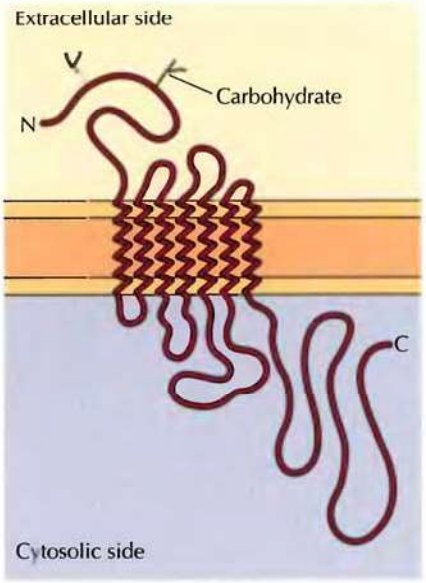


FIGURE 15.11 Structure of a G protein-coupled receptor The G protein-coupled receptor is characterized by seven transmembrane α helices.

■ **The G protein-coupled receptors responsible for our sense of smell (odorant receptors) are encoded by more than 500 genes in the human genome.**

intracellular proteins. These proteins then transmit signals from the receptor to a series of additional intracellular targets, frequently including transcription factors. Ligand binding to a receptor on the surface of the cell thus initiates a chain of intracellular reactions, ultimately reaching the target cell nucleus and resulting in programmed changes in gene expression. The functions of the major classes of cell surface receptors are discussed here, with the pathways of intracellular signaling downstream of these receptors being considered in the next section of this chapter.

G Protein-Coupled Receptors

The largest family of cell surface receptors transmits signals to intracellular targets via the intermediary action of guanine nucleotide-binding proteins called **G proteins**. More than a thousand such **G protein-coupled receptors** have been identified, including the receptors for eicosanoids, many neurotransmitters, neuropeptides, and peptide hormones. In addition, the G protein-coupled receptor family includes a large number of receptors that are responsible for smell, sight, and taste.

The G protein-coupled receptors are structurally and functionally related proteins characterized by seven membrane-spanning α helices (Figure 15.11). The binding of ligands to the extracellular domain of these receptors induces a conformational change that allows the cytosolic domain of the receptor to bind to a G protein associated with the inner face of the plasma membrane. This interaction activates the G protein, which then dissociates from the receptor and carries the signal to an intracellular target, which may be either an enzyme or an ion channel.

The discovery of G proteins came from studies of hormones (such as epinephrine) that regulate the synthesis of cyclic AMP (cAMP) in their target cells. As discussed later in the chapter, cAMP is an important second messenger that mediates cellular responses to a variety of hormones. In the 1970s Martin Rodbell and his colleagues made the key observation that GTP is required for hormonal stimulation of adenylyl cyclase (the enzyme responsible for cAMP formation). This finding led to the discovery that a guanine nucleotide-binding protein (called a G protein) is an intermediary in adenylyl cyclase activation (Figure 15.12). Since then an array of G proteins have been found to act as physiological switches that regulate the activities of a variety of intracellular targets in response to extracellular signals.

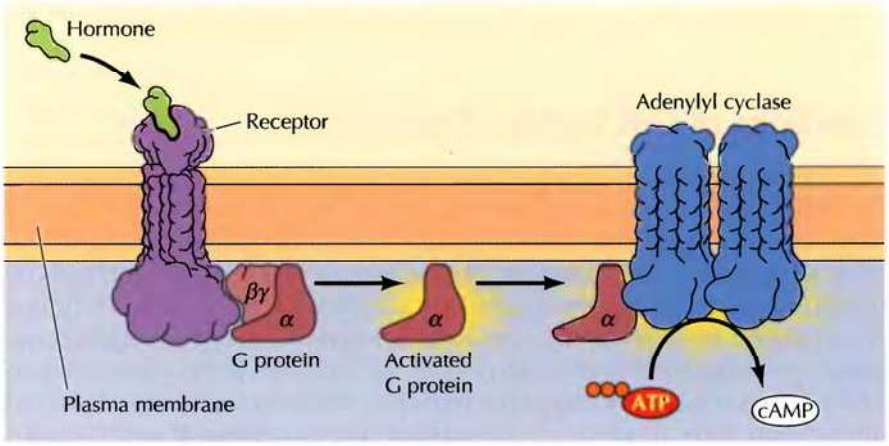
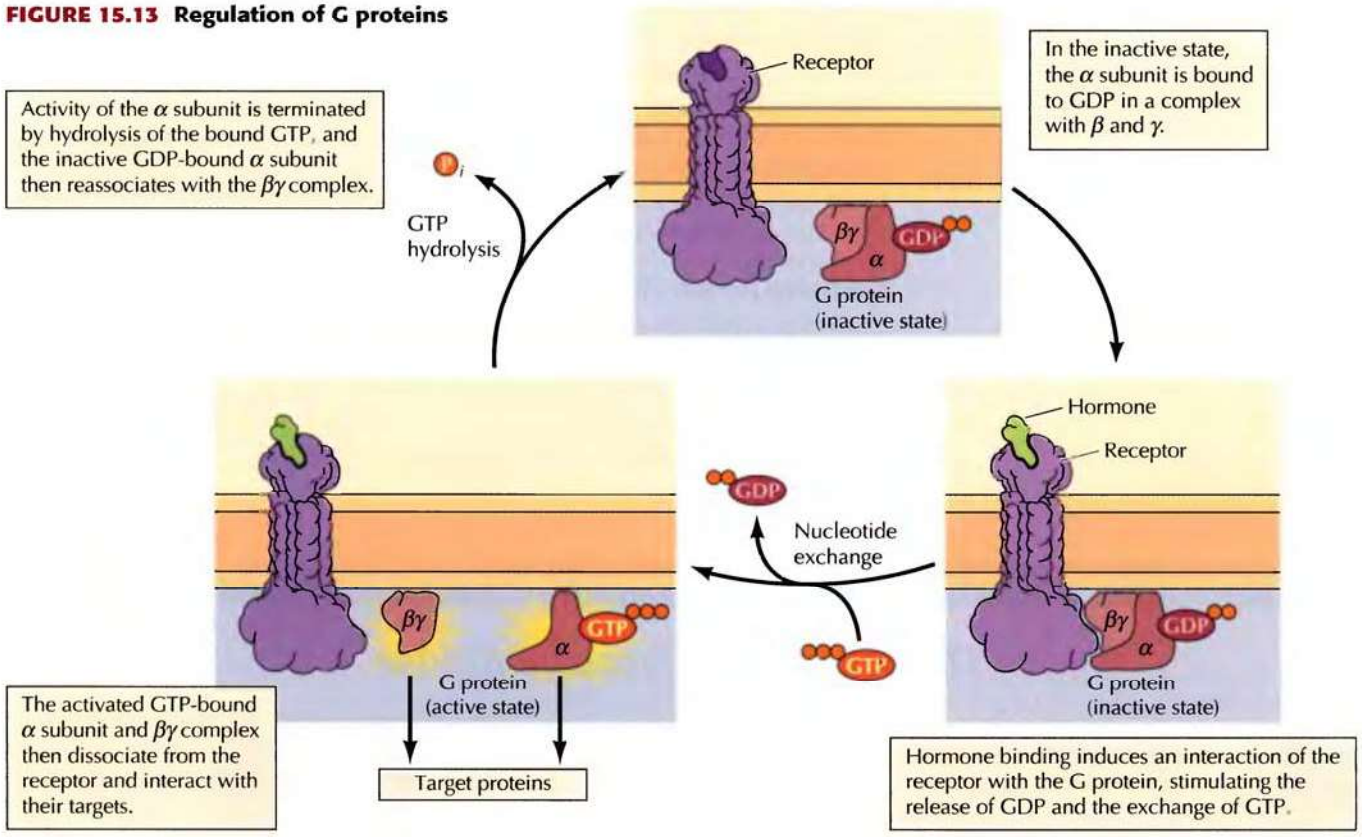


FIGURE 15.12 Hormonal activation of adenylyl cyclase Binding of hormone promotes the interaction of the receptor with a G protein. The activated G protein α subunit then dissociates from the receptor and stimulates adenylyl cyclase, which catalyzes the conversion of ATP to cAMP.

FIGURE 15.13 Regulation of G proteins



G proteins consist of three subunits designated α, β, and γ (Figure 15.13). They are frequently called **heterotrimeric G proteins** to distinguish them from other guanine nucleotide-binding proteins, such as the Ras proteins discussed later in the chapter. The α subunit binds guanine nucleotides, which regulate G protein activity. In the resting state, α is bound to GDP in a complex with β and γ. Hormone binding induces a conformational change in the receptor, such that the cytosolic domain of the receptor interacts with the G protein and stimulates the release of bound GDP and its exchange for GTP. The activated GTP-bound α subunit then dissociates from β and γ, which remain together and function as a $\beta\gamma$ complex. Both the active GTP-bound α subunit and the $\beta\gamma$ complex then interact with their targets to elicit an intracellular response. The activity of the α subunit is terminated by hydrolysis of the bound GTP, and the inactive α subunit (now with GDP bound) then reassociates with the $\beta\gamma$ complex, ready for the cycle to start anew.

Mammalian genomes encode 20 different α subunits, 5 β subunits, and 12 γ subunits. Different G proteins associate with different receptors, so this array of G proteins couples receptors to distinct intracellular targets. For example, the G protein associated with the epinephrine receptor is called G_s because its α subunit stimulates adenylyl cyclase (see Figure 15.12). Other G protein α and $\beta\gamma$ subunits act instead to inhibit adenylyl cyclase or to regulate the activities of other target enzymes.

In addition to regulating target enzymes, both the α and $\beta\gamma$ subunits of some G proteins directly regulate ion channels. A good example is provided

15.2 WEBSITE ANIMATION

Signal Transduction

The largest family of cell surface receptors transmits signals inside the cell by activating G proteins, which bind GTP and then activate effector proteins.

by the action of the neurotransmitter acetylcholine on heart muscle, which is distinct from its effects on nerve and skeletal muscle. The nicotinic acetylcholine receptor on nerve and skeletal muscle cells is a ligand-gated ion channel (see Figure 13.23). Heart muscle cells have a different acetylcholine receptor, which is G protein-coupled. This G protein is designated G_i because its α subunit *i*nhibits adenylyl cyclase. In addition, the G_i $\beta\gamma$ subunits act directly to open K^+ channels in the plasma membrane, which has the effect of slowing heart muscle contraction.

Receptor Protein-Tyrosine Kinases

In contrast to the G protein-coupled receptors, other cell surface receptors are directly linked to intracellular enzymes. The largest family of such enzyme-linked receptors is the **receptor protein-tyrosine kinases**, which phosphorylate their substrate proteins on tyrosine residues. This family includes the receptors for most polypeptide growth factors, so protein-tyrosine

KEY EXPERIMENT

The Src Protein-Tyrosine Kinase

Transforming Gene Product of Rous Sarcoma Virus Phosphorylates Tyrosine

Tony Hunter and Bartholomew M. Sefton
The Salk Institute, San Diego, CA
Proceedings of the National Academy of Science, USA, 1980, Volume 77, pages 1311–1315

Tony Hunter

Bartholomew Sefton

The Context

Following its isolation in 1911 Rous sarcoma virus (RSV) became the first virus that was generally accepted to cause tumors in animals (see the Molecular Medicine box in Chapter 1). Several features of RSV then made it an attractive model for studying the development of cancer. In particular, the small size of the RSV genome offered the hope of identifying specific viral genes responsible for inducing the abnormal proliferation that is characteristic of cancer cells. This goal was reached in the 1970s when it was established that a single RSV gene (called *src* for *sar*coma) is required for tumor induction. Importantly, a closely related *src* gene was also found to be part of the normal genetic complement of a variety of vertebrates, including humans. Since the viral Src protein is responsible for driving the uncontrolled proliferation of cancer cells, it appeared that understanding Src function would yield crucial insights into the molecular bases of both cancer induction and the regulation of normal cell proliferation.

In 1977 Ray Erikson and his colleagues identified the Src protein by immunoprecipitation (see Figure 4.30) with antisera from animals bearing RSV-induced tumors. Shortly thereafter, it was found that incubation of Src immunoprecipitates with radioactive ATP resulted in phosphorylation of the immunoglobulin molecules. Src therefore appeared to be a protein kinase, clearly implicating protein phosphorylation in the control of cell proliferation.

All previously studied protein kinases phosphorylated serine or threonine residues, which were also the only phosphoamino acids to have been detected in animal cells. However, Walter Eckhardt and Tony Hunter had observed in 1979 that the oncogenic protein of another animal tumor virus (polyomavirus) was phosphorylated on a tyrosine residue. Hunter and Sefton therefore tested the possibility that Src might phosphorylate tyrosine, rather than serine/threonine, residues in its substrate proteins. Their experiments demonstrated that Src does indeed function as a protein-tyrosine kinase—an activity now recognized as playing a central role in cell signaling pathways.

phosphorylation has been particularly well studied as a signaling mechanism involved in the control of animal cell growth and differentiation. Indeed, the first protein-tyrosine kinase was discovered in 1980 during studies of the oncogenic proteins of animal tumor viruses—in particular, Rous sarcoma virus—by Tony Hunter and Bartholomew Sefton. The EGF receptor was then found to function as a protein-tyrosine kinase by Stanley Cohen and his colleagues, clearly establishing protein-tyrosine phosphorylation as a key signaling mechanism in the response of cells to growth factor stimulation.

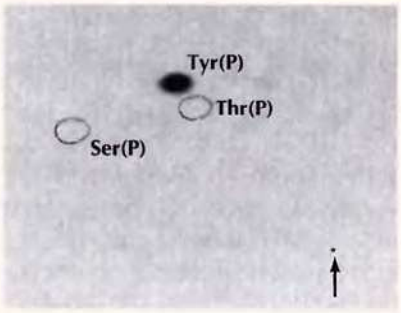
The human genome encodes 59 receptor protein-tyrosine kinases, including the receptors for EGF, NGF, PDGF, insulin, and many other growth factors. All these receptors share a common structural organization: an N-terminal extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with protein-tyrosine kinase activity (Figure 15.14). Most of the receptor protein-tyrosine kinases consist of single polypeptides, although the insulin receptor and some

KEY EXPERIMENT

The Experiments

Hunter and Sefton identified the amino acid phosphorylated by Src by incubating Src immunoprecipitates with ^{32}P-labeled ATP. The amino acid that was phosphorylated by Src in the substrate protein (in this case, immunoglobulin) therefore became radioactively labeled. The immunoglobulin was then isolated and hydrolyzed to yield individual amino acids, which were analyzed by electrophoresis and chromatography methods that separated phosphotyrosine, phosphoserine, and phosphothreonine (see figure). The radioactive amino acid detected in these experiments was phosphotyrosine, indicating that Src specifically phosphorylates tyrosine residues.

Further experiments showed that the normal cell Src protein, as well as viral Src, functions as a protein-tyrosine kinase in immunoprecipitation assays. In addition, Hunter and Sefton extended these *in vitro* experiments by demonstrating the presence of phosphotyrosine in proteins extracted from whole cells. In normal cells, phosphotyrosine accounted for only about 0.03% of total phosphoamino acids (the rest being phosphoserine and phosphothreonine), explaining why it had previously escaped detection. However, phosphotyrosine was about



Identification of phosphotyrosine in immunoglobulin phosphorylated by Src. An immunoprecipitate containing RSV Src was incubated with [^{32}P]-ATP. The immunoglobulin was then isolated and hydrolyzed. Amino acids in the hydrolysate were separated by electrophoresis and chromatography on a cellulose thin-layer plate. The positions of ^{32}P-labeled amino acids were determined by exposing the plate to X-ray film. Broken lines indicate the positions of unlabeled phosphoamino acids that were included as markers. Note that the principal ^{32}P-labeled amino acid is phosphotyrosine.

ten times more abundant in cells that were infected with RSV, suggesting that increased protein-tyrosine kinase activity of the viral Src protein was responsible for its ability to induce abnormal cell proliferation.

The Impact

The discovery that Src was a protein-tyrosine kinase both identified a new protein kinase activity and established this activity as being related to the control of cell proliferation. The results of Hunter and Sefton were followed by demonstrations that many other tumor virus proteins also function as protein-tyrosine kinases, generalizing the link between protein-tyrosine phosphorylation and the abnormal proliferation of cancer cells. Stanley Cohen and his colleagues further found that the EGF receptor is a protein-tyrosine kinase, directly implicating protein-tyrosine phosphorylation in the control of normal cell proliferation. Continuing studies have identified numerous additional receptor and nonreceptor protein-tyrosine kinases that function in a variety of cell signaling pathways. Studies of the mechanism by which a virus causes cancer in chickens thus revealed a previously unknown enzymatic activity that plays a central role in the signaling pathways that regulate animal cell growth, survival, and differentiation. Moreover, as discussed in Chapter 18, protein-tyrosine kinases encoded by oncogenes have provided the most promising targets to date for development of specific drugs against cancer cells.

