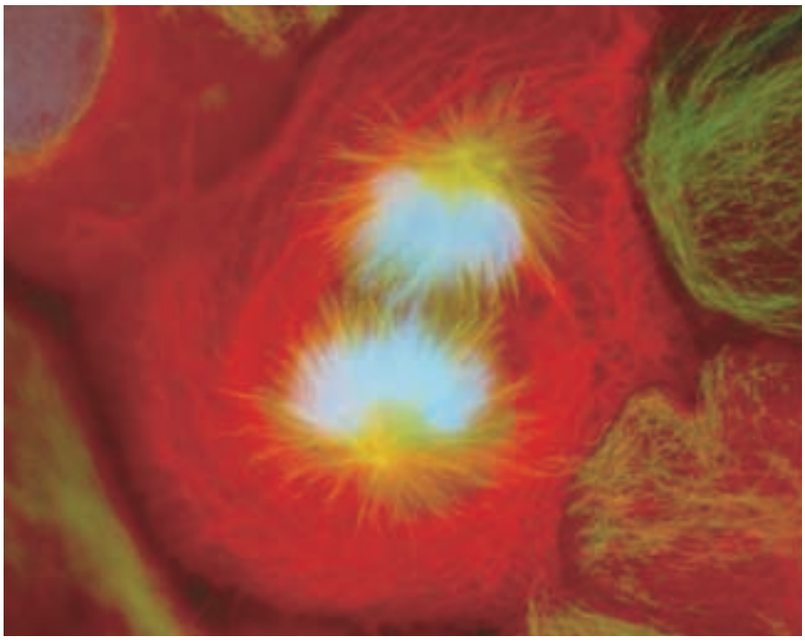
Part IV

Cell Regulation



Chapter 13 *Cell Signaling*

ALL CELLS RECEIVE AND RESPOND TO SIGNALS from their surroundings. Even the simplest bacteria sense and swim toward high concentrations of nutrients, such as glucose or amino acids. Many unicellular eukaryotes also respond to signaling molecules secreted by other cells, allowing cell-cell communication. Mating between yeast cells, for example, is signaled by peptides that are secreted by one cell and bind to receptors on the surface of another. It is in multicellular organisms, however, that cell-cell communication reaches its highest level of sophistication. Whereas the cells of prokaryotes and unicellular eukaryotes are largely autonomous, the behavior of each individual cell in multicellular plants and animals must be carefully regulated to meet the needs of the organism as a whole. This is accomplished by a variety of signaling molecules that are secreted or expressed on the surface of one cell and bind to receptors expressed by other cells, thereby integrating and coordinating the functions of the many individual cells that make up organisms as complex as human beings.

The binding of most signaling molecules to their receptors initiates a series of intracellular reactions that regulate virtually all aspects of cell behavior, including metabolism, movement, proliferation, survival, and differentiation. Understanding the molecular mechanisms responsible for these pathways of cell signaling has thus become a major area of research in contemporary cell biology. Interest in this area is further heightened by the fact that many cancers arise as a result of a breakdown in the signaling pathways that control normal cell proliferation and survival. Conversely, many of our current insights into cell signaling mechanisms have come from the study of cancer cells—a striking example of the fruitful interplay between medicine and basic research in cell and molecular biology.

Signaling Molecules and Their Receptors

Many different kinds of molecules transmit information between the cells of multicellular organisms. Although all these molecules act as ligands that bind to receptors expressed by their target cells, there is considerable variation in the structure and function of the different types of molecules that serve as signal transmitters. Structurally, the signaling molecules used by plants and animals

range in complexity from simple gases to proteins. Some of these molecules carry signals over long distances, whereas others act locally to convey information between neighboring cells. In addition, signaling molecules differ in their mode of action on their target cells. Some signaling molecules are able to cross the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus, whereas most bind to receptors expressed on the target cell surface. The sections that follow discuss the major types of signaling molecules and the receptors with which they interact. Subsequent discussion in this chapter focuses on the mechanisms by which cell surface receptors then function to regulate cell behavior.

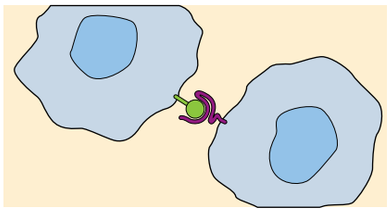
Modes of Cell-Cell Signaling

Cell signaling can result either from the direct interaction of a cell with its neighbor or from the action of secreted signaling molecules (Figure 13.1). Signaling by direct cell-cell (or cell-matrix) interactions plays a critical role in regulating the behavior of cells in animal tissues. For example, the integrins and cadherins (which were discussed in the previous chapter) function not only as cell adhesion molecules but also as signaling molecules that regulate cell proliferation and survival in response to cell-cell and cell-matrix contacts. In addition, cells express a variety of cell surface receptors that interact with signaling molecules on the surface of neighboring cells. Signaling via such direct cell-cell interactions plays a critical role in regulating the many interactions between different types of cells that take place during embryonic development, as well as in the maintenance of adult tissues.

The multiple varieties of signaling by secreted molecules are frequently divided into three general categories based on the distance over which signals are transmitted. In **endocrine signaling**, the signaling molecules (**hormones**) are secreted by specialized endocrine cells and carried through the circulation to act on target cells at distant body sites. A classic example is provided by the steroid hormone estrogen, which is produced by the ovary and stimulates development and maintenance of the female reproductive system and secondary sex characteristics. In animals, more than 50 different hormones are produced by endocrine glands, including the pituitary, thyroid, parathyroid, pancreas, adrenal glands, and gonads.

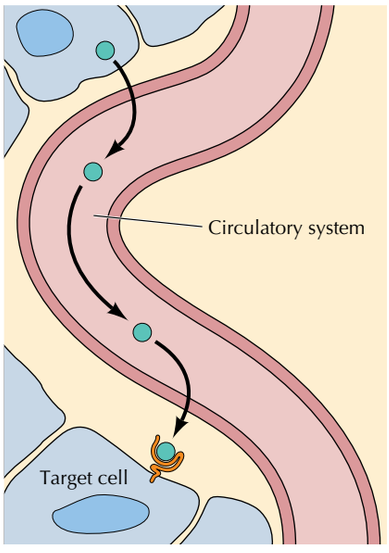
In contrast to hormones, some signaling molecules act locally to affect the behavior of nearby cells. In **paracrine signaling**, a molecule released by one cell acts on neighboring target cells. An example is provided by the action of neurotransmitters in carrying signals between nerve cells at a synapse. Finally, some cells respond to signaling molecules that they themselves produce. One important example of such **autocrine signaling** is the response of cells of the vertebrate immune system to foreign antigens. Certain types of T lymphocytes respond to antigenic stimulation by synthesizing a growth factor that drives their own proliferation, thereby increasing the number of responsive T lymphocytes and amplifying the immune response. It is also noteworthy that abnormal autocrine signaling frequently contributes to the uncontrolled growth of cancer cells (see Chapter 15). In

Direct Cell-Cell Signaling

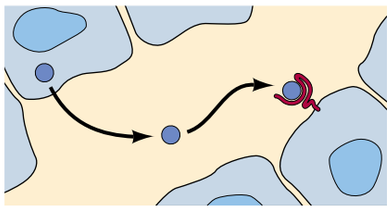


Signaling by Secreted Molecules

(A) Endocrine signaling



(B) Paracrine signaling



(C) Autocrine signaling

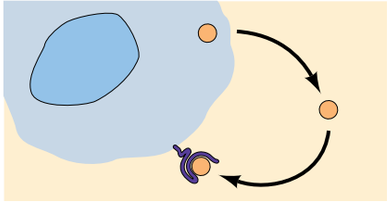


Figure 13.1 Modes of cell-cell signaling
Cell signaling can take place either through direct cell-cell contacts or through the action of secreted signaling molecules. (A) In endocrine signaling, hormones are carried through the circulatory system to act on distant target cells. (B) In paracrine signaling, a molecule released from one cell acts locally to affect nearby target cells. (C) In autocrine signaling, a cell produces a signaling molecule to which it also responds.

this situation, a cancer cell produces a growth factor to which it also responds, thereby continuously driving its own unregulated proliferation.

Steroid Hormones and the Nuclear Receptor Superfamily

As already noted, all signaling molecules act by binding to receptors expressed by their target cells. In many cases, these receptors are expressed on the target cell surface, but some receptors are intracellular proteins located in the cytosol or the nucleus. These intracellular receptors respond to small hydrophobic signaling molecules that are able to diffuse across the plasma membrane. The **steroid hormones** are the classic examples of this group of signaling molecules, which also includes thyroid hormone, vitamin D_3, and retinoic acid (Figure 13.2).

The steroid hormones (including testosterone, estrogen, progesterone, the corticosteroids, and ecdysone) are all synthesized from cholesterol. **Testosterone**, **estrogen**, and **progesterone** are the sex steroids, which are produced by the gonads. The **corticosteroids** are produced by the adrenal gland. They include the **glucocorticoids**, which act on a variety of cells to stimulate production of glucose, and the **mineralocorticoids**, which act on the kidney to regulate salt and water balance. **Ecdysone** is an insect hormone that plays a key role in development by triggering the metamorphosis of larvae to adults. The **brassinosteroids** are plant-specific steroid hormones that control a number of developmental processes, including cell growth and differentiation.

Although thyroid hormone, vitamin D_3, and retinoic acid are both structurally and functionally distinct from the steroids, they share a common

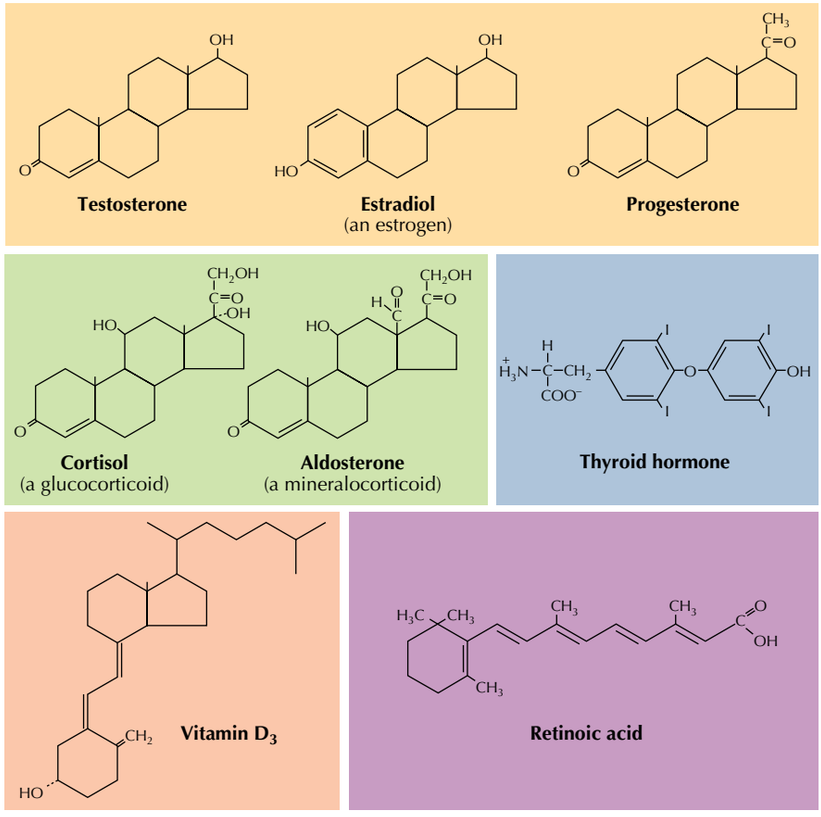


Figure 13.2 Structure of steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid
The steroids include the sex hormones (testosterone, estrogen, and progesterone), glucocorticoids, and mineralocorticoids.

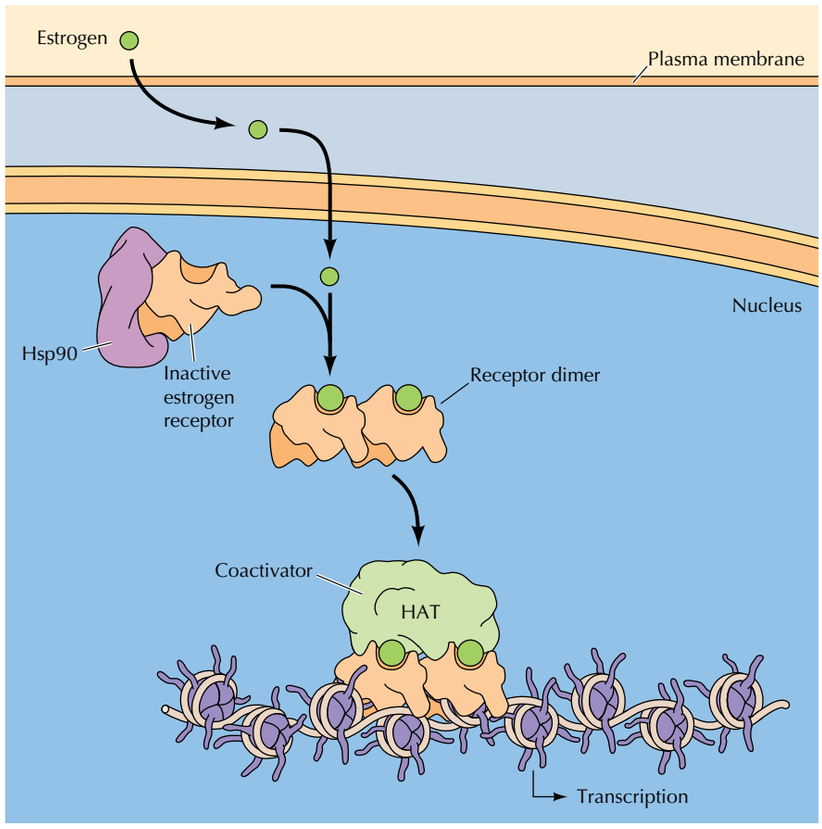


Figure 13.3 Estrogen action
Estrogen diffuses across the plasma membrane and bind to its receptor in the nucleus. In the absence of hormone, estrogen receptor is bound to Hsp90. Estrogen binding displaces the receptor from Hsp90 and allows the formation of receptor dimers, which bind DNA, associate with coactivators with histone acetyltransferase (HAT) activity, and stimulate transcription of their target genes.

mechanism of action in their target cells. **Thyroid hormone** is synthesized from tyrosine in the thyroid gland; it plays important roles in development and regulation of metabolism. **Vitamin D_3** regulates Ca^{2+} metabolism and bone growth. **Retinoic acid** and related compounds (**retinoids**) synthesized from vitamin A play important roles in vertebrate development.

Because of their hydrophobic character, the steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid are able to enter cells by diffusing across the plasma membrane. Once inside the cell, they bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors, which are members of a family of proteins known as the **nuclear receptor superfamily**, are transcription factors that contain related domains for ligand binding, DNA binding, and transcriptional activation. Ligand binding regulates their function as activators or repressors of their target genes, so the steroid hormones and related molecules directly regulate gene expression.

Ligand binding has distinct effects on different receptors. Some members of the steroid receptor superfamily are unable to bind to DNA in the absence of hormone. Estrogen receptor, for example, is bound to Hsp90 chaperones in the absence of hormone (Figure 13.3) The binding of estrogen induces a conformational change in the receptor, displacing Hsp90 and leading to the formation of receptor dimers that bind to regulatory DNA sequences and activate transcription of target genes. In other cases, the receptor binds DNA in either the presence or absence of hormone, but hormone binding alters the activity of the receptor as a transcriptional regulatory molecule. For example, in the absence of hormone, thyroid hormone receptor is associated with a corepressor complex and represses transcription of its target genes (Figure 13.4). Hormone binding induces a conforma-

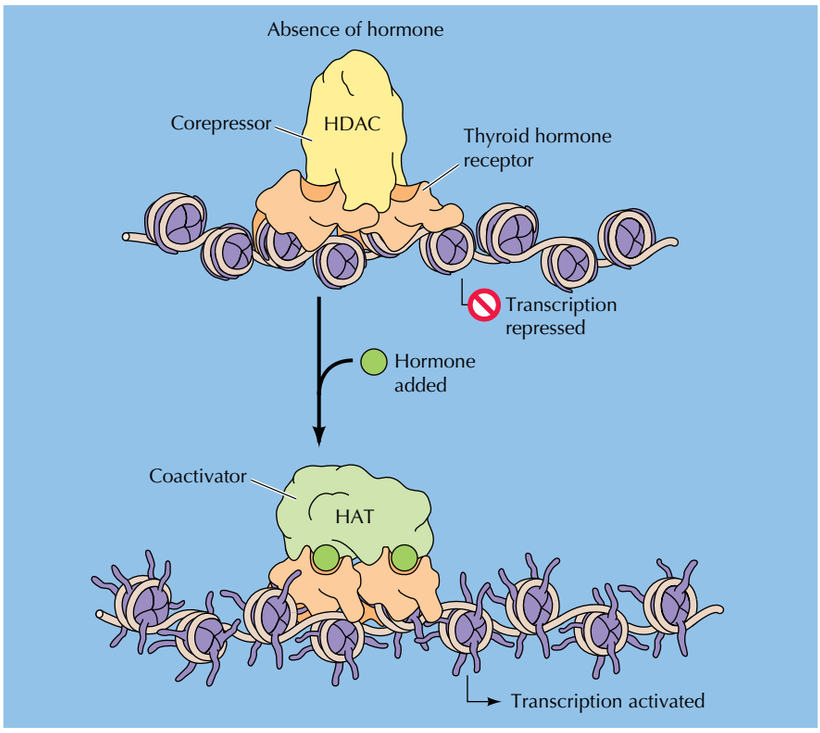


Figure 13.4 Gene regulation by the thyroid hormone receptor
Thyroid hormone receptor binds DNA in either the presence or absence of hormone. However, hormone binding changes the function of the receptor from a repressor to an activator of target gene transcription. In the absence of hormone, the receptor associates with corepressors with histone deacetylase (HDAC) activity. In the presence of hormone, the receptor associates with coactivators with histone acetyltransferase (HAT) activity.

