the hormone to the receptor causes the complex to shift from the cytoplasm to the nucleus (figure 9.5). As the ligand–receptor complex makes it all the way to the nucleus of the cell, these receptors are often called **nuclear receptors.**

Steroid receptor action

The primary function of steroid hormone receptors, as well as receptors for a number of other small, lipid-soluble signal molecules such as vitamin D and thyroid hormone, is to act as regulators of gene expression (see chapter 16).

All of these receptors have similar structures; the genes that code for them appear to be the evolutionary descendants of a single ancestral gene. Because of their structural similarities, they are all part of the *nuclear receptor superfamily.*

Each of these receptors has three functional domains:

1. a hormone-binding domain,
2. a DNA-binding domain, and
3. a domain that can interact with coactivators to affect the level of gene transcription.

In its inactive state, the receptor typically cannot bind to DNA because an inhibitor protein occupies the DNA-binding site. When the signal molecule binds to the hormone-binding site, the conformation of the receptor changes, releasing the inhibitor and exposing the DNA-binding site, allowing the receptor to attach to specific nucleotide sequences on the DNA (figure 9.5). This binding activates (or, in a few instances, suppresses) particular genes, usually located adjacent to the hormone-binding sequences. In the case of cortisol, which is a glucocorticoid hormone that can increase levels of glucose in cells, a number of different genes involved in the synthesis of glucose have binding sites for the hormone receptor complex.

The lipid-soluble ligands that intracellular receptors recognize tend to persist in the blood far longer than water-soluble signals. Most water-soluble hormones break down within minutes, and neurotransmitters break down within seconds or even milliseconds. In contrast, a steroid hormone such as cortisol or estrogen persists for hours.

Specificity and the role of coactivators

The target cell's response to a lipid-soluble cell signal can vary enormously, depending on the nature of the cell. This characteristic is true even when different target cells have the same intracellular receptor. Given that the receptor proteins bind to specific DNA sequences, which are the same in all cells, this may seem puzzling. It is explained in part by the fact that the receptors act in concert with **coactivators,** and the number and nature of these molecules can differ from cell to cell. Thus, a cell's response depends on not only the receptors but also the coactivators present.

The hormone estrogen has different effects in uterine tissue than in mammary tissue. This differential response is mediated by coactivators and not by the presence or absence of a receptor in the two tissues. In mammary tissue, a critical coactivator is lacking and the hormone–receptor complex instead interacts with another protein that acts to reduce gene expression. In uterine tissue, the coactivator is present, and the expression of genes that encode proteins involved in preparing the uterus for pregnancy are turned on.

Other intracellular receptors act as enzymes

A very interesting example of a receptor acting as an enzyme is found in the receptor for nitric oxide (NO). This small gas molecule diffuses readily out of the cells where it is produced and passes directly into neighboring cells, where it binds to the enzyme guanylyl cyclase. Binding of NO activates this enzyme, enabling it to catalyze the synthesis of *cyclic guanosine monophosphate (cGMP),* an intracellular messenger molecule that produces cell-specific responses such as the relaxation of smooth muscle cells.

When the brain sends a nerve signal to relax the smooth muscle cells lining the walls of vertebrate blood vessels, acetylcholine released by the nerve cell binds to receptors on epithelial cells. This causes an increase in intracellular Ca^{2+} in the epithelial cell that stimulates nitric oxide synthase to produce NO. The NO diffuses into the smooth muscle, where it increases the level of cGMP, leading to relaxation. This relaxation allows the vessel to expand and thereby increases blood flow. This explains the use of nitroglycerin to treat the pain of angina caused by constricted blood vessels to the heart. The nitroglycerin is converted by cells to NO, which then acts to relax the blood vessels.

The drug sildenafil (better known as Viagra) also functions via this signal transduction pathway by binding to and inhibiting the enzyme cGMP phosphodiesterase, which breaks down cGMP. This keeps levels of cGMP high, thereby stimulating production of NO. The reason for Viagra's selective effect is that it binds to a form of cGMP phosphodiesterase found in cells in the penis. This allows relaxation of smooth muscle in erectile tissue, thereby increasing blood flow.