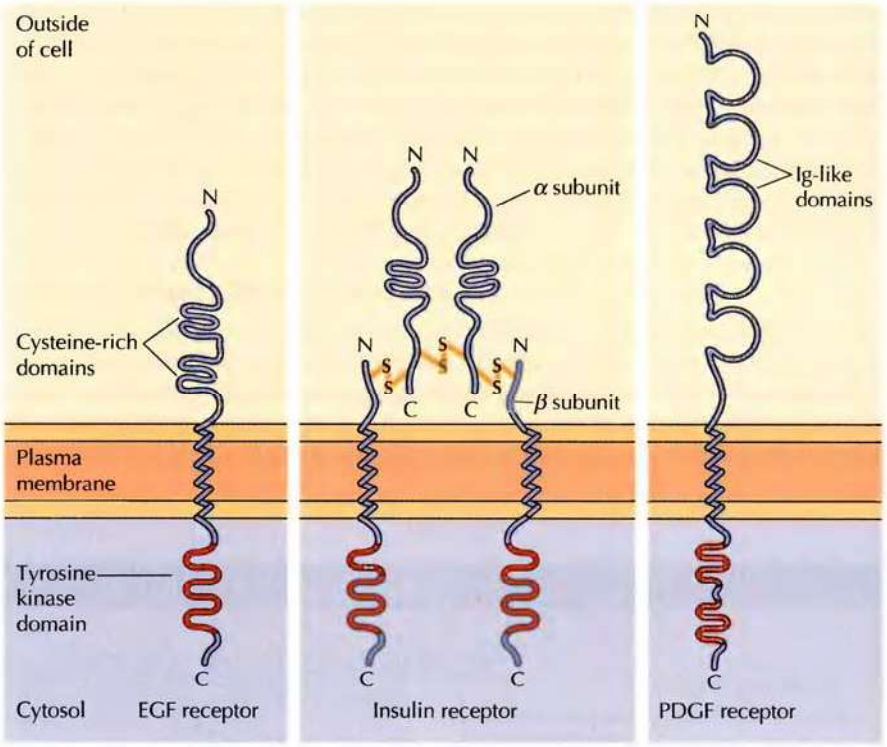


FIGURE 15.14 Organization of receptor protein-tyrosine kinases Each receptor consists of an N-terminal extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with protein-tyrosine kinase activity. The structures of three distinct subfamilies of receptor protein-tyrosine kinases are shown. The EGF receptor and insulin receptor both have cysteine-rich extracellular domains, whereas the PDGF receptor has immunoglobulin (Ig)-like domains. The PDGF receptor is also noteworthy in that its kinase domain is interrupted by an insert of approximately a hundred amino acids unrelated to those found in most other protein-tyrosine kinase catalytic domains. The insulin receptor is unusual in being a dimer of two pairs of polypeptide chains (designated α and β).

related receptors are dimers consisting of two polypeptide chains. The binding of ligands (e.g., growth factors) to the extracellular domains of these receptors activates their cytosolic kinase domains, resulting in phosphorylation of both the receptors themselves and intracellular target proteins that propagate the signal initiated by growth factor binding.

The first step in signaling from most receptor protein-tyrosine kinases is ligand-induced receptor dimerization (Figure 15.15). Some growth factors, such as PDGF and NGF, are themselves dimers consisting of two identical

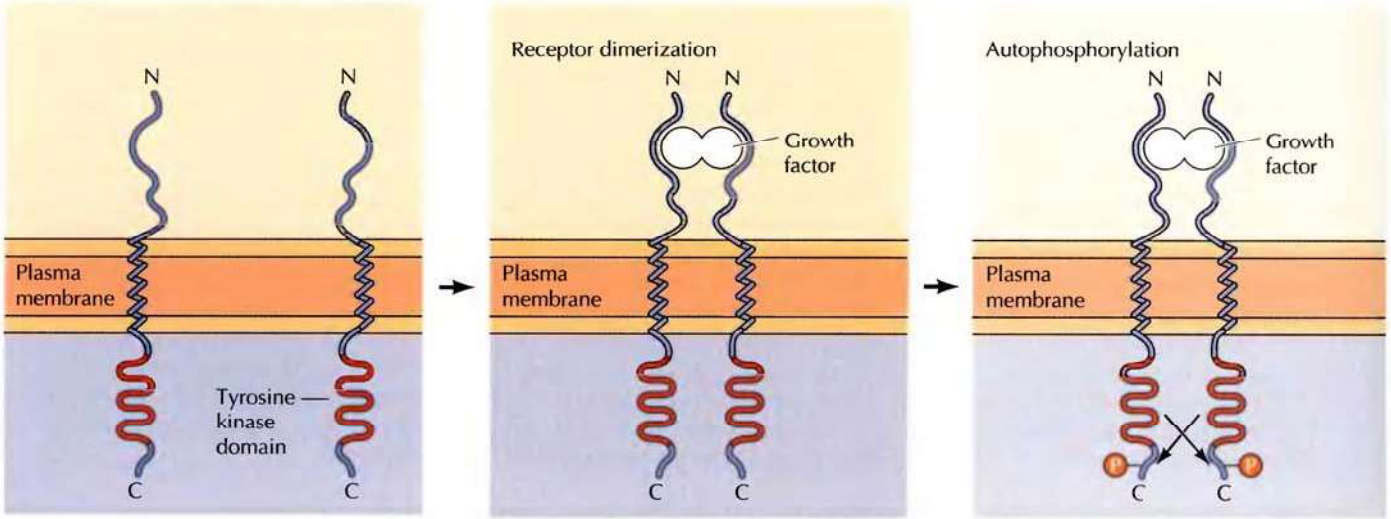


FIGURE 15.15 Dimerization and autophosphorylation of receptor protein-tyrosine kinases Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation as the two polypeptide chains phosphorylate one another.

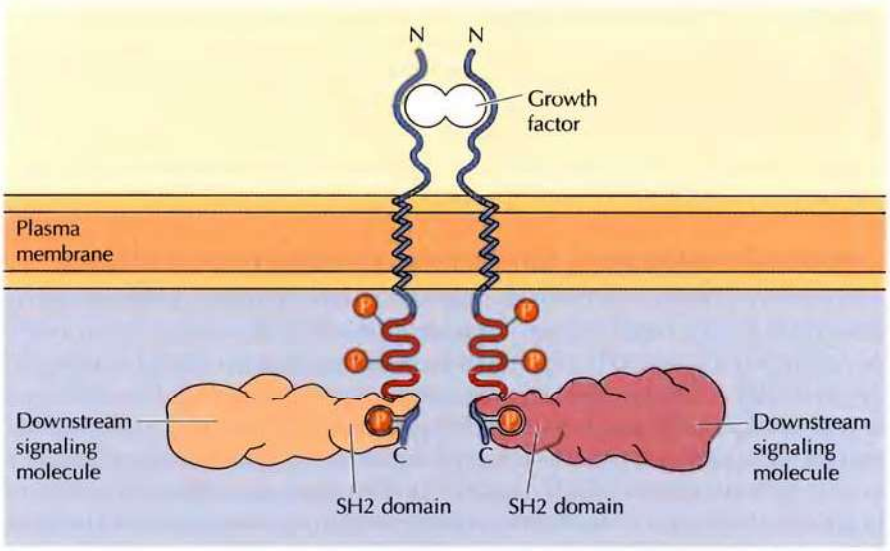


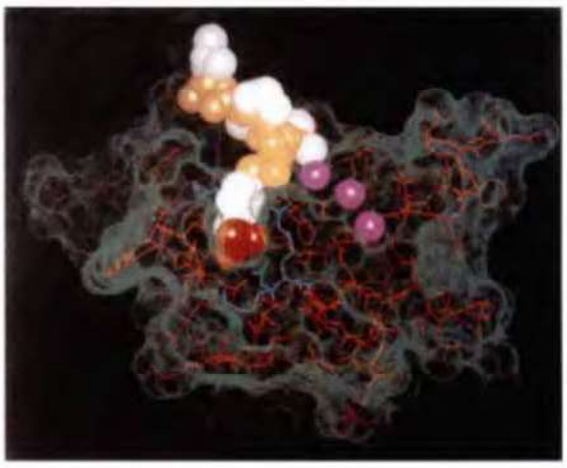
FIGURE 15.16 Association of downstream signaling molecules with receptor protein-tyrosine kinases SH2 domains bind to specific phosphotyrosine-containing peptides of the activated receptors.

polypeptide chains; these growth factors directly induce dimerization by simultaneously binding to two different receptor molecules. Other growth factors (such as EGF) are monomers but lead to receptor dimerization as a result of inducing conformational changes that promote protein-protein interactions between different receptor polypeptides.

Ligand-induced dimerization then leads to **autophosphorylation** of the receptor as the dimerized polypeptide chains cross-phosphorylate one another (see Figure 15.15). Such autophosphorylation plays two key roles in signaling from these receptors. First, phosphorylation of tyrosine residues within the catalytic domain increases protein kinase activity. Second, phosphorylation of tyrosine residues outside of the catalytic domain creates specific binding sites for additional proteins that transmit intracellular signals downstream of the activated receptors.

The association of these downstream signaling molecules with receptor protein-tyrosine kinases is mediated by protein domains that bind to specific phosphotyrosine-containing peptides (Figure 15.16). The first of these domains to be characterized are called **SH2 domains** (for *S*rc *h*omology 2) because they were initially recognized in protein-tyrosine kinases related to Src, the oncogenic protein of Rous sarcoma virus. SH2 domains consist of approximately 100 amino acids and bind to specific short peptide sequences containing phosphotyrosine residues (Figure 15.17). Other proteins bind to phosphotyrosine-containing peptides via **PTB domains** (for *p*hospho*t*yrosine-*b*inding). The resulting association of SH2- or PTB-containing proteins with activated receptor protein-tyrosine kinases can have several effects: It localizes these proteins to the plasma membrane, leads to their association with other proteins, promotes their phosphorylation, and stimulates their enzymatic activities. The association of these proteins with autophosphorylated receptors thus represents the first step in the intracellular transmission of signals initiated by the binding of growth factors to the cell surface.

FIGURE 15.17 Complex between an SH2 domain and a phosphotyrosine peptide The polypeptide chain of the Src SH2 domain is shown in red with its surface indicated by green dots. Purple spheres indicate a groove on the surface. The three amino acid residues that interact with the phosphotyrosine are shown in blue. The phosphotyrosine-containing peptide is shown as a space-filling model. Yellow and white spheres indicate the backbone and side-chain atoms, respectively, and the phosphate group is shown in red. (From G. Waksman and 13 others, 1992. *Nature* 358: 646.)



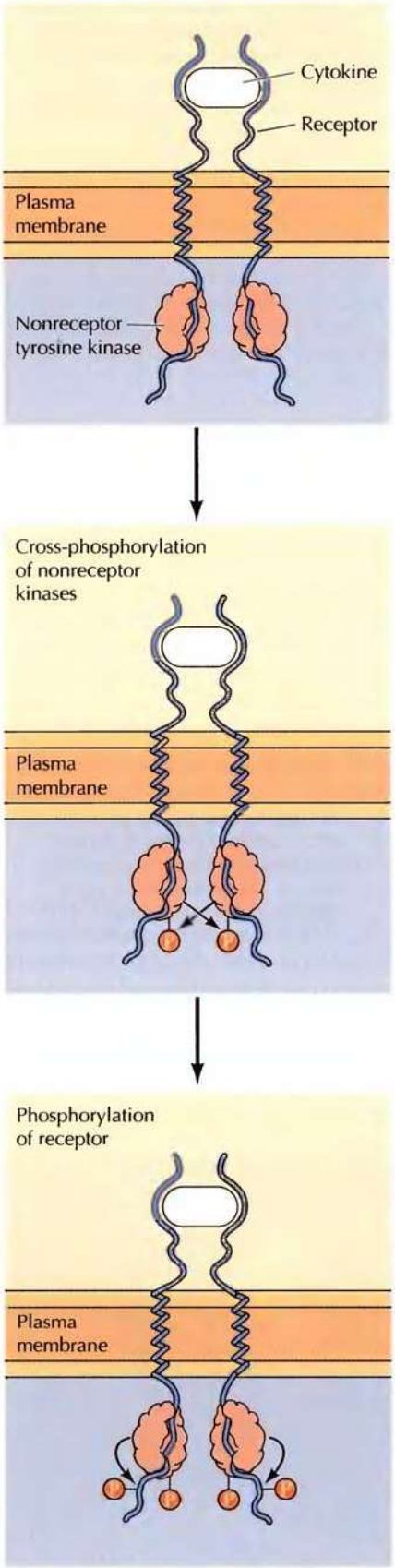


FIGURE 15.18 Signaling from cytokine receptors Ligand binding induces receptor dimerization and leads to the activation of associated nonreceptor protein-tyrosine kinases as a result of cross-phosphorylation. The activated kinases then phosphorylate tyrosine residues of the receptor, creating phosphotyrosine-binding sites for downstream signaling molecules.

Cytokine Receptors and Nonreceptor Protein-Tyrosine Kinases

Rather than possessing intrinsic enzymatic activity, many receptors act by stimulating intracellular protein-tyrosine kinases with which they are noncovalently associated. This family of receptors (called the **cytokine receptor superfamily**) includes the receptors for most cytokines (e.g., interleukin-2 and erythropoietin) and for some polypeptide hormones (e.g., growth hormone). Like receptor protein-tyrosine kinases, the cytokine receptors contain N-terminal extracellular ligand-binding domains, single transmembrane α helices, and C-terminal cytosolic domains. However, the cytosolic domains of the cytokine receptors are devoid of any known catalytic activity. Instead, the cytokine receptors function in association with **nonreceptor protein-tyrosine kinases**, which are activated as a result of ligand binding.

The first step in signaling from cytokine receptors is thought to be ligand-induced receptor dimerization and cross-phosphorylation of the associated nonreceptor protein-tyrosine kinases (Figure 15.18). These activated kinases then phosphorylate the receptor, providing phosphotyrosine-binding sites for the recruitment of downstream signaling molecules that contain SH2 domains. Combinations of cytokine receptors plus associated nonreceptor protein-tyrosine kinases thus function analogously to the receptor protein-tyrosine kinases discussed in the previous section.

The kinases associated with cytokine receptors belong to the **Janus kinase** (or **JAK**) family, which consists of four related nonreceptor protein-tyrosine kinases. Members of the JAK family appear to be universally required for signaling from cytokine receptors, indicating that JAK family kinases play a critical role in coupling these receptors to the tyrosine phosphorylation of intracellular targets.

Additional nonreceptor protein-tyrosine kinases belong to the **Src** family, which consists of Src and eight closely related proteins. As already noted, Src was initially identified as the oncogenic protein of Rous sarcoma virus and was the first protein shown to possess protein-tyrosine kinase activity, so it has played a pivotal role in experiments leading to our current understanding of cell signaling. Members of the Src family play key roles in signaling downstream of receptor protein-tyrosine kinases, from antigen receptors on B and T lymphocytes, and (as discussed later in this chapter) from integrins at sites of cell attachment to the extracellular matrix.

Receptors Linked to Other Enzymatic Activities

Although the vast majority of enzyme-linked receptors stimulate protein-tyrosine phosphorylation, some receptors are associated with other enzymatic activities. These receptors include protein-tyrosine phosphatases, protein-serine/threonine kinases, and guanylyl cyclases.

Protein-tyrosine phosphatases remove phosphate groups from phosphotyrosine residues, thus acting to counterbalance the effects of protein-tyrosine kinases. In many cases, protein-tyrosine phosphatases play negative regulatory roles in cell signaling pathways by terminating the signals initiated by protein-tyrosine phosphorylation. However, some protein-tyro-

sine phosphatases are cell surface receptors whose enzymatic activities play a positive role in cell signaling: 21 such receptor protein-tyrosine phosphatases are encoded in the human genome. A good example is provided by a receptor called CD45, which is expressed on the surface of T and B lymphocytes. Following antigen stimulation, CD45 dephosphorylates a specific phosphotyrosine that inhibits the enzymatic activity of Src family members. Thus the CD45 protein-tyrosine phosphatase acts (somewhat paradoxically) to stimulate nonreceptor protein-tyrosine kinases.

■ **Cytokine receptors are used by human immunodeficiency virus (HIV) as cell surface receptors for infection of immune cells.**

The receptors for **transforming growth factor β (TGF-β)** and related polypeptides are protein kinases that phosphorylate serine or threonine, rather than tyrosine, residues on their substrate proteins. TGF-β is the prototype of a family of polypeptide growth factors that control proliferation and differentiation of a variety of cell types. The cloning of the first receptor for a member of the TGF-β family in 1991 revealed that it is the prototype of a unique receptor family with a cytosolic **protein-serine/threonine kinase** domain. Since then, receptors for additional TGF-β family members have similarly been found to be protein-serine/threonine kinases. The binding of ligand to these receptors results in the association of two distinct types of polypeptide chains, which are encoded by different members of the TGF-β receptor family, to form heterodimers in which one of the receptor kinases phosphorylates the other. The activated TGF-β receptors then phosphorylate members of a family of transcription factors called Smads, which translocate to the nucleus and stimulate expression of target genes.