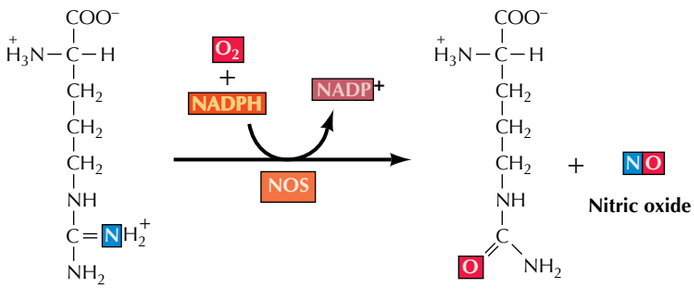
tional change that results in the interaction of the receptor with coactivators rather than corepressors, leading to transcriptional activation of thyroid hormone-inducible genes.

Nitric Oxide and Carbon Monoxide

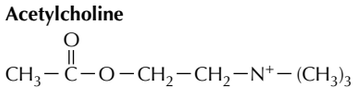
The simple gas nitric oxide (NO) is a major paracrine signaling molecule in the nervous, immune, and circulatory systems. Like the steroid hormones, NO is able to diffuse directly across the plasma membrane of its target cells. The molecular basis of NO action, however, is distinct from that of steroid action; rather than binding to a receptor that regulates transcription, NO alters the activity of intracellular target enzymes.

Nitric oxide is synthesized from the amino acid arginine by the enzyme nitric oxide synthase (Figure 13.5). Once synthesized, NO diffuses out of the cell and can act locally to affect nearby cells. Its action is restricted to such local effects because NO is extremely unstable, with a half-life of only a few seconds. The major intracellular target of NO is guanylyl cyclase. NO binds to a heme group at the active site of this enzyme, stimulating synthesis of the second messenger cyclic GMP (discussed later in this chapter). In addition, NO may directly modify some target proteins by nitrosylation of cysteine residues. A well-characterized example of NO action is signaling the dilation of blood vessels. The first step in this process is the release of neurotransmitters, such as acetylcholine, from the termini of nerve cells in the blood vessel wall. These neurotransmitters act on endothelial cells to stimulate NO synthesis. NO then diffuses to neighboring smooth muscle cells where it activates guanylyl cyclase, resulting in synthesis of cyclic GMP, which induces muscle cell relaxation and blood vessel dilation. For example, NO is responsible for signaling the dilation of blood vessels that leads to penile erection. It is also interesting to note that the medical use of nitroglycerin in treatment of heart disease is based on its conversion to NO, which dilates coronary blood vessels and increases blood flow to the heart.

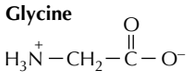
Figure 13.5 Synthesis of nitric oxide
The enzyme nitric oxide synthase (NOS) catalyzes the formation of nitric oxide from arginine.



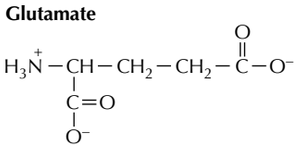
Acetylcholine



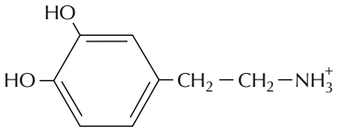
Glycine



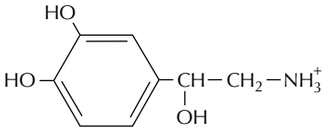
Glutamate



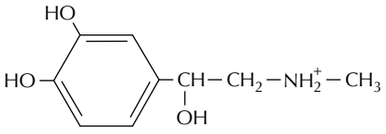
Dopamine



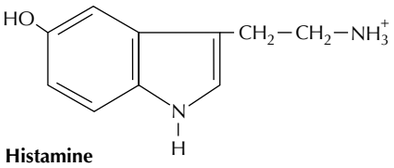
Norepinephrine



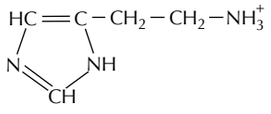
Epinephrine



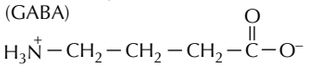
Serotonin



Histamine



γ-Aminobutyric acid
(GABA)



Another simple gas, carbon monoxide (CO), also functions as a signaling molecule in the nervous system. CO is closely related to NO and appears to act similarly as a neurotransmitter and mediator of blood vessel dilation. The synthesis of CO in brain cells, like that of NO, is stimulated by neurotransmitters. In addition, CO can stimulate guanylate cyclase, which may also represent the major physiological target of CO signaling.

Neurotransmitters

The **neurotransmitters** carry signals between neurons or from neurons to other types of target cells (such as muscle cells). They are a diverse group of small hydrophilic molecules including acetylcholine, dopamine, epinephrine (adrenaline), serotonin, histamine, glutamate, glycine, and γ-aminobutyric acid (GABA) (Figure 13.6). The release of neurotransmitters is signaled by the arrival of an action potential at the terminus of a neuron (see Figure 12.22). The neurotransmitters then diffuse across the synaptic cleft and bind to receptors on the target cell surface. Note that some neurotransmitters can also act as hormones. For example, epinephrine functions both as a neurotransmitter and as a hormone produced by the adrenal gland to signal glycogen breakdown in muscle cells.

Because the neurotransmitters are hydrophilic molecules, they are unable to cross the plasma membrane of their target cells. Therefore, in contrast to steroid hormones and NO or CO, the neurotransmitters act by binding to cell surface receptors. Many neurotransmitter receptors are ligand-gated ion channels, such as the acetylcholine receptor discussed in the preceding chapter (see Figure 12.23). Neurotransmitter binding to these receptors induces a conformational change that opens ion channels, directly resulting in changes in ion flux in the target cell. Other neurotransmitter receptors are coupled to G proteins—a major group of signaling molecules (discussed later in this chapter) that link cell surface receptors to a variety of intracellular responses. In the case of neurotransmitter receptors, the associated G proteins frequently act to indirectly regulate ion channel activity.

Peptide Hormones and Growth Factors

The widest variety of signaling molecules in animals are peptides, ranging in size from only a few to more than a hundred amino acids. This group of signaling molecules includes peptide hormones, neuropeptides, and a diverse array of polypeptide growth factors (Table 13.1). Well-known examples of **peptide hormones** include insulin, glucagon, and the hormones pro-

Figure 13.6 Structure of representative neurotransmitters
The neurotransmitters are hydrophilic molecules that bind to cell surface receptors.

TABLE 13.1 Representative Peptide Hormones, Neuropeptides, and Growth Factors

Signaling molecule	Size[a]	Activities[b]
Peptide hormones		
Insulin	A = 21, B = 30	Regulation of glucose uptake; stimulation of cell proliferation
Glucagon	29	Stimulation of glucose synthesis
Growth hormone	191	General stimulation of growth
Follicle-stimulating hormone (FSH)	α = 92, β = 118	Stimulation of the growth of oocytes and ovarian follicles
Prolactin	198	Stimulation of milk production
Neuropeptides and neurohormones		
Substance P	11	Sensory synaptic transmission
Oxytocin	9	Stimulation of smooth muscle contraction
Vasopressin	9	Stimulation of water reabsorption in the kidney
Enkephalin	5	Analgesic
β-Endorphin	31	Analgesic
Growth factors		
Nerve growth factor (NGF)	118	Differentiation and survival of neurons
Epidermal growth factor (EGF)	53	Proliferation of many types of cells
Platelet-derived growth factor (PDGF)	A = 125, B = 109	Proliferation of fibroblasts and other cell types
Interleukin-2	133	Proliferation of T lymphocytes
Erythropoietin	166	Development of red blood cells

[a] Size is indicated in number of amino acids. Some hormones and growth factors consist of two different polypeptide chains, which are designated either A and B or α and β.
[b] Most of these hormones and growth factors possess other activities in addition to those indicated.

duced by the pituitary gland (growth hormone, follicle-stimulating hormone, prolactin, and others).

Neuropeptides are secreted by some neurons instead of the small-molecule neurotransmitters discussed in the previous section. Some of these peptides, such as the **enkephalins** and **endorphins**, function not only as neurotransmitters at synapses but also as **neurohormones** that act on distant cells. The enkephalins and endorphins have been widely studied because of their activity as natural analgesics that decrease pain responses in the central nervous system. Discovered during studies of drug addiction, they are naturally occurring compounds that bind to the same receptors on the surface of brain cells as morphine does.

The polypeptide **growth factors** include a wide variety of signaling molecules that control animal cell growth and differentiation. The first of these factors (**nerve growth factor,** or **NGF**) was discovered by Rita Levi-Montalcini in the 1950s. NGF is a member of a family of polypeptides (called **neurotrophins**) that regulate the development and survival of neurons. During the course of experiments on NGF, Stanley Cohen serendipitously discovered an unrelated factor (called **epidermal growth factor**, or **EGF**) that stimulates cell proliferation. EGF, a 53-amino-acid polypeptide (Figure 13.7), has served as the prototype of a large array of growth factors that play critical roles in controlling animal cell proliferation, both during embryonic development and in adult organisms.

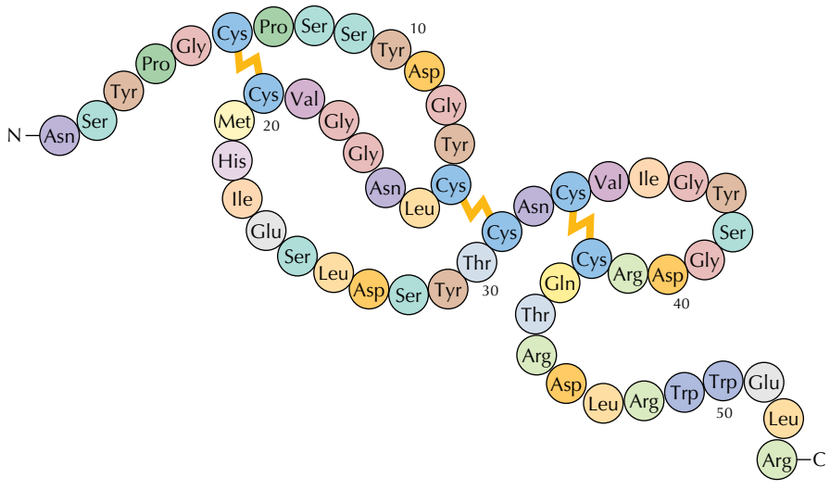


Figure 13.7 Structure of epidermal growth factor (EGF)
EGF is a single polypeptide chain of 53 amino acids. Disulfide bonds between cysteine residues are indicated. (After G. Carpenter and S. Cohen, 1979. *Ann. Rev. Biochem.* 48: 193.)

A good example of growth factor action is provided by the activity of **platelet-derived growth factor** (**PDGF**) in wound healing. PDGF is stored in blood platelets and released during blood clotting at the site of a wound. It then stimulates the proliferation of fibroblasts in the vicinity of the clot, thereby contributing to regrowth of the damaged tissue. Members of another large group of polypeptide growth factors (called **cytokines**) regulate the development and differentiation of blood cells and control the activities of lymphocytes during the immune response. Other polypeptide growth factors (**membrane-anchored growth factors**) remain associated with the plasma membrane rather than being secreted into extracellular fluids, therefore functioning specifically as signaling molecules during direct cell-cell interactions.

Peptide hormones, neuropeptides, and growth factors are unable to cross the plasma membrane of their target cells, so they act by binding to cell surface receptors, as discussed later in this chapter. As might be expected from the critical roles of polypeptide growth factors in controlling cell proliferation, abnormalities in growth factor signaling are the basis for a variety of diseases, including many kinds of cancer. For example, abnormal expression of a close relative of the EGF receptor is an important factor in the development of many human breast and ovarian cancers.

Eicosanoids

Several types of lipids serve as signaling molecules that, in contrast to the steroid hormones, act by binding to cell surface receptors. The most important of these molecules are members of a class of lipids called the **eicosanoids**, which includes **prostaglandins**, **prostacyclin**, **thromboxanes**, and **leukotrienes** (Figure 13.8). The eicosanoids are rapidly broken down and therefore act locally in autocrine or paracrine signaling pathways. They stimulate a variety of responses in their target cells, including blood platelet aggregation, inflammation, and smooth-muscle contraction.

All eicosanoids are synthesized from arachidonic acid, which is formed from phospholipids. The first step in the pathway leading to synthesis of either prostaglandins or thromboxanes is the conversion of arachidonic acid to prostaglandin H_2. Interestingly, the enzyme that catalyzes this reaction (cyclooxygenase) is the target of aspirin and other nonsteroidal anti-inflammatory drugs. By inhibiting synthesis of the prostaglandins, aspirin reduces inflammation and pain. By inhibiting synthesis of thromboxane, aspirin also reduces platelet aggregation and blood clotting. Because of this activity, small daily doses of aspirin are frequently prescribed for prevention of

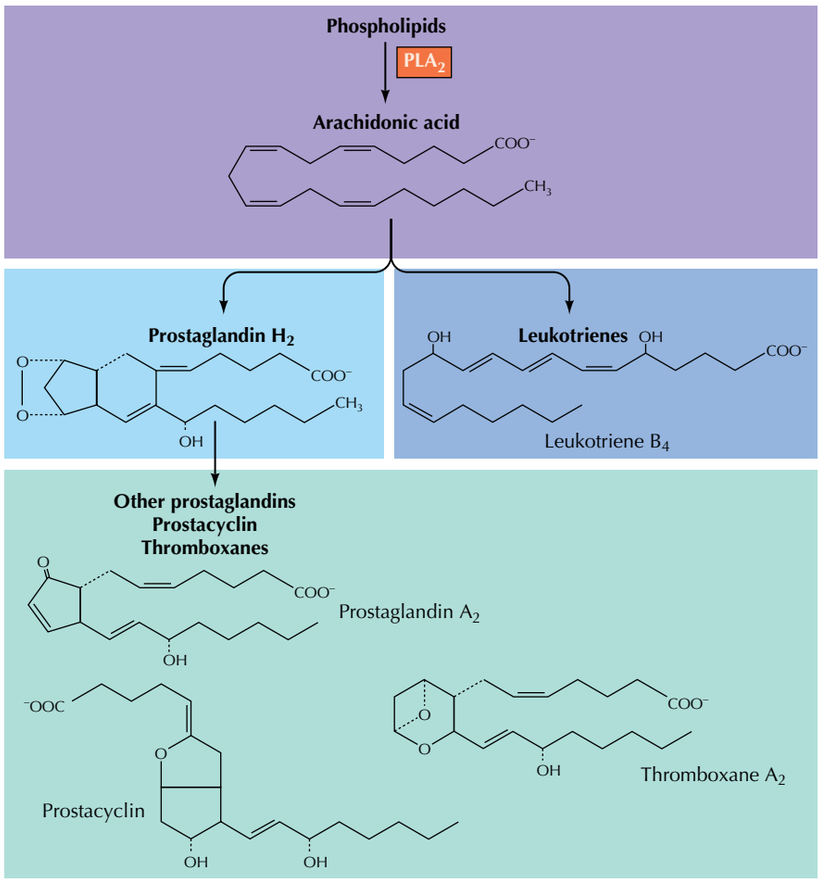


Figure 13.8 Synthesis and structure of eicosanoids
The eicosanoids include the prostaglandins, prostacyclin, thromboxanes, and leukotrienes. They are synthesized from arachidonic acid, which is formed by the hydrolysis of phospholipids catalyzed by phospholipase A_2 (PLA_2). Arachidonic acid can then be metabolized via two alternative pathways; one pathway leads to synthesis of prostaglandins, prostacyclin, and thromboxanes, while the other pathway leads to synthesis of leukotrienes.

strokes. In addition, aspirin and nonsteroidal anti-inflammatory drugs have been found to reduce the frequency of colon cancer in both animal models and humans, apparently by inhibiting the synthesis of prostaglandins that act to stimulate cell proliferation and promote cancer development.

Plant Hormones

Plant growth and development are regulated by a group of small molecules called **plant hormones**. The levels of these molecules within the plant are typically modified by environmental factors, such as light or infection, so they coordinate the responses of tissues in different parts of the plant to environmental signals.

The plant hormones are classically divided into five major groups: **auxins**, **gibberellins**, **cytokinins**, **abscisic acid**, and **ethylene** (Figure 13.9), although several additional plant hormones (such as the plant steroid hormones) have recently been discovered. The first plant hormone to be identified was auxin, with the early experiments leading to its discovery having been performed by Charles Darwin in the 1880s. One of the effects of auxins is to induce plant cell elongation by weakening the cell wall (see Figure 12.50). In addition, auxins regulate many other aspects of plant development, including cell division and differentiation. The other plant hormones likewise have multiple effects in their target tissues, including stem elongation (gibberellins), fruit ripening (ethylene), cell division (cytokinins), and the onset of dormancy (abscisic acid).

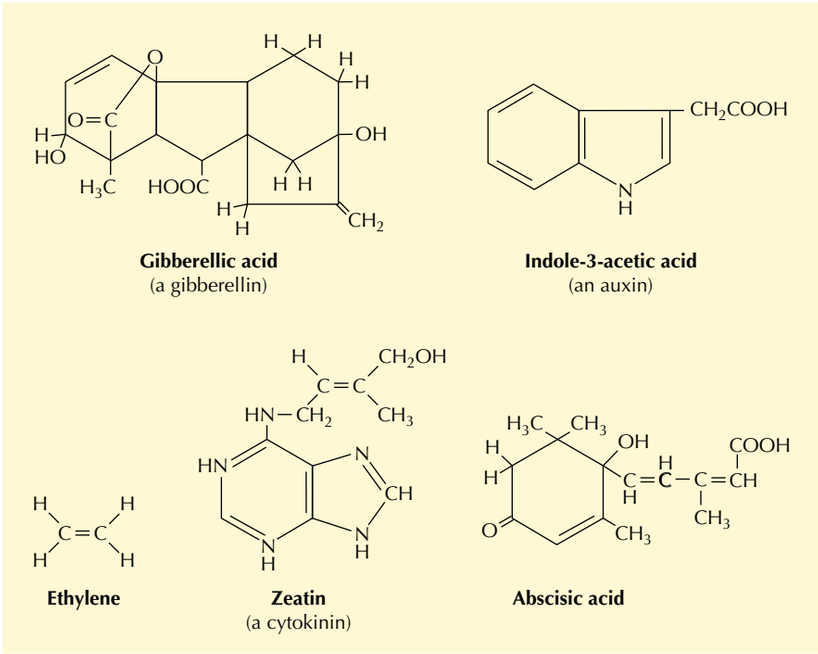


Figure 13.9 Structure of plant hormones

The signaling pathways triggered by these hormones in plants use a variety of mechanisms that are conserved in animal cells, as well as a number of elements that are unique to plants. For example, one well understood pathway, which has been elucidated by genetic analysis of *Arabidopsis thaliana*, signals the response of plant cells to ethylene. Elements of this pathway include an ethylene receptor on the plant cell surface, a protein kinase related to the Raf protein kinases of animal cells, and a novel transcription factor that regulates expression of ethylene-responsive genes.

