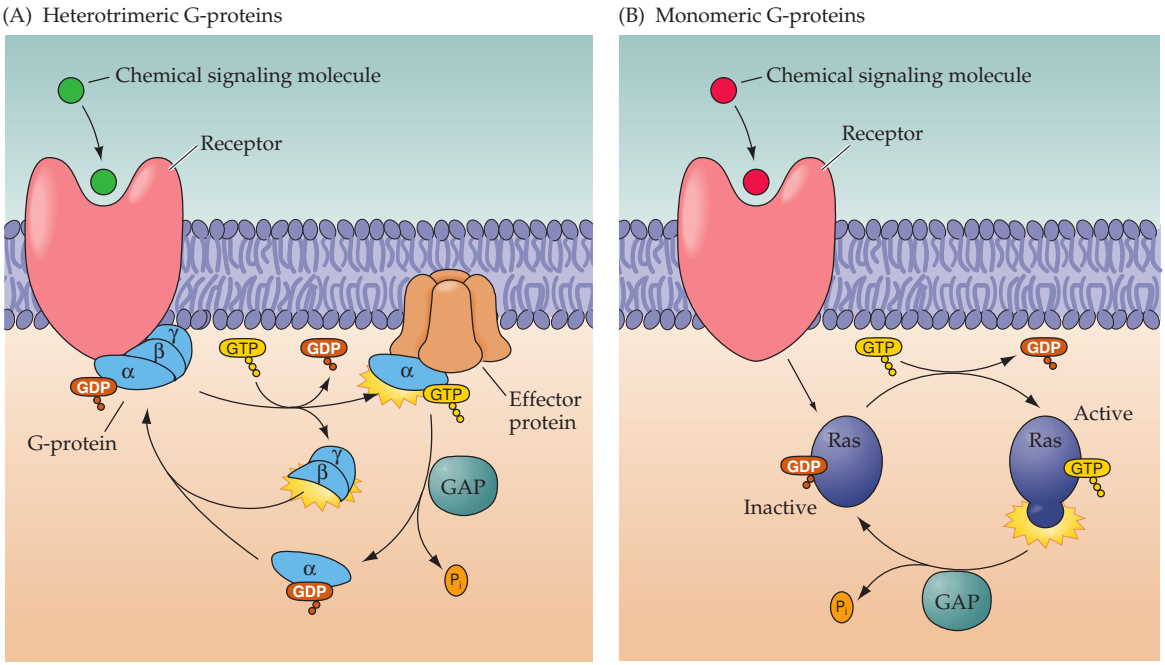


Figure 7.5 Types of GTP-binding protein. (A) Heterotrimeric G-proteins are composed of three distinct subunits (α, β, and γ). Receptor activation causes the binding of the G-protein and the α subunit to exchange GDP for GTP, leading to a dissociation of the α and βγ subunits. The biological actions of these G-proteins are terminated by hydrolysis of GTP, which is enhanced by GTPase-activating (GAP) proteins. (B) Monomeric G-proteins use similar mechanisms to relay signals from activated cell surface receptors to intracellular targets. Binding of GTP stimulates the biological actions of these G-proteins, and their activity is terminated by hydrolysis of GTP, which is also regulated by GAP proteins.

large number of small GTPases have been identified and can be sorted into five different subfamilies with different functions. For instance, some are involved in vesicle trafficking in the presynaptic terminal or elsewhere in the neuron, while others play a central role in protein and RNA trafficking in and out of the nucleus.

Termination of signaling by both heterotrimeric and monomeric G-proteins is determined by hydrolysis of GTP to GDP. The rate of GTP hydrolysis is an important property of a particular G-protein that can be regulated by other proteins, termed GTPase-activating proteins (GAPs). By replacing GTP with GDP, GAPs return G-proteins to their inactive form. GAPs were first recognized as regulators of small G-proteins, but recently similar proteins have been found to regulate the α subunits of heterotrimeric G-proteins. Hence, monomeric and trimeric G-proteins function as molecular timers that are active in their GTP-bound state, and become inactive when they have hydrolized the bound GTP to GDP (Figure 7.5B).

Activated G-proteins alter the function of many downstream effectors. Most of these effectors are enzymes that produce intracellular second messengers. Effector enzymes include adenylyl cyclase, guanylyl cyclase, phospholipase C, and others (Figure 7.6). The second messengers produced by these enzymes trigger the complex biochemical signaling cascades discussed in the next section. Because each of these cascades is activated by specific G-protein subunits, the pathways activated by a particular receptor are determined by the specific identity of the G-protein subunits associated with it.

As well as activating effector molecules, G-proteins can also directly bind to and activate ion channels. For example, some neurons, as well as heart muscle cells, have G-protein-coupled receptors that bind acetylcholine. Because these receptors are also activated by the agonist muscarine, they are usually called muscarinic receptors (see Chapters 6 and 20). Activation of muscarinic receptors can open K^+ channels, thereby inhibiting the rate at which the neuron fires action potentials, or slowing the heartbeat of muscle

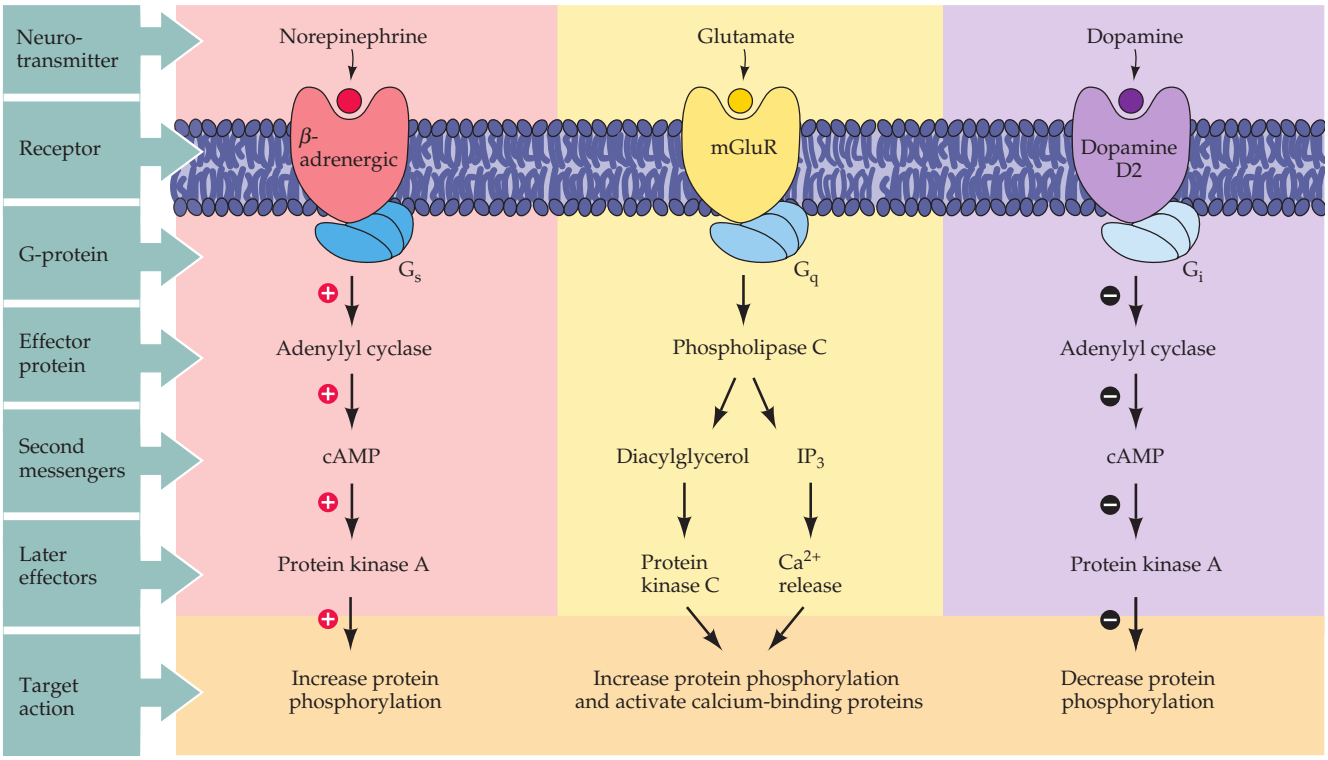


Figure 7.6 Effector pathways associated with G-protein-coupled receptors. In all three examples shown here, binding of a neurotransmitter to such a receptor leads to activation of a G-protein and subsequent recruitment of second messenger pathways. G_s, G_q, and G_i refer to three different types of heterotrimeric G-protein.