Some peptide ligands bind to receptors whose cytosolic domains are guanylyl cyclases, which catalyze formation of cyclic GMP. As discussed earlier, nitric oxide also acts by stimulating guanylyl cyclase, but the target of nitric oxide is an intracellular enzyme rather than a transmembrane receptor. The receptor **guanylyl cyclases** have an extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic domain with catalytic activity. Ligand binding stimulates cyclase activity, leading to the formation of cyclic GMP—a second messenger whose intracellular effects are discussed in the next section of this chapter.

Other receptors bind to cytoplasmic proteins with additional biochemical activities. For example, the cytokine tumor necrosis factor (TNF) induces cell death, perhaps (as discussed in Chapter 17) as a way of eliminating damaged or unwanted cells from tissues. The receptors for TNF and related death-signaling molecules are associated with specific proteases, which are activated in response to ligand binding. Activation of these receptor-associated proteases triggers the activation of additional downstream proteases, ultimately leading to degradation of a variety of intracellular proteins and death of the cell.

Pathways of Intracellular Signal Transduction

Many cell surface receptors stimulate intracellular target enzymes, which may be either directly linked or indirectly coupled to receptors by G proteins. These intracellular enzymes serve as downstream signaling elements that propagate and amplify the signal initiated by ligand binding. In most cases, a chain of reactions transmits signals from the cell surface to a variety of intracellular targets—a process called **intracellular signal transduction**. The targets of such signaling pathways frequently include transcription factors that function to regulate gene expression. Intracellular signaling pathways thus connect the cell surface to the nucleus, leading to changes in gene expression in response to extracellular stimuli.

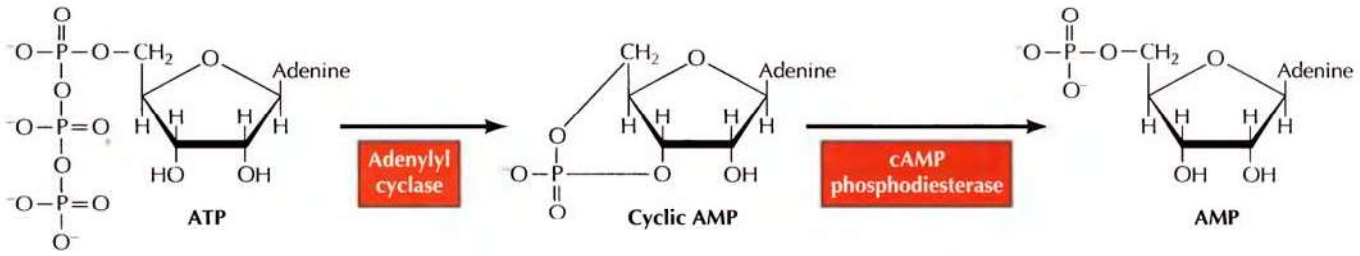


FIGURE 15.19 Synthesis and degradation of cAMP Cyclic AMP is synthesized from ATP by adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase.

The cAMP Pathway: Second Messengers and Protein Phosphorylation

Intracellular signaling was first elucidated by studies of the action of hormones such as epinephrine, which signals the breakdown of glycogen to glucose in anticipation of muscular activity. In 1958 Earl Sutherland discovered that the action of epinephrine was mediated by an increase in the intracellular concentration of **cyclic AMP (cAMP)**, leading to the concept that cAMP is a **second messenger** in hormonal signaling (the first messenger being the hormone itself). Cyclic AMP is formed from ATP by the action of **adenylyl cyclase** and degraded to AMP by **cAMP phosphodiesterase** (Figure 15.19). As discussed earlier, the epinephrine receptor is coupled to adenylyl cyclase via a G protein that stimulates enzymatic activity, thereby increasing the intracellular concentration of cAMP (see Figure 15.12).

How does cAMP then signal the breakdown of glycogen? This and most other effects of cAMP in animal cells are mediated by the action of **cAMP-dependent protein kinase**, or **protein kinase A**, an enzyme discovered by Donal Walsh and Ed Krebs in 1968. The inactive form of protein kinase A is a tetramer consisting of two catalytic and two regulatory subunits (Figure 15.20). Cyclic AMP binds to the regulatory subunits, leading to their dissociation from the catalytic subunits. The free catalytic subunits are then enzymatically active and able to phosphorylate serine residues on their target proteins.

In the regulation of glycogen metabolism, protein kinase A phosphorylates two key target enzymes (Figure 15.21). The first is another protein kinase, phosphorylase kinase, which is phosphorylated and activated by protein kinase A. Phosphorylase kinase in turn phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate. In addition, protein kinase A phosphorylates the enzyme glycogen synthase, which catalyzes glycogen synthesis. In this case, however, phosphorylation inhibits enzymatic activity. Elevation of cAMP and activation of protein kinase A thus blocks further glycogen synthesis at the same time as it stimulates glycogen breakdown.

The chain of reactions leading from the epinephrine receptor to glycogen phosphorylase provides a good illustration of signal amplification during intracellular signal transduction. Each molecule of epinephrine activates only a single receptor. However, each receptor may activate up to a hundred molecules of G_s. Each molecule of G_s then stimulates the enzymatic

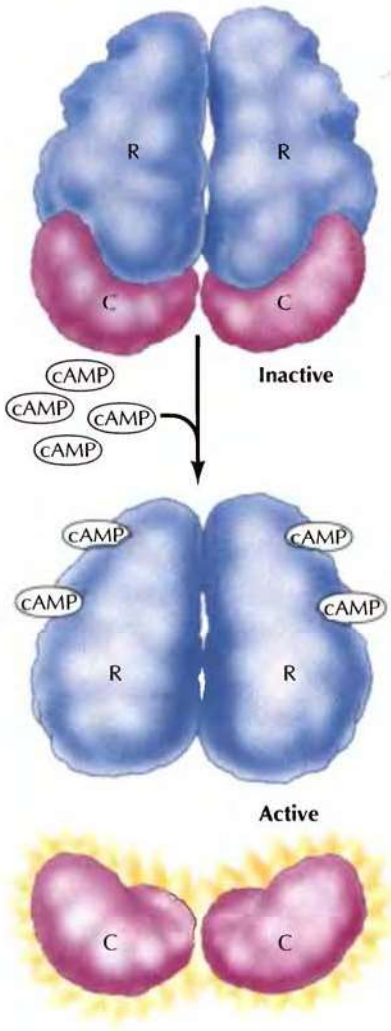


FIGURE 15.20 Regulation of protein kinase A The inactive form of protein kinase A consists of two regulatory (R) and two catalytic (C) subunits. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which are then enzymatically active.

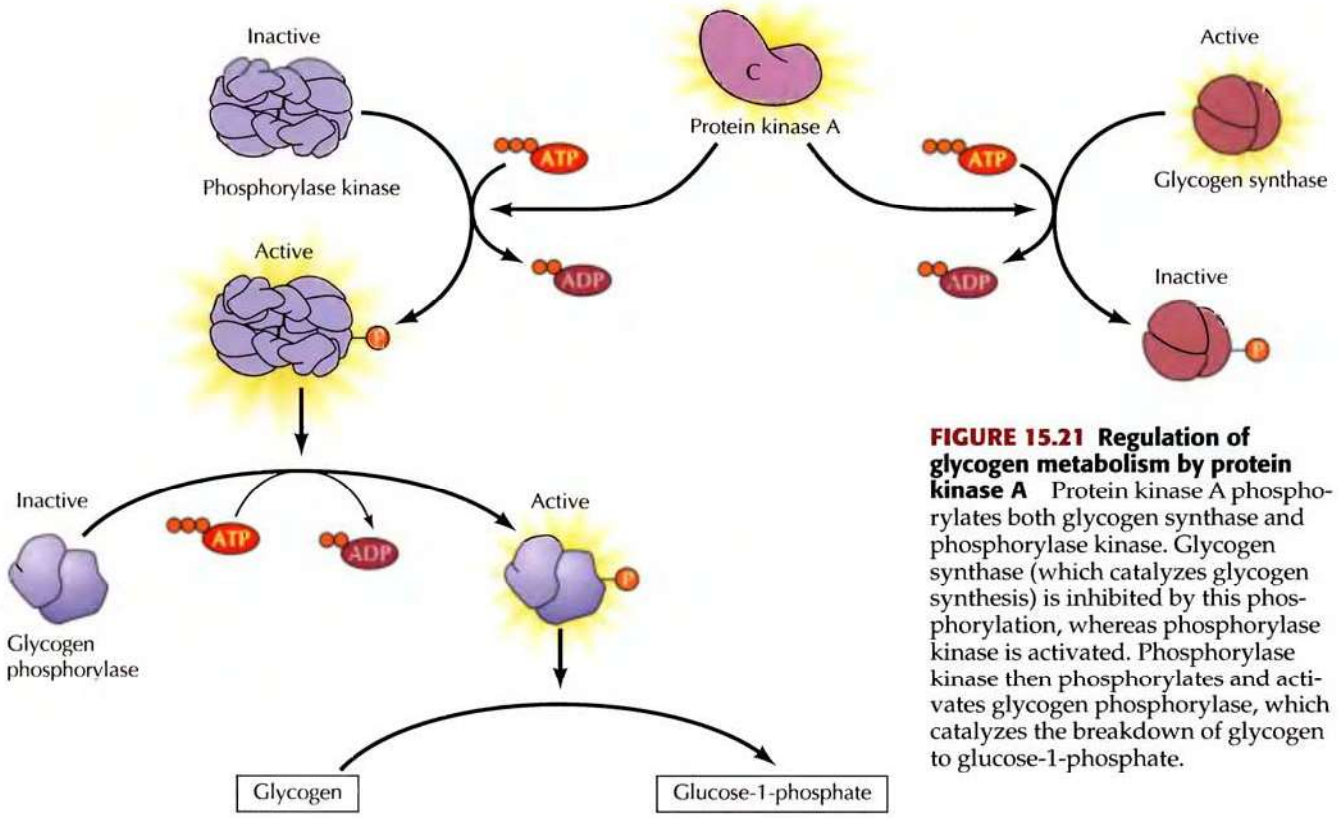


FIGURE 15.21 Regulation of glycogen metabolism by protein kinase A Protein kinase A phosphorylates both glycogen synthase and phosphorylase kinase. Glycogen synthase (which catalyzes glycogen synthesis) is inhibited by this phosphorylation, whereas phosphorylase kinase is activated. Phosphorylase kinase then phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate.

activity of adenylyl cyclase, which can catalyze the synthesis of many molecules of cAMP. Signal amplification continues as each molecule of protein kinase A phosphorylates many molecules of phosphorylase kinase, which in turn phosphorylates many molecules of glycogen phosphorylase. Hormone binding to a small number of receptors thus leads to activation of a much larger number of intracellular target enzymes.

In many animal cells, increases in cAMP activate the transcription of specific target genes that contain a regulatory sequence called the **cAMP response element**, or **CRE** (Figure 15.22). In this case, the signal is carried from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus following its release from the regulatory subunit. Within the nucleus, protein kinase A phosphorylates a transcription factor called **CREB** (for CRE-*b*inding protein), leading to the recruitment of coactivators and transcription of cAMP-inducible genes. Such regulation of gene expression by cAMP plays important roles in controlling the proliferation, survival, and differentiation of a wide variety of animal cells, as well as being implicated in learning and memory.

It is important to recognize that protein kinases, such as protein kinase A, do not function in isolation within the cell. To the contrary, protein phosphorylation is rapidly reversed by the action of protein phosphatases. Some protein phosphatases are transmembrane receptors, as discussed in the preceding section. A number of others are cytosolic enzymes that remove phosphate groups from either phosphorylated tyrosine or serine/threonine residues in their substrate proteins. These protein phosphatases serve to terminate the responses initiated by receptor activation of protein kinases. For example, the serine residues of proteins that are phosphorylated by protein

15.3 WEBSITE ANIMATION

Signal Amplification

In a signal transduction cascade, each enzyme activated at a stage in the cascade may activate 100 molecules of the next enzyme, quickly amplifying the response to the receptor-bound ligand.

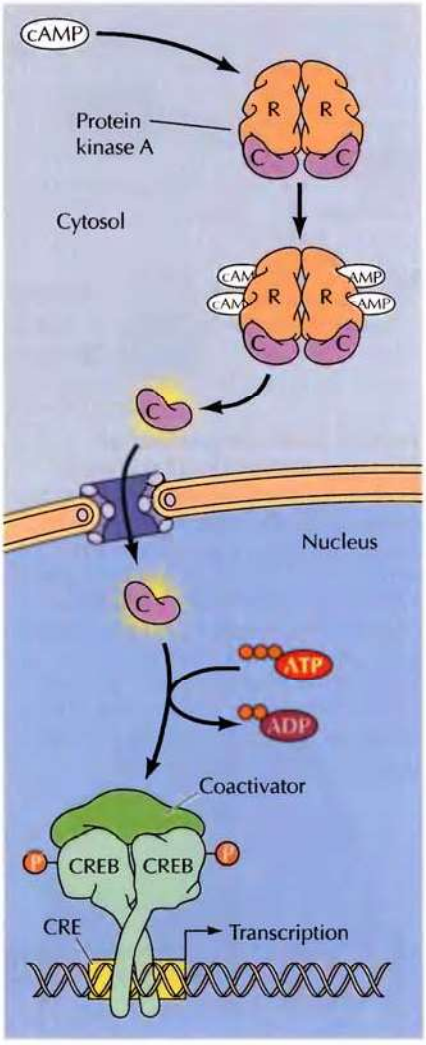


FIGURE 15.22 Cyclic AMP-inducible gene expression The free catalytic subunit of protein kinase A translocates to the nucleus and phosphorylates the transcription factor CREB (CRE-binding protein), leading to the recruitment of coactivators and expression of cAMP-inducible genes.

kinase A are usually dephosphorylated by the action of a phosphatase called protein phosphatase 1 (Figure 15.23). The levels of phosphorylation of protein kinase A substrates (such as phosphorylase kinase and CREB) are thus determined by a balance between the intracellular activities of protein kinase A and protein phosphatases.

Although most effects of cAMP are mediated by protein kinase A, cAMP can also directly regulate ion channels, independent of protein phosphorylation. Cyclic AMP functions in this way as a second messenger involved in sensing smells. Many of the odorant receptors in sensory neurons in the nose are G protein-coupled receptors that stimulate adenylyl cyclase, leading to an increase in intracellular cAMP. Rather than stimulating protein kinase A, cAMP in this system directly opens Na^+ channels in the plasma membrane, leading to membrane depolarization and initiation of a nerve impulse.

Cyclic GMP

Cyclic GMP (cGMP) is also an important second messenger in animal cells, although its roles are not as clearly understood as those of cAMP. Cyclic GMP is formed from GTP by guanylyl cyclases and degraded to GMP by a phosphodiesterase. As discussed earlier in this chapter, guanylyl cyclases are activated by nitric oxide and carbon monoxide as well as by peptide ligands. Stimulation of these guanylyl cyclases leads to elevated levels of cGMP, which then mediate biological responses, such as blood vessel dilation. The action of cGMP is frequently mediated by activation of cGMP-dependent protein kinases, although cGMP also regulates ion channels and phosphodiesterases.

One well-characterized role of cGMP is in the vertebrate eye, where it serves as the second messenger responsible for converting the visual signals received as light to nerve impulses. The photoreceptor in rod cells of the retina is a G protein-coupled receptor called **rhodopsin** (Figure 15.24). Rhodopsin is activated as a result of the absorption of light by the associated small molecule 11-*cis*-retinal, which then isomerizes to all-*trans*-retinal, inducing a conformational change in the rhodopsin protein. Rhodopsin

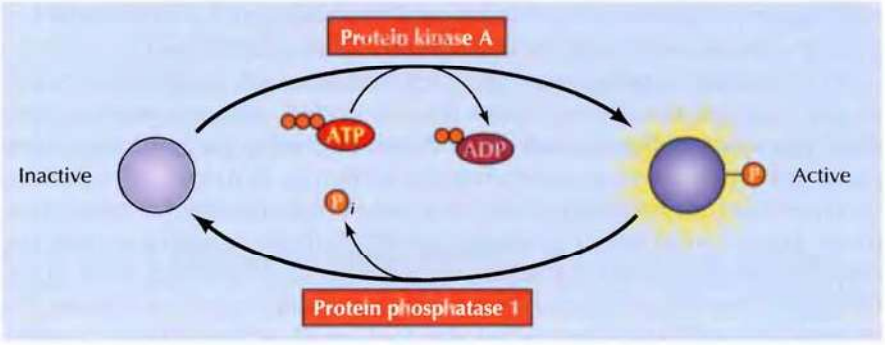


FIGURE 15.23 Regulation of protein phosphorylation by protein kinase A and protein phosphatase 1 The phosphorylation of target proteins by protein kinase A is reversed by the action of protein phosphatase 1.