Functions of Cell Surface Receptors

As already reviewed, most ligands responsible for cell-cell signaling (including neurotransmitters, peptide hormones, and growth factors) bind to receptors on the surface of their target cells. Consequently, a major challenge in understanding cell-cell signaling is unraveling the mechanisms by which cell surface receptors transmit the signals initiated by ligand binding. As discussed in Chapter 12, some neurotransmitter receptors are ligand-gated ion channels that directly control ion flux across the plasma membrane. Other cell surface receptors, including the receptors for peptide hormones and growth factors, act instead by regulating the activity of 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.

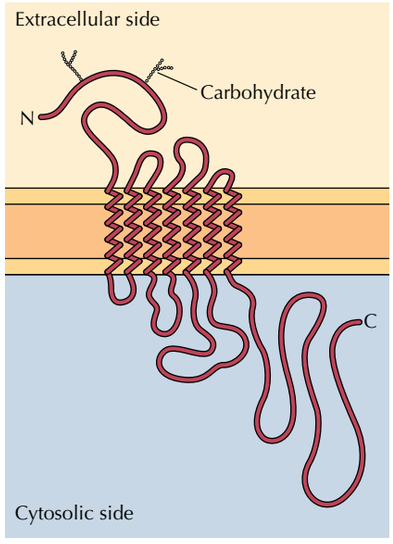


Figure 13.10 Structure of a G protein-coupled receptor
The G protein-coupled receptors are characterized by seven transmembrane α helices.

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 13.10). 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 13.11). 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.

G proteins consist of three subunits, designated α, β, and γ (Figure 13.12). 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

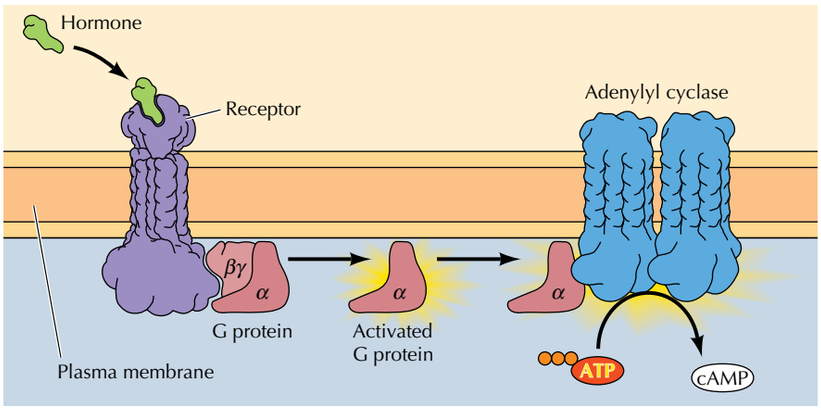


Figure 13.11 Hormonal activation of adenylyl cyclase
Binding of hormone promotes the interaction of the receptor with a G protein. The activated G protein α subunit then dissociates from the receptor and stimulates adenylyl cyclase, which catalyzes the conversion of ATP to cAMP.

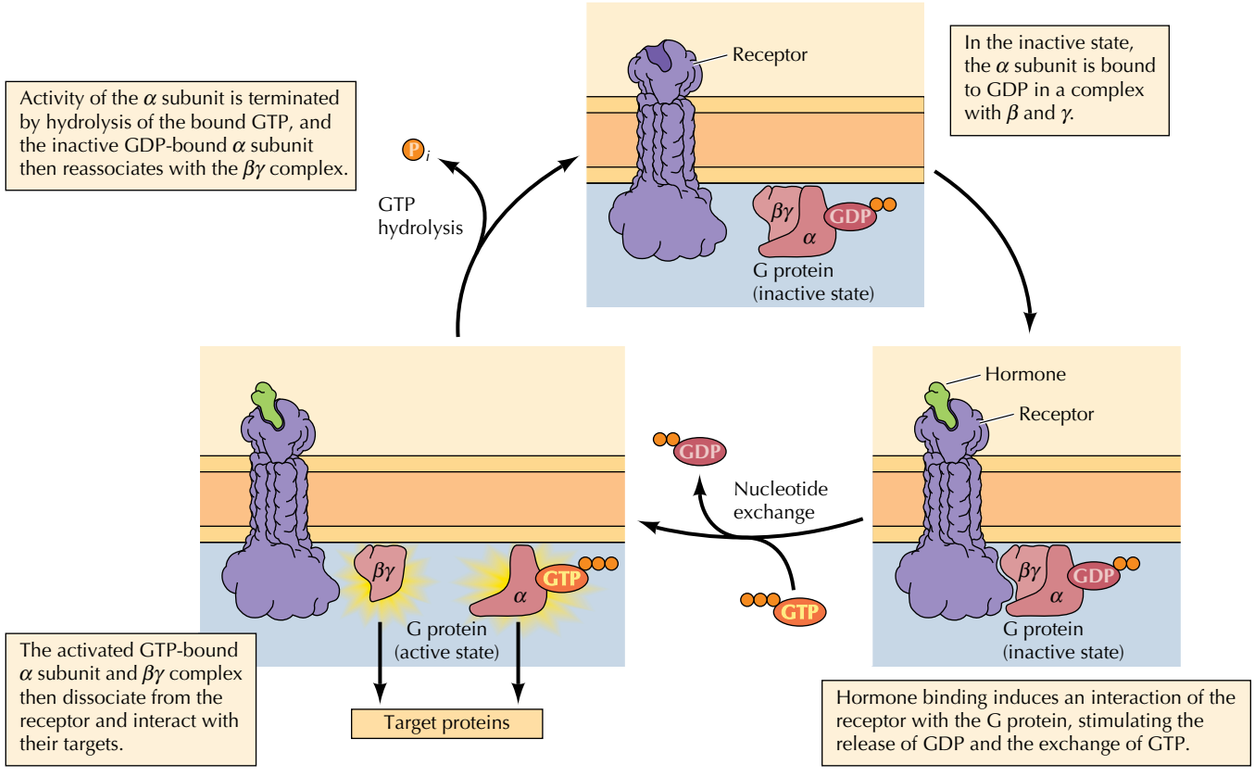


Figure 13.12 Regulation of G proteins

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 at least 20 different α subunits, 6 β subunits, and 11 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 *s*timulates adenylyl cyclase (see Figure 13.11). 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 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 12.23). 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 are 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 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 58 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 13.13). Most of the receptor protein-tyrosine kinases consist of single polypeptides, although the insulin receptor and some 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.

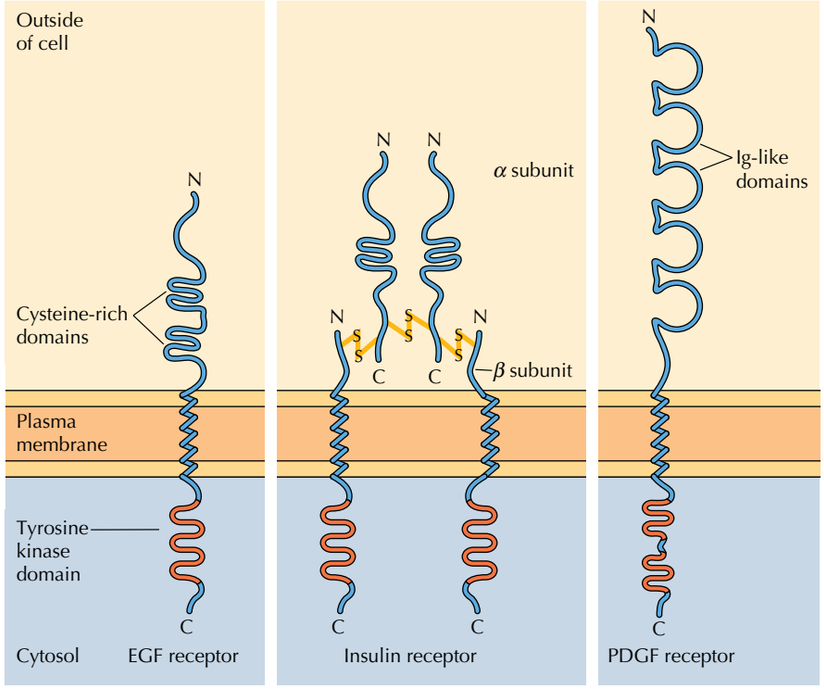
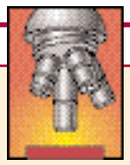


Figure 13.13 Organization of receptor protein-tyrosine kinases Each receptor consists of an N-terminal extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with protein-tyrosine kinase activity. The structures of three distinct subfamilies of receptor 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).



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

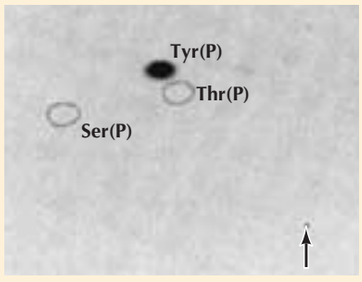
Bartholomew Sefton

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 *sarc*oma) 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 3.30) 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.



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.

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 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

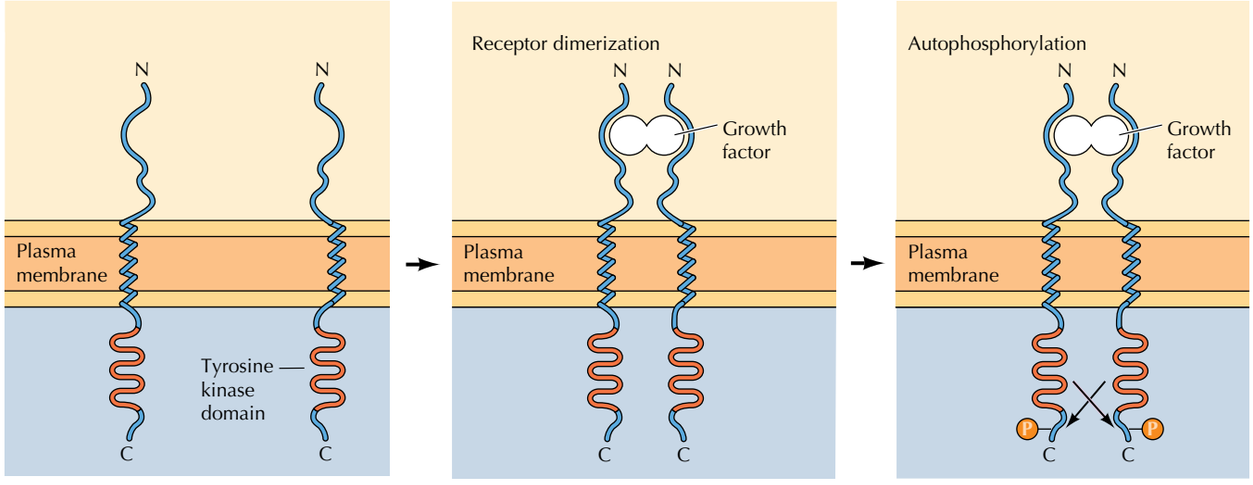
The discovery that Src was a protein-tyrosine kinase both identified a new protein kinase activity and established this activity as being related to the control of cell proliferation. The results of Hunter and Sefton were followed by demonstrations that many other tumor virus proteins also function as 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.

The first step in signaling from most receptor protein-tyrosine kinases is ligand-induced receptor dimerization (Figure 13.14). Some growth factors, such as PDGF and NGF, are themselves dimers consisting of two identical 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 13.14). 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 spe-

Figure 13.14 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.



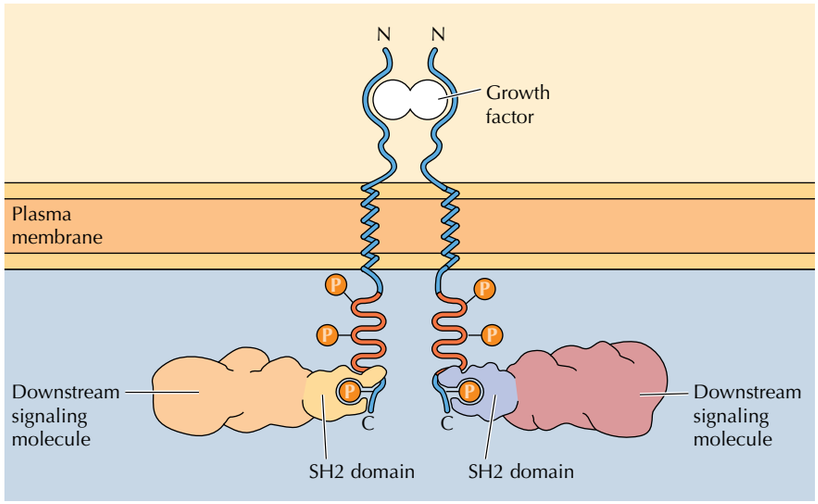


Figure 13.15 Association of downstream signaling molecules with receptor protein-tyrosine kinases
SH2 domains bind to specific phosphotyrosine-containing peptides of the activated receptors.

cific phosphotyrosine-containing peptides (Figure 13.15). 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 a hundred amino acids and bind to specific short peptide sequences containing phosphotyrosine residues (Figure 13.16). 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.

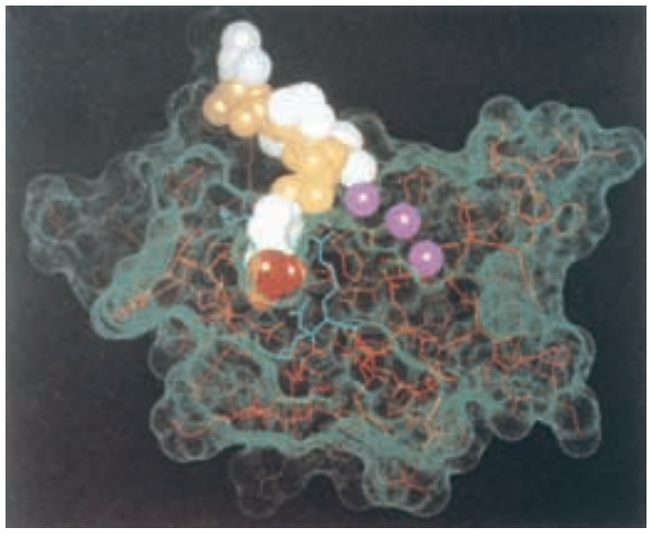


Figure 13.16 Complex between an SH2 domain and a phosphotyrosine peptide
The polypeptide chain of the Src SH2 domain is shown in red with its surface indicated by green dots. Purple spheres indicate a groove on the surface. The three amino acid residues that interact with the phosphotyrosine are shown 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.)

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 13.17). 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-

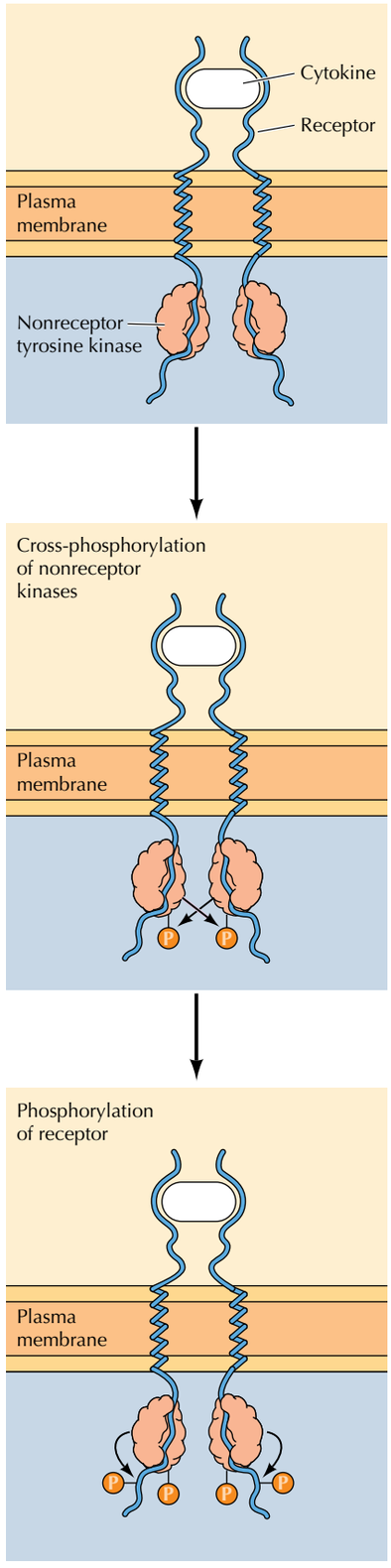


Figure 13.17 Signaling from cytokine receptors
Ligand binding induces receptor dimerization and leads to the activation of associated nonreceptor protein-tyrosine kinases as a result of cross-phosphorylation. The activated kinases then phosphorylate tyrosine residues of the receptor, creating phosphotyrosine-binding sites for downstream signaling molecules.

tyrosine phosphatases are cell surface receptors whose enzymatic activities play a positive role in cell signaling. 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.

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, generally inhibiting proliferation of their target cells. 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 later in this chapter) 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

Most 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.