cells. These inhibitory responses are believed to be the result of βγ subunits of G-proteins binding to the K^+ channels. The activation of α subunits can also lead to the rapid closing of voltage-gated Ca^{2+} and Na^+ channels. Because these channels carry inward currents involved in generating action potentials, closing them makes it more difficult for target cells to fire (see Chapters 3 and 4).

In summary, the binding of chemical signals to their receptors activates cascades of signal transduction events in the cytosol of target cells. Within such cascades, G-proteins serve a pivotal function as the molecular transducing elements that couple membrane receptors to their molecular effectors within the cell. The diversity of G-proteins and their downstream targets leads to many types of physiological responses. By directly regulating the gating of ion channels, G-proteins can influence the membrane potential of target cells.

Second Messengers

Neurons use many different second messengers as intracellular signals. These messengers differ in the mechanism by which they are produced and removed, as well as their downstream targets and effects (Figure 7.7A). This section summarizes the attributes of some of the principal second messengers.

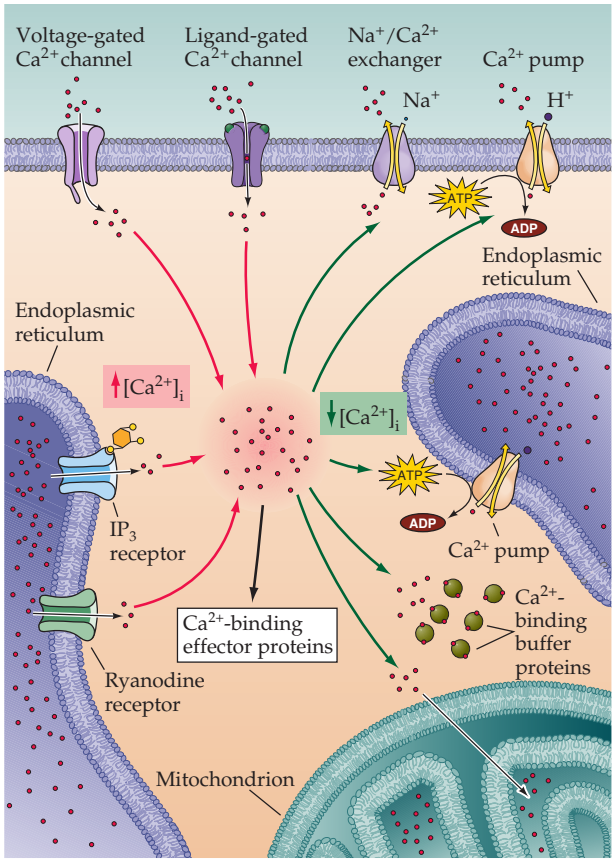
• *Calcium.* The calcium ion (Ca^{2+}) is perhaps the most common intracellular messenger in neurons. Indeed, few neuronal functions are immune to the influence—direct or indirect—of Ca^{2+}. In all cases, information is transmitted by a transient rise in the cytoplasmic calcium concentration, which

(A)

Second messenger	Sources	Intracellular targets	Removal mechanisms
Ca^{2+}	Plasma membrane: Voltage-gated Ca^{2+} channels Various ligand-gated channels Endoplasmic reticulum: IP_3 receptors Ryanodine receptors	Calmodulin Protein kinases Protein phosphatases Ion channels Synaptotagmin Many other Ca^{2+}-binding proteins	Plasma membrane: Na^+/Ca^{2+} exchanger Ca^{2+} pump Endoplasmic reticulum: Ca^{2+} pump Mitochondria
Cyclic AMP	Adenylyl cyclase acts on ATP	Protein kinase A Cyclic nucleotide-gated channels	cAMP phosphodiesterase
Cyclic GMP	Guanylyl cyclase acts on GTP	Protein kinase G Cyclic nucleotide-gated channels	cGMP phosphodiesterase
IP_3	Phospholipase C acts on PIP_2	IP_3 receptors on endoplasmic reticulum	Phosphatases
Diacylglycerol	Phospholipase C acts on PIP_2	Protein kinase C	Various enzymes

Figure 7.7 Neuronal second messengers. (A) Mechanisms responsible for producing and removing second messengers, as well as the downstream targets of these messengers. (B) Proteins involved in delivering calcium to the cytoplasm and in removing calcium from the cytoplasm. (C) Mechanisms of production and degradation of cyclic nucleotides. (D) Pathways involved in production and removal of diacylglycerol (DAG) and IP_3.

(B)



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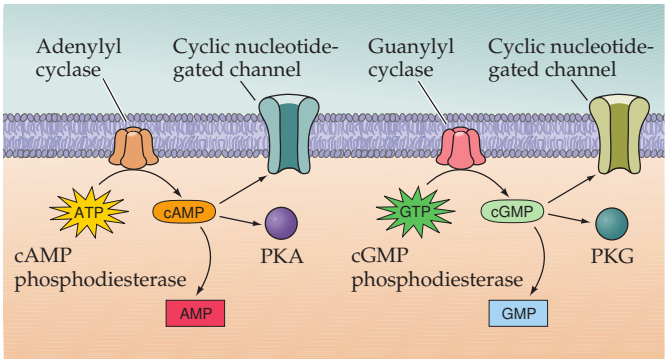

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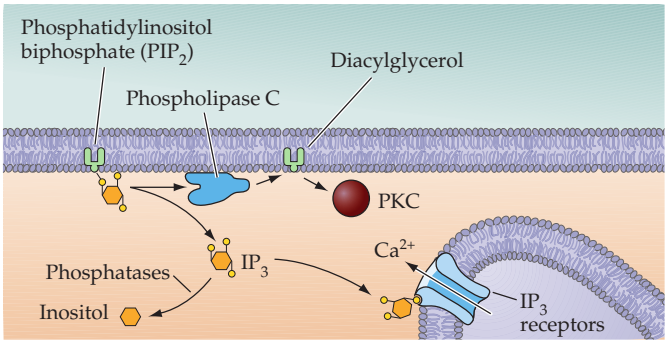


allows Ca^{2+} to bind to a large number of Ca^{2+}-binding proteins that serve as molecular targets. One of the most thoroughly studied targets of Ca^{2+} is **calmodulin**, a Ca^{2+}-binding protein abundant in the cytosol of all cells. Binding of Ca^{2+} to calmodulin activates this protein, which then initiates its effects by binding to still other downstream targets, such as protein kinases.