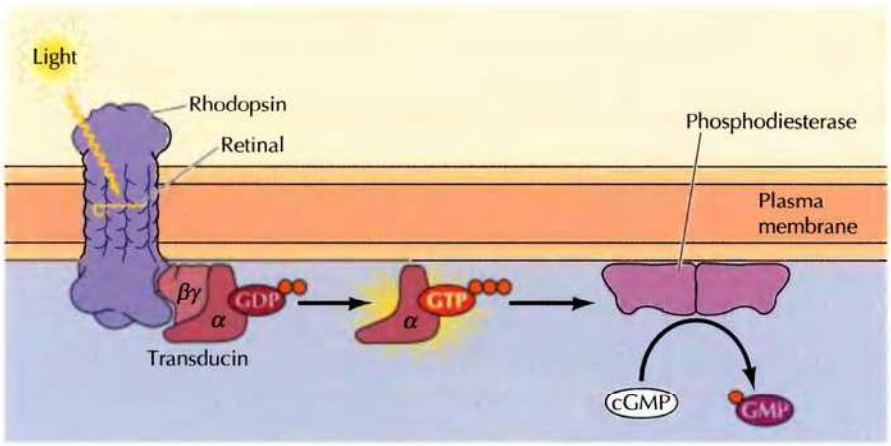


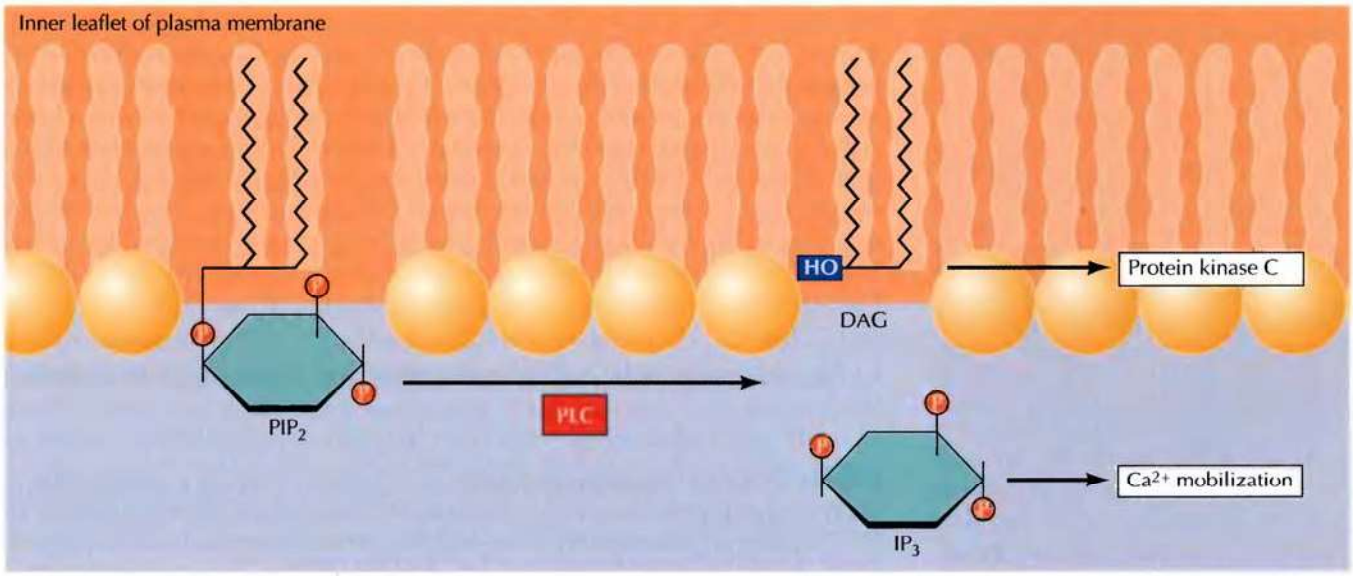
FIGURE 15.24 Role of cGMP in photoreception Absorption of light by retinal activates the G protein-coupled receptor rhodopsin. The α subunit of transducin then stimulates cGMP phosphodiesterase, leading to a decrease in intracellular levels of cGMP.

then activates the G protein **transducin**, and the α subunit of transducin stimulates the activity of **cGMP phosphodiesterase**, leading to a decrease in the intracellular level of cGMP. This change in cGMP level in retinal rod cells is translated to a nerve impulse by a direct effect of cGMP on ion channels in the plasma membrane, similar to the action of cAMP in sensing smells.

Phospholipids and Ca^{2+}

One of the most widespread pathways of intracellular signaling is based on the use of second messengers derived from the membrane phospholipid **phosphatidylinositol 4,5-bisphosphate (PIP_2)**. PIP_2 is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipid bilayer (see Figure 13.2). A variety of hormones and growth factors stimulate the hydrolysis of PIP_2 by **phospholipase C**—a reaction that produces two distinct second messengers, **diacylglycerol** and **inositol 1,4,5-trisphosphate (IP_3)** (Figure 15.25). Diacylglycerol and IP_3 stimulate distinct down-

FIGURE 15.25 Hydrolysis of PIP_2 Phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3). Diacylglycerol activates members of the protein kinase C family, and IP_3 signals the release of Ca^{2+} from intracellular stores.



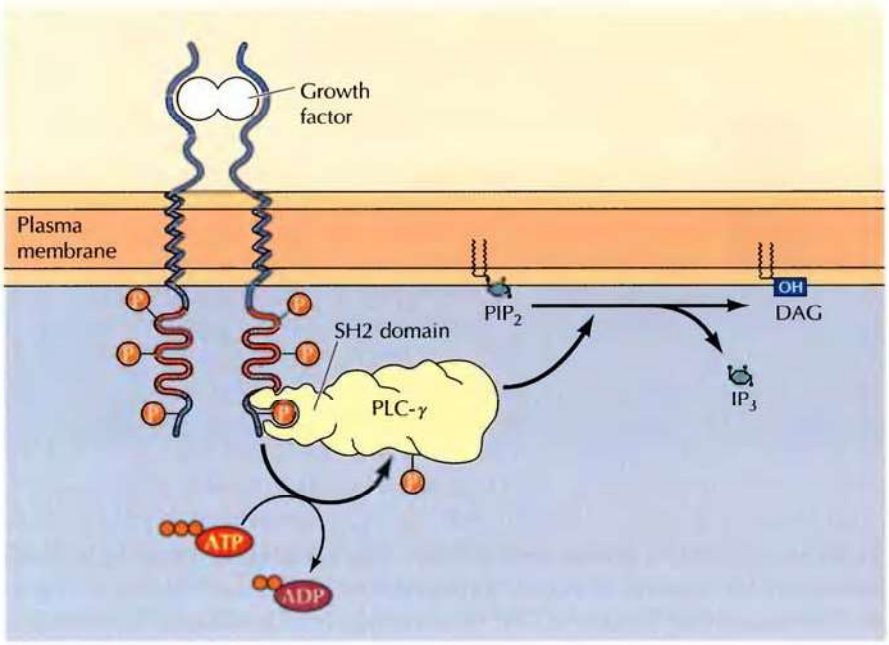
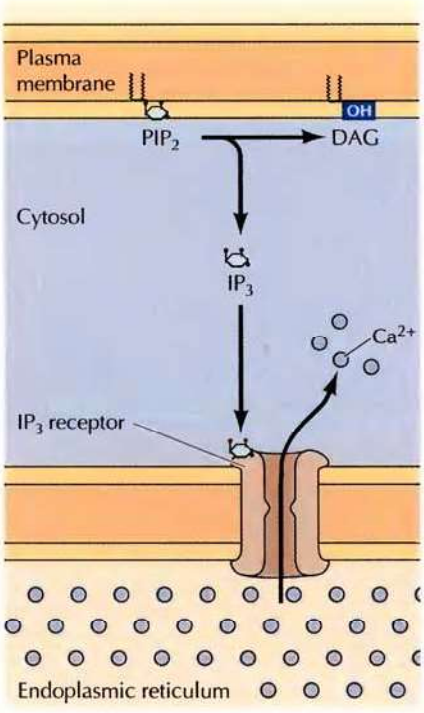


FIGURE 15.26 Activation of phospholipase C by protein-tyrosine kinases Phospholipase C-γ (PLC-γ) binds to activated receptor protein-tyrosine kinases via its SH2 domains. Tyrosine phosphorylation increases PLC-γ activity, stimulating the hydrolysis of PIP_2.

■ **Toxic snake venoms contain phospholipases. Hydrolysis of phospholipids by venom from rattlesnakes and cobras leads to the rupture of red blood cell membranes.**



stream signaling pathways (protein kinase C and Ca^{2+} mobilization, respectively), so PIP_2 hydrolysis triggers a two-armed cascade of intracellular signaling.

It is noteworthy that the hydrolysis of PIP_2 is activated downstream of both G protein-coupled receptors and protein-tyrosine kinases. This occurs because one form of phospholipase C (PLC-β) is stimulated by G proteins, whereas a second form of phospholipase C (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases (Figure 15.26). This interaction localizes PLC-γ to the plasma membrane as well as leading to its tyrosine phosphorylation, which increases its catalytic activity.

The diacylglycerol produced by hydrolysis of PIP_2 remains associated with the plasma membrane and activates protein-serine/threonine kinases belonging to the **protein kinase C** family, many of which play important roles in the control of cell growth and differentiation. The other second messenger produced by PIP_2 cleavage, IP_3, is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca^{2+} from intracellular stores (Figure 15.27). As noted in Chapter 13, the cytosolic concentration of Ca^{2+} is maintained at an extremely low level (about 0.1 μM) as a result of Ca^{2+} pumps that actively export Ca^{2+} from the cell. Ca^{2+} is pumped not only across the plasma membrane but also into the endoplasmic reticulum, which therefore serves as an intracellular Ca^{2+} store. IP_3 acts to release Ca^{2+} from the endoplasmic reticulum by binding to receptors that are ligand-gated Ca^{2+} channels. As a result, cytosolic Ca^{2+} levels increase to about 1 μM, which affects the activities of a variety of target proteins, including

FIGURE 15.27 Ca^{2+} mobilization by IP_3 Ca^{2+} is pumped from the cytosol into the endoplasmic reticulum, which serves as an intracellular Ca^{2+} store. IP_3 binds to receptors that are ligand-gated Ca^{2+} channels in the endoplasmic reticulum membrane, thereby allowing the efflux of Ca^{2+} into the cytosol.

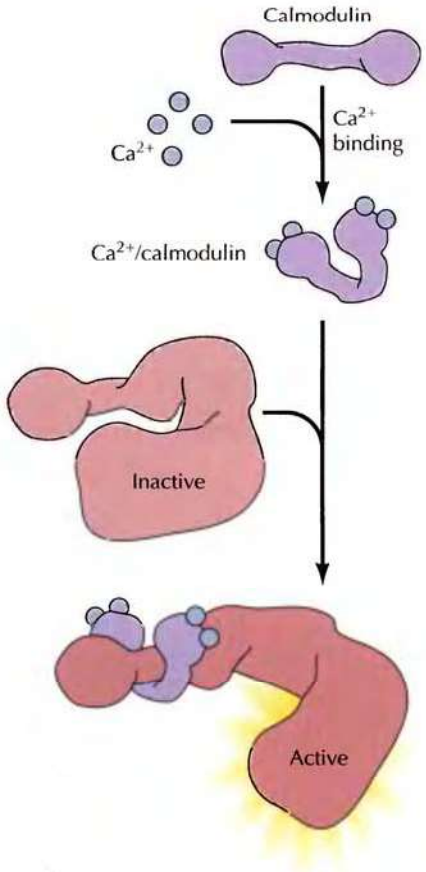


FIGURE 15.28 Function of calmodulin Calmodulin is a dumbbell-shaped protein with four Ca^{2+}-binding sites. The active Ca^{2+}/calmodulin complex binds to a variety of target proteins, including Ca^{2+}/ calmodulin-dependent protein kinases.

protein kinases and phosphatases. For example, some members of the protein kinase C family require Ca^{2+} as well as diacylglycerol for their activation, so these protein kinases are regulated jointly by both arms of the PIP_2 signaling pathway. In most cells, the transient increase in intracellular Ca^{2+} resulting from production of IP_3 triggers a more sustained increase caused by the entry of extracellular Ca^{2+} through channels in the plasma membrane. This entry of Ca^{2+} from outside the cell serves both to prolong the signal initiated by release of Ca^{2+} from the endoplasmic reticulum and to allow the stores of Ca^{2+} within the endoplasmic reticulum to be replenished.

Many of the effects of Ca^{2+} are mediated by the Ca^{2+}-binding protein **calmodulin**, which is activated when the concentration of cytosolic Ca^{2+} increases to about 0.5 μM (Figure 15.28). Ca^{2+}/calmodulin then binds to a variety of target proteins, including protein kinases. One example of such a Ca^{2+}/calmodulin-dependent protein kinase is myosin light-chain kinase, which signals actin-myosin contraction by phosphorylating one of the myosin light chains (see Figure 12.31). Other protein kinases that are activated by Ca^{2+}/calmodulin include members of the **CaM kinase** family, which phosphorylate a number of different proteins, including metabolic enzymes, ion channels, and transcription factors. One form of CaM kinase is particularly abundant in the nervous system where it regulates the synthesis and release of neurotransmitters. In addition, CaM kinases can regulate gene expression by phosphorylating transcription factors. Interestingly, one of the transcription factors phosphorylated by CaM kinase is CREB, which is phosphorylated at the same site by protein kinase A. This phosphorylation of CREB illustrates one of many intersections between the Ca^{2+} and cAMP signaling pathways. Other examples include the regulation of adenylyl cyclases and phosphodiesterases by Ca^{2+}/calmodulin, the regulation of Ca^{2+} channels by cAMP, and the phosphorylation of a number of target proteins by both protein kinase A and Ca^{2+}/calmodulin-dependent protein kinases. The cAMP and Ca^{2+} signaling pathways thus function coordinately to regulate many cellular responses.

The entry of extracellular Ca^{2+} is particularly important in the electrically excitable cells of nerve and muscle in which voltage-gated Ca^{2+} channels in the plasma membrane are opened by membrane depolarization (Figure 15.29). The resulting increases in intracellular Ca^{2+} then trigger the further release of Ca^{2+} from intracellular stores by activating distinct Ca^{2+} channels known as **ryanodine receptors**. One effect of increases in intracellular Ca^{2+} in neurons is to trigger the release of neurotransmitters, so Ca^{2+} plays a crit-

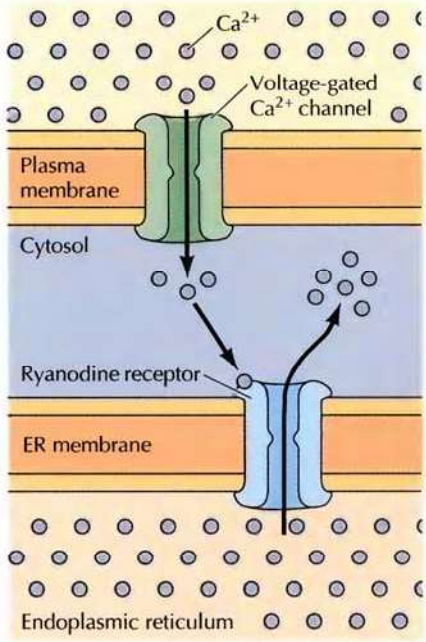


FIGURE 15.29 Regulation of intracellular Ca^{2+} in electrically excitable cells Membrane depolarization leads to the opening of voltage-gated Ca^{2+} channels in the plasma membrane causing the influx of Ca^{2+} from extracellular fluids. The resulting increase in intracellular Ca^{2+} then signals the further release of Ca^{2+} from intracellular stores by opening distinct Ca^{2+} channels (ryanodine receptors) in the endoplasmic reticulum membrane. In muscle cells, ryanodine receptors in the sarcoplasmic reticulum may also be opened directly in response to membrane depolarization.

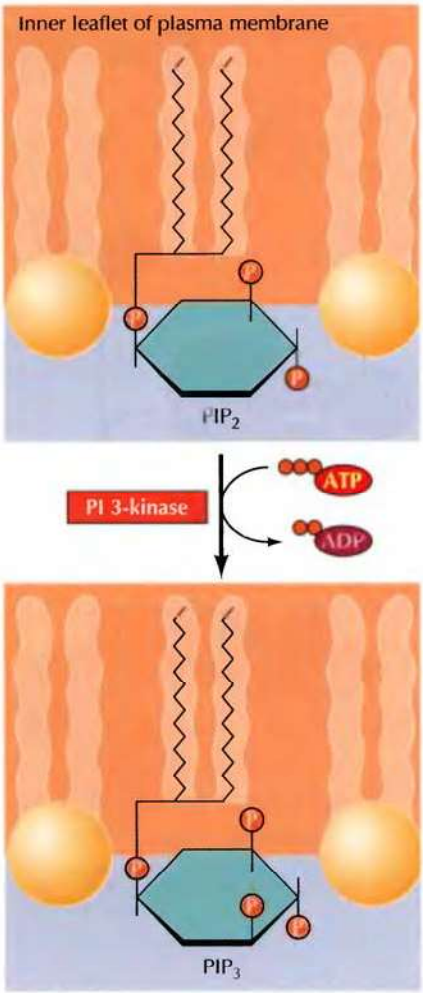


FIGURE 15.30 Activity of PI 3-kinase PI 3-kinase phosphorylates the 3 position of inositol, converting PIP_2 to PIP_3.

ical role in converting electric to chemical signals in the nervous system. In muscle cells Ca^{2+} is stored in the sarcoplasmic reticulum from which it is released by the opening of ryanodine receptors in response to changes in membrane potential. This release of stored Ca^{2+} leads to large increases in cytosolic Ca^{2+}, which trigger muscle contraction (see Chapter 12). Cells thus utilize a variety of mechanisms to regulate intracellular Ca^{2+} levels, making Ca^{2+} a versatile second messenger that controls a wide range of cellular processes.