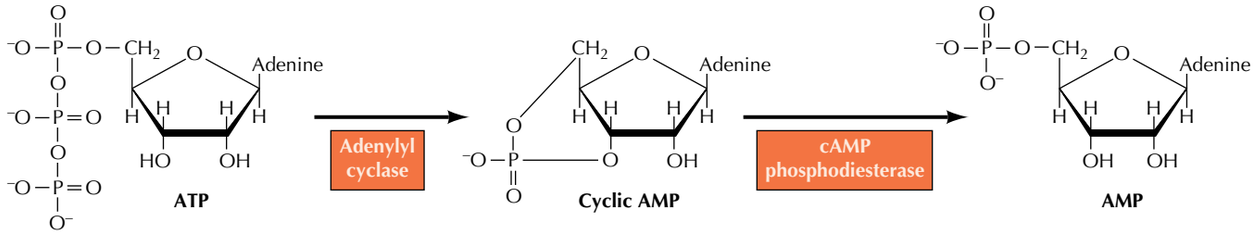


Figure 13.18 Synthesis and degradation of cAMP
Cyclic AMP is synthesized from ATP by adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase.

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 13.18). 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 13.11).

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 13.19). 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 13.20). 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 hun-

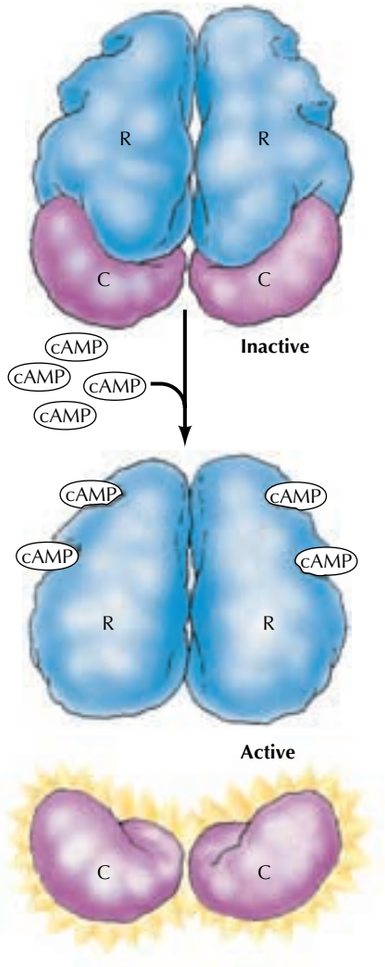


Figure 13.19 Regulation of protein kinase A
The inactive form of protein kinase A consists of two regulatory (R) and two catalytic (C) subunits. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which are then enzymatically active.

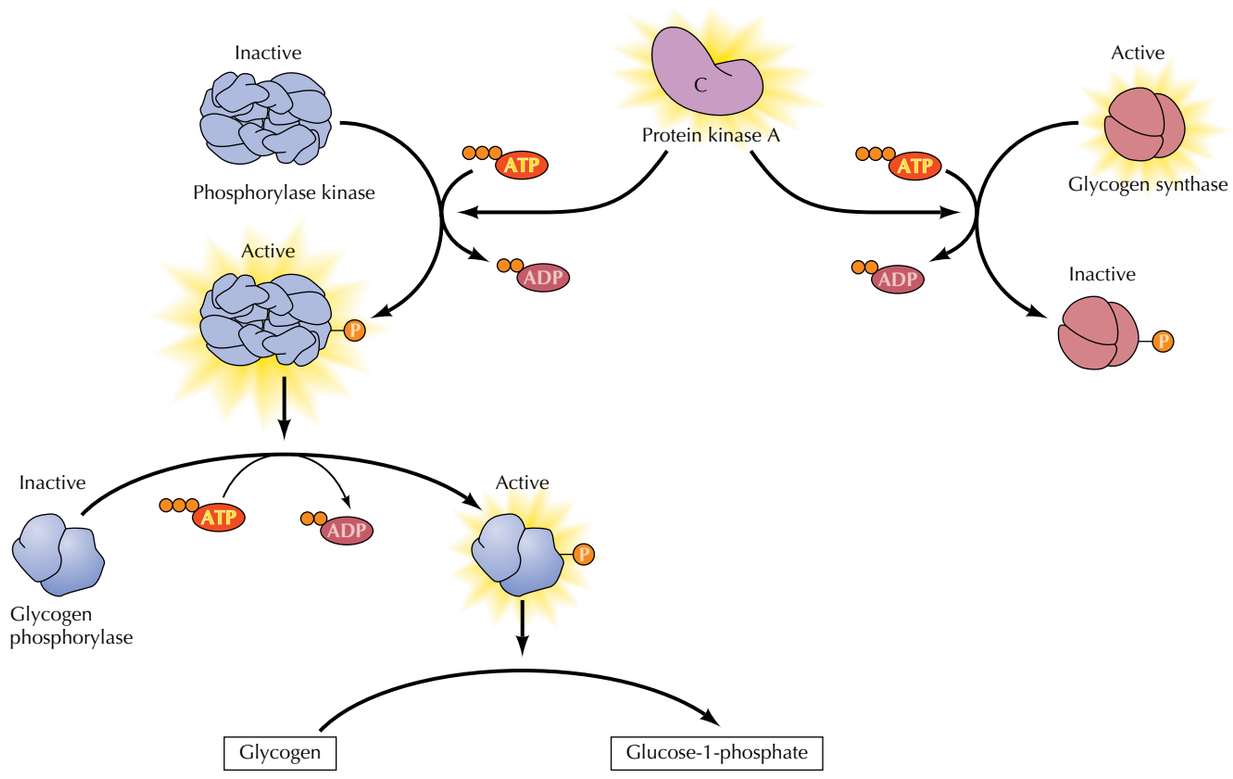


Figure 13.20 Regulation of glycogen metabolism by protein kinase A
Protein kinase A phosphorylates both glycogen synthase and phosphorylase kinase. Glycogen synthase (which catalyzes glycogen synthesis) is inhibited by this phosphorylation, whereas phosphorylase kinase is activated. Phosphorylase kinase then phosphorylates and activates glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate.

dred molecules of G_s. Each molecule of G_s then stimulates the enzymatic 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 phosphorylate 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 13.21). 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 activation 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.

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

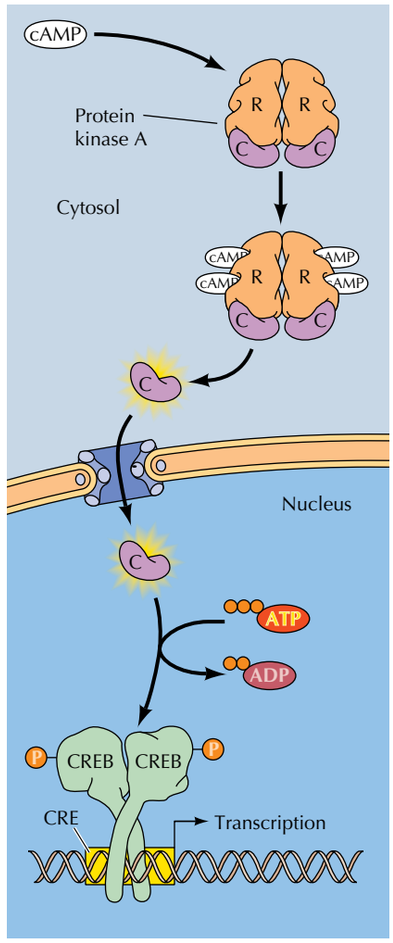


Figure 13.21 Cyclic AMP-inducible gene expression
The free catalytic subunit of protein kinase A translocates to the nucleus and phosphorylates the transcription factor CREB (CRE-binding protein), leading to expression of cAMP-inducible genes.

kinase A are usually dephosphorylated by the action of a phosphatase called protein phosphatase 1 (Figure 13.22). 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 a cGMP-dependent protein kinase, although cGMP can also act to regulate other targets, including ion channels.

The best-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 13.23). 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 then activates the G protein **transducin**, and the α subunit of transducin stimulates the activity of **cGMP phosphodiesterase**, leading to a decrease

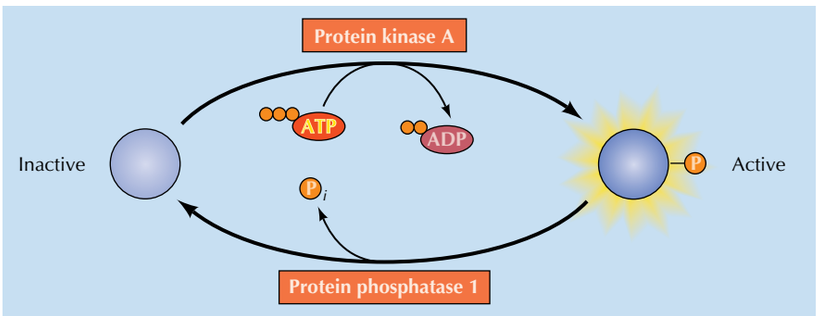


Figure 13.22 Regulation of protein phosphorylation by protein kinase A and protein phosphatase 1
The phosphorylation of target proteins by protein kinase A is reversed by the action of protein phosphatase 1.

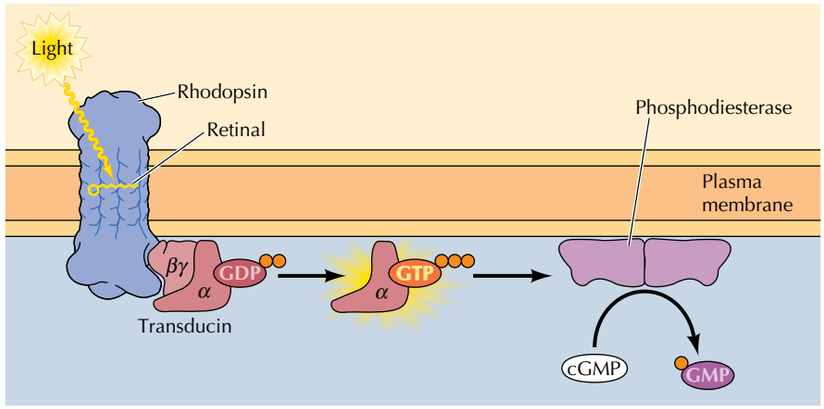


Figure 13.23 Role of cGMP in photoreception
Absorption of light by retinal activates the G protein-coupled receptor rhodopsin. The α subunit of transducin then stimulates cGMP phosphodiesterase, leading to a decrease in intracellular levels of cGMP.

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 12.2). 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 13.24). Diacylglycerol and IP_3 stimulate distinct downstream signaling pathways (protein kinase C and Ca^{2+} mobilization, respectively), so PIP_2 hydrolysis triggers a two-armed cascade of intracellular signaling.

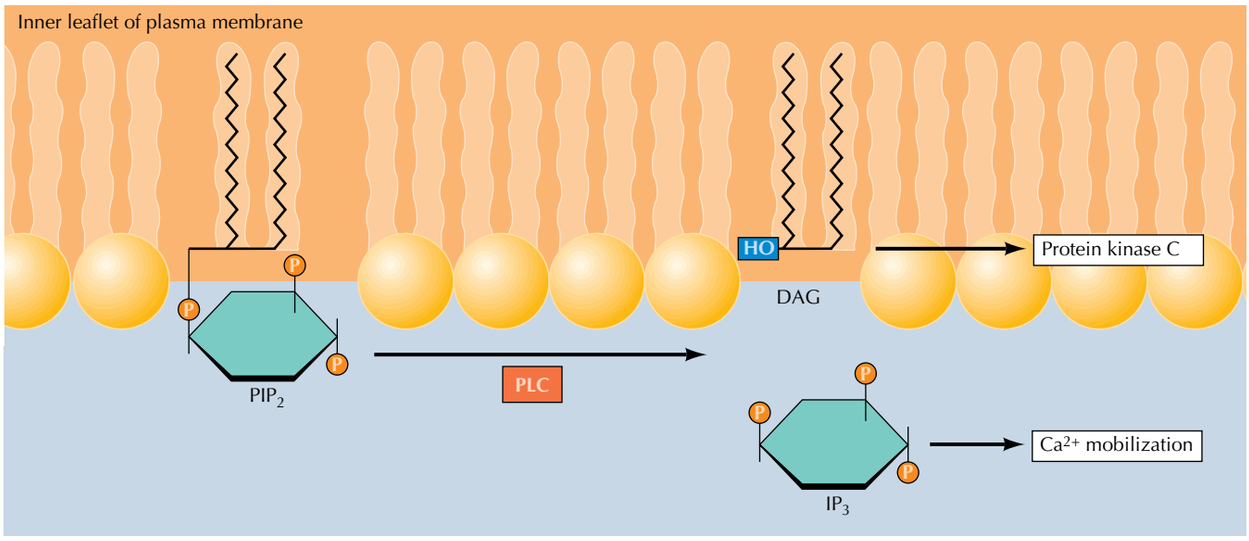


Figure 13.24 Hydrolysis of PIP_2
Phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3). Diacylglycerol activates members of the protein kinase C family, and IP_3 signals the release of Ca^{2+} from intracellular stores.

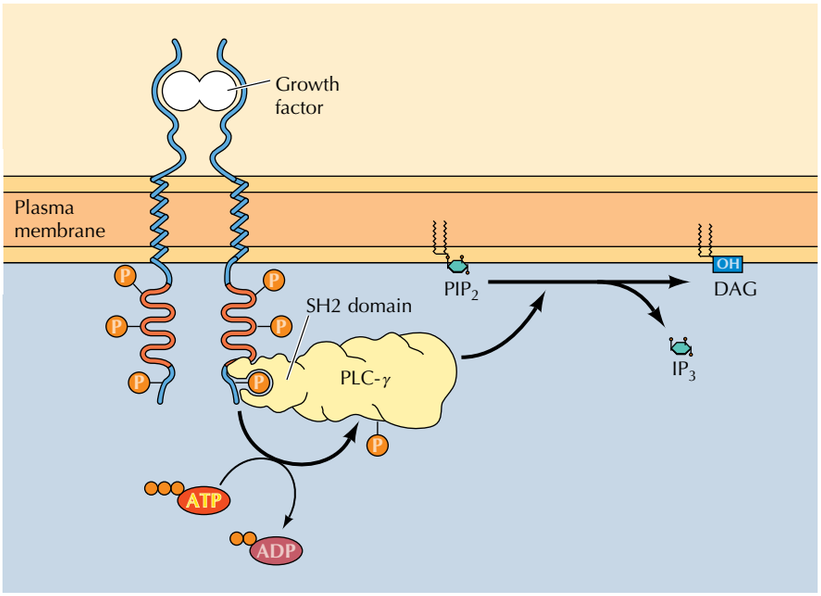


Figure 13.25 Activation of phospholipase C by protein-tyrosine kinases
Phospholipase C-γ (PLC-γ) binds to activated receptor protein-tyrosine kinases via its SH2 domains. Tyrosine phosphorylation increases PLC-γ activity, stimulating the hydrolysis of PIP_2.

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 (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases (Figure 13.25). 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 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. A good illustration of this role of protein kinase C is provided by the action of **phorbol esters** (Figure 13.26), which have been studied extensively because they promote the growth of tumors in animals. This tumor-promoting activity of the phorbol esters is based on their ability to stimulate protein kinase C by acting as analogs of diacylglycerol. Protein kinase C then activates other intracellular targets, including transcription factors, leading to changes in gene expression and stimulation of cell proliferation.

Whereas diacylglycerol remains associated with the plasma membrane, 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 13.27). As noted in Chapter 12, 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

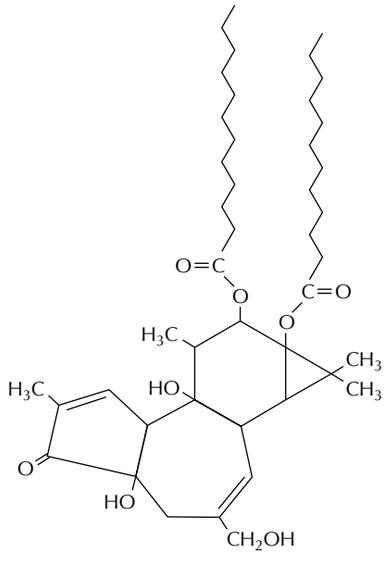


Figure 13.26 Structure of a phorbol ester
Phorbol esters stimulate protein kinase C by acting as analogs of diacylglycerol.

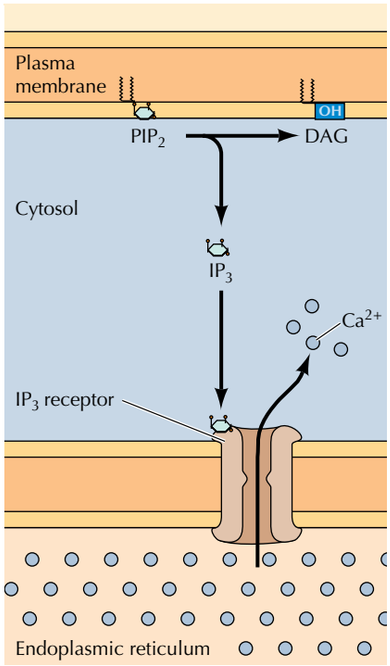


Figure 13.27 Ca^{2+} mobilization by IP_3
Ca^{2+} is pumped from the cytosol into the endoplasmic reticulum, which serves as an intracellular Ca^{2+} store. IP_3 binds to receptors that are ligand-gated Ca^{2+} channels in the endoplasmic reticulum membrane, thereby allowing the efflux of Ca^{2+} to the cytosol.

proteins, including 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 13.28). 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 11.29). 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 13.29). 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 critical 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

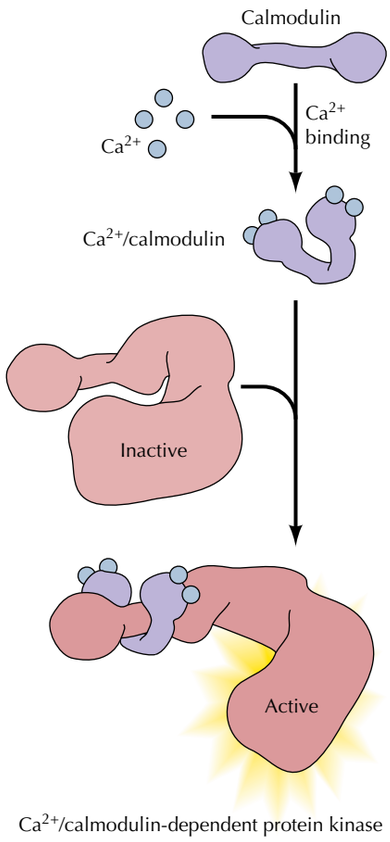


Figure 13.28 Function of calmodulin
Calmodulin is a dumbbell-shaped protein with four Ca^{2+}-binding sites. The active Ca^{2+}/calmodulin complex binds to a variety of target proteins, including Ca^{2+}/calmodulin-dependent protein kinases.