Learning Outcomes Review 9.3

Hydrophobic signaling molecules can cross the membrane and bind to intracellular receptors. The steroid hormone receptors act by directly influencing gene expression. On binding hormone, the hormone–receptor complex moves into the nucleus to turn on (or sometimes turn off) gene expression. This may also require a coactivator that functions with the hormone–receptor complex. Thus, the cell's response to a hormone depends on the presence of a receptor and coactivators as well.

- ***Would these types of intracellular receptors be fast acting, or have effects of longer duration?***

9.4 Signal Transduction Through Receptor Kinases

Learning Outcomes

1. *Compare the function of RTKs to steroid hormone receptors.*
2. *Describe how information crosses the membrane in RTKs.*
3. *Explain the role of kinase cascades in signal transduction.*

In section 9.1, you read that protein kinases phosphorylate proteins to alter protein function and that the most common kinases act on the amino acids serine, threonine, and tyrosine.

The **receptor tyrosine kinases (RTKs)** influence the cell cycle, cell migration, cell metabolism, and cell proliferation—virtually all aspects of the cell are affected by signaling through these receptors. Alterations to the function of these receptors and their signaling pathways can lead to cancers in humans and other animals.

Some of the earliest examples of cancer-causing genes, or oncogenes, involve RTK function (discussed in chapter 10). The avian erythroblastosis virus carries an altered form of the epidermal growth factor receptor that lacks most of its extracellular domain. When this virus infects a cell the altered receptors produced are stuck in the "on" state. The continuous signaling from this receptor leads to cells that have lost the normal controls over growth.

Receptor tyrosine kinases are a large class of membrane receptors in animal cells that recognize hydrophilic ligands. Plants possess receptors with a similar overall structure and function, but they are serine–threonine kinases. These plant receptors have been named **plant receptor kinases.**

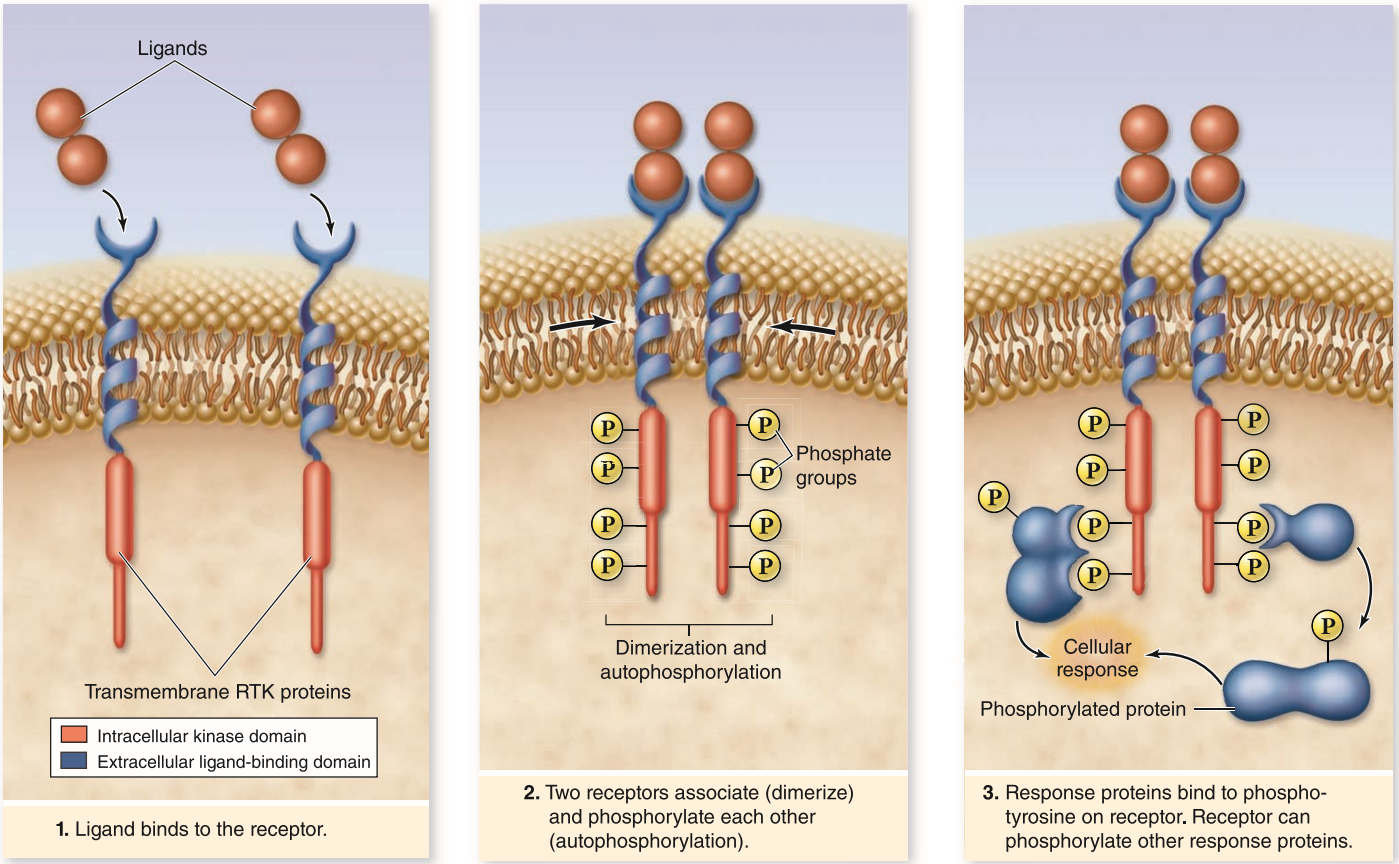
Because these receptors are performing similar functions in plant and animal cells but differ in their substrates, the duplication and divergence of each kind of receptor kinase probably occurred after the plant–animal divergence. The proliferation of these types of signaling molecules is thought to coincide with the independent evolution of multicellularity in each group.