The PI 3-Kinase/Akt and mTOR Pathways

PIP_2 not only serves as the source of diacylglycerol and IP_3 but is also the starting point of a distinct second messenger pathway that plays a key role in regulating cell growth and survival. In this pathway, PIP_2 is phosphorylated on the 3 position of inositol by the enzyme **phosphatidylinositide (PI) 3-kinase** (Figure 15.30). Like phospholipase C, one form of PI 3-kinase is activated by G proteins while a second form has SH2 domains and is activated by association with receptor protein-tyrosine kinases. Phosphorylation of PIP_2 yields the second messenger **phosphatidylinositol 3,4,5-trisphosphate (PIP_3)**.

A key target of PIP_3, which is critical for signaling cell proliferation and survival, is a protein-serine/threonine kinase called **Akt**. PIP_3 binds to a domain of Akt known as the pleckstrin homology domain (Figure 15.31). This interaction recruits Akt to the inner face of the plasma membrane where it is phosphorylated and activated by another protein kinase (called PDK1) that also contains a pleckstrin homology domain and binds PIP_3. The formation of PIP_3 thus results in the association of both Akt and PDK1 with the plasma membrane, leading to phosphorylation and activation of Akt. Activation of Akt also requires phosphorylation at a second site by a distinct protein kinase, which has recently been identified as a form of mTOR complexed with a protein called rictor. The mTOR/rictor complex is itself stimulated by growth factors, but its mechanism of activation remains to be understood.

Once activated, Akt phosphorylates a number of target proteins, including proteins that are direct regulators of cell proliferation and survival (discussed in Chapter 17), transcription factors, and other protein kinases. The critical transcription factors targeted by Akt include members of the Forkhead or FOXO family (Figure 15.32). Phosphorylation of FOXO by Akt creates a binding site for cytosolic chaperone proteins (14-3-3 proteins) that sequester FOXO in an inactive form in the cytoplasm. In the absence of growth factor signaling and Akt activity, FOXO is released from 14-3-3 and translocates to the nucleus, stimulating transcription of genes that inhibit cell proliferation or induce cell death. Another target of Akt is the protein kinase GSK-3β, which regulates metabolism as well as cell proliferation and survival. Like FOXO, GSK-3β is inhibited by Akt phosphorylation. The targets of GSK-3β include several transcription factors and the translation initiation factor eIF-2B. Phosphorylation of eIF-2B leads to a global downregulation of translation initiation (see Figure 8.20), so GSK-3β provides a link between growth factor signaling and control of cellular protein synthesis.

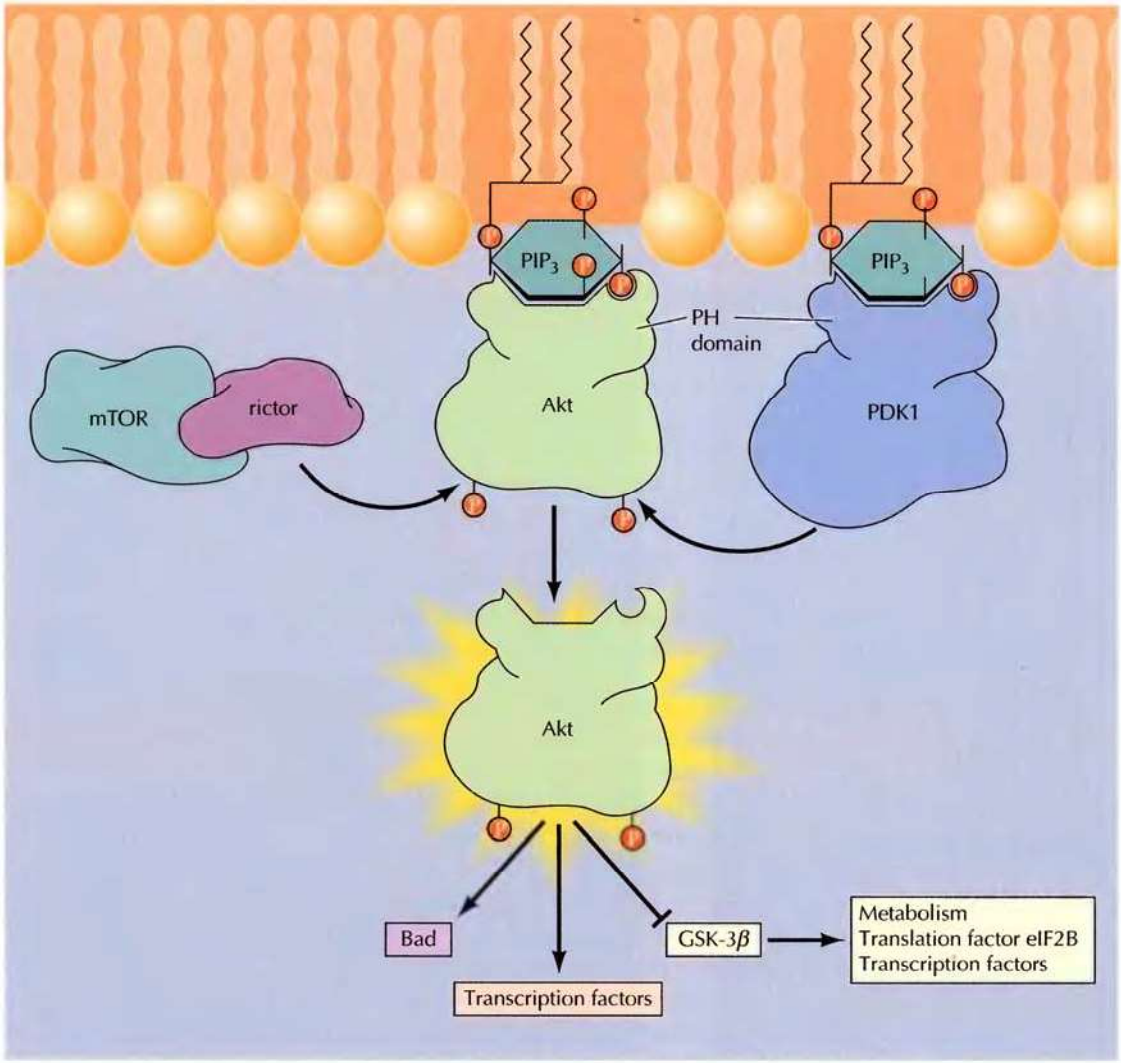


FIGURE 15.31 The PI 3-kinase/Akt pathway Akt is recruited to the plasma membrane by binding to PIP_3 via its pleckstrin homology (PH) domain. It is then activated as a result of phosphorylation by another protein kinase (PDK1) that also binds PIP_3, as well as by the mTOR/rictor complex. Akt then phosphorylates a number of target proteins, including direct regulators of cell survival (Bad, see Chapter 17), several transcription factors, and the protein kinase GSK-3β (which is inhibited by Akt phosphorylation). GSK-3β phosphorylates metabolic enzymes, transcription factors, and the translation initiation factor eIF-2B.

The **mTOR** pathway is a central regulator of cell growth that couples the control of protein synthesis to the availability of growth factors, nutrients, and energy (Figure 15.33). This is accomplished via the regulation of mTOR by multiple signals, including the PI 3-kinase/Akt pathway. The mTOR protein kinase exists in two distinct complexes in cells in which mTOR is associated with either rictor or raptor. As discussed above, the mTOR/rictor complex is one of the protein kinases that phosphorylates and activates Akt (see Figure 15.31). In contrast, the mTOR/raptor complex is activated downstream of Akt and functions to regulate cell size, at least in part by

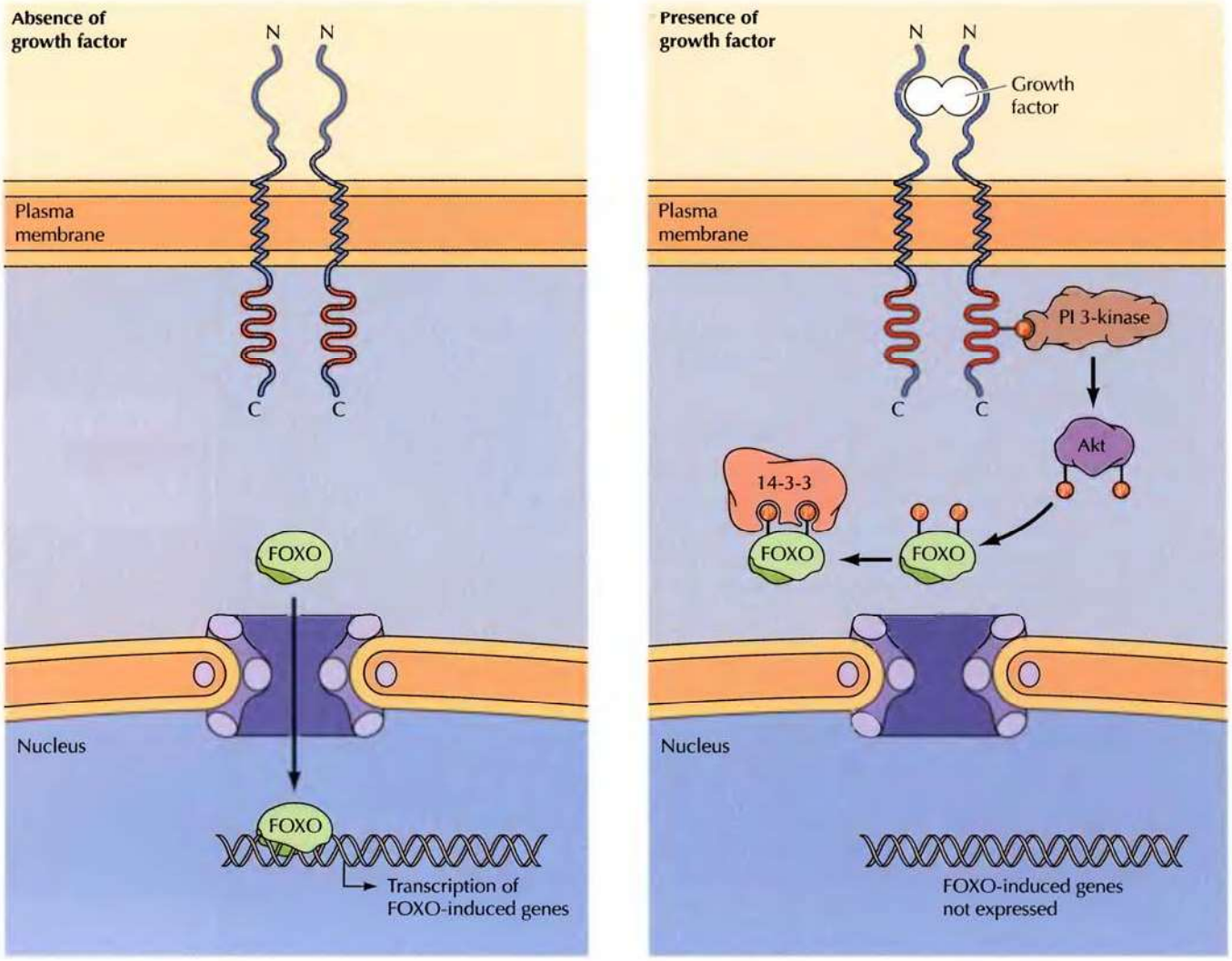


FIGURE 15.32 Regulation of FOXO In the absence of growth factor stimulation, the FOXO transcription factor translocates to the nucleus and induces target gene expression. Growth factor stimulation leads to activation of Akt, which phosphorylates FOXO. This creates binding sites for the cytosolic chaperone 14-3-3, which sequesters FOXO in an inactive form in the cytoplasm.

controlling protein synthesis. The mTOR/raptor complex is regulated by the Ras-related GTP-binding protein Rheb, which is in turn regulated by the GTPase-activating protein complex TSC1/2. Akt phosphorylates TSC2, leading to activation of mTOR/raptor in response to growth factor stimulation. In addition, TSC2 is regulated by another protein kinase called the AMP/activated kinase (AMPK). AMPK senses the energy state of the cell and is activated by a high ratio of AMP to ATP. Under these conditions, AMPK phosphorylates TSC2, leading to inhibition of mTOR/raptor when cellular energy stores are depleted. TSC2 is also regulated by the availability of amino acids, although the mechanism responsible remains to be established.

The mTOR/raptor complex phosphorylates at least two well-characterized targets that function to regulate protein synthesis: S6 kinase and eIF4E binding protein-1 (4E-BP1). S6 kinase controls translation by phosphorylating the ribosomal protein S6 as well as other proteins involved in translational regulation. The eIF4E binding protein controls translation by interacting with initiation factor eIF4E, which binds to the 5′ cap of mRNAs. In the absence of mTOR signaling, nonphosphorylated 4E-BPs bind to eIF4E and inhibit translation by interfering with the interaction of eIF4E with eIF4G (see Figure 8.11). Phosphorylation of 4E-BP1 by mTOR prevents its interaction with eIF4E, leading to increased rates of translation initiation.

■ **Rapamycin, an antibiotic produced by certain fungi, is a specific inhibitor of the mTOR/raptor complex and is used as an immunosuppressive drug in organ transplants.**

MAP Kinase Pathways

The MAP kinase pathway refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eukaryotic cells ranging from yeasts to humans. The central elements in the pathway are a family of protein-serine/threonine kinases called the **MAP kinases** (for *m*itogen-*a*ctivated *p*rotein kinases) that are activated in response to a variety of growth factors and other signaling molecules. In yeasts, MAP kinase pathways control a variety of cellular responses, including mating, cell shape, and sporulation. In higher eukaryotes (including *C. elegans*, *Drosophila*, frogs, and mammals), MAP kinases are ubiquitous regulators of cell growth and differentiation.

The MAP kinases that were initially characterized in mammalian cells belong to the **ERK** (*e*xtracellular signal-*r*egulated *k*inase) family. ERK activation plays a central role in signaling cell proliferation induced by growth factors that act through either protein-tyrosine kinase or G protein-coupled receptors. Protein kinase C can also activate the ERK pathway, and both the Ca^{2+} and cAMP pathways intersect with ERK signaling, either stimulating or inhibiting the ERK pathway in different types of cells.

Activation of ERK is mediated by two upstream protein kinases, which are coupled to growth factor receptors by the **Ras** GTP-binding protein (Figure 15.34). Activation of Ras leads to activation of the **Raf** protein-serine/threonine kinase, which phosphorylates and activates a second protein kinase called **MEK** (for *M*AP kinase/*E*RK *k*inase). MEK is a dual-specificity protein kinase that activates members of the ERK family by phosphorylation of both threonine and tyrosine residues separated by one amino acid (e.g., threonine-183 and tyrosine-185 of ERK2). Once activated, ERK phosphorylates a variety of targets, including other protein kinases and transcription factors.

The central role of the ERK pathway in mammalian cells emerged from studies of the Ras proteins, which were first identified as the oncogenic proteins of tumor viruses that cause sarcomas in rats (hence the name Ras, from *ra*t *s*arcoma virus). Interest in Ras intensified considerably in 1982 when mutations in *ras* genes were first implicated in the development of

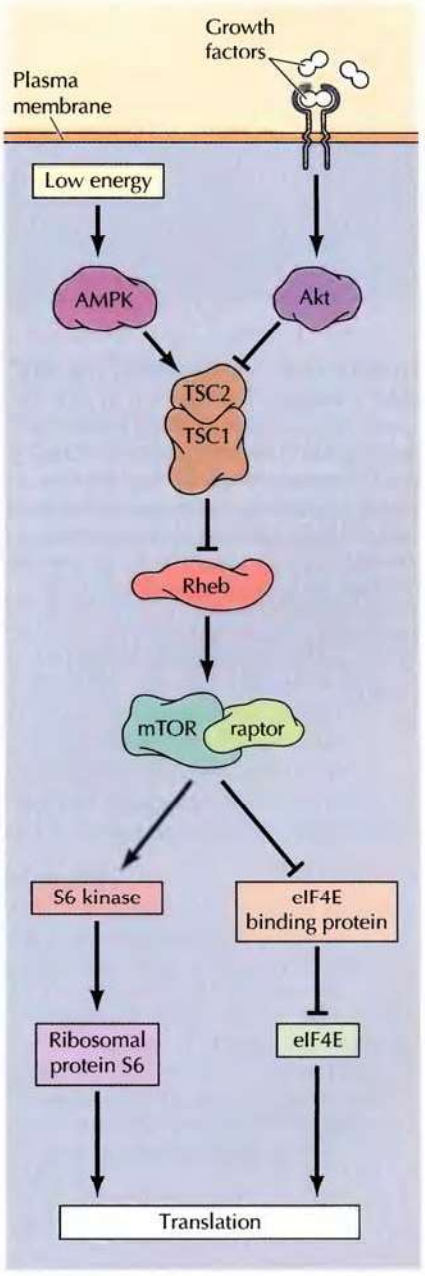


FIGURE 15.33 The mTOR pathway The mTOR/raptor protein kinase is activated by Rheb, which is inhibited by the TSC1/2 complex. Akt inhibits TSC1/2, leading to activation of Rheb and mTOR/raptor in response to growth factor stimulation. In contrast, AMPK activates TSC1/2, leading to inhibition of Rheb and mTOR/raptor if cellular energy stores are depleted. mTOR/raptor stimulates translation by phosphorylating S6 kinase (which phosphorylates ribosomal protein S6) and by phosphorylating eIF4E binding protein, relieving inhibition of translation initiation factor eIF4E.