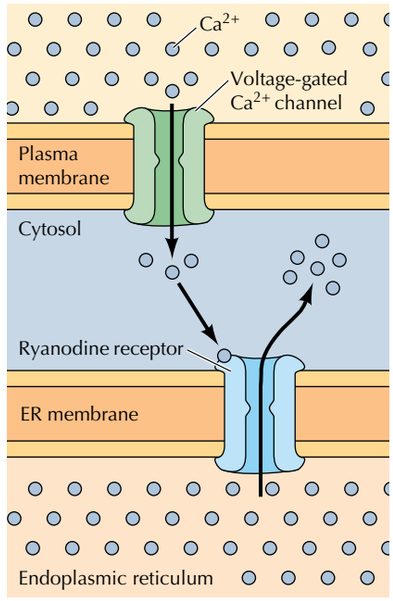


Figure 13.29 Regulation of intracellular Ca^{2+} in electrically excitable cells
Membrane depolarization leads to the opening of voltage-gated Ca^{2+} channels in the plasma membrane, causing the influx of Ca^{2+} from extracellular fluids. The resulting increase in intracellular Ca^{2+} then signals 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.

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 11). 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.

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 survival. In this pathway, PIP_2 is phosphorylated on the 3 position of inositol by the enzyme **phosphatidylinositide (PI) 3-kinase** (Figure 13.30). Like phospholipase C, one form of PI 3-kinase is activated by G proteins, while a second has SH2 domains and is activated by association with receptor protein-tyrosine kinases. Phosphorylation of PIP_2 yields **phosphatidylinositol 3,4,5-trisphosphate (PIP_3)**, which functions as a distinct second messenger. A key target of PIP_3, which is critical for signaling cell survival, is a protein-serine/threonine kinase called **Akt**. PIP_3 binds to a domain of Akt known as the pleckstrin homology domain (Figure 13.31). 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. Once activated, Akt phosphorylates a number of target proteins, including proteins that are direct regulators of cell survival, transcription factors, and other protein kinases.

Ras, Raf, and the MAP Kinase Pathway

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

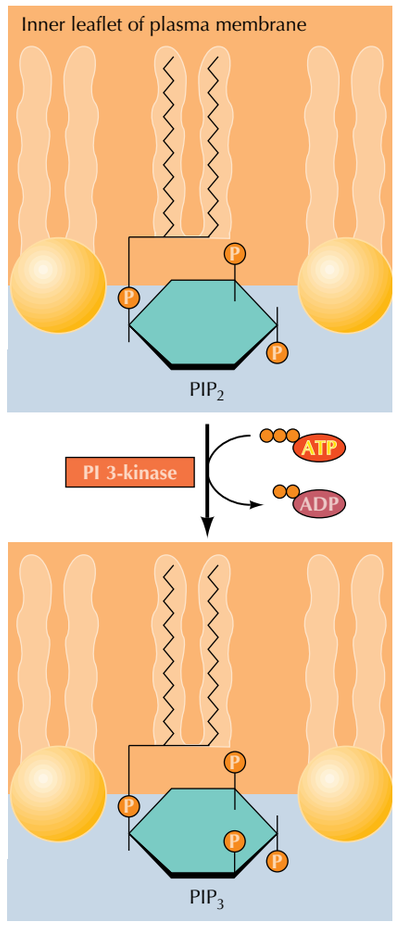


Figure 13.30 Activity of PI 3-kinase
PI 3-kinase phosphorylates the 3 position of inositol, converting PIP_2 to PIP_3.

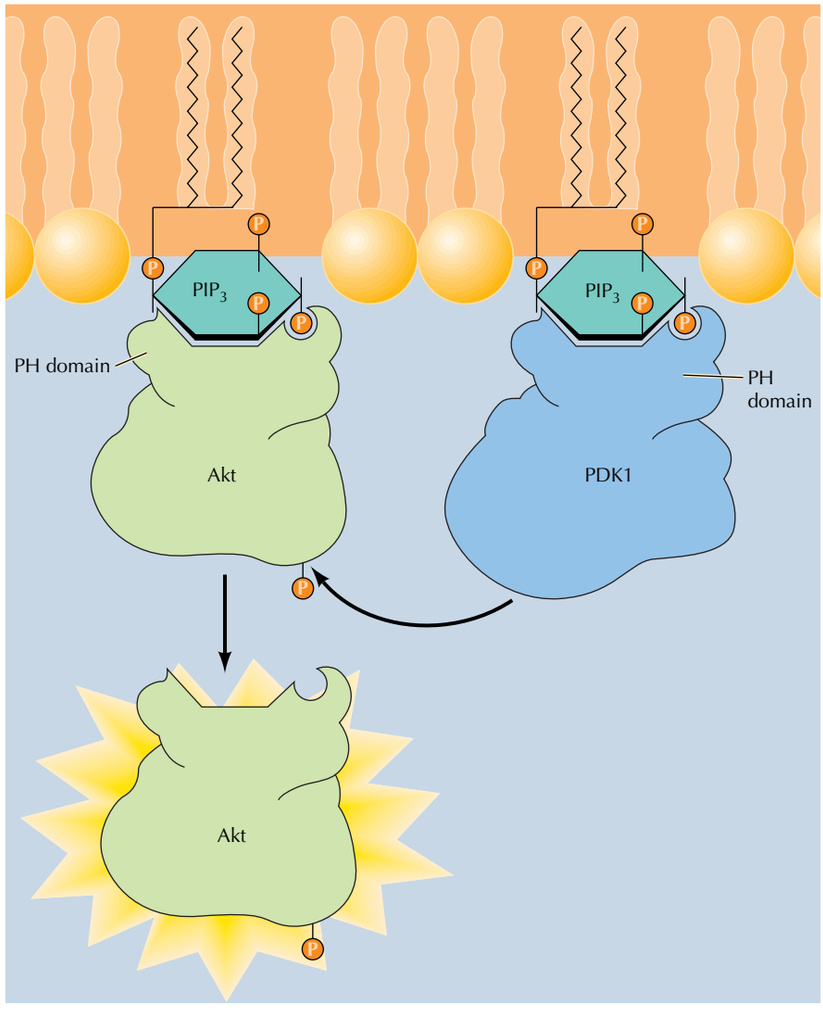


Figure 13.31 Activation of the Akt protein kinase
Akt is recruited to the plasma membrane by binding to PIP_3 via its pleckstrin homology (PH) domain. It is then activated as a result of phosphorylation by another protein kinase (PDK1) that also binds PIP_3.

receptors. Protein kinase C can also activate the ERK pathway, which contributes to the stimulation of cell proliferation induced by phorbol ester tumor promoters. In addition, 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 a GTP-binding protein called **Ras** (Figure 13.32). 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

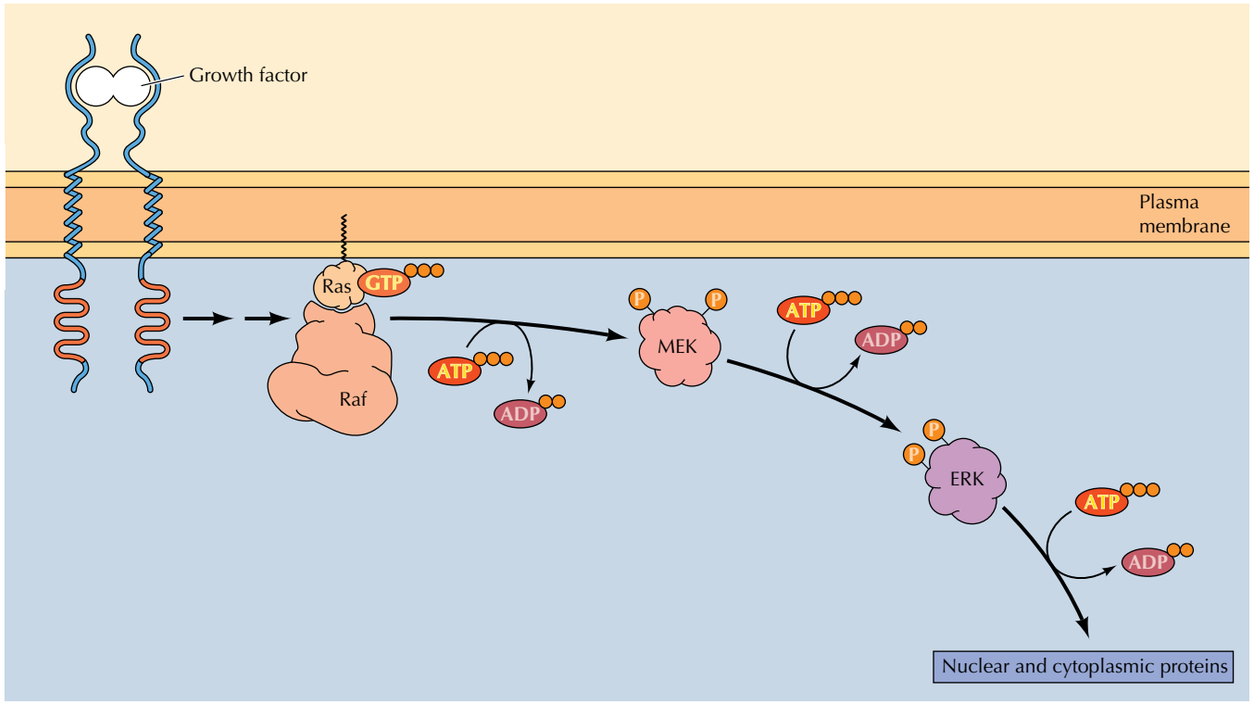


Figure 13.32 Activation of the ERK MAP kinases
Stimulation of growth factor receptors leads to activation of the small GTP-binding protein Ras, which interacts with the Raf protein kinase. Raf phosphorylates and activates MEK, a dual-specificity protein kinase that activates ERK by phosphorylation on both threonine and tyrosine residues (Thr-183 and Tyr-185). ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins.

*ra*t sarcoma virus). Interest in Ras intensified considerably in 1982, when mutations in *ras* genes were first implicated in the development of human cancers (discussed in Chapter 15). 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 13.33). 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. While the Ras proteins regulate cell growth and differentiation, other subfamilies of small GTP-binding proteins control distinct cellular activities. For example, the

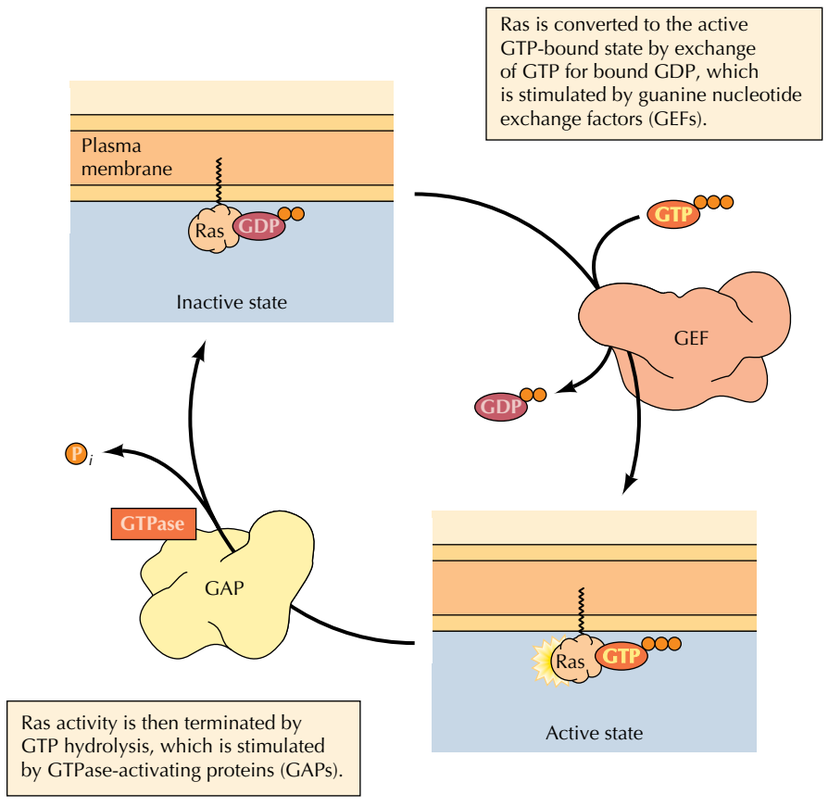


Figure 13.33 Regulation of Ras proteins
Ras proteins alternate between inactive GDP-bound and active GTP-bound states.

largest subfamily of small GTP-binding proteins (the Rab proteins) function to regulate vesicle trafficking, as discussed in Chapter 9. Other small GTP-binding proteins are involved in the import and export of proteins from the nucleus (the Ran protein, discussed in Chapter 8) 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 13.34). 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 12.9). 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, where it is activated as a result of phosphorylation by both protein-tyrosine and protein-serine/threonine kinases.

As already noted, activation of Raf initiates a protein kinase cascade leading to ERK activation. ERK then phosphorylates a variety of target pro-

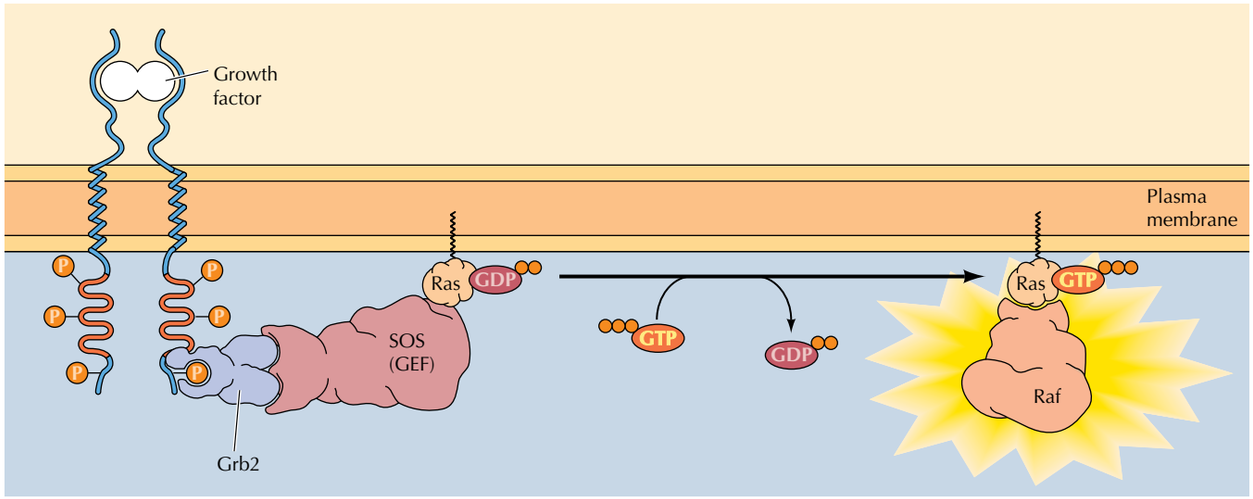


Figure 13.34 Ras activation downstream of receptor protein-tyrosine kinases
A complex of Grb2 and the guanine nucleotide exchange factor Sos binds to a phosphotyrosine-containing sequence in the activated receptor via the Grb2 SH2 domain. This interaction recruits Sos to the plasma membrane, where it can stimulate Ras GDP/GTP exchange. The activated Ras-GTP complex then binds to the Raf protein kinase.

teins, including other protein kinases. Importantly, a fraction of activated ERK translocates to the nucleus, where it regulates transcription factors by phosphorylation (Figure 13.35). 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, thereby establishing new programs of gene expression.

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 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 13.36). 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 expres-

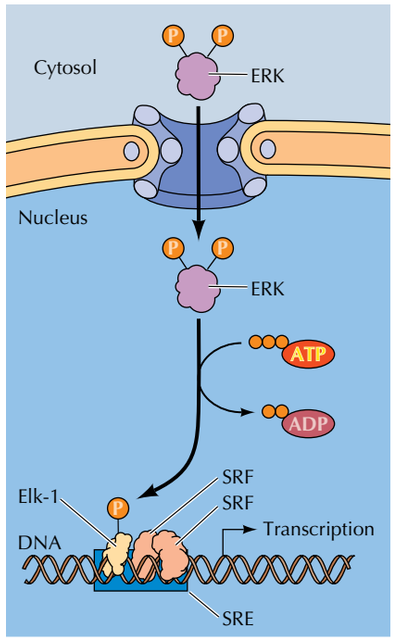


Figure 13.35 Induction of immediate-early genes by ERK
Activated ERK translocates to the nucleus, where it phosphorylates the transcription factor Elk-1. Elk-1 binds to the serum response element (SRE) in a complex with serum response factor (SRF). Phosphorylation stimulates the activity of Elk-1 as a transcriptional activator, leading to immediate-early gene induction.