In this section, we will concentrate on the RTK family of receptors that has been extensively studied in a variety of animal cells.

RTKs are activated by autophosphorylation

Receptor tyrosine kinases have a relatively simple structure consisting of a single transmembrane domain, an extracellular ligand-binding domain, and an intracellular kinase domain. This kinase domain contains the catalytic site of the receptor, which acts as a protein kinase that adds phosphate groups to tyrosines. On ligand binding to a specific receptor, two of these receptor–ligand complexes associate together (often referred to as dimerization) and phosphorylate each other, a process called *autophosphorylation* (figure 9.6).

The autophosphorylation event transmits across the membrane the signal that began with the binding of the ligand to the receptor. The next step, propagation of the signal in the cytoplasm, can take a variety of different forms. These forms include activation of the tyrosine kinase domain to phosphorylate other



1. Ligand binds to the receptor.

2. Two receptors associate (dimerize) and phosphorylate each other (autophosphorylation).

3. Response proteins bind to phosphotyrosine on receptor. Receptor can phosphorylate other response proteins.

Figure 9.6 Activation of a receptor tyrosine kinase (RTK). These membrane receptors bind hormones or growth factors that are hydrophilic and cannot cross the membrane. The receptor is a transmembrane protein with an extracellular ligand-binding domain and an intracellular kinase domain. Signal transduction pathways begin with response proteins binding to phosphotyrosine on receptor, and by receptor phosphorylation of response proteins.

intracellular targets or interaction of other proteins with the phosphorylated receptor.

The cellular response after activation depends on the possible response proteins in the cell. Two different cells can have the same receptor yet a different response, depending on what response proteins are present in the cytoplasm. For example, fibroblast growth factor stimulates cell division in fibroblasts but stimulates nerve cells to differentiate rather than to divide.

Phosphotyrosine domains mediate protein–protein interactions

One way that the signal from the receptor can be propagated in the cytoplasm is via proteins that bind specifically to phosphorylated tyrosines in the receptor. When the receptor is activated, regions of the protein outside of the catalytic site are phosphorylated. This creates "docking" sites for proteins that bind specifically to phosphotyrosine. The proteins that bind to these phosphorylated tyrosines can initiate intracellular events to convert the signal from the ligand into a response (figure 9.6).

The insulin receptor

The use of docking proteins is illustrated by the insulin receptor. The hormone insulin is part of the body's control system to maintain a constant level of blood glucose. The role of insulin is to lower blood glucose, acting by binding to an RTK. Another protein called the *insulin response protein* binds to the phosphorylated receptor and is itself phosphorylated. The insulin response protein passes the signal on by binding to additional proteins that lead to the activation of the enzyme glycogen synthase, which converts glucose to glycogen (figure 9.7), thereby lowering blood glucose. Other proteins activated by the insulin receptor act to inhibit the synthesis of enzymes involved in making glucose, and to increase the number of glucose transporter proteins in the plasma membrane.