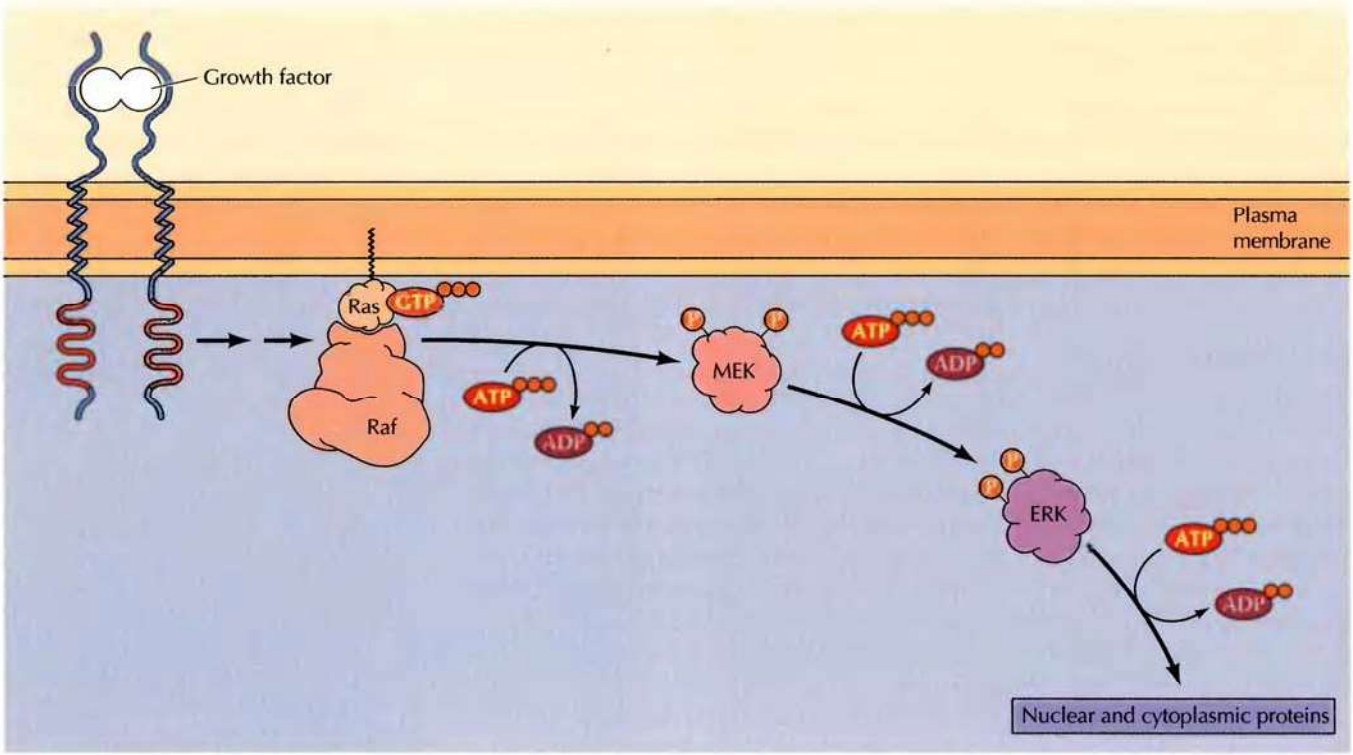


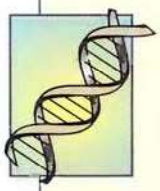
FIGURE 15.34 Activation of the ERK MAP kinases Stimulation of growth factor receptors leads to activation of the small GTP-binding protein Ras, which interacts with the Raf protein kinase. Raf phosphorylates and activates MEK, a dual-specificity protein kinase that activates ERK by phosphorylation on both threonine and tyrosine residues (Thr-183 and Tyr-185). ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins.

human cancers (discussed in Chapter 18). The importance of Ras in intracellular signaling was then indicated by experiments showing that microinjection of active Ras protein directly induces proliferation of normal mammalian cells. Conversely, interference with Ras function by either microinjection of anti-Ras antibody or expression of a dominant negative Ras mutant blocks growth factor-induced cell proliferation. Thus Ras is not only capable of inducing the abnormal growth characteristic of cancer cells but also appears to be required for the response of normal cells to growth factor stimulation.

The Ras proteins are guanine nucleotide-binding proteins that function analogously to the α subunits of G proteins, alternating between inactive GDP-bound and active GTP-bound forms (Figure 15.35). In contrast to the G protein α subunits, however, Ras functions as a monomer rather than in association with $\beta\gamma$ subunits. Ras activation is mediated by **guanine nucleotide exchange factors** that stimulate the release of bound GDP and its exchange for GTP. Activity of the Ras-GTP complex is then terminated by GTP hydrolysis, which is stimulated by the interaction of Ras-GTP with **GTPase-activating proteins**. It is interesting to note that the mutations of *ras* genes in human cancers have the effect of inhibiting GTP hydrolysis by the Ras proteins. These mutated Ras proteins therefore remain continuously in the active GTP-bound form, driving the unregulated proliferation of cancer cells even in the absence of growth factor stimulation.

The Ras proteins are prototypes of a large family of approximately 50 related proteins frequently called small GTP-binding proteins because Ras and its relatives are about half the size of G protein α subunits. One member of this family is Rheb, which regulates mTOR signaling (see Figure 15.33). Other subfamilies of small GTP-binding proteins control a vast array

Cancer: Signal Transduction and the ras Oncogenes



The Disease

Cancer claims the lives of approximately one out of every four Americans, accounting for almost 600,000 deaths each year in the United States. There are more than a hundred different kinds of cancer, but some are more common than others. In this country the most common lethal cancers are those of the lung and colon/rectum, which together account for about 40% of all cancer deaths. Other major contributors to cancer mortality include cancers of the breast, pancreas, and prostate, which are responsible for approximately 7.2%, 5.6%, and 5.3% of U.S. cancer deaths, respectively.

The common feature of all cancers is the unrestrained proliferation of cancer cells, which eventually spread throughout the body, invading normal tissues and organs and leading to death of the patient. Surgery and radiotherapy are effective treatments for localized cancers but are unable to reach cancer cells that have spread to distant body sites. Treatment of these cancers therefore requires the use of chemotherapeutic drugs. Unfortunately, the commonly available chemotherapeutic agents are not specific for cancer cells. Most act by either damaging DNA or interfering with DNA synthesis, so they also kill rapidly dividing normal cells, such as the epithelial cells that line the digestive tract and the blood-forming cells of the bone marrow. The resulting toxicity of these drugs limits their effectiveness, and many cancers are not eliminated by doses of chemotherapy that can be tolerated by the patient. Consequently, although major progress has been made in cancer treatment, nearly half of all patients diagnosed with cancer ultimately die of their disease.

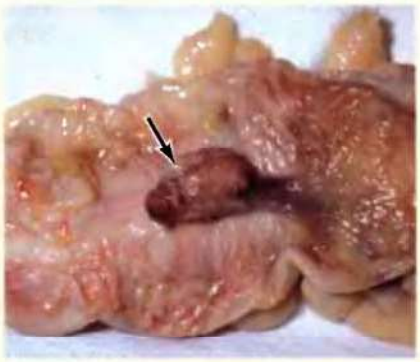
Molecular and Cellular Basis

The identification of viral genes that can convert normal cells to cancer cells, such as the *src* gene of RSV, provided the first demonstration that cancers can result from the action of specific genes (oncogenes). The subsequent discovery that viral oncogenes are related to genes of normal cells then engendered the hypothesis that non-virus-induced cancers (including most human cancers) might arise as a result of mutations in normal cell genes, giving rise to oncogenes of cellular rather than viral origin. Such cellular oncogenes were first identified in human cancers in 1981. The following year, human oncogenes of bladder, lung, and colon cancers were found to be related to the *ras* genes previously identified in rat sarcoma viruses.

Although many different genes are now known to play critical roles in cancer development, mutations of the *ras* genes remain one of the most common genetic abnormalities in human tumors. Mutated *ras* oncogenes are found in about 20% of all human cancers, including approximately 25% of lung cancers, 50% of colon cancers, and more than 90% of pancreatic cancers. Moreover, the action of *ras* oncogenes has clearly linked the development of human cancer to abnormalities in the signaling pathways that regulate cell proliferation. The mutations that convert normal *ras* genes to oncogenes substantially decrease GTP hydrolysis by the Ras proteins. Consequently, the mutated oncogenic Ras proteins remain locked in the active GTP-bound form, rather than alternating normally between inactive and active states in response to extracellular signals. The oncogenic Ras proteins thus continuously stimulate the ERK signaling pathway and drive cell proliferation, even in the absence of the growth factors that would be required to activate Ras and signal proliferation of normal cells.

Prevention and Treatment

The discovery of mutated oncogenes in human cancers raised the possibility of developing drugs specifically targeted against the oncogene proteins. In principle, such drugs might act selectively against cancer cells with less toxicity toward normal cells than that of conventional chemotherapeutic agents. Because *ras* is frequently mutated in human cancers,



A human colon polyp (an early stage of colon cancer). The *ras* oncogenes contribute to the development of about half of all colon cancers. (E. P. Ewing, Jr., Centers for Disease Control.)

the Ras proteins and other elements of Ras signaling pathways have attracted considerable interest as potential drug targets.

As discussed in Chapter 18, effective drugs that selectively target cancer cells have been recently developed against some protein-tyrosine kinase oncogenes, including the EGF receptor, that act upstream of Ras. A variety of additional drugs are under investigation as potential cancer treatments, including drugs targeted against Ras itself and against protein kinases activated downstream of Ras, such as Raf. The identification of oncogenes in human tumors has thus opened new strategies to rational development of drugs that act effectively and selectively against human cancer cells by targeting the signaling pathways that are responsible for cancer development.

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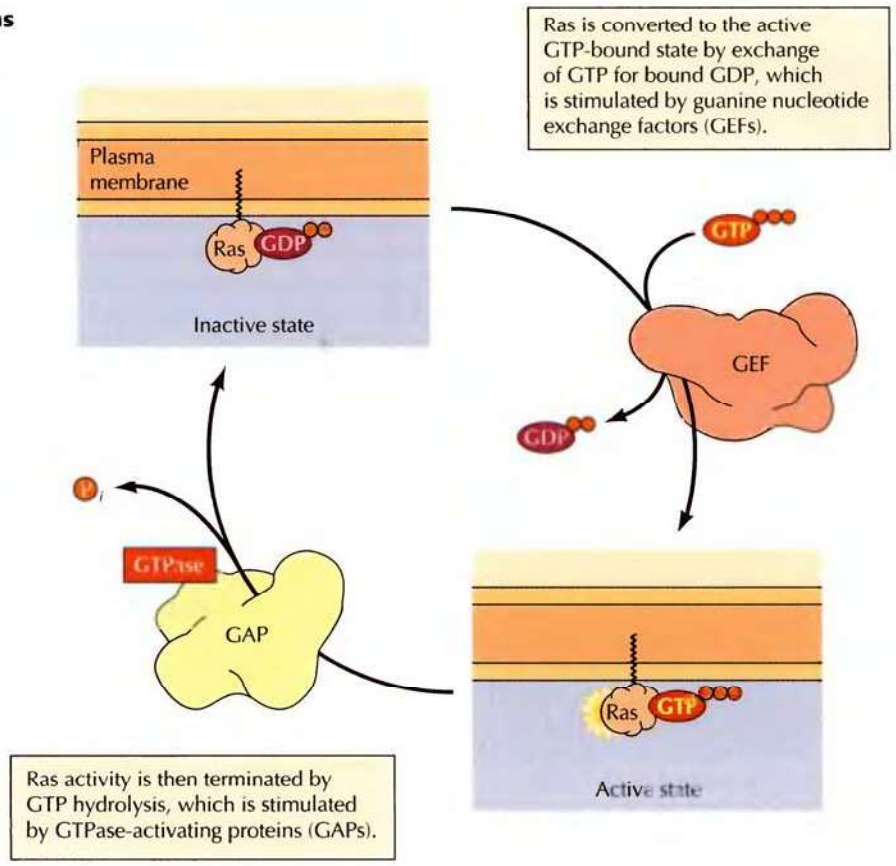


FIGURE 15.35 Regulation of Ras proteins Ras proteins alternate between inactive GDP-bound and active GTP-bound states.

of cellular activities. For example, the largest subfamily of small GTP-binding proteins (the Rab proteins) functions to regulate vesicle trafficking, as discussed in Chapter 10. Other small GTP-binding proteins are involved in the import and export of proteins from the nucleus (the Ran protein, discussed in Chapter 9) and organization of the cytoskeleton (the Rho subfamily, discussed later in this chapter).

The best understood mode of Ras activation is that mediated by receptor protein-tyrosine kinases (Figure 15.36). Autophosphorylation of these receptors results in their association with Ras guanine nucleotide exchange factors as a result of SH2-mediated protein interactions. One well-characterized example is provided by the guanine nucleotide exchange factor Sos, which is bound to the SH2-containing protein Grb2 in the cytosol of unstimulated cells. Tyrosine phosphorylation of receptors (or of other receptor-associated proteins) creates a binding site for the Grb2 SH2 domains. Association of Grb2 with activated receptors localizes Sos to the plasma membrane where it is able to interact with Ras proteins, which are anchored to the inner leaflet of the plasma membrane by lipids attached to the Ras C terminus (see Figure 13.9). Sos then stimulates guanine nucleotide exchange resulting in formation of the active Ras-GTP complex. In its active GTP-bound form Ras interacts with a number of effector proteins, including the Raf protein-serine/threonine kinase. This interaction with Ras recruits Raf from the cytosol to the plasma membrane and initiates Raf activation, which also involves phosphorylation of Raf by both protein-tyrosine and protein-serine/threonine kinases.

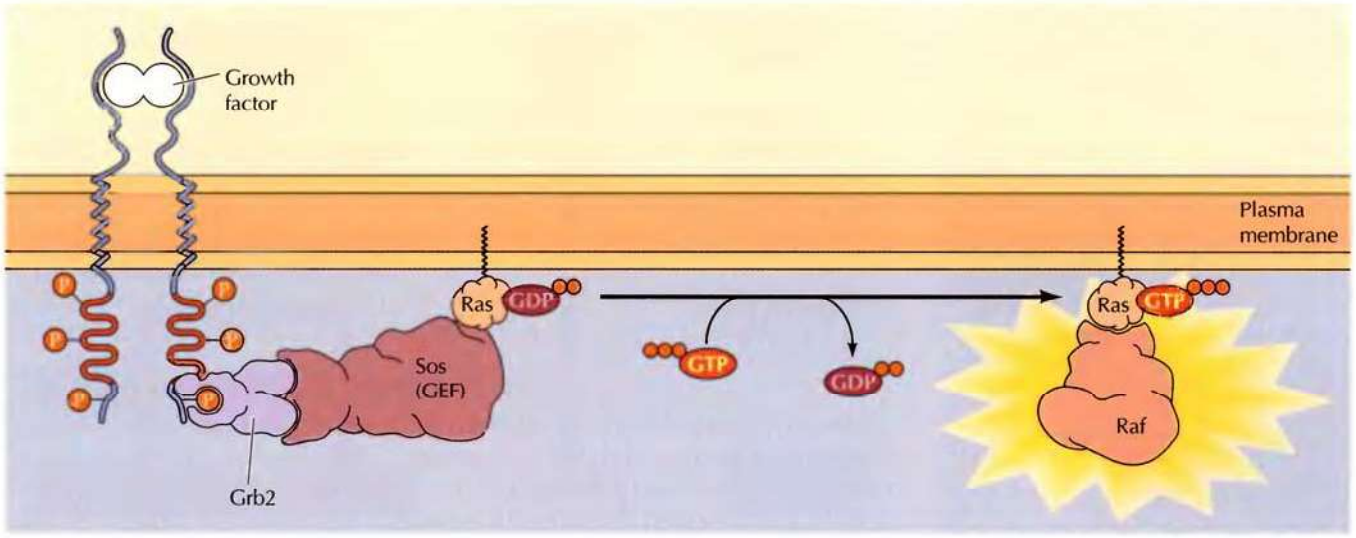


FIGURE 15.36 Ras activation downstream of receptor protein-tyrosine kinases A complex of Grb2 and the guanine nucleotide exchange factor Sos binds to a phosphotyrosine-containing sequence in the activated receptor via the Grb2 SH2 domain. This interaction recruits Sos to the plasma membrane where it can stimulate Ras GDP/GTP exchange. The activated Ras-GTP complex then binds to the Raf protein kinase.