Ordinarily the concentration of Ca^{2+} ions in the cytosol is extremely low, typically 50–100 nanomolar (10^{-9} *M*). The concentration of Ca^{2+} ions outside neurons—in the bloodstream or cerebrospinal fluid, for instance—is several orders of magnitude higher, typically several millimolar (10^{-3} *M*). This steep Ca^{2+} gradient is maintained by a number of mechanisms (Figure 7.7B). Most important in this maintenance are two proteins that translocate Ca^{2+} from the cytosol to the extracellular medium: an ATPase called the **calcium pump**, and an **Na^+/Ca^{2+} exchanger**, which is a protein that replaces intracellular Ca^{2+} with extracellular sodium ions (see Chapter 4). In addition to these plasma membrane mechanisms, Ca^{2+} is also pumped into the endoplasmic reticulum and mitochondria. These organelles can thus serve as storage depots of Ca^{2+} ions that are later released to participate in signaling events. Finally, nerve cells contain other Ca^{2+}-binding proteins—such as **calbindin**—that serve as Ca^{2+} buffers. Such buffers reversibly bind Ca^{2+} and thus blunt the magnitude and kinetics of Ca^{2+} signals within neurons.

The Ca^{2+} ions that act as intracellular signals enter cytosol by means of one or more types of Ca^{2+}-permeable ion channels (see Chapter 4). These can be voltage-gated Ca^{2+} channels or ligand-gated channels in the plasma membrane, both of which allow Ca^{2+} to flow down the Ca^{2+} gradient and into the cell from the extracellular medium. In addition, other channels allow Ca^{2+} to be released from the interior of the endoplasmic reticulum into the cytosol. These intracellular Ca^{2+}-releasing channels are gated, so they can be opened or closed in response to various intracellular signals. One such channel is the **inositol trisphosphate (IP_3) receptor.** As the name implies, these channels are regulated by IP_3, a second messenger described in more detail below. A second type of intracellular Ca^{2+}-releasing channel is the **ryanodine receptor,** named after a drug that binds to and partially opens these receptors. Among the biological signals that activate ryanodine receptors are cytoplasmic Ca^{2+} and, at least in muscle cells, depolarization of the plasma membrane.

These various mechanisms for elevating and removing Ca^{2+} ions allow precise control of both the timing and location of Ca^{2+} signaling within neurons, which in turn permit Ca^{2+} to control many different signaling events. For example, voltage-gated Ca^{2+} channels allow Ca^{2+} concentrations to rise very rapidly and locally within presynaptic terminals to trigger neurotransmitter release, as already described in Chapter 5. Slower and more widespread rises in Ca^{2+} concentration regulate a wide variety of other responses, including gene expression in the cell nucleus.

• *Cyclic nucleotides.* Another important group of second messengers are the cyclic nucleotides, specifically cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Figure 7.7C). Cyclic AMP is a derivative of the common cellular energy storage molecule, ATP. Cyclic AMP is produced when G-proteins activate adenylyl cyclase in the plasma membrane. This enzyme converts ATP into cAMP by removing two phosphate groups from the ATP. Cyclic GMP is similarly produced from GTP by the action of guanylyl cyclase. Once the intracellular concentration of cAMP or cGMP is elevated, these nucleotides can bind to two different classes of targets. The most common targets of cyclic nucleotide action are protein kinases, either the cAMP-dependent protein kinase (PKA) or the cGMP-dependent

protein kinase (PKG). These enzymes mediate many physiological responses by phosphorylating target proteins, as described in the following section. In addition, cAMP and cGMP can bind to certain ligand-gated ion channels, thereby influencing neuronal signaling. These cyclic nucleotide-gated channels are particularly important in phototransduction and other sensory transduction processes, such as olfaction. Cyclic nucleotide signals are degraded by phosphodiesterases, enzymes that cleave phosphodiester bonds and convert cAMP into AMP or cGMP into GMP.

- *Diacylglycerol and IP_3*. Remarkably, membrane lipids can also be converted into intracellular second messengers (Figure 7.7D). The two most important messengers of this type are produced from phosphatidylinositol bisphosphate (PIP_2). This lipid component is cleaved by phospholipase C, an enzyme activated by certain G-proteins and by calcium ions. Phospholipase C splits the PIP_2 into two smaller molecules that each act as second messengers. One of these messengers is diacylglycerol (DAG), a molecule that remains within the membrane and activates protein kinase C, which phosphorylates substrate proteins in both the plasma membrane and elsewhere. The other messenger is inositol trisphosphate (IP_3), a molecule that leaves the cell membrane and diffuses within the cytosol. IP_3 binds to IP_3 receptors, channels that release calcium from the endoplasmic reticulum. Thus, the action of IP_3 is to produce yet another second messenger (perhaps a third messenger, in this case!) that triggers a whole spectrum of reactions in the cytosol. The actions of DAG and IP_3 are terminated by enzymes that convert these two molecules into inert forms that can be recycled to produce new molecules of PIP_2.

Second Messenger Targets: Protein Kinases and Phosphatases

As already mentioned, second messengers typically regulate neuronal functions by modulating the phosphorylation state of intracellular proteins (Figure 7.8). Phosphorylation (the addition of phosphate groups) rapidly and reversibly changes protein function. Proteins are phosphorylated by a wide variety of **protein kinases**; phosphate groups are removed by other enzymes called **protein phosphatases**. The degree of phosphorylation of a target protein thus reflects a balance between the competing actions of protein kinases and phosphatases, thus integrating a host of cellular signaling pathways. The substrates of protein kinases and phosphatases include enzymes, neurotransmitter receptors, ion channels, and structural proteins.

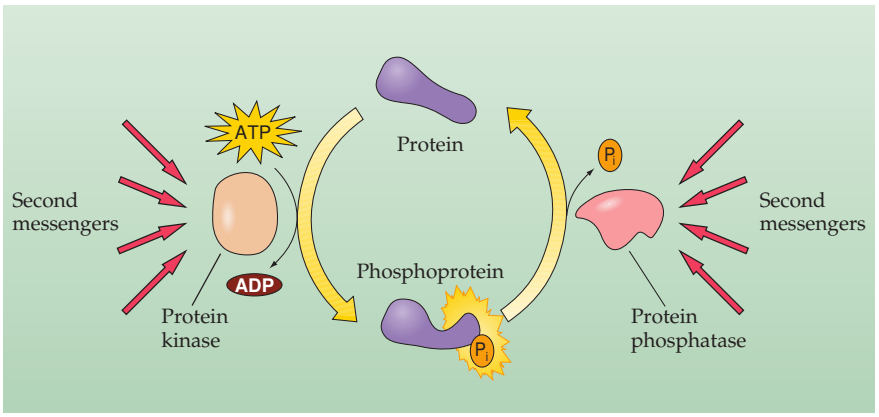


Figure 7.8 Regulation of cellular proteins by phosphorylation. Protein kinases transfer phosphate groups (P_i) from ATP to serine, threonine, or tyrosine residues on substrate proteins. This phosphorylation reversibly alters the structure and function of cellular proteins. Removal of the phosphate groups is catalyzed by protein phosphatases. Both kinases and phosphatases are regulated by a variety of intracellular second messengers.

Protein kinases and phosphatases typically act either on the serine and threonine residues (Ser/Thr kinases or phosphatases) or the tyrosine residues (Tyr kinases or phosphatases) of their substrates. Some of these enzymes act specifically on only one or a handful of protein targets, while others are multifunctional and have a broad range of substrate proteins. The activity of protein kinases and phosphatases can be regulated either by second messengers, such as cAMP or Ca^{2+}, or by extracellular chemical signals, such as growth factors (see Chapter 22). Typically, second messengers activate Ser/Thr kinases, whereas extracellular signals activate Tyr kinases. Although thousands of protein kinases are expressed in the brain, a relatively small number function as regulators of neuronal signaling.