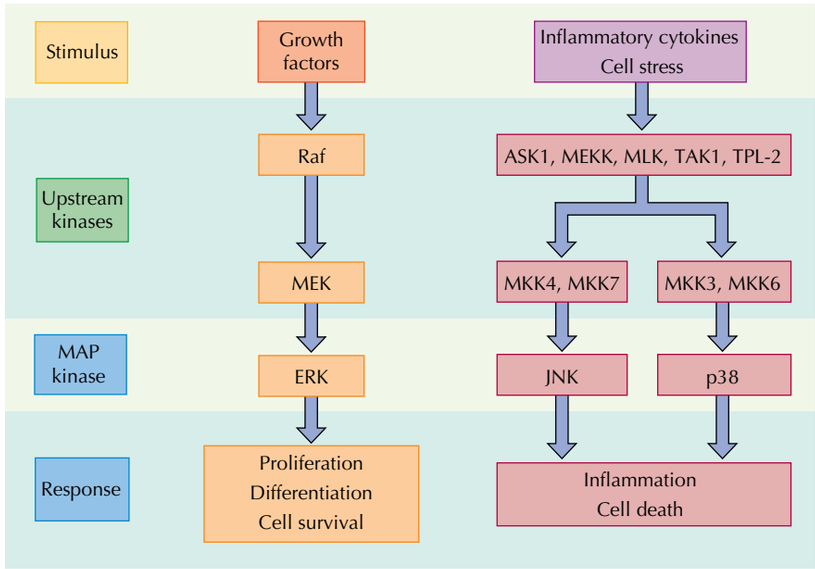


Figure 13.36 Pathways of MAP kinase activation in mammalian cells
In addition to ERK, mammalian cells contain JNK and p38 MAP kinases. Activation of JNK and p38 is mediated by protein kinase cascades parallel to that responsible for ERK activation. The protein kinase cascades leading to JNK and p38 activation appear to be preferentially activated by inflammatory cytokines or cellular stress and generally lead to inflammation and cell death.

sion. 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 13.37). As a result of the specific association of these protein kinases on the JIP-1 scaffold, activation of MLK by an upstream stimulus 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 casettes, 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 interaction with scaffold proteins is thought to play an important role in determining the specificity of signaling pathways within the cell.

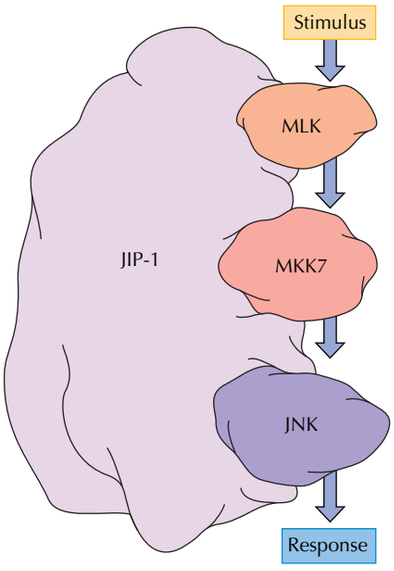


Figure 13.37 A scaffold protein for the JNK MAP kinase cascade
The JIP-1 scaffold protein binds MLK, MKK7, and JNK, organizing these components of the JNK pathway into a signaling cassette.

The JAK/STAT Pathway

The MAP kinase pathway provides an indirect connection between the cell surface and the nucleus, in which a cascade of protein kinases ultimately leads to transcription factor phosphorylation. An alternative pathway, known as the **JAK/STAT pathway**, provides a much more immediate connection between protein-tyrosine kinases and transcription factors. In this pathway protein-tyrosine phosphorylation directly affects transcription factor localization and function (Figure 13.38).

The key elements in this pathway are the **STAT proteins** (signal *t*ransducers and *a*ctivators of *t*ranscription), which were originally identified in studies of cytokine receptor signaling. The STAT proteins are a family of 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.

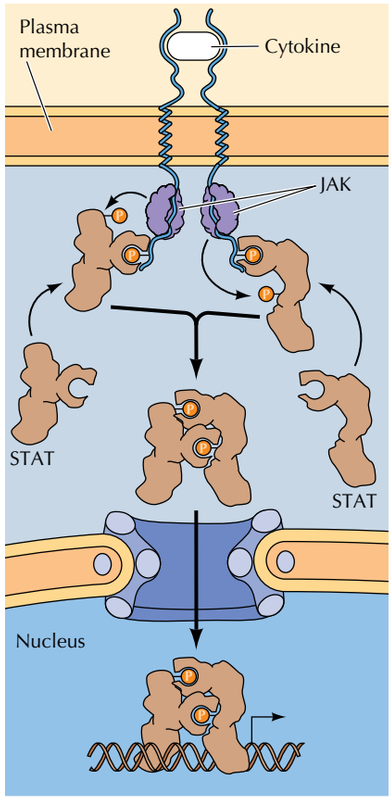


Figure 13.38 The JAK/STAT pathway
The STAT proteins are transcription factors that contain SH2 domains that mediate their binding to phosphotyrosine-containing sequences. In unstimulated cells, STAT proteins are inactive in the cytosol. Stimulation of cytokine receptors leads to the binding of STAT proteins, 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.

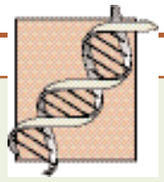
Signal Transduction and the Cytoskeleton

The preceding sections focused on signaling pathways that regulate changes in metabolism or gene expression 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 11 and 12, 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 12.62). In addition to this structural role, the integrins serve as receptors that activate intracellular signaling pathways, thereby controlling gene expression 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 13.39). 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. 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



MOLECULAR MEDICINE

Cancer: Signal Transduction and the ras *Oncogenes*

The Disease

Cancer claims the lives of approximately one out of every four Americans, accounting for more than 500,000 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, which together account for about 40% of all cancer deaths. Other major contributors to cancer mortality include cancers of the breast, prostate, and pancreas, which are responsible for approximately 7.2%, 5.2%, and 5.4% 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 currently 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.

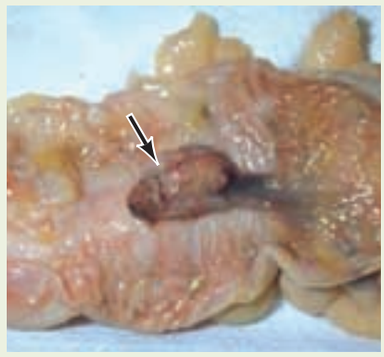
Molecular and Cellular Basis

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. Shortly thereafter, 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 raises 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, the Ras proteins have attracted considerable interest as potential drug targets.



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.)

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-Sos complex. Recruitment of the Sos guanine nucleotide exchange factor leads to activation of Ras,

An important aspect of Ras function is the targeting of Ras proteins to the plasma membrane by the post-translational addition of lipid (a farnesyl isoprenoid) to their C terminus (see Figure 7.32). Although farnesylation is not unique to Ras, it is a relatively uncommon modification of cellular proteins, leading several research groups to develop inhibitors of the enzyme farnesyl transferase as potential Ras-targeted drugs. Such drugs have now been found not only to inhibit Ras membrane localization and function, but also to display substantial activity against human tumor cells. Although some of the activity of these compounds may be directed against targets other than Ras, several farnesyl transferase inhibitors are being evaluated in clinical trials, so direct data on the potential of these drugs in the treatment of human cancers should be forthcoming in the near future.

Reference

Prendergast, G. C. and A. Oliff. 2000. Farnesyltransferase inhibitors: antineoplastic properties, mechanisms of action, and clinical prospects. *Semin. Cancer Biol*. 10: 443–452.

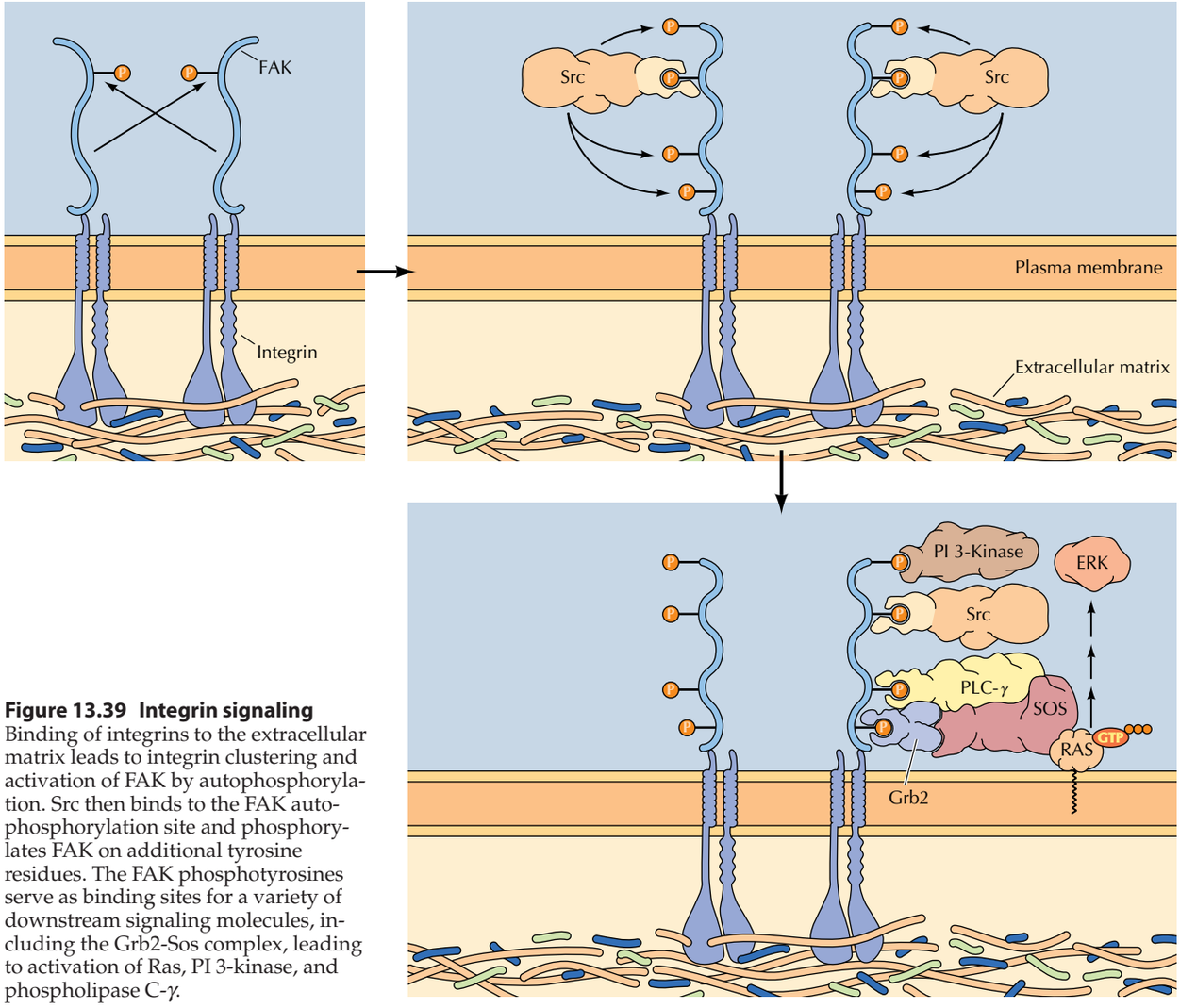


Figure 13.39 Integrin signaling
Binding of integrins to the extracellular matrix leads to integrin clustering and activation of FAK by autophosphorylation. Src then binds to the FAK autophosphorylation site and phosphorylates FAK on additional tyrosine residues. The FAK phosphotyrosines serve as binding 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.

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

Cellular responses to extracellular signals, including growth factors, frequently include changes in cell movement and cell shape. For example, growth factor-induced alterations in cell motility (as well as in cell proliferation) play critical roles in processes such as wound healing and embryonic development. As discussed in Chapter 11, these aspects of cell behavior are governed principally by the actin cytoskeleton. In particular, many types of cell movement are based on the dynamic assembly and disassembly of actin filaments underlying the plasma membrane. Remodeling of the actin cytoskeleton therefore represents a key element of the response of many cells to growth factors and other extracellular stimuli.

Members of the Rho subfamily of small GTP-binding proteins (including **Rho**, **Rac**, and **Cdc42**) play central roles in regulating the organization of the actin cytoskeleton and thus control a variety of cell processes, including cell motility, cell adhesion, and cytokinesis. The role of Rho family members in regulating different aspects of actin remodeling was first elucidated by studies of the response of fibroblasts to growth factor stimulation (Figure 13.40). The cytoskeletal alterations resulting from growth factor stimulation include the production of cell surface protrusions (filopodia, lamellipodia, and membrane ruffles) as well as the formation of focal adhesions and stress fibers. Microinjection of cells with specific mutants of different Rho family members has shown that Cdc42 induces the formation of filopodia, Rac mediates the formation of lamellipodia, and Rho is responsible for the formation of stress fibers.

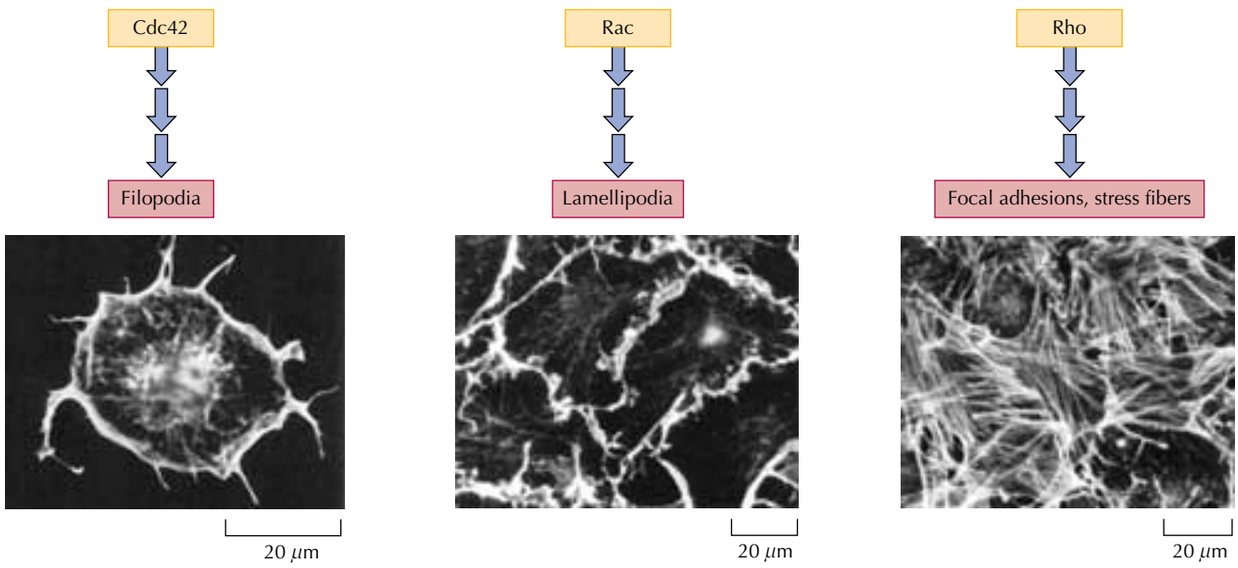


Figure 13.40 Regulation of actin remodeling by Rho family proteins
Different members of the Rho family regulate the polymerization of actin to produce filopodia (Cdc42), lamellipodia (Rac), and stress fibers (Rho). Fluorescence micrographs illustrate the distribution of actin following microinjection of fibroblasts with Cdc42, Rac, and Rho. (From C. D. Nobes and A. Hall, 1995. *Cell* 81: 53.)

Further studies have demonstrated that the activities of Rho family members are not restricted to fibroblasts: they play similar roles in regulating the actin cytoskeleton in all types of eukaryotic cells. For example, Rho is required for cytokinesis (cell division following mitosis), which is mediated by an actin-myosin contractile ring (see Figure 11.28). In neurons, Rac, Cdc42 and Rho regulate the extension and retraction of axons during development of the nervous system. In smooth muscle cells, Rho contributes to regulation of contraction. In epithelial cells, Rho family members regulate the formation of adherens junctions, which involve the linkage of cadherins to the actin cytoskeleton (see Figure 12.64). Members of the Rho family thus serve as universal regulators of the actin cytoskeleton, linking extracellular signals to changes in cell shape and movement. In addition, Rho family members can activate MAP kinase signaling pathways, leading to changes in gene expression, as well as affecting other cellular activities such as vesicular transport and cell polarity.

A large number of proteins have been identified as potential targets of Rho, Rac, and Cdc42, and the cellular roles of many of these candidate target proteins remain to be elucidated. One of the key targets of Rho in regulating cytoskeletal alterations is a protein-serine/threonine kinase called PKN (Figure 13.41). Activation of PKN increases the phosphorylation of the light chain of myosin II by two mechanisms: PKN not only directly phosphorylates the myosin light chain but also phosphorylates and inhibits myosin light chain phosphatase. The resulting increase in myosin light chain phosphorylation activates myosin and leads to the assembly of actin-myosin filaments, resulting in cytoskeletal alterations such as the formation of stress fibers and focal adhesions, adherens junctions, and cytokinesis. Both Rac and Cdc42 lead to the formation of cell surface protrusions (filopodia and lamellipodia) by stimulating actin polymerization. This appears to be mediated by several targets of Rac and Cdc42 that can associate with the Arp2/3 complex (see Figure 11.5) to induce the formation of actin filaments, but the details of these interactions are not yet understood.

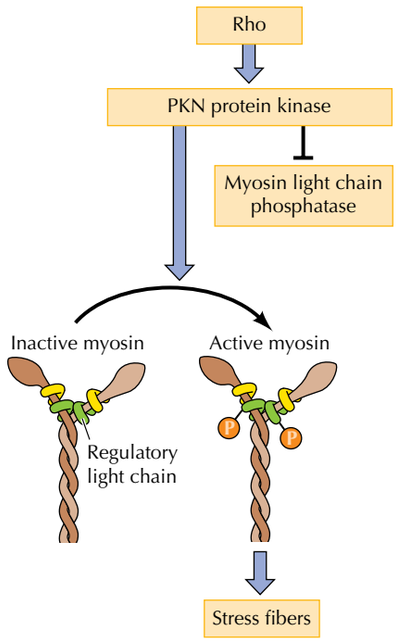


Figure 13.41 Regulation of myosin light chain phosphorylation by Rho
Rho activates the protein kinase PKN, which phosphorylates the regulatory light chain of myosin II and inhibits myosin light chain phosphatase. The resulting increase in phosphorylation of the light chain activates myosin II, leading to assembly of actin-myosin filaments and the formation of stress fibers.

Signaling in Development and Differentiation

Understanding the molecular mechanisms that govern animal development is one of the major challenges of contemporary cell and molecular biology. Starting from only a single cell, the fertilized egg, all the diverse cell types of the body are produced and organized into tissues and organs. Both cell differentiation and the development of body structures must be regulated by intricate pathways of cell-cell signaling that coordinate the activities of individual cells and ultimately give rise to organisms as complex as human beings. Although a comprehensive discussion of developmental biology is beyond the scope of this book, it is noteworthy that considerable progress has been made in elucidating the signaling pathways responsible for many aspects of development and differentiation. Three examples of such signaling pathways are discussed here.