As already noted, activation of Raf initiates a protein kinase cascade leading to ERK activation. ERK then phosphorylates a variety of target proteins, including other protein kinases. In addition, ERK regulates the mTOR pathway by phosphorylating TSC2 (see Figure 15.33). Importantly, a fraction of activated ERK translocates to the nucleus where it regulates transcription factors by phosphorylation (Figure 15.37). In this regard, it is notable that a primary response to growth factor stimulation is the rapid transcriptional induction of a family of approximately 100 genes called **immediate-early genes**. The induction of a number of immediate-early genes is mediated by a regulatory sequence called the **serum response element (SRE)**, which is recognized by a complex of transcription factors including the **serum response factor (SRF)** and **Elk-1**. ERK phosphorylates and activates Elk-1, providing a direct link between the ERK family of MAP kinases and immediate-early gene induction. Many immediate-early genes themselves encode transcription factors, so their induction in response to growth factor stimulation leads to altered expression of a battery of other downstream genes called **secondary response genes**. As discussed in Chapter 16, these alterations in gene expression directly link ERK signaling to the stimulation of cell proliferation induced by growth factors.

Both yeasts and mammalian cells have multiple MAP kinase pathways that control distinct cellular responses. Each cascade consists of three protein kinases: a terminal MAP kinase and two upstream kinases (analogous to Raf and MEK) that regulate its activity. In the yeast *S. cerevisiae* five different MAP kinase cascades regulate mating, sporulation, filamentation, cell wall remodeling, and response to high osmolarity. In mammalian cells, three major groups of MAP kinases have been identified. In addition to

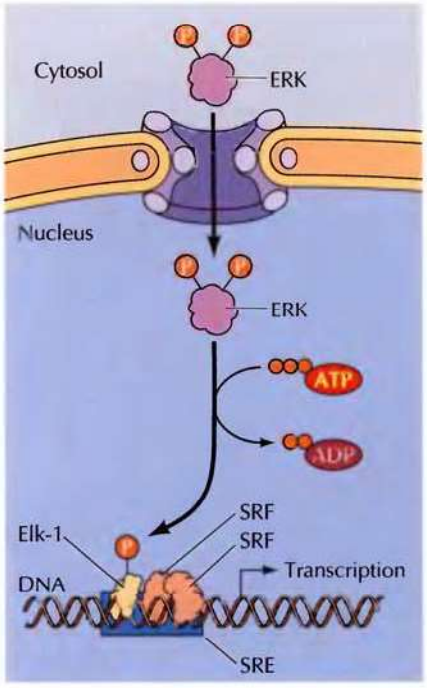


FIGURE 15.37 Induction of immediate-early genes by ERK Activated ERK translocates to the nucleus where it phosphorylates the transcription factor Elk-1. Elk-1 binds to the serum response element (SRE) in a complex with serum response factor (SRF). Phosphorylation stimulates the activity of Elk-1 as a transcriptional activator, leading to immediate-early gene induction.

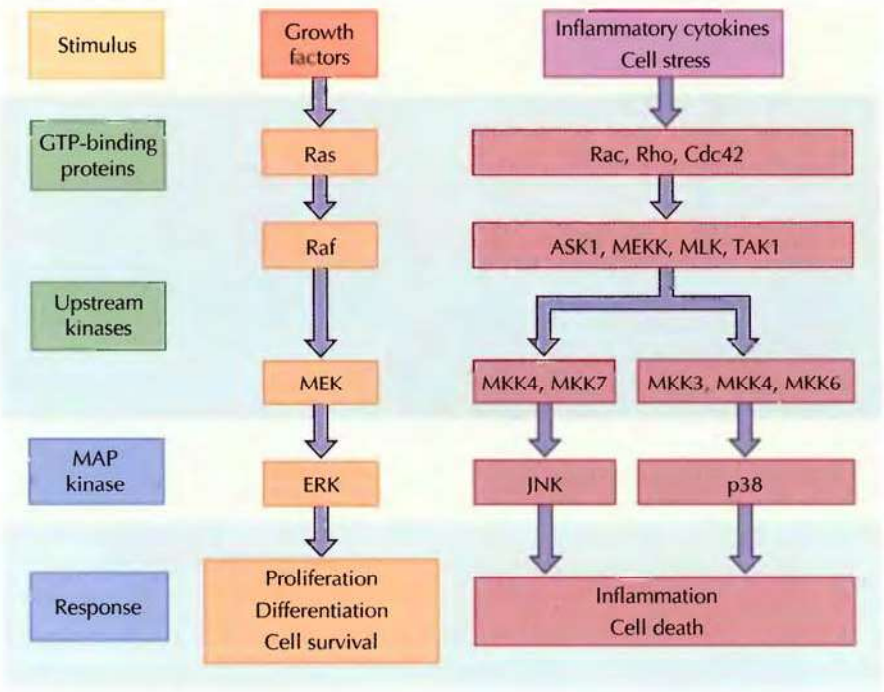


FIGURE 15.38 Pathways of MAP kinase activation in mammalian cells In addition to ERK, mammalian cells contain JNK and p38 MAP kinases. Activation of JNK and p38 is mediated by members of the Rho subfamily of small GTP-binding proteins (Rac, Rho, and Cdc42), which stimulate protein kinase cascades parallel to that responsible for ERK activation. The protein kinase cascades leading to JNK and p38 activation appear to be preferentially activated by inflammatory cytokines or cellular stress and generally lead to inflammation and cell death.

members of the ERK family these include the JNK and p38 MAP kinases, which are preferentially activated in response to inflammatory cytokines and cellular stress (e.g., ultraviolet irradiation) (Figure 15.38). The JNK and p38 MAP kinase cascades are activated by members of the Rho subfamily of small GTP-binding proteins (including Rac, Rho, and Cdc42) rather than by Ras. Whereas ERK signaling principally leads to cell proliferation, survival, and differentiation, the JNK and p38 MAP kinase pathways often lead to inflammation and cell death. Like ERK, the JNK and p38 MAP kinases can translocate to the nucleus and phosphorylate transcription factors that regulate gene expression. Multiple MAP kinase pathways thus function in all types of eukaryotic cells to control cellular responses to diverse environmental signals.

The specificity of MAP kinase signaling is maintained at least in part by the organization of the components of each MAP kinase cascade as complexes that are associated with **scaffold proteins**. For example, the JIP-1 scaffold protein organizes the JNK MAP kinase and its upstream activators MLK and MKK7 into a signaling cassette (Figure 15.39). As a result of the specific association of these protein kinases on the JIP-1 scaffold, activation of MLK by Rac leads to specific and efficient activation of MKK7, which in turn activates JNK. Distinct scaffold proteins are involved not only in the organization of other MAP kinase signaling cassettes but also in the association of other downstream signaling molecules with their receptors. The physical association of signaling pathway components as a result of their

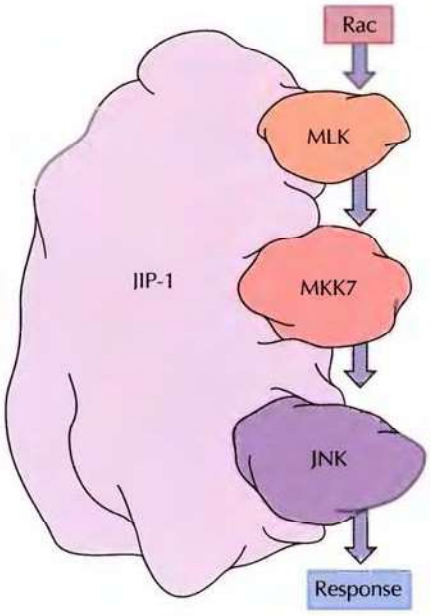


FIGURE 15.39 A scaffold protein for the JNK MAP kinase cascade The JIP-1 scaffold protein binds MLK, MKK7, and JNK, organizing these components of the JNK pathway into a signaling cassette.

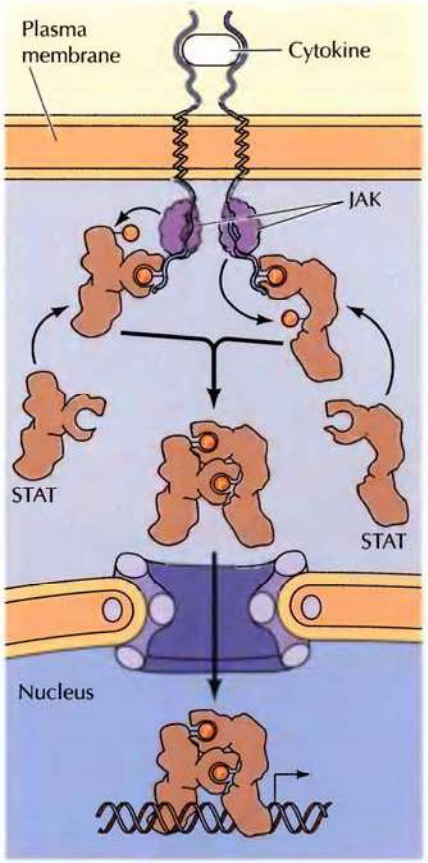


FIGURE 15.40 The JAK/STAT pathway The STAT proteins are transcription factors that contain SH2 domains that mediate their binding to phosphotyrosine-containing sequences. In unstimulated cells, STAT proteins are inactive in the cytosol. Stimulation of cytokine receptors leads to the binding of STAT proteins where they are phosphorylated by the receptor-associated JAK protein-tyrosine kinases. The phosphorylated STAT proteins then dimerize and translocate to the nucleus where they activate the transcription of target genes.

interaction with scaffold proteins is thought to play an important role in determining the specificity of signaling pathways within the cell.

The JAK/STAT and TGF-β/Smad Pathways

The PI 3-kinase and MAP kinase pathways are examples of indirect connections between the cell surface and the nucleus in which a cascade of protein kinases ultimately leads to transcription factor phosphorylation. The JAK/STAT and TGF-β/Smad pathways illustrate more direct connections between growth factor receptors and transcription factors in which the targeted transcription factors are phosphorylated directly by receptor-associated protein kinases.

The key elements in the **JAK/STAT pathway** are the **STAT proteins** (signal *t*ransducers and *a*ctivators of *t*ranscription), which were originally identified in studies of cytokine receptor signaling (Figure 15.40). The STAT proteins are a family of seven transcription factors that contain SH2 domains. They are inactive in unstimulated cells where they are localized to the cytoplasm. Stimulation of cytokine receptors leads to recruitment of STAT proteins, which bind via their SH2 domains to phosphotyrosine-containing sequences in the cytoplasmic domains of receptor polypeptides. Following their association with activated receptors the STAT proteins are phosphorylated by members of the JAK family of nonreceptor protein-tyrosine kinases, which are associated with cytokine receptors. Tyrosine phosphorylation promotes the dimerization of STAT proteins, which then translocate to the nucleus where they stimulate transcription of their target genes.

Further studies have shown that STAT proteins are also activated downstream of receptor protein-tyrosine kinases where their phosphorylation may be catalyzed either by the receptors themselves or by associated nonreceptor kinases. The STAT transcription factors thus serve as direct links between both cytokine and growth factor receptors on the cell surface and regulation of gene expression in the nucleus.

The receptors for members of the TGF-β family of growth factors are protein-serine/threonine kinases, which directly phosphorylate transcription factors of the **Smad** family (Figure 15.41). The receptors are composed of type I and type II polypeptides, which become associated following ligand binding. The type II receptor then phosphorylates the type I receptor, which in turn phosphorylates a Smad protein. The phosphorylated Smads then translocate to the nucleus and regulate gene expression. It should be noted that there are 42 different members of the TGF-β family in humans, which

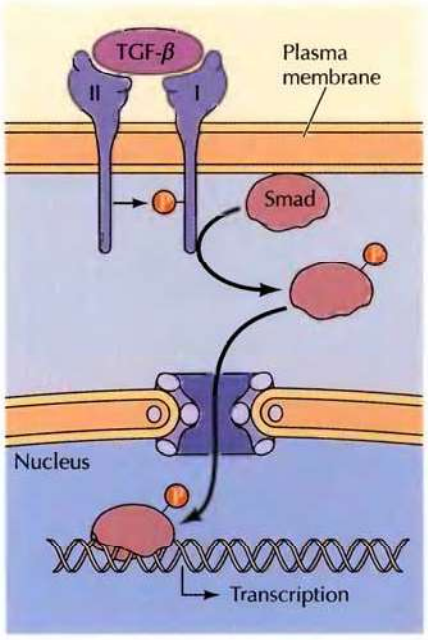


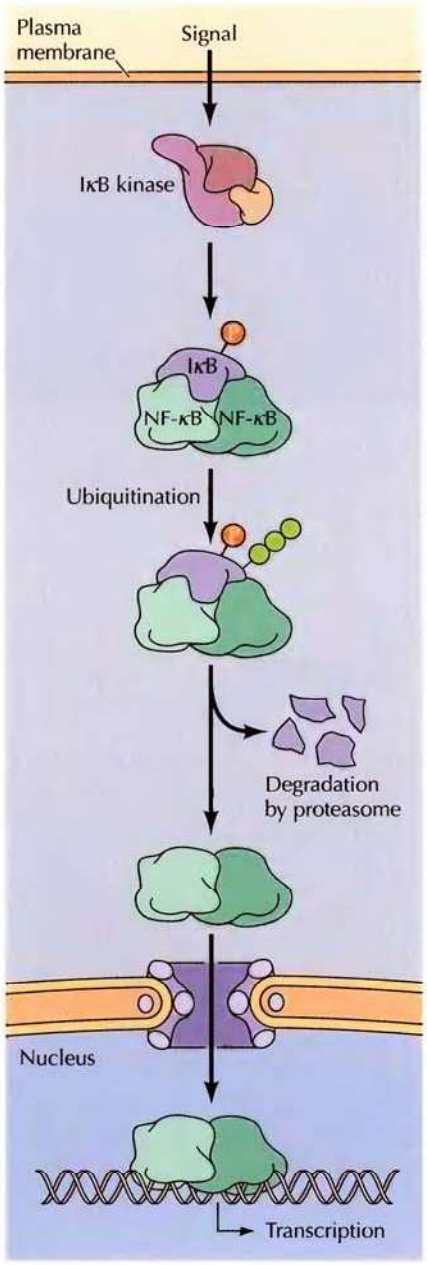
FIGURE 15.41 Signaling from TGF-β receptors TGF-β receptors are dimers of type I and II polypeptides. The type II receptor phosphorylates and activates type I, which then phosphorylates a Smad protein. Phosphorylated Smads translocate to the nucleus and activate transcription of target genes.

■ **Growth hormone functions by activating the JAK/STAT pathway.**

elicit different responses in their target cells. This is accomplished by combinatorial interactions of seven different type I receptors and five type II receptors, which lead to activation of different members of the Smad family (a total of 8 family members). Smad family members can also be phosphorylated by ERK, and this intersection between the TGFβ/Smad pathway and ERK plays a critical role in embryonic development.

NF-κB Signaling

NF-κB signaling is another example of a signaling pathway that directly targets a specific family of transcription factors. The **NF-κB** family consists of five transcription factors that play key roles in the immune system and in inflammation as well as in regulation of proliferation and survival of many types of animal cells. Members of this transcription factor family are activated in response to a variety of stimuli, including cytokines, growth factors, viral infection, and DNA damage. In unstimulated cells NF-κB proteins are bound to inhibitory **IκB** proteins that maintain NF-κB in an inactive state in the cytosol (Figure 15.42). Activation of NF-κB results from signals that activate the IκB kinase, which phosphorylates IκB. This phosphorylation targets IκB for ubiquitination and degradation by the proteasome, freeing NF-κB to translocate to the nucleus and induce expression of its target genes.



The Hedgehog, Wnt, and Notch Pathways

The **Hedgehog** and **Wnt** pathways are closely connected signaling systems that play key roles in determining cell fate during embryonic development. Both Hedgehog and Wnt pathways were first described in *Drosophila*, but members of the Hedgehog and Wnt families have been found to control a wide range of events that establish cell patterning during the development of both vertebrate and invertebrate embryos. Examples of the processes regulated by these signaling pathways include the determination of cell types and establishment of cell patterning during the development of limbs, the nervous system, the skeleton, lungs, hair, teeth, and gonads.

The *hedgehog* genes (one in *Drosophila* and three in mammals) encode secreted proteins that are modified by the addition of lipids. The functional receptor for Hedgehog consists of two transmembrane proteins, Patched and Smoothened (Figure 15.43). Hedgehog binds to Patched, which acts as a negative regulator of Smoothened. The binding of Hedgehog to Patched allows Smoothened to propagate an intracellular signal, leading to activation of a transcription factor called Cubitus interruptus (Ci) in *Drosophila* (Gli in mammals). In the absence of Hedgehog, Ci is maintained in a complex with a protein kinase called Fused and a kinesin-related protein called Coastal-2, which anchors the complex to microtubules. Within this complex, Ci is either completely degraded or cleaved to generate a transcriptional repressor (Ci75). Hedgehog signaling promotes the interaction of Smoothened with Coastal-2, leading to the release of full-length Ci (Ci155), which is then able to translocate to the nucleus and activate transcription of its target genes.