• *cAMP-dependent protein kinase (PKA).* The primary effector of cAMP is the cAMP-dependent protein kinase (PKA). PKA is a tetrameric complex of two catalytic subunits and two inhibitory (regulatory) subunits. cAMP activates PKA by binding to the regulatory subunits and causing them to release active catalytic subunits. Such displacement of inhibitory domains is a general mechanism for activation of several protein kinases by second messengers (Figure 7.9A). The catalytic subunit of PKA phosphorylates serine and threonine residues of many different target proteins. Although this subunit is similar to the catalytic domains of other protein kinases, distinct amino acids allow the PKA to bind to specific target proteins, thus allowing only those targets to be phosphorylated in response to intracellular cAMP signals.

• *Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII).* Ca^{2+} ions binding to calmodulin can regulate protein phosphorylation/dephosphorylation. In neurons, the most abundant Ca^{2+}/calmodulin-dependent protein kinase is CaMKII, a multifunctional Ser/Thr protein kinase. CaMKII is composed of approximately 14 subunits, which in the brain are the α and β types. Each subunit contains a catalytic domain and a regulatory domain, as well as other domains that allow the enzyme to oligomerize and target to the proper region within the cell. Ca^{2+}/calmodulin activates CaMKII by displacing the inhibitory domain from the catalytic site (Figure 7.9B). CaMKII phosphorylates a large number of substrates, including ion channels and other proteins involved in intracellular signal transduction.

• *Protein kinase C (PKC).* Another important group of Ser/Thr protein kinases is protein kinase C (PKC). PKCs are diverse monomeric kinases activated by the second messengers DAG and Ca^{2+}. DAG causes PKC to move from the cytosol to the plasma membrane, where it also binds Ca^{2+} and phosphatidylserine, a membrane phospholipid (Figure 7.9C). These events relieve autoinhibition and cause PKC to phosphorylate various protein substrates. PKC also diffuses to sites other than the plasma membrane—such as the cytoskeleton, perinuclear sites, and the nucleus—where it phosphorylates still other substrate proteins. Prolonged activation of PKC can be accomplished with phorbol esters, tumor-promoting compounds that activate PKC by mimicking DAG.

• *Protein tyrosine kinases.* Two classes of protein kinases transfer phosphate groups to tyrosine residues on substrate proteins. Receptor tyrosine kinases are transmembrane proteins with an extracellular domain that binds to protein ligands (growth factors, neurotrophic factors, or cytokines) and an intracellular catalytic domain that phosphorylates the relevant substrate proteins. Non-receptor tyrosine kinases are cytoplasmic or membrane-associated enzymes that are indirectly activated by extracellular signals. Tyrosine phosphorylation is less common than Ser/Thr phosphorylation, and it often serves to recruit signaling molecules to the phosphorylated protein. Tyrosine

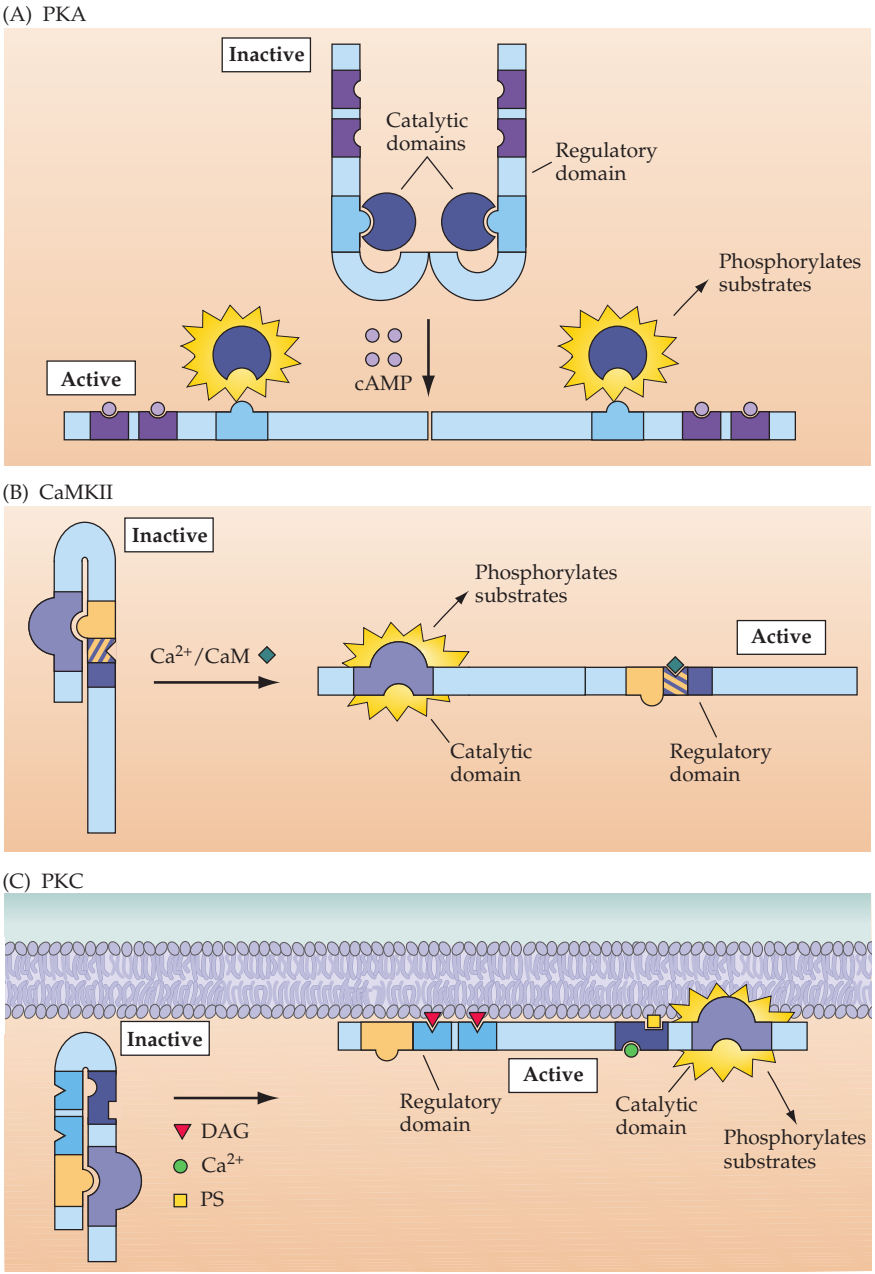


Figure 7.9 Mechanism of activation of protein kinases. Protein kinases contain several specialized domains with specific functions. Each of the kinases has homologous catalytic domains responsible for transferring phosphate groups to substrate proteins. These catalytic domains are kept inactive by the presence of an autoinhibitory domain that occupies the catalytic site. Binding of second messengers, such as cAMP, DAG, and Ca^{2+}, to the appropriate regulatory domain of the kinase removes the autoinhibitory domain and allows the catalytic domain to be activated. For some kinases, such as PKC and CaMKII, the autoinhibitory and catalytic domains are part of the same molecule. For other kinases, such as PKA, the autoinhibitory domain is a separate subunit.

kinases are particularly important for cell growth and differentiation (see Chapters 21 and 22).

• *Mitogen-activated protein kinase (MAPK).* In addition to protein kinases that are directly activated by second messengers, some of these molecules can be activated by other signals, such as phosphorylation by another protein kinase. Important examples of such protein kinases are the mitogen-activated protein kinases (MAPKs), also called extracellular signal-regulated kinases (ERKs). MAPKs were first identified as participants in the control of cell growth and are now known to have many other signaling functions.