Adapter proteins

Another class of proteins, **adapter proteins,** can also bind to phosphotyrosines. These proteins themselves do not participate in signal transduction but act as a link between the receptor and proteins that initiate downstream signaling events. For example, the Ras protein discussed later in this section is activated by adapter proteins binding to a receptor.

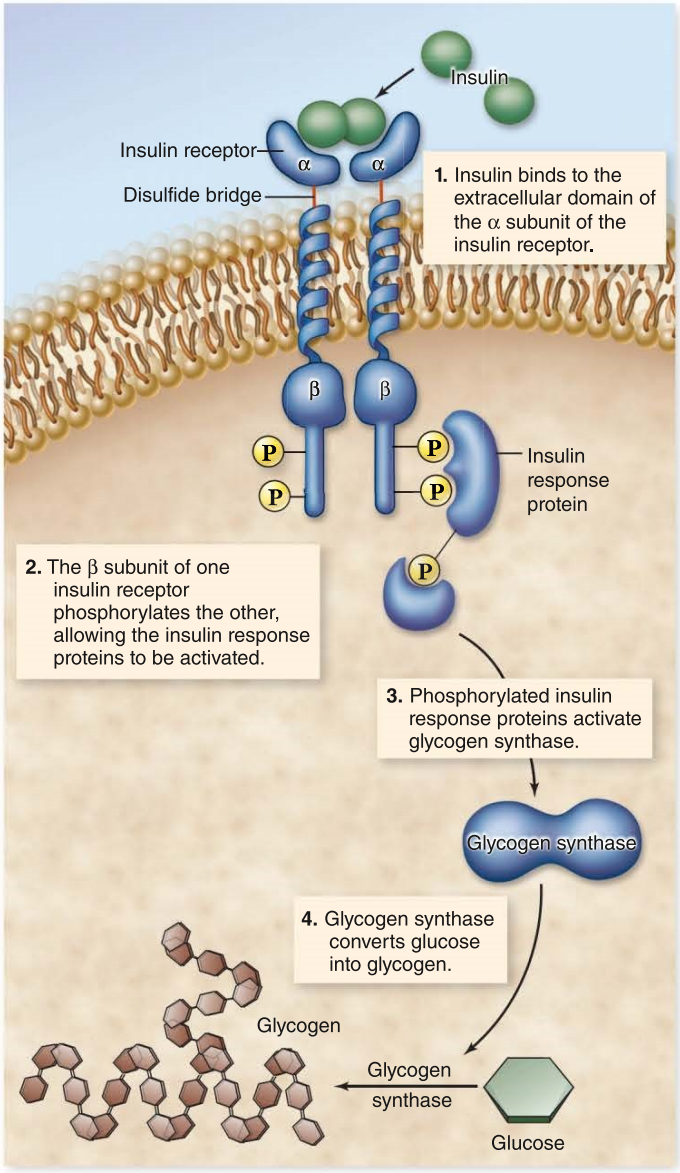


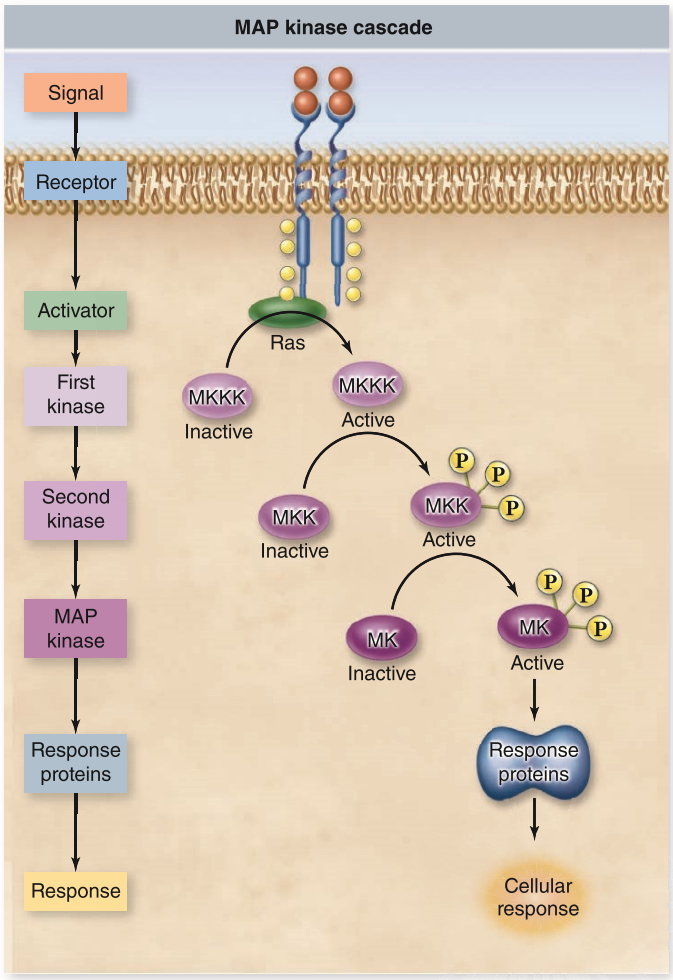
Figure 9.7 The insulin receptor. The insulin receptor is a receptor tyrosine kinase that initiates a variety of cellular responses related to glucose metabolism. One signal transduction pathway that this receptor mediates leads to the activation of the enzyme glycogen synthase. This enzyme converts glucose to glycogen.

Protein kinase cascades can amplify a signal

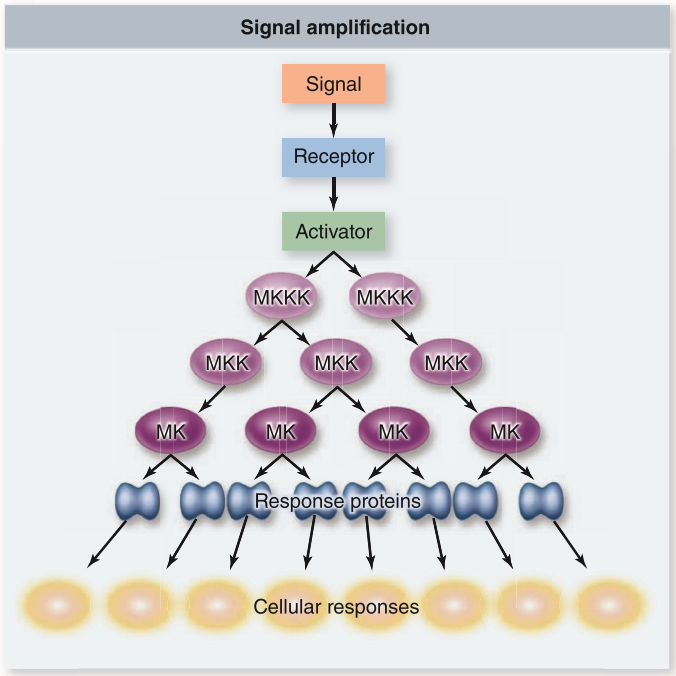
One important class of cytoplasmic kinases are **mitogen-activated protein (MAP) kinases.** A *mitogen* is a chemical that stimulates cell division by activating the normal pathways that control division. The MAP kinases are activated by a signaling module called a *phosphorylation cascade* or a **kinase cascade.** This module is a series of protein kinases that phosphorylate each other in succession. The final step in the cascade is the activation by phosphorylation of MAP kinase itself (figure 9.8).

One function of a kinase cascade is to amplify the original signal. Because each step in the cascade is an enzyme, it can act on a number of substrate molecules. With each enzyme in the cascade acting on many substrates this produces a large amount of the final product (figure 9.8). This allows a small number of initial signaling molecules to produce a large response.

The cellular response to this cascade in any particular cell depends on the targets of the MAP kinase, but usually involves phosphorylating transcription factors that then activate gene expression (see chapter 16). An example of this kind of signaling through growth factor receptors is provided in chapter 10 and



a.



b.