FIGURE 15.42 NF-κB signaling In the inactive state, homo- or heterodimers of NF-κB are bound to IκB in the cytoplasm. A variety of signals leads to activation of the IκB kinase, which phosphorylates IκB. This phosphorylation marks IκB for ubiquitination and degradation by the proteasome, allowing NF-κB to translocate to the nucleus and activate transcription of target genes.

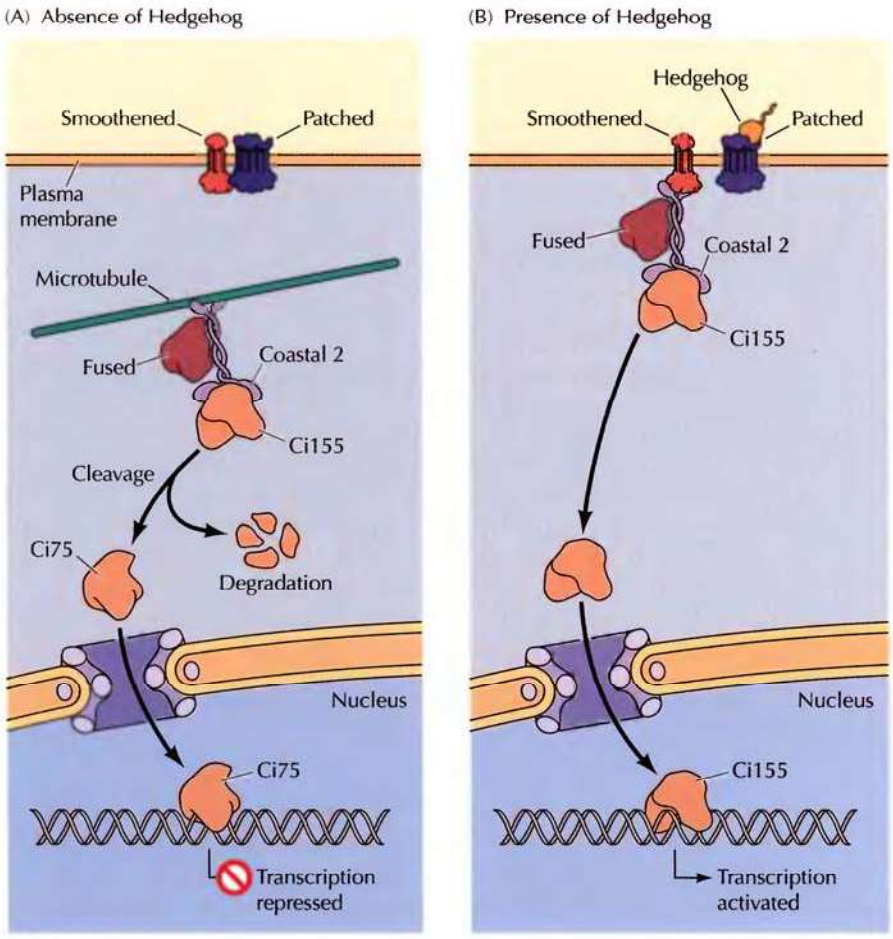


FIGURE 15.43 Hedgehog signaling In the absence of Hedgehog, the transcription factor Ci155 is anchored to microtubules by the kinesin-related protein Coastal-2 in association with the protein kinase Fused. Within this complex, Ci155 is degraded or cleaved to generate a transcriptional repressor (Ci75). The Hedgehog polypeptide binds to Patched on the surface of a target cell, relieving the inhibition of Smoothened by Patched and promoting the interaction of Smoothened with Coastal-2. This leads to the release of full-length Ci (Ci155), which translocates to the nucleus and activates transcription of its target genes.

The Wnt proteins are a family of secreted growth factors that bind to receptors of the LRP and Frizzled family, which are related to Smoothened (Figure 15.44). Signaling from LRP and Frizzled leads to activation of a cytoplasmic protein called Dishevelled, and inhibition of a complex of the proteins axin, APC, and the protein kinase GSK-3β. Within this complex, GSK-3β phosphorylates β-catenin, leading to its ubiquitination and degradation, so Wnt signaling results in increased β-catenin levels. β-catenin was discussed in Chapter 14 as a transmembrane protein that links cadherins to actin at adherens junctions (see Figure 14.22). Importantly, linking cadherins to actin is only one role of β-catenin. In Wnt signaling, β-catenin acts as a direct regulator of gene expression by forming a complex with members of the Tcf/LEF family of transcription factors. The association of β-catenin activates Tcf/LEF family members, leading to the expression of tar-

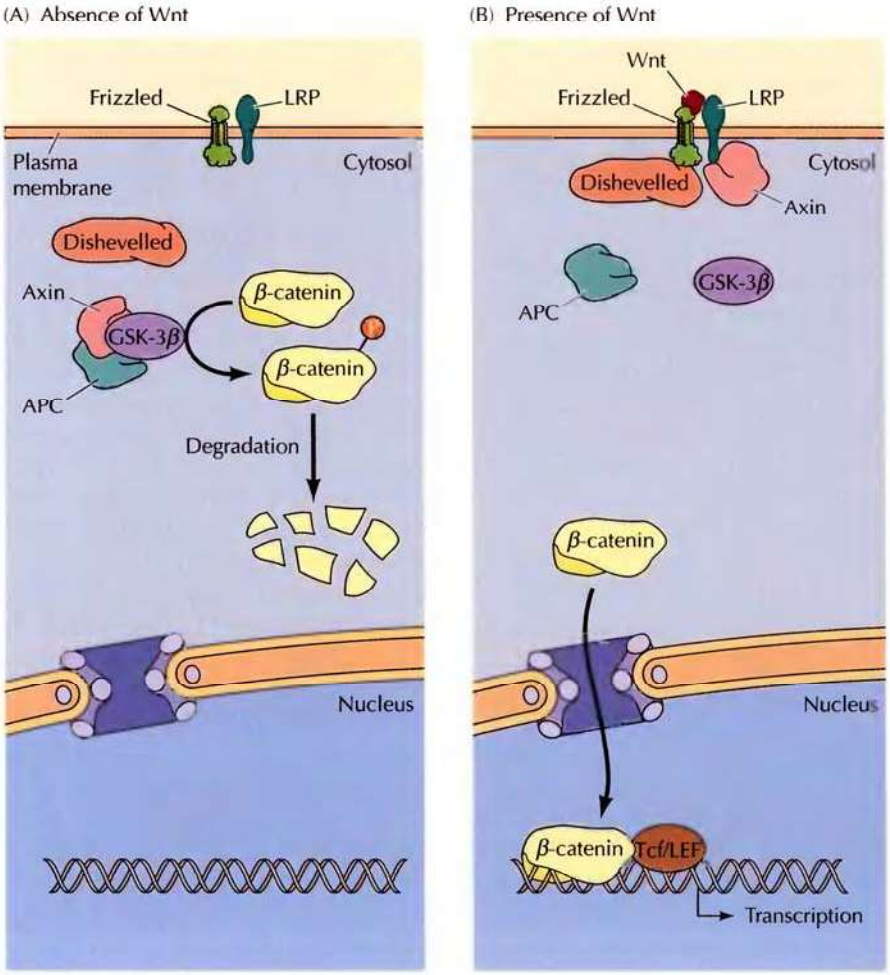


FIGURE 15.44 The Wnt pathway In the absence of Wnt, β-catenin is phosphorylated by GSK-3β in a complex with axin and APC, leading to its ubiquitination and degradation. Wnt polypeptides bind to Frizzled and LRP cell surface receptors, which activate Dishevelled and lead to inhibition of the axin/APC/GSK-3β complex. This results in stabilization of β-catenin, which translocates to the nucleus and forms a complex with Tcf/LEF transcription factors.

get genes encoding other cell signaling molecules and a variety of transcription factors that control cell fate.

The **Notch** pathway is another highly conserved signaling pathway that controls cell fate during animal development. Notch signaling is an example of direct cell-cell interactions during development. Notch is a large protein with a single transmembrane domain that serves as a receptor for signaling by transmembrane proteins (e.g., Delta) on the surface of adjacent cells (Figure 15.45). Stimulation of Notch initiates a novel and direct pathway of transcriptional activation. In particular, ligand binding leads to proteolytic cleavage of Notch, and the intracellular domain of Notch is then translocated into the nucleus. The Notch intracellular domain then interacts with a transcription factor (called Su[H] in *Drosophila*, or CSL in mammals) and converts it from a repressor to an activator of its target genes. As in the

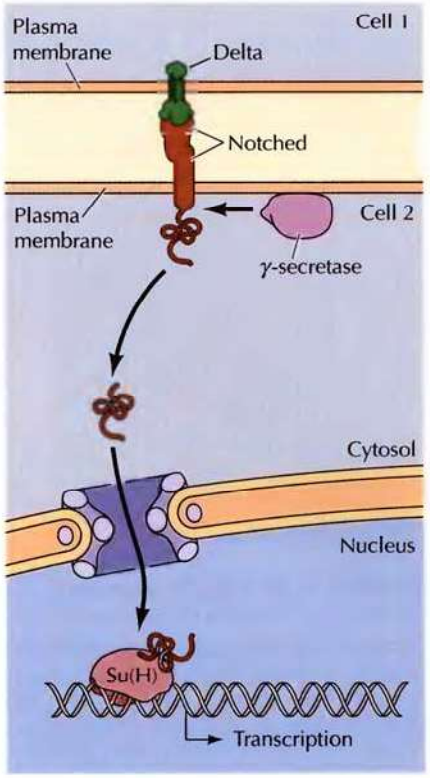


FIGURE 15.45 Notch signaling Notch serves as a receptor for direct cell-cell signaling by transmembrane proteins (e.g., Delta) on neighboring cells. The binding of Delta leads to proteolytic cleavage of Notch by γ-secretase. This releases the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor (Su[H] or CSL) to induce gene expression.

Wnt signaling pathway, the Notch target genes include genes encoding other transcriptional regulatory proteins, which act to determine cell fate.

Signal Transduction and the Cytoskeleton

The preceding sections focused on signaling pathways that regulate changes in metabolism, gene expression, and cell behavior in response to hormones and growth factors. However, the functions of most cells are also directly affected by cell adhesion and the organization of the cytoskeleton. The receptors responsible for cell adhesion thus act to initiate intracellular signaling pathways that regulate other aspects of cell behavior, including gene expression. Conversely, growth factors frequently act to induce cytoskeletal alterations resulting in cell movement or changes in cell shape. Components of the cytoskeleton thus act as both receptors and targets in cell signaling pathways, integrating cell shape and movement with other cellular responses.

Integrins and Signal Transduction

As discussed in Chapters 12 and 14, the integrins are the major receptors responsible for the attachment of cells to the extracellular matrix. At two types of cell-matrix junctions (focal adhesions and hemidesmosomes), the integrins also interact with components of the cytoskeleton to provide a stable linkage between the extracellular matrix and adherent cells (see Figure 14.19). In addition to this structural role, the integrins serve as receptors that activate intracellular signaling pathways thereby controlling cell movement and other aspects of cell behavior (including cell proliferation and survival) in response to adhesive interactions.

Like members of the cytokine receptor superfamily, the integrins have short cytoplasmic tails that lack any intrinsic enzymatic activity. However, protein-tyrosine phosphorylation is an early response to the interaction of integrins with extracellular matrix components, suggesting that the integrins are linked to nonreceptor protein-tyrosine kinases. One mode of signaling downstream of integrins involves activation of a nonreceptor protein-tyrosine kinase called **FAK** (*f*ocal *a*dhesion *k*inase) (Figure 15.46). As its name implies, FAK is localized to focal adhesions and rapidly becomes tyrosine-phosphorylated following the binding of integrin to extracellular matrix components, such as fibronectin. Like other protein-tyrosine kinases, the activation of FAK involves autophosphorylation induced by the clustering of integrins bound to the extracellular matrix as well as by their interactions with actin. Autophosphorylation of FAK creates docking sites for signaling molecules containing SH2 domains, including members of the Src family of nonreceptor protein-tyrosine kinases that phosphorylate additional sites on FAK. As discussed earlier for growth factor receptors, tyrosine phosphorylation of FAK creates binding sites for the SH2 domains of other downstream signaling molecules, including phospholipase C-γ, PI 3-kinase, and the Grb2-

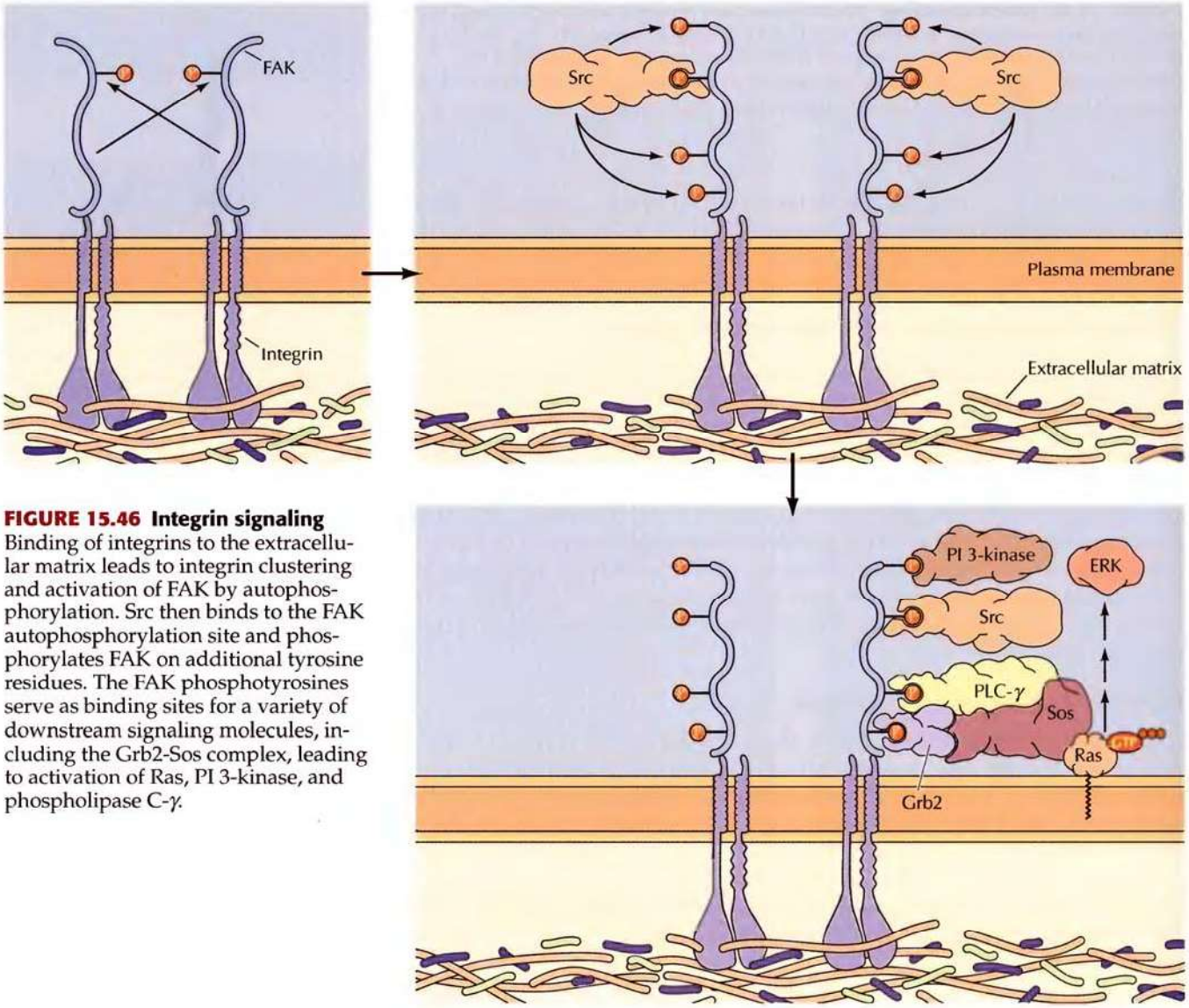


FIGURE 15.46 Integrin signaling Binding of integrins to the extracellular matrix leads to integrin clustering and activation of FAK by autophosphorylation. Src then binds to the FAK autophosphorylation site and phosphorylates FAK on additional tyrosine residues. The FAK phosphotyrosines serve as binding sites for a variety of downstream signaling molecules, including the Grb2-Sos complex, leading to activation of Ras, PI 3-kinase, and phospholipase C-γ.

Sos complex. Recruitment of the Sos guanine nucleotide exchange factor leads to activation of Ras, which in turn couples integrins to activation of the ERK pathway. Integrin activation of the FAK and Src nonreceptor protein-tyrosine kinases thus links cell adhesion to the same downstream signaling pathways that regulate gene expression, cell proliferation, and cell survival downstream of growth factor receptors. In addition, integrins can interact with and stimulate the activities of receptor protein-tyrosine kinases, such as the EGF receptor, leading to a parallel activation of the signaling pathways stimulated by growth factors and by cell adhesion.

Regulation of the Actin Cytoskeleton

Signaling from integrins as well as from growth factor receptors also plays a central role in the control of cell movement by regulating the dynamic behavior of the actin cytoskeleton. Cellular responses to growth factors as well as cell adhesion receptors frequently include changes in cell motility,