MAPKs are normally inactive in neurons but become activated when they are phosphorylated by other kinases. In fact, MAPKs are part of a kinase cascade in which one protein kinase phosphorylates and activates the next protein kinase in the cascade. The extracellular signals that trigger these kinase cascades are often extracellular growth factors that bind to receptor tyrosine kinases that, in turn, activate monomeric G-proteins such as ras. Once activated, MAPKs can phosphorylate transcription factors, proteins that regulate gene expression. Among the wide variety of other MAPK substrates are various enzymes, including other protein kinases, and cytoskeletal proteins.

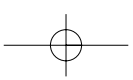
The best-characterized protein phosphatases are the Ser/Thr phosphatases PP1, PP2A, and PP2B (also called calcineurin). In general, protein phosphatases display less substrate specificity than protein kinases. Their limited specificity may arise from the fact that the catalytic subunits of the three major protein phosphatases are highly homologous, though each still associates with specific targeting or regulatory subunits. PP1 dephosphorylates a wide array of substrate proteins and is probably the most prevalent Ser/Thr protein phosphatase in mammalian cells. PP1 activity is regulated by several inhibitory proteins expressed in neurons. PP2A is a multisubunit enzyme with a broad range of substrates that overlap with PP1. PP2B, or calcineurin, is present at high levels in neurons. A distinctive feature of this phosphatase is its activation by Ca^{2+}/calmodulin. PP2B is composed of a catalytic and a regulatory subunit. Ca^{2+}/calmodulin activates PP2B primarily by binding to the catalytic subunit and displacing the inhibitory regulatory domain. PP2B generally does not have the same molecular targets as CaMKII, even though both enzymes are activated by Ca^{2+}/calmodulin.

In summary, activation of membrane receptors can elicit complex cascades of enzyme activation, resulting in second messenger production and protein phosphorylation or dephosphorylation. These cytoplasmic signals produce a variety of rapid physiological responses by transiently regulating enzyme activity, ion channels, cytoskeletal proteins, and many other cellular processes. In addition, such signals can propagate to the nucleus to cause long-lasting changes in gene expression.

Nuclear Signaling

Second messengers elicit prolonged changes in neuronal function by promoting the synthesis of new RNA and protein. The resulting accumulation of new proteins requires at least 30–60 minutes, a time frame that is orders of magnitude slower than the responses mediated by ion fluxes or phosphorylation. Likewise, the reversal of such events requires hours to days. In some cases, genetic "switches" can be thrown to permanently alter a neuron, as in neuronal differentiation (see Chapter 21).

The amount of protein present in cells is determined primarily by the rate of transcription of DNA into RNA (Figure 7.10). The first step in RNA synthesis is the decondensation of the structure of chromatin to provide binding sites for the RNA polymerase complex and for **transcriptional activator proteins**, also called **transcription factors**. Transcriptional activator proteins attach to binding sites that are present on the DNA molecule near the start of the target gene sequence; they also bind to other proteins that promote unwrapping of DNA. The net result of these actions is to allow RNA polymerase, an enzyme complex, to assemble on the **promoter** region of the DNA and begin transcription. In addition to clearing the promoter for RNA polymerase, activator proteins can stimulate transcription by interacting



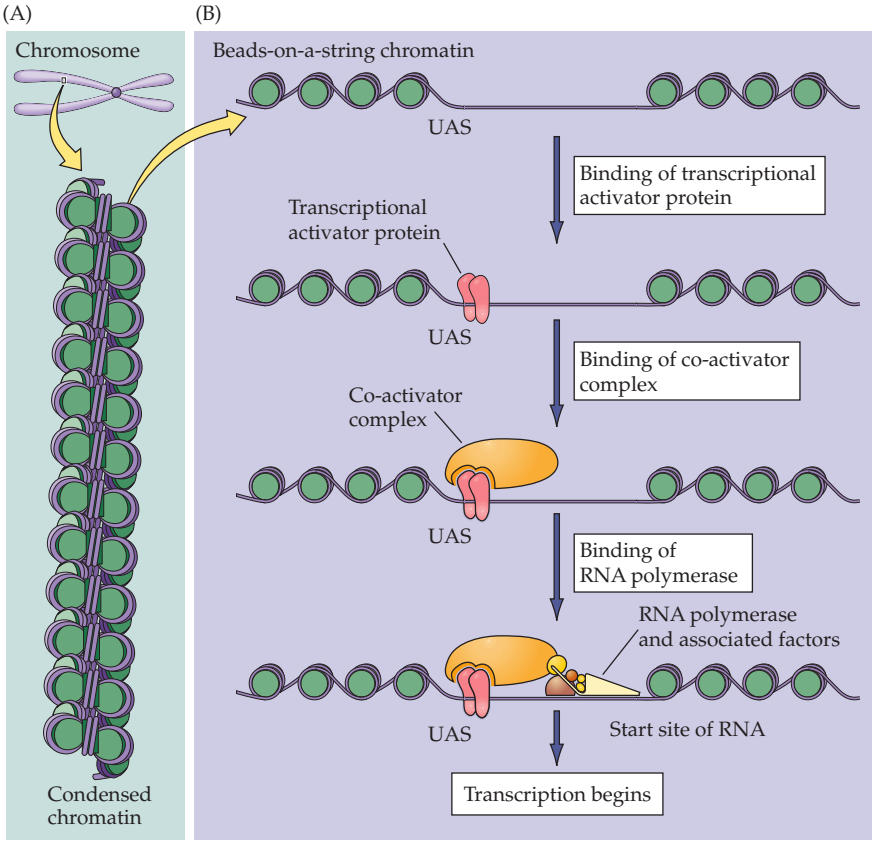


Figure 7.10 Steps involved in transcription of DNA into RNA. Condensed chromatin (A) is decondensed into a beads-on-a-DNA-string array (B) in which an upstream activator site (UAS) is free of proteins and is bound by a sequence-specific transcriptional activator protein (transcription factor). The transcriptional activator protein then binds co-activator complexes that enable the RNA polymerase with its associated factors to bind at the start site of transcription and initiate RNA synthesis.

with the RNA polymerase complex or by interacting with other activator proteins that influence the polymerase.

Intracellular signal transduction cascades regulate gene expression by converting transcriptional activator proteins from an inactive state to an active state in which they are able to bind to DNA. This conversion comes about in several ways. The key activator proteins and the mechanisms that allow them to regulate gene expression in response to signaling events are briefly summarized in the following sections.

• *CREB.* The *c*AMP *r*esponse *e*lement *b*inding protein, usually abbreviated **CREB**, is a ubiquitous transcriptional activator (Figure 7.11). CREB is normally bound to its binding site on DNA (called the cAMP response element, or CRE), either as a homodimer or bound to another, closely related transcription factor. In unstimulated cells, CREB is not phosphorylated and has little or no transcriptional activity. However, phosphorylation of CREB greatly potentiates transcription. Several signaling pathways are capable of causing CREB to be phosphorylated. Both PKA and the ras pathway, for example, can phosphorylate CREB. CREB can also be phosphorylated in response to increased intracellular calcium, in which case the CRE site is also called the CaRE (calcium response element) site. The calcium-dependent phosphorylation of CREB is primarily caused by Ca^{2+}/calmodulin kinase IV (a relative of CaMKII) and by MAP kinase, which leads to prolonged CREB phosphorylation. CREB phosphorylation must be maintained long enough for transcription to ensue, even though neuronal electrical activity only tran-