Figure 9.8 MAP kinase cascade leads to signal amplification. ***a.*** Phosphorylation cascade is shown as a flowchart on the left. The corresponding cellular events are shown on the right, beginning with the receptor in the plasma membrane. Each kinase is named starting with the last, the MAP kinase (MK), which is phosphorylated by a MAP kinase kinase (MKK), which is in turn phosphorylated by a MAP kinase kinase kinase (MKKK). The cascade is linked to the receptor protein by an activator protein. ***b.*** At each step the enzymatic action of the kinase on multiple substrates leads to amplification of the signal.

illustrates how signal transduction initiated by a growth factor can control the process of cell division through a kinase cascade.

Scaffold proteins organize kinase cascades

The proteins in a kinase cascade need to act sequentially to be effective. One way the efficiency of this process can be increased is to organize them in the cytoplasm. Proteins called *scaffold proteins* are thought to organize the components of a kinase cascade into a single protein complex, the ultimate in a signaling module. The scaffold protein binds to each individual kinase such that they are spatially organized for optimal function (figure 9.9).

The advantages of this kind of organization are many. A physically arranged sequence is clearly more efficient than one that depends on diffusion to produce the appropriate order of events. This organization also allows the segregation of signaling modules in different cytoplasmic locations.

The disadvantage of this kind of organization is that it reduces the amplification effect of the kinase cascade. Enzymes held in one place are not free to find new substrate molecules, but must rely on substrates being nearby.

The best-studied example of a scaffold protein comes from mating behavior in budding yeast. Yeast cells respond to mating pheromones with changes in cell morphology and gene expression, mediated by a protein kinase cascade. A protein called Ste5 was originally identified as a protein required for mating behavior, but no enzymatic activity could be detected for this protein. It has now been shown that this protein interacts with all of the members of the kinase cascade and acts as a scaffold protein that organizes the cascade and insulates it from other signaling pathways.

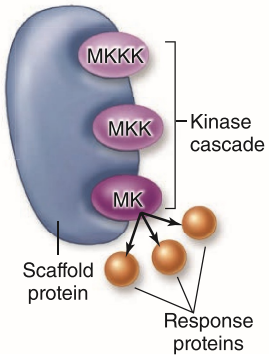


Figure 9.9 Kinase cascade can be organized by scaffold proteins. The scaffold protein binds to each kinase in the cascade, organizing them so each substrate is next to its enzyme. This organization also sequesters the kinases from other signaling pathways in the cytoplasm.

Ras is a small G protein that acts as a molecular switch

The link between the RTK and the MAP kinase cascade is a small GTP-binding protein (G protein) called **Ras.** Like all G proteins, Ras is active when bound to GTP, and inactive when bound to GDP. The Ras protein is mutated in many human tumors, indicative of its central role in linking growth factor receptors to their cellular response.

Ras was the first protein identified in a large superfamily of small G proteins with over 150 members in the human genome. The superfamily consists of five subgroups, one of which is the Ras family. These small G proteins are found in eukaryotes from yeast to vertebrates, indicating their ancient origin.

The roles of these small G proteins vary, affecting cell proliferation, the cytoskeleton, membrane transport, and nuclear transport. They are an excellent example of how gene duplication and diversification allow evolution to create modular units with diverse functions. The common feature of all members of the family is to act as a molecular switch linking external signals to internal signal transduction pathways (figure 9.10).

The Ras switch is flipped by exchanging GDP for GTP, and by Ras hydrolyzing GTP to GDP. The switch is sensitive to outside signals that regulate the activation and deactivation of Ras. Guanine nucleotide exchange factors (GEFs) activate Ras by stimulating the exchange of GDP for GTP. When a growth factor receptor is activated, it binds to an adapter protein that acts as a GEF. The activated Ras protein then activates the first kinase in the MAP kinase cascade (see figure 9.8 and chapter 10).