The Receptor Tyrosine Kinase/Ras/Raf/ERK Pathway in Drosophila *and* C. elegans

Signaling by receptor tyrosine kinases that activate the Ras/Raf/ERK pathway regulates development and differentiation of many types of cells. A well-studied example in vertebrates is provided by the differentiation of neurons, which is mediated by activation of the nerve growth factor receptor (a receptor tyrosine kinase) and subsequent stimulation of the

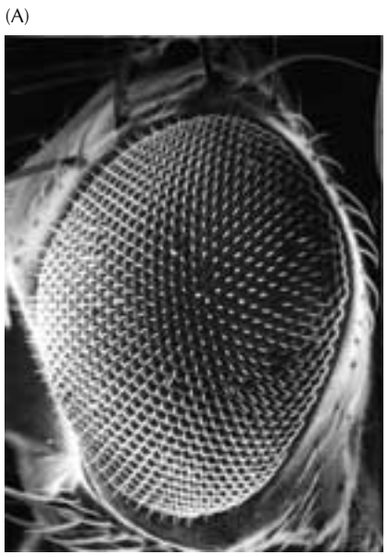


Figure 13.42 The *Drosophila* compound eye
(A) Scanning electron micrograph showing the compound eye, composed of about 800 individual units. (B) Each unit contains eight photoreceptor neurons (designated R1 through R8) that develop in a fixed order and pattern. (A, courtesy of T. Venkatesh, City College of New York.)

Ras/Raf/ERK pathway. However, the key role of this pathway in development has been demonstrated most clearly by genetic analysis of the model organisms *Drosophila* and *C. elegans*.

Signaling by the Ras/Raf/ERK pathway plays a key role in development of the compound eye of *Drosophila*, which also illustrates the role of direct cell-cell signaling in differentiation. The *Drosophila* compound eye consists of about 800 individual units, each of which contains eight photoreceptor neurons (R1 through R8) and 12 lens cells (Figure 13.42). The photoreceptor neurons develop in a fixed order, beginning with the differentiation of R8. R8 then induces two neighboring cells to become the R2 and R5 photoreceptors. Next, R2 induces neighboring cells to become R1 and R3, and R5 induces neighboring cells to become R4 and R6. The final step is differentiation of R7, which is induced by interaction with R8. Lens cells then develop from those cells that do not differentiate into photoreceptors.

The signaling pathway leading to development of the R7 cell has been characterized in detail, based on the isolation of mutant flies in which R7 fails to develop (Figure 13.43). One of these mutants (*sevenless*) results from defects in a gene encoding a receptor protein-tyrosine kinase that is expressed by precursors of R7 cells. Another mutant (called *boss*, which is short for *bride-of-sevenless*) results from defects in a gene encoding a cell surface protein expressed by R8 cells. Boss is the ligand for Sevenless, so direct cell-cell interaction between R8 and a precursor cell activates the Sevenless protein-tyrosine kinase. Further studies have shown that cell differentiation induced by signaling from Sevenless also requires Ras and Raf, leading to activation of the ERK MAP kinase and resulting phosphorylation of transcription factors that mediate R7 differentiation.

Development of the vulva in the nematode *C. elegans* is another example in which the role of the Ras/Raf/ERK pathway has been elucidated by genetic analysis. In this system, a single cell first differentiates to become a gonadal anchor cell, which attaches the vulva to the uterus. The anchor cell then induces the differentiation of three precursor cells, which proliferate to form the 22 cells of the vulva.

Isolation of mutants in which the vulva fails to develop has allowed the characterization of several genes that are necessary for vulval induction, thereby delineating the pathway by which the anchor cell signals differenti-

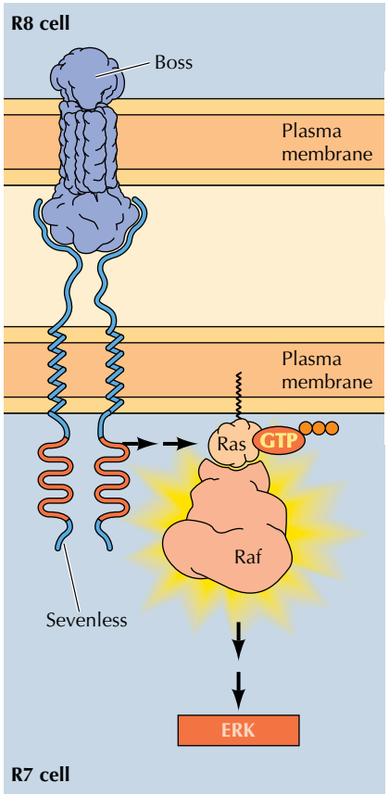


Figure 13.43 Induction of R7 differentiation
Differentiation of the R7 photoreceptor neuron is induced by contact of a precursor cell with R8. The Boss protein on the R8 cell surface is the ligand for the Sevenless receptor protein-tyrosine kinase, which is expressed by R7 precursor cells. Stimulation of Sevenless activates the Ras/Raf/ ERK pathway.

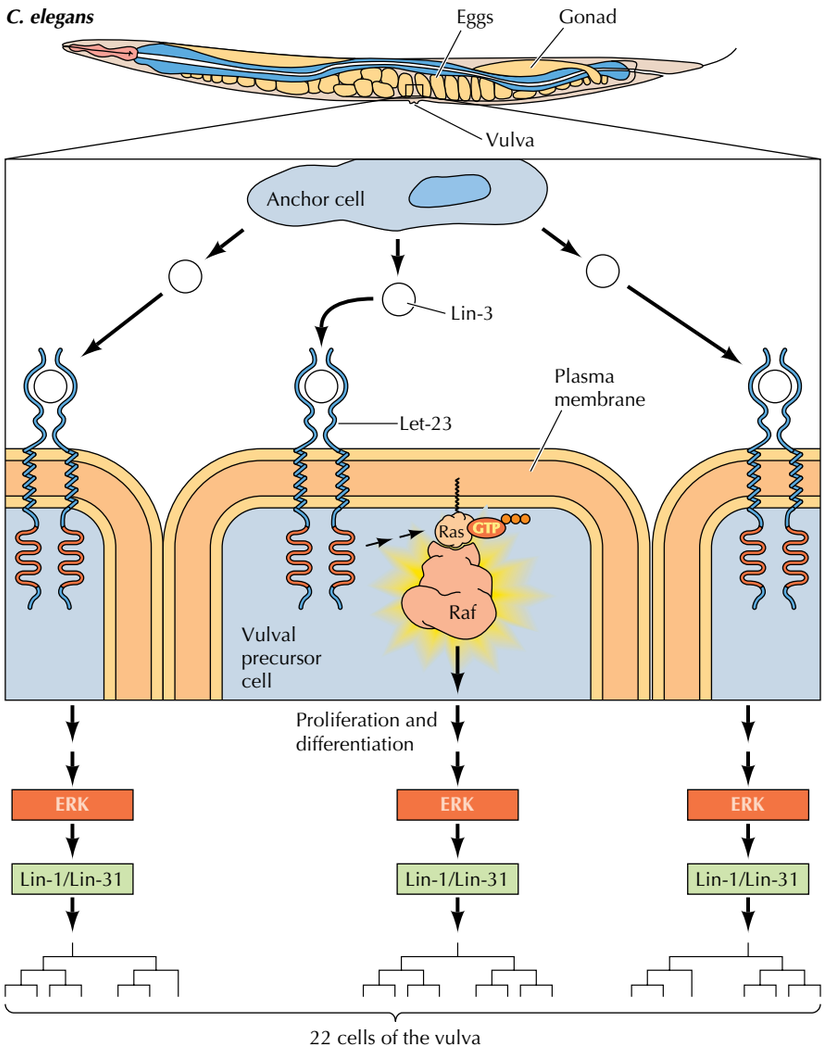


Figure 13.44 Induction of the vulva in *C. elegans*
The gonadal anchor cell secretes Lin-3, which is related to EGF. Lin-3 stimulates Let-23, a receptor protein-tyrosine kinase expressed by vulval precursor cells. Activated Let-23 stimulates the Ras/Raf/ERK pathway. ERK phosphorylates two transcription factors (Lin-1 and Lin-31), which induce three vulval precursor cells to proliferate and differentiate, forming the 22 cells of the vulva.

ation of the vulval precursor cells (Figure 13.44). One of these genes, *lin-3*, encodes a protein related to the mammalian growth factor EGF. The Lin-3 protein is secreted by anchor cells and binds to a receptor (Let-23) expressed on the surface of vulval precursor cells. Let-23 is a receptor protein-tyrosine kinase related to the mammalian EGF receptor. Other genes required for vulval induction include *let-60* and *lin-45*, which encode *C. elegans* Ras and Raf proteins, respectively. Vulval development in *C. elegans* thus involves growth factor stimulation of a receptor protein-tyrosine kinase and subsequent activation of the Ras/Raf signaling pathway. This leads to activation of the ERK MAP kinase, which phosphorylates two transcription factors (Lin-1 and Lin-31) responsible for vulval induction.

Hedgehog and Wnt

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

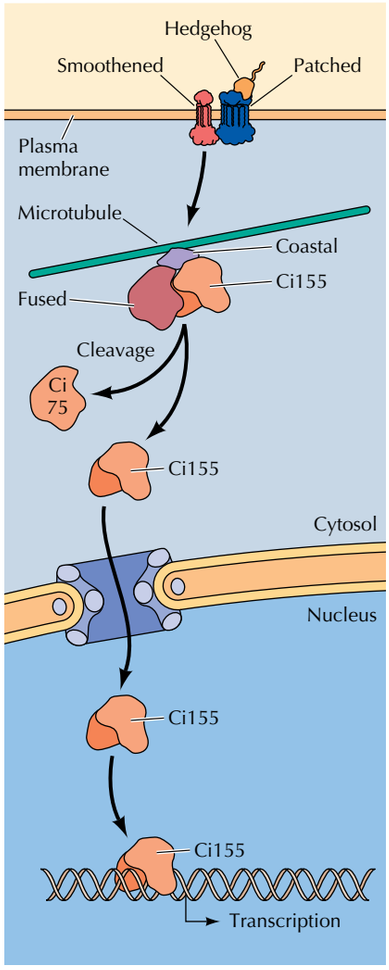


Figure 13.45 Hedgehog signaling
The Hedgehog polypeptide, modified by addition of lipid, binds to Patched on the surface of a target cell. This relieves the inhibition of Smoothened by Patched, allowing Smoothened to propagate an intracellular signal. Signaling from Smoothened disrupts a complex in which the transcription factor Cubitis interruptus (Ci) is anchored to microtubules by the kinesin-related protein Coastal, in association with the protein kinase Fused. Within this complex, Ci is cleaved to generate a transcriptional repressor (Ci75). Disruption of the complex allows full-length Ci (Ci155) to translocate to the nucleus where it activates transcription of its target genes.

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 13.45). Hedgehog binds to Patched, which acts a negative regulator of Smoothened. The binding of Hedgehog to Patched allows Smoothened to propagate an intracellular signal. Smoothened has seven transmembrane α helices, and is thus similar in structure to G protein-coupled receptors (see Figure 13.10). However, Smoothened is not known to be linked to a G protein, and the mechanism of signaling from Smoothened is not yet understood. The target for Smoothened signaling is a transcription factor called Cubitus interruptus (Ci) in *Drosophila* (Gli in mammals), which is in a complex with a protein kinase called Fused and a kinesin-related protein called Coastal. In the absence of Smoothened signaling, this complex is associated with microtubules, apparently by the interaction of Coastal with tubulin, and Ci is cleaved to generate a transcriptional repressor (Ci75). Activation of Smoothened leads to dissociation of the complex from microtubules and translocation of full-length Ci (Ci155) to the nucleus, where it activates transcription of its target genes. Interestingly, the targets of Ci include genes encoding members of the Wnt family, providing a direct link between the Hedgehog and Wnt signaling pathways.

The Wnt proteins are a family of secreted growth factors that bind to receptors of the Frizzled family (Figure 13.46). The Frizzled receptors are related to Smoothened and similarly have seven transmembrane α helices, although it is not known whether they are coupled to G proteins. Signaling from Frizzled leads to phosphorylation of a cytoplasmic protein called Dishevelled and inhibition of the protein kinase glycogen synthase kinase-3 (GSK-3). GSK-3 phosphorylates and promotes the degradation of β-catenin, which was discussed in Chapter 11 as a transmembrane protein that links cadherins to actin at adherens junctions (see Figure 11.15). 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 converts Tcf/LEF family members from transcriptional repressors to activators, leading to the expression of target genes encoding other cell signaling molecules and a variety of transcription factors that control cell fate.

Notch Signaling

The **Notch** pathway is another highly conserved signaling pathway that controls cell fate during animal development. Like the signaling pathway leading to differentiation of the R7 photoreceptor neuron in *Drosophila*, Notch signaling is an example of direct cell-cell interactions during development. It functions at all stages of development to regulate cell proliferation, survival, and differentiation in organisms ranging from *Drosophila* and *C. elegans* to humans.

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 13.47). Stimulation of Notch initiates a novel and direct pathway of transcriptional activation. In particular, ligand

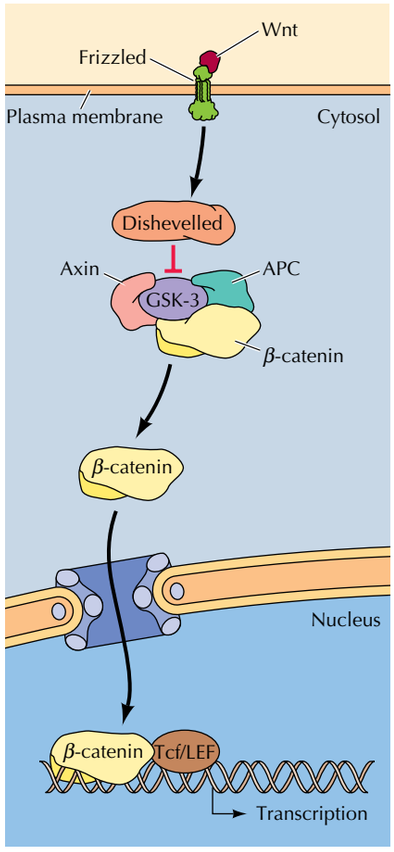


Figure 13.46 The Wnt pathway
Wnt polypeptides bind to cell surface receptors of the Frizzled family. Signaling from Frizzled leads to inhibition of the protein kinase GSK-3, resulting in stabilization of β-catenin, which forms a complex with Tcf/LEF transcription factors. This converts Tcf/LEF family members from repressors to activators, stimulating expression of target genes.

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

Regulation of Programmed Cell Death

Programmed cell death is a normal physiological form of cell death that plays a key role in both the maintenance of adult tissues and in embryonic development. In adults, programmed cell death is responsible for balancing cell proliferation and maintaining constant cell numbers in tissues undergoing cell turnover. For example, about 5×10^{11} blood cells are eliminated by programmed cell death daily in humans, balancing their continual production in the bone marrow. In addition, programmed cell death provides a defense mechanism by which damaged and potentially dangerous cells can be eliminated for the good of the organism as a whole. Virus-infected cells frequently undergo programmed cell death, thereby preventing the production of new virus particles and limiting spread of the virus through the host organism. Other types of insults, such as DNA damage, also induce programmed cell death. In the case of DNA damage, programmed cell death may eliminate cells carrying potentially harmful mutations, including cells with mutations that might lead to the development of cancer.

During development, programmed cell death plays a key role by eliminating unwanted cells from a variety of tissues. For example, programmed cell death is responsible for the elimination of larval tissues during amphibian and insect metamorphosis, as well as for the elimination of tissue between the digits during the formation of fingers and toes. Another well-characterized example of programmed cell death is provided by development of the mammalian nervous system. Neurons are produced in excess, and up to 50% of developing neurons are eliminated by programmed cell death. Those that survive are selected for having made the correct connections with their target cells, which secrete growth factors that signal cell survival by blocking the neuronal cell death program. The survival of many other types of cells in animals is similarly dependent on growth factors or contacts with neighboring cells or the extracellular matrix, so programmed cell death is thought to play an important role in regulating the associations

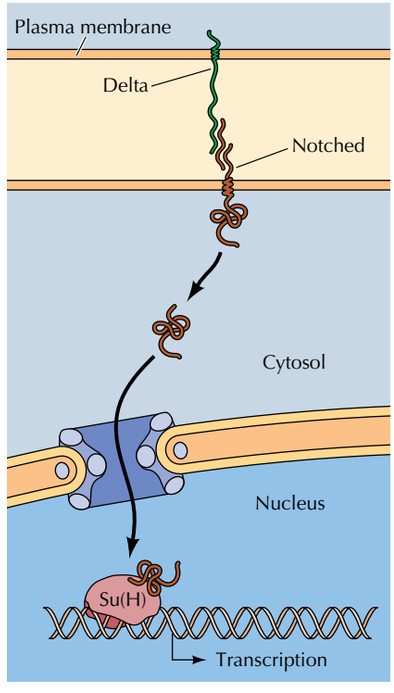
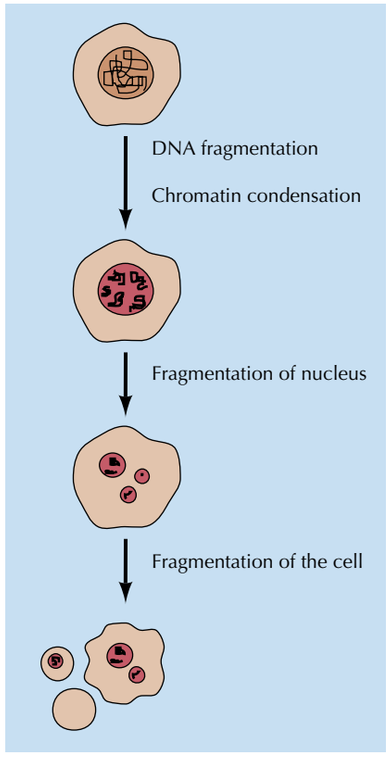
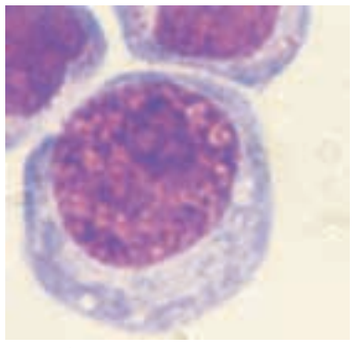


Figure 13.47 Notch signaling
Notch serves as a receptor for direct cell-cell signaling by transmembrane proteins (e.g., Delta) on neighboring cells. The binding of Delta leads to proteolytic cleavage of Notch, releasing the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor (Su(H) or CSL) to induce gene expression.