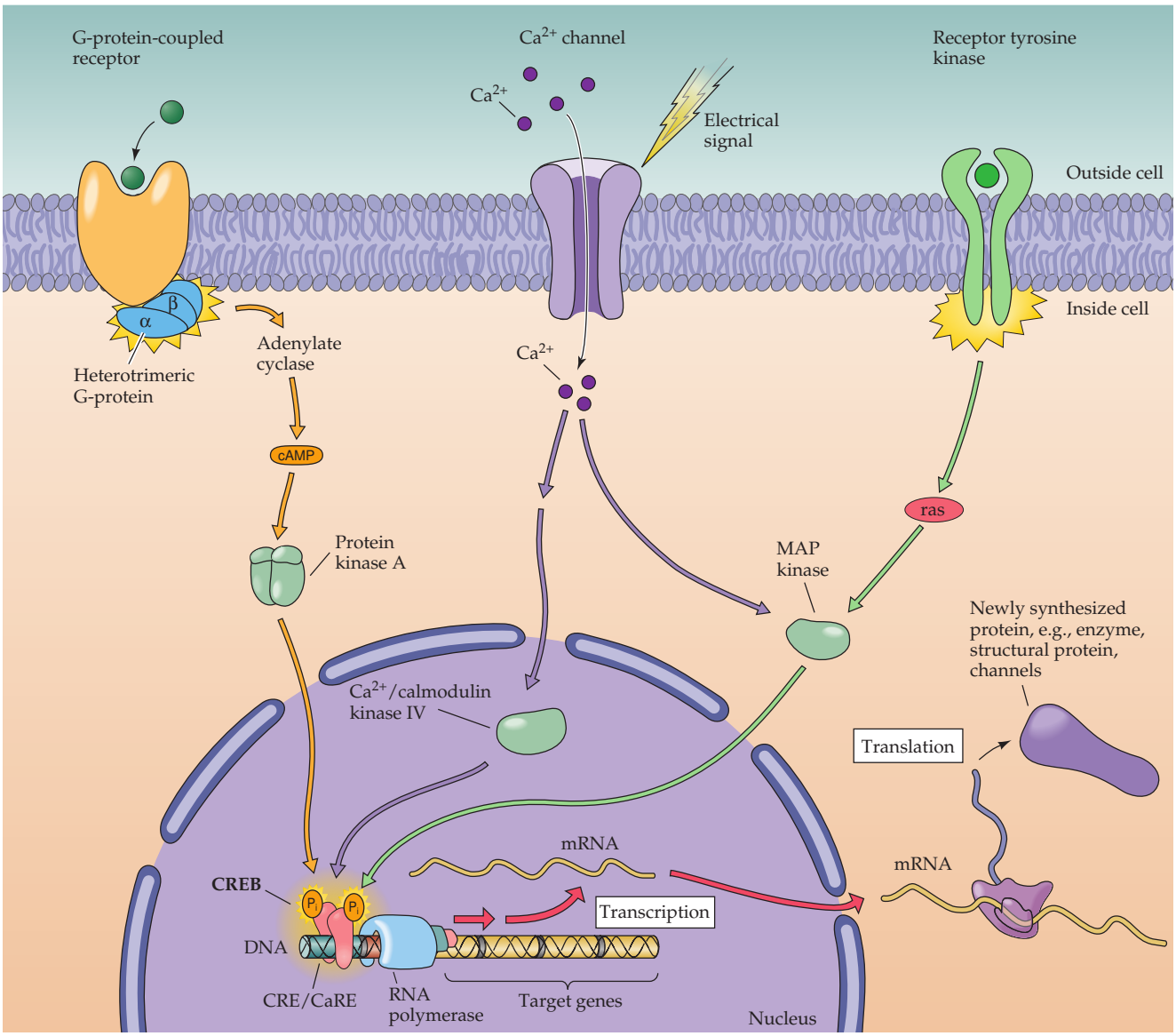


Figure 7.11 Transcriptional regulation by CREB. Multiple signaling pathways converge by activating kinases that phosphorylate CREB. These include PKA, Ca^{2+}/calmodulin kinase IV, and MAP kinase. Phosphorylation of CREB allows it to bind co-activators (not shown in the figure), which then stimulate RNA polymerase to begin synthesis of RNA. RNA is then processed and exported to the cytoplasm, where it serves as mRNA for translation into protein.

siently raises intracellular calcium concentration. Such signaling cascades can potentiate CREB-mediated transcription by inhibiting a protein phosphatase that dephosphorylates CREB. CREB is thus an example of the convergence of multiple signaling pathways onto a single transcriptional activator.

Many genes whose transcription is regulated by CREB have been identified. CREB-sensitive genes include the immediate early gene, *c-fos* (see below), the neurotrophin BDNF (see Chapter 22), the enzyme tyrosine hydroxylase (which is important for synthesis of catecholamine neurotransmitters; see Chapter 6), and many neuropeptides (including somatostatin, enkephalin, and corticotropin releasing hormone). CREB also is thought to mediate long-lasting changes in brain function. For example, CREB has been implicated in spatial learning, behavioral sensitization, long-term memory of odorant-conditioned behavior, and long-term synaptic plasticity (see Chapters 23 and 24).

• *Nuclear receptors.* Nuclear receptors for membrane-permeant ligands also are transcriptional activators. The receptor for glucocorticoid hormones illustrates one mode of action of such receptors. In the absence of glucocorticoid hormones, the receptors are located in the cytoplasm. Binding of glucocorticoids causes the receptor to unfold and move to the nucleus, where it binds a specific recognition site on the DNA. This DNA binding activates the relevant RNA polymerase complex to initiate transcription and subsequent gene expression. Thus, a critical regulatory event for steroid receptors is their translocation to the nucleus to allow DNA binding.

The receptors for thyroid hormone (TH) and other non-steroid nuclear receptors illustrate a second mode of regulation. In the absence of TH, the receptor is bound to DNA and serves as a potent repressor of transcription. Upon binding TH, the receptor undergoes a conformational change that ultimately opens the promoter for polymerase binding. Hence, TH binding switches the receptor from being a repressor to being an activator of transcription.

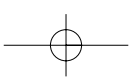
• *c-fos.* A different strategy of gene regulation is apparent in the function of the transcriptional activator protein, **c-fos**. In resting cells, c-fos is present at a very low concentration. However, stimulation of the target cell causes c-fos to be synthesized, and the amount of this protein rises dramatically over 30–60 minutes. Therefore, *c-fos* is considered to be an **immediate early gene** because its synthesis is directly triggered by the stimulus. Once synthesized, c-fos protein can act as a transcriptional activator to induce synthesis of second-order genes. These are termed **delayed response genes** because their activity is delayed by the fact that an immediate early gene—*c-fos* in this case—needs to be activated first.

Multiple signals converge on *c-fos*, activating different transcription factors that bind to at least three distinct sites in the promoter region of the gene. The regulatory region of the *c-fos* gene contains a binding site that mediates transcriptional induction by cytokines and ciliary neurotropic factor. Another site is targeted by growth factors such as neurotrophins through ras and protein kinase C, and a CRE/CaRE that can bind to CREB and thereby respond to cAMP or calcium entry resulting from electrical activity. In addition to synergistic interactions among these *c-fos* sites, transcriptional signals can be integrated by converging on the same activator, such as CREB.

Nuclear signaling events typically result in the generation of a large and relatively stable complex composed of a functional transcriptional activator protein, additional proteins that bind to the activator protein, and the RNA polymerase and associated proteins bound at the start site of transcription. Most of the relevant signaling events act to "seed" this complex by generating an active transcriptional activator protein by phosphorylation, by inducing a conformational change in the activator upon ligand binding, by fostering nuclear localization, by removing an inhibitor, or simply by making more activator protein.

Examples of Neuronal Signal Transduction

Understanding the general properties of signal transduction processes at the plasma membrane, in the cytosol, and within the nucleus make it possible to consider how these processes work in concert to mediate specific functions in the brain. Three important signal transduction pathways illustrate some of the roles of intracellular signal transduction processes in the nervous system.