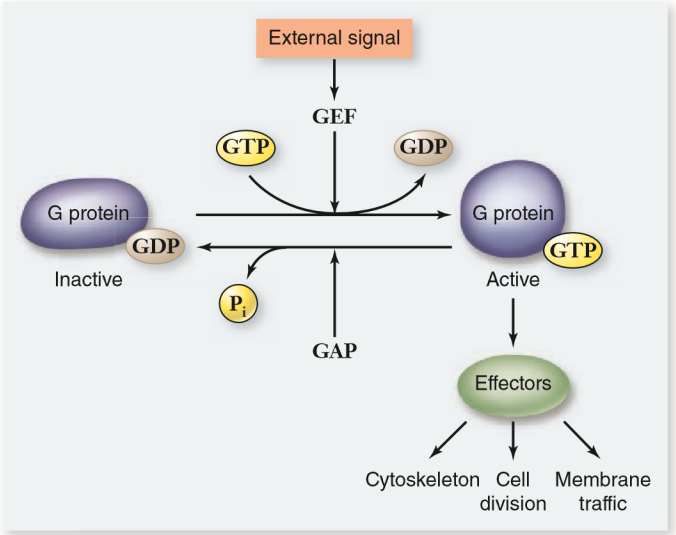


Figure 9.10 Small G proteins act as molecular switches. Small G proteins, such as Ras, link external signals to internal signal transduction pathways. External signals activate guanine nucleotide exchange proteins (GEF), which activate the G protein. The G protein can be inactivated by its weak intrinsic GTPase activity, which can be stimulated by activating proteins (GAP).

The action of Ras can be terminated by its intrinsic GTPase activity. This can be stimulated by a GAP protein, which provides the opportunity to fine-tune signaling based on the duration of Ras activity. The importance of these proteins is shown by mutations in GAP proteins that can lead to a predisposition for specific cancers such as neurofibromatosis.

RTKs are inactivated by internalization

It is important to cells that signaling pathways are only activated transiently. Continued activation could render the cell unable to respond to other signals or to respond inappropriately to a signal that is no longer relevant. Consequently, inactivation is as important for the control of signaling as activation. Receptor tyrosine kinases can be inactivated by two basic mechanisms—dephosphorylation and internalization. Internalization is by endocytosis, in which the receptor is taken up into the cytoplasm in a vesicle where it can be degraded or recycled.

The enzymes in the kinase cascade are all controlled by dephosphorylation by phosphatase enzymes. This leads to termination of the response at both the level of the receptor and the response proteins.

Learning Outcomes Review 9.4

Receptor tyrosine kinases (RTKs) are membrane receptors that can phosphorylate tyrosine. When activated, they autophosphorylate, creating binding domains for other proteins. These proteins transmit the signal inside the cell. One form of signaling pathway involves the MAP kinase cascade, a series of kinases that each activate the next in the series. This ends with a MAP kinase that activates transcription factors to alter gene expression.

- *Ras protein is mutated in many human cancers. What are possible reasons for this?*

9.5 Signal Transduction Through G Protein–Coupled Receptors

Learning Outcomes

1. *Contrast signaling through GPCRs and RTKs.*
2. *Relate the function of second messengers to signal transduction pathways.*

The single largest category of receptor type in animal cells is **G protein–coupled receptors (GPCRs),** so named because the receptors act by coupling with a G protein. These receptors bind diverse ligands, including ions, organic odorants, peptides, proteins, and lipids. Light-sensing receptors are also part of this family, so we could even count photons as "ligands."

This superfamily of proteins also has a characteristic structure with seven transmembrane domains that anchor the receptors in the membrane. This arrangement of seven transmembrane domains is highly conserved and is used to search for new members in sequenced genomes. The analysis of many animal genomes indicates that GPCRs are the largest gene family in most animals. They have been found in virtually all types of eukaryotic organisms, indicating an ancient origin with duplication and divergence leading to a wide array of signaling pathways.

The latest count of genes encoding GPCRs in the human genome is 865, with about half of these encoding odorant receptors involved in the sense of taste and smell. In the mouse, over 1000 different odorant receptors are involved in the sense of smell. The family of GPCRs has been subdivided into five groups based on structure and function: Rhodopsin, Secretin, Adhesion, Glutamate, and Frizzled/Taste 2. The names refer to the first discovered member of each group; for example, Rhodopsin is the GPCR involved in light sensing in mammals. In this section, we will concentrate on the basic mechanism of activation and some of the possible signal transduction pathways.

G proteins link receptors with effector proteins

The function of the G protein in signaling by GPCRs is to provide a link between a receptor that receives signals and effector proteins that produce cellular responses. The G protein functions as a switch that is turned on by the receptor. In its "on" state, the G protein activates effector proteins to cause a cellular response.