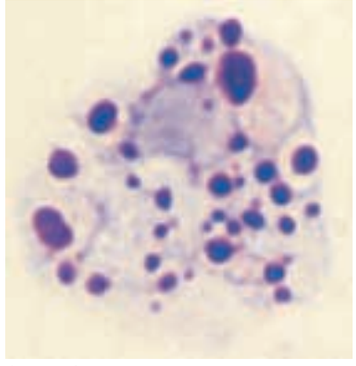
(A)



(B)



Normal



Apoptotic

between cells in tissues. Regulation of programmed cell death is mediated by the integrated activity of a variety of signaling pathways, some acting to induce cell death and others to promote cell survival.

Caspases and Apoptosis

In contrast to the accidental death of cells that results from an acute injury, programmed cell death is an active process characterized by a distinct morphological change known as **apoptosis** (Figure 13.48). During apoptosis, chromosomal DNA is usually fragmented as a result of cleavage between nucleosomes. The chromatin condenses and the nucleus then breaks up into small pieces. Finally, the cell itself shrinks and breaks up into membrane-enclosed fragments called apoptotic bodies. Such apoptotic cells and cell fragments are readily recognized and phagocytosed by both macrophages and neighboring cells, so cells that die by apoptosis are efficiently removed from tissues. In contrast, cells that die as a result of acute injury swell and lyse, releasing their contents into the extracellular space and causing inflammation.

Studies of programmed cell death during the development of *C. elegans* initially identified three genes that play key roles in regulating and executing apoptosis. During normal nematode development, 131 somatic cells out of a total of 1090 are eliminated by programmed cell death. Two genes, *ced-3* and *ced-4*, are required for apoptosis to occur; if either of these genes is inactivated, the normal programmed cell deaths do not take place. A third gene, *ced-9*, functions as a negative regulator of apoptosis. If *ced-9* is inactivated by mutation, the cells that would normally survive fail to do so. Instead, they also undergo apoptosis, leading to death of the developing animal. Conversely, if *ced-9* is expressed at an abnormally high level, the normal programmed cell deaths fail to occur.

Genes related to *ced-3*, *ced-4*, and *ced-9* have been identified in mammals and found to encode proteins that represent conserved effectors and regulators of apoptosis induced by a variety of stimuli. Ced-3 is the prototype of a family of more than a dozen proteases, known as **caspases** because they have cysteine (C) residues at their active sites and cleave after aspartic acid (Asp) residues in their substrate proteins. The caspases are the ultimate effectors or executioners of programmed cell death, bringing about the events of apoptosis by cleaving nearly 100 different cell target proteins. Key targets of the caspases include an inhibitor of a DNase which, when activated, is responsible for fragmentation of nuclear DNA. In addition, caspases cleave nuclear lamins, leading to fragmentation of the nucleus, and cytoskeletal proteins, leading to disruption of the cytoskeleton, membrane blebbing, and cell fragmentation.

The caspases are synthesized as inactive precursors that are usually converted to the active form by proteolytic cleavage, catalyzed by other caspases. The activation of an initiator caspase therefore starts off a chain reaction leading to activation of additional downstream caspases and death of the cell. Regulation of caspases is thus central to determining cell survival. Ced-4 and its mammalian homolog (Apaf-1) bind to caspases and promote

Figure 13.48 Apoptosis
(A) Diagrammatic representation of the events of apoptosis. (B) Light micrographs of normal and apoptotic human leukemia cells, illustrating chromatin condensation and nuclear fragmentation during apoptosis. (B, courtesy of D. R. Green/La Jolla Institute for Allergy and Immunology.)

their activation. In contrast, Ced-9 inhibits caspase activation. Mammals encode a whole family of proteins (called the **Bcl-2** family) that are related to Ced-9. Some members of the Bcl-2 family, including Bcl-2 itself, function analogously to Ced-9 as inhibitors of caspase activation and programmed cell death. Other members of the Bcl-2 family, however, induce caspase activation and promote cell death. Caspases are also regulated by a family of proteins called **IAPs**, for *i*nhibitor of *a*poptosis *p*roteins, which suppress apoptosis by directly inhibiting caspase activity.

In mammalian cells, members of the Bcl-2 family act at mitochondria, which play a central role in controlling programmed cell death (Figure 13.49). One of the key initiator caspases in mammalian cells (caspase-9) is activated, like Ced-3 in *C. elegans*, by forming a complex with the Ced-4 homolog Apaf-1. In mammals, formation of this complex also requires cytochrome *c*, which is released from mitochondria by stimuli that trigger apoptosis. Under normal conditions of cell survival, cytochrome *c* is localized to the mitochondrial intermembrane space (see Figure 10.8) while Apaf-1 and caspase-9 are found in the cytosol, so caspase-9 remains inactive. However, many stimuli that trigger cell death, including DNA damage and growth factor deprivation, lead to damage of the mitochondria and release of cytochrome *c* to the cytosol. In the cytosol, cytochrome *c* binds to Apaf-1 and triggers the formation of a multisubunit Apaf-1/caspase-9 complex called the **apoptosome**, in which caspase-9 is activated. Caspase-9 then cleaves and activates other downstream effector caspases, such as caspase-3, eventually resulting in cell death. Members of the Bcl-2 family act in the mitochondrial membrane to regulate mitochondrial integrity and cytochrome *c* release. Bcl-2 family members that inhibit apoptosis (such as Bcl-2 itself) pre-

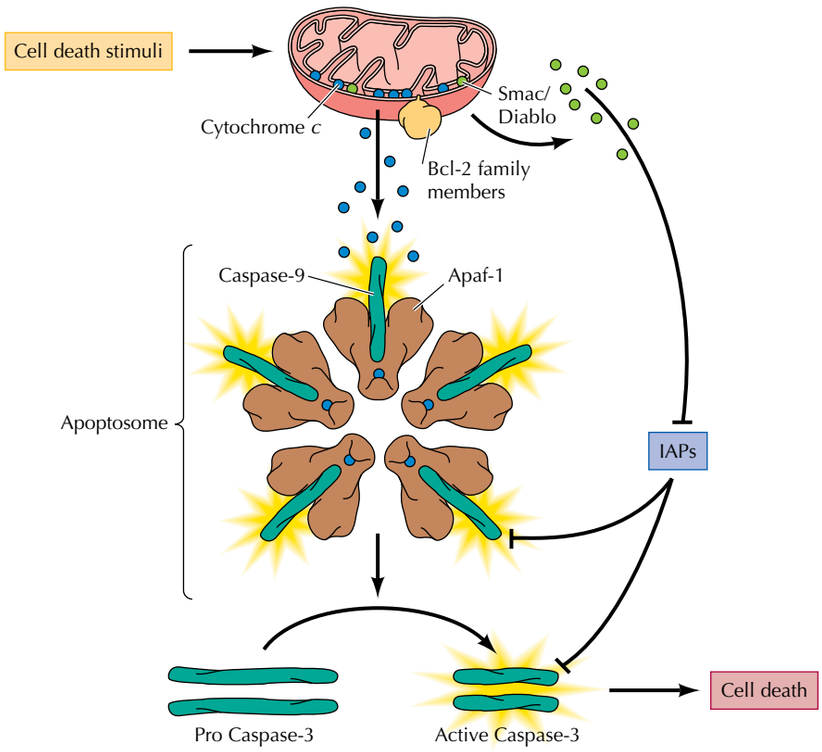


Figure 13.49 Regulators and effectors of apoptosis
In mammalian cells, many cell death signals induce apoptosis as a result of damage to mitochondria, resulting in the release of cytochrome *c* and other pro-apoptotic molecules such as Smac/Diablo from the intermembrane space. Members of the Bcl-2 family act at the mitochondrial outer membrane to regulate mitochondrial integrity. Release of cytochrome *c* from mitochondria leads to the formation of complexes (apoptosomes) containing Apaf-1 and caspase-9, in which caspase-9 is activated. Caspase-9 then activates downstream caspases, such as caspase-3, by proteolytic cleavage. Smac/Diablo promote cell death by interfering with the action of IAPs, which are inhibitors of the caspases.

vent cytochrome *c* release, whereas Bcl-2 family members that promote cell death act by inducing mitochondrial damage, cytochrome *c* release, and caspase activation. Interestingly, mitochondrial damage results not only in the release of cytochrome *c*, but also of other molecules that promote apoptosis. These include a protein (called Smac/Diablo) that stimulates caspase activity by interfering with the action of IAPs. As discussed below, caspases, members of the Bcl-2 family, and IAPs are critical targets of the signaling pathways that control survival of mammalian cells.

Cell Death Receptors and Caspase Activation

Some secreted polypeptides signal programmed cell death by activating receptors that directly induce apoptosis of the target cell. These cell death signals are polypeptides belonging to the **tumor necrosis factor (TNF)** family. They bind to members of the TNF receptor family, which can signal apoptosis in a variety of cell types. One of the best characterized members of this family is the cell surface receptor called Fas, which plays important roles in controlling cell death in the immune system. For example, apoptosis induced by activation of Fas is responsible for killing target cells of the immune system, such as cancer cells or virus-infected cells, as well as for eliminating excess lymphocytes at the end of an immune response.

The cell death receptors signal apoptosis by directly activating caspases (Figure 13.50). TNF and related family members consist of three identical polypeptide chains, and their binding induces receptor trimerization. The cytoplasmic portions of the receptors bind adaptor molecules that in turn bind an upstream caspase called caspase-8. This leads to activation of caspase-8 as a result of self-cleavage, and the activated molecules of caspase-8 can then activate other downstream caspases, thereby initiating a caspase cascade that results in death of the cell.

Caspase-8 not only cleaves other caspases, but it also cleaves a member of the Bcl-2 family called Bid. Bid is one of the Bcl-2 family members that induces rather than protects against apoptosis. Normally, it is retained in inactive form in the cytosol. However, cleavage by caspase-8 allows Bid to translocate to mitochondria where it disrupts the membrane and releases cytochrome *c* into the cytosol. This leads to activation of caspase-9, further amplifying the caspase cascade initiated by direct activation of caspase-8 at cell death receptors.

Signaling Cell Survival

Signaling by TNF and related polypeptides is an active process in which stimulation of cell death receptors induces apoptosis. Other signaling pathways act in the opposite direction to promote cell survival by inhibiting apoptosis. These signaling pathways control the fate of a wide variety of cells whose survival is dependent on extracellular growth factors or cell-cell interactions. Indeed, most cells in higher animals are programmed to undergo apoptosis unless cell death is actively suppressed by survival signals from other cells.

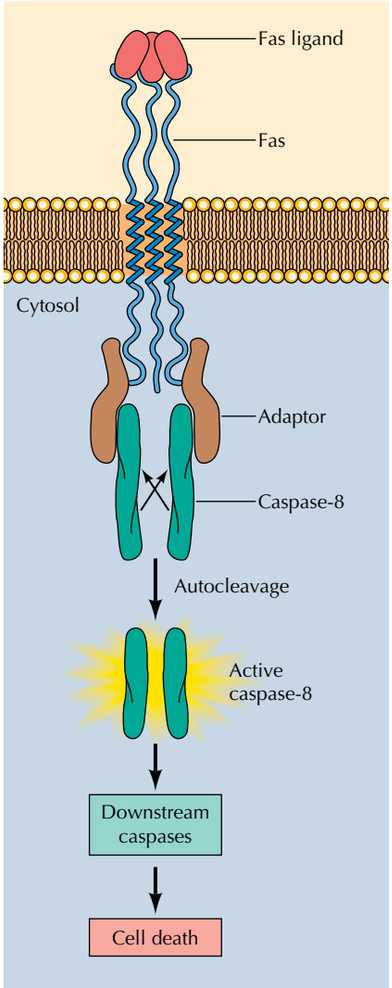
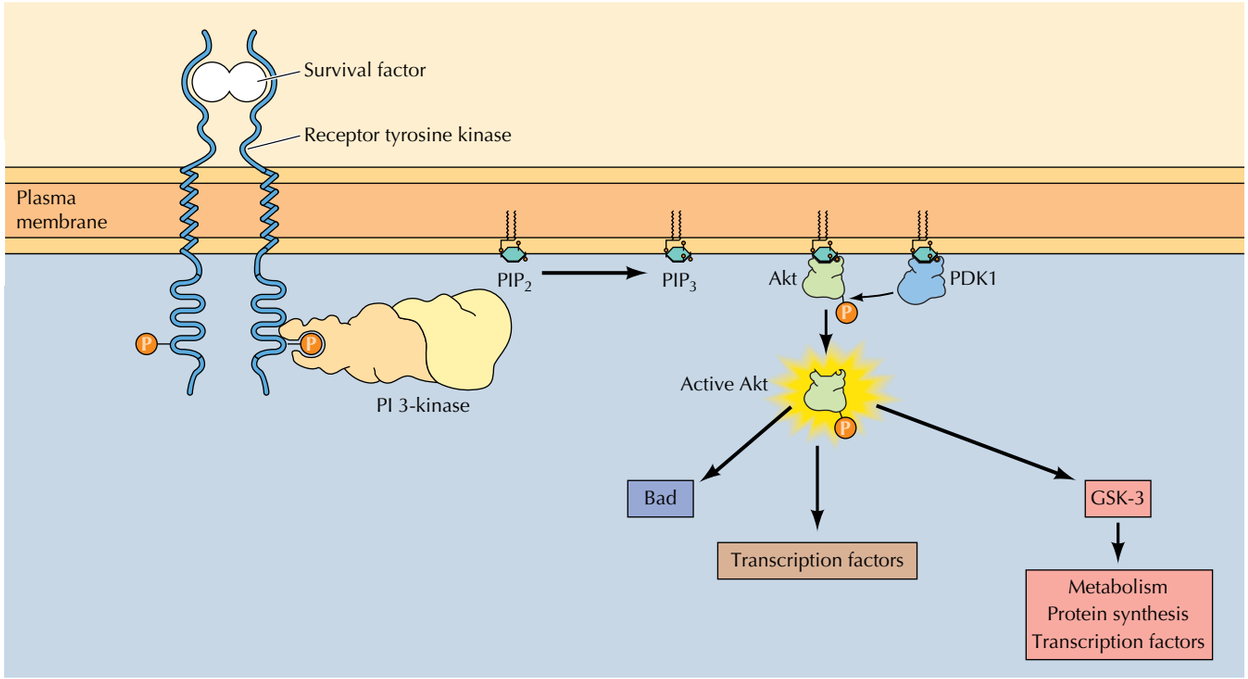


Figure 13.50 Cell death receptors
Binding of ligand to the Fas receptor induces apoptosis by direct activation of caspase-8. Fas ligand consists of three polypeptide chains, so its binding induces receptor trimerization. Caspase-8 bound to the receptor via adaptor molecules is then activated by autocleavage, leading to activation of downstream caspases and cell death.

As already noted, a well-characterized example of programmed cell death in development is provided by the vertebrate nervous system. About 50% of neurons die by apoptosis, with the survivors having received sufficient amounts of survival signals from their target cells. These survival signals are polypeptide growth factors related to nerve growth factor (NGF), which induces both neuronal survival and differentiation by activating a receptor protein-tyrosine kinase. Other types of cells are similarly dependent upon growth factors or cell contacts that activate nonreceptor protein-tyrosine kinases associated with integrins.

One of the major intracellular signaling pathways responsible for promoting cell survival is initiated by the enzyme PI 3-kinase, which is activated by either protein-tyrosine kinases or G protein-coupled receptors. PI 3-kinase phosphorylates the membrane phospholipid PIP_2 to form PIP_3, which activates the protein-serine/threonine kinase Akt (see Figure 13.31). Akt then phosphorylates a number of proteins that regulate apoptosis (Figure 13.51). One substrate for Akt is a member of the Bcl-2 family called Bad. Bad is one of the Bcl-2 family members (like Bid) that induces cell death by stimulating the release of cytochrome *c* from mitochondria. Phosphorylation of Bad by Akt creates a binding site for proteins that sequester Bad in the cytosol, thereby preventing the translocation of Bad to the mitochondrial membrane. Akt also phosphorylates a variety of transcription factors that regulate cell survival by controlling the expression of target genes that include members of the Bcl-2 family. In addition, Akt phosphorylates another protein kinase (GSK-3) that affects apoptosis, potentially by regulating both the transcription and translation of its target genes. The PI 3-kinase/Akt pathway thus regulates cell survival through a variety of downstream targets, which may include IAPs and their regulators in addition to Bcl-2 family members.

Figure 13.51 The PI 3-kinase pathway and cell survival
Survival factors such as NGF activate receptor protein-tyrosine kinases, leading to activation of PI 3-kinase and formation of PIP_3. PIP_3 recruits the protein kinase Akt to the plasma membrane where it is activated as a result of phosphorylation by PDK1. Akt then appears to phosphorylate a number of proteins that contribute to cell survival. The targets of Akt that have been implicated in suppression of apoptosis include the Bcl-2 family member Bad, several transcription factors, and the protein kinase GSK-3, which affects cell metabolism and protein synthesis as well as phosphorylating additional transcription factors.



Cell survival is mediated not only by PI 3-kinase/Akt signaling, but also by other signaling pathways including the Ras/Raf/ERK pathway. One mechanism through which this pathway inhibits apoptosis involves the phosphorylation and activation of a protein kinase called RSK by ERK. Like Akt, RSK phosphorylates the