• *NGF/TrkA*. The first of these is signaling by the **nerve growth factor** (**NGF**). This protein is a member of the neurotrophin growth factor family and is required for the differentiation, survival, and synaptic connectivity of sympathetic and sensory neurons (see Chapter 22). NGF works by binding to a high-affinity tyrosine kinase receptor, TrkA, found on the plasma membrane of these target cells (Figure 7.12). NGF binding causes TrkA receptors to dimerize, and the intrinsic tyrosine kinase activity of each receptor then phosphorylates its partner receptor. Phosphorylated TrkA receptors trigger the ras cascade, resulting in the activation of multiple protein kinases. Some of these kinases translocate to the nucleus to activate transcriptional activators, such as CREB. This ras-based component of the NGF pathway is primarily responsible for inducing and maintaining differentiation of NGF-sensitive neurons. Phosphorylation of TrkA also causes this receptor to stimulate the activity of phospholipase C, which increases production of IP_3 and DAG. IP_3 induces release of Ca^{2+} from the endoplasmic reticulum, and diacylglycerol activates PKC. These two second messengers appear to target many of the same downstream effectors as ras. Finally, activation of TrkA receptors also causes activation of other protein kinases (such as Akt kinase) that inhibit cell death. This pathway, therefore, primarily mediates the NGF-dependent survival of sympathetic and sensory neurons described in Chapter 22.

• *Long-term depression (LTD)*. The interplay between several intracellular signals can be observed at the excitatory synapses that innervate Purkinje

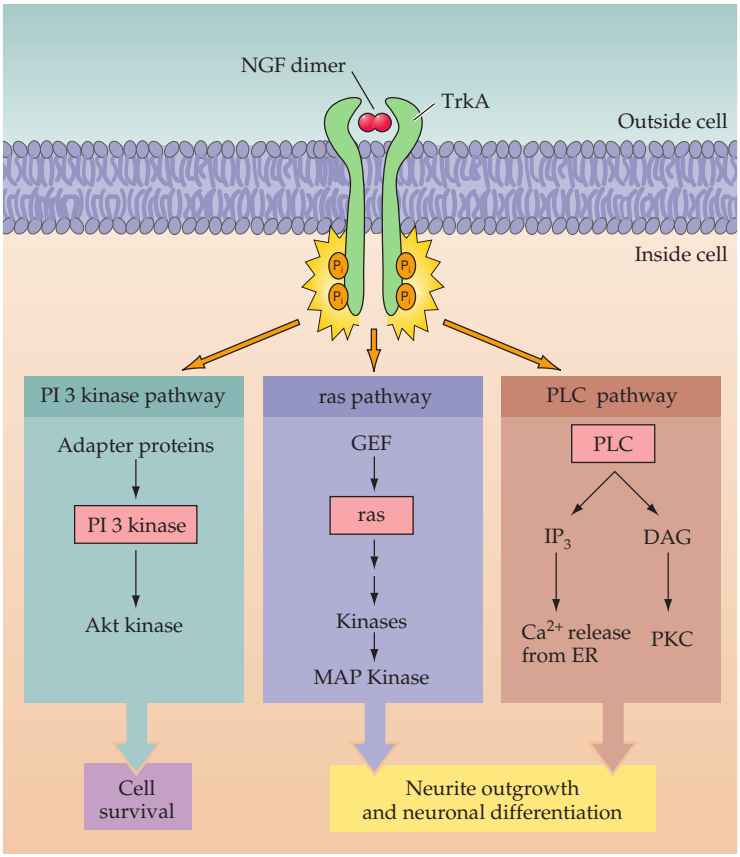


Figure 7.12 Mechanism of action of NGF. NGF binds to a high-affinity tyrosine kinase receptor, TrkA, on the plasma membrane to induce phosphorylation of TrkA at two different tyrosine residues. These phosphorylated tyrosines serve to tether various adapter proteins or phospholipase C (PLC), which, in turn, activate three major signaling pathways: the PI 3 kinase pathway leading to activation of Akt kinase, the ras pathway leading to MAP kinases, and the PLC pathway leading to release of intracellular Ca^{2+} and activation of PKC. The ras and PLC pathways primarily stimulate processes responsible for neuronal differentiation, while the PI 3 kinase pathway is primarily involved in cell survival.

cells in the cerebellum. These synapses are central to information flow through the cerebellar cortex, which in turn helps coordinate motor movements (see Chapter 18). One of the synapses is between the parallel fibers (PFs) and their Purkinje cell targets. LTD is a form of synaptic plasticity that causes the PF synapses to become less effective (see Chapter 24). When PFs are active, they release the neurotransmitter glutamate onto the dendrites of Purkinje cells. This activates AMPA-type receptors, which are ligand-gated ion channels (see Chapter 6), and causes a small EPSP that briefly depolarizes the Purkinje cell. In addition to this electrical signal, PF synaptic transmission also generates two second messengers within the Purkinje cell (Figure 7.13). The glutamate released by PFs activates metabotropic glutamate receptors, which stimulates phospholipase C to produce IP_3 and DAG. When the PF synapses alone are active, these intracellular signals are insufficient to open IP_3 receptors or to stimulate PKC.

LTD is induced when PF synapses are activated at the same time as the glutamatergic climbing fiber synapses that also innervate Purkinje cells. The climbing fiber synapses produce large EPSPs that strongly depolarize the membrane potential of the Purkinje cell. This depolarization allows Ca^{2+} to

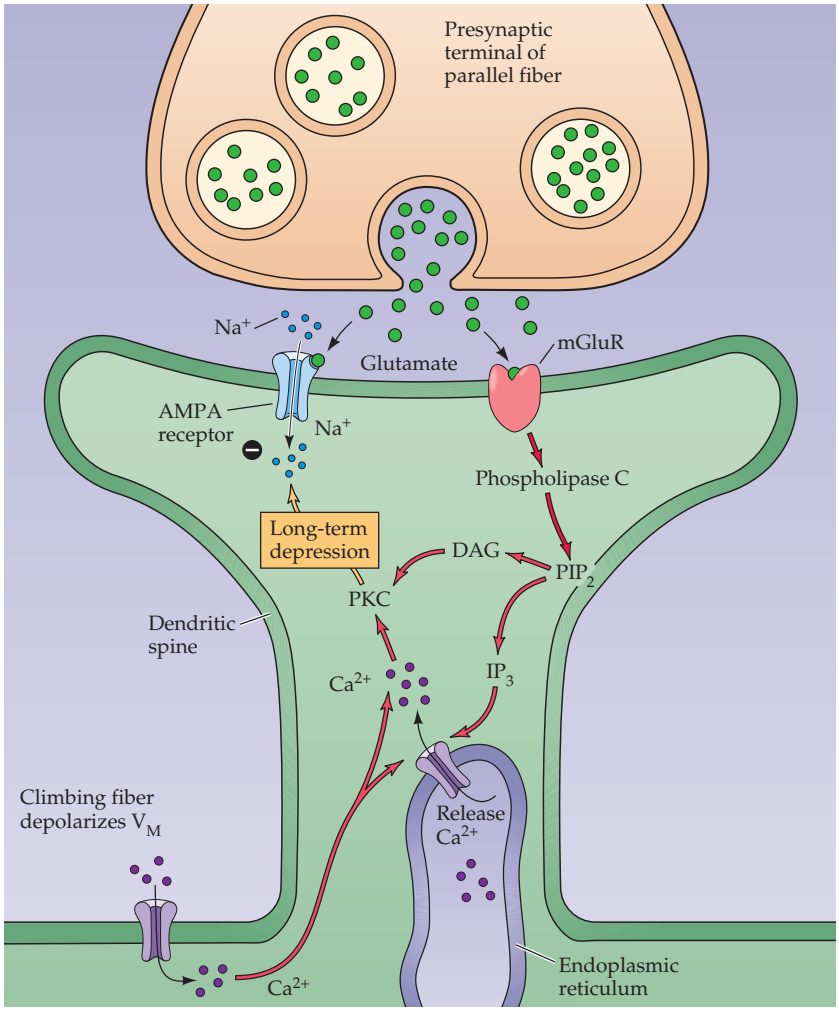


Figure 7.13 Signaling at cerebellar parallel fiber synapses. Glutamate released by parallel fibers activates both AMPA-type and metabotropic receptors. The latter produces IP_3 and DAG within the Purkinje cell. When paired with a rise in Ca^{2+} associated with activity of climbing fiber synapses, the IP_3 causes Ca^{2+} to be released from the endoplasmic reticulum, while Ca^{2+} and DAG together activate protein kinase C. These signals together change the properties of AMPA receptors to produce LTD.