All G proteins are active when bound to GTP and inactive when bound to GDP. The main difference between the G proteins in GPCRs and the small G proteins described in section 9.4 is that these G proteins are composed of three subunits, called α, β, and γ. As a result, they are often called *heterotrimeric G proteins.* When a ligand binds to a GPCR and activates its associated G protein, the G protein exchanges GDP for GTP and dissociates into two parts consisting of the G_α subunit bound to GTP, and the G_β and G_γ subunits together ($G_{\beta\gamma}$). The signal can then be transmitted by either the G_α or the $G_{\beta\gamma}$ components stimulating effector proteins. The hydrolysis of bound GTP to GDP by G_α causes reassociation of the heterotrimer, restoring the "off" state of the system (figure 9.11).

The effector proteins are usually enzymes. An effector protein might be a protein kinase that phosphorylates proteins to directly propagate the signal, or it may produce a second messenger to initiate a signal transduction pathway.

Effector proteins produce multiple second messengers

Often, the effector proteins activated by G proteins produce a second messenger. Two of the most common effectors are *adenylyl cyclase* and *phospholipase C,* which produce cAMP and IP_3 plus DAG, respectively.

Cyclic AMP

All animal cells studied thus far use cAMP as a second messenger (see chapter 45). When a signaling molecule binds to a GPCR that uses the enzyme **adenylyl cyclase** as an effector, a large amount of cAMP is produced within the cell

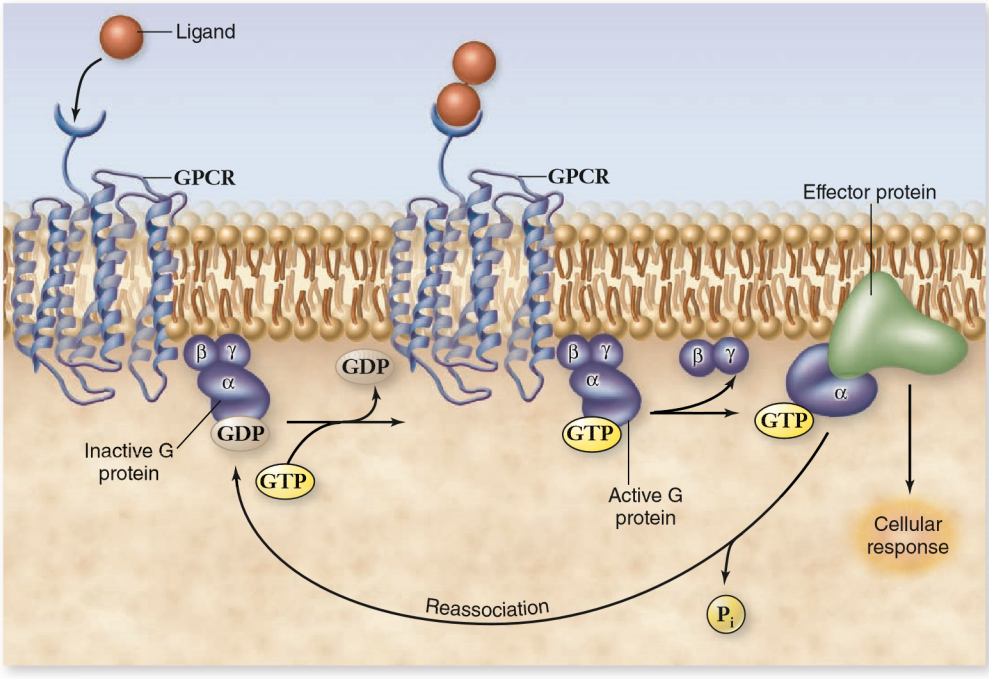


Figure 9.11 The action of G protein–coupled receptors. G protein–coupled receptors act through a heterotrimeric G protein that links the receptor to an effector protein. When ligand binds to the receptor, it activates an associated G protein, exchanging GDP for GTP. The active G protein complex dissociates into G_α and $G_{\beta\gamma}$. The G_α subunit (bound to GTP) is shown activating an effector protein. The effector protein may act directly on cellular proteins or produce a second messenger to cause a cellular response. G_α can hydrolyze GTP inactivating the system, then reassociate with $G_{\beta\gamma}$.