enter the Purkinje cell via voltage-gated Ca^{2+} channels. When both synapses are simultaneously activated, the rise in intracellular Ca^{2+} concentration caused by the climbing fiber synapse enhances the sensitivity of IP_3 receptors to the IP_3 produced by PF synapses and allows the IP_3 receptors within the Purkinje cell to open. This releases Ca^{2+} from the endoplasmic reticulum and further elevates Ca^{2+} concentration locally near the PF synapses. This larger rise in Ca^{2+}, in conjunction with the DAG produced by the PF synapses, activates PKC. PKC in turn phosphorylates a number of substrate proteins. Ultimately, these signaling processes change AMPA-type receptors at the PF synapse, so that these receptors produce smaller electrical signals in response to the glutamate released from the PFs. This weakening of the PF synapse is the final cause of LTD.

In short, transmission at Purkinje cell synapses produces brief electrical signals and chemical signals that last much longer. The temporal interplay between these signals allows LTD to occur only when both PF and climbing fiber synapses are active. The actions of IP_3, DAG and Ca^{2+} also are restricted to small parts of the Purkinje cell dendrite, which is a more limited spatial range than the EPSPs, which spread throughout the entire dendrite and cell body of the Purkinje cell. Thus, in contrast to the electrical signals, the second messenger signals can impart precise information about the location of active synapses and allow LTD to occur only in the vicinity of active PFs.

- *Phosphorylation of tyrosine hydroxylase*. A third example of intracellular signaling in the nervous system is the regulation of the enzyme tyrosine hydroxylase. Tyrosine hydroxylase governs the synthesis of the catecholamine neurotransmitters: dopamine, norepinephrine, and epinephrine (see Chapter 6). A number of signals, including electrical activity, other neurotransmitters, and NGF, increase the rate of catecholamine synthesis by increasing the catalytic activity of tyrosine hydroxylase (Figure 7.14). The rapid increase of tyrosine hydroxylase activity is largely due to phosphorylation of this enzyme.

Tyrosine hydroxylase is a substrate for several protein kinases, including PKA, CaMKII, MAP kinase, and PKC. Phosphorylation causes conformational changes that increase the catalytic activity of tyrosine hydroxylase. Stimuli that elevate cAMP, Ca^{2+}, or DAG can all increase tyrosine hydroxylase activity and thus increase the rate of catecholamine biosynthesis. This regulation by several different signals allows for close control of tyrosine hydroxylase activity, and illustrates how several different pathways can converge to influence a key enzyme involved in synaptic transmission.

Summary

A diversity of signal transduction pathways exist within all neurons. Activation of these pathways typically is initiated by chemical signals such as neurotransmitters and hormones. These molecules bind to receptors that include ligand-gated ion channels, G-protein-coupled receptors and tyrosine kinase receptors. Many of these receptors activate either heterotrimeric or monomeric G-proteins that regulate intracellular enzyme cascades and/or ion channels. A common outcome of the activation of these receptors is the production of second messengers, such as cAMP, Ca^{2+}, and IP_3, that bind to effector enzymes. Particularly important effectors are protein kinases and phosphatases that regulate the phosphorylation state of their substrates, and thus their function. These substrates can be metabolic enzymes or other signal transduction molecules, such as ion channels, protein kinases, or transcription factors that regulate gene expression. Examples of transcription

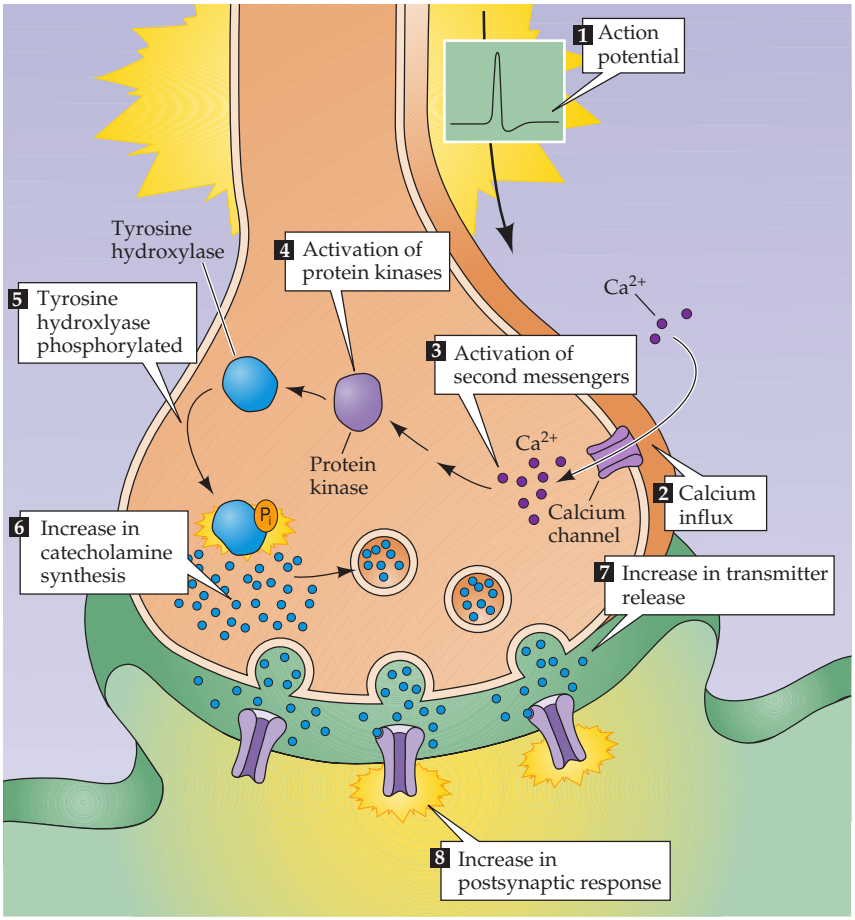


Figure 7.14 Regulation of tyrosine hydroxylase by protein phosphorylation. This enzyme governs the synthesis of the catecholamine neurotransmitters and is stimulated by a number of intracellular signals. In the example shown here, neuronal electrical activity (1) causes influx of Ca^{2+} (2). The resultant rise in intracellular Ca^{2+} concentration (3) activates protein kinases (4), which phosphorylates tyrosine hydroxylase (5) to stimulate catecholamine synthesis (6). This, in turn, increases release of catecholamines (7) and enhances the postsynaptic response produced by the synapse (8).

factors include CREB, steroid hormone receptors, and c-fos. This plethora of molecular components allows intracellular signal transduction pathways to generate responses over a wide range of times and distances, greatly augmenting and refining the information-processing ability of neuronal circuits and ultimately systems.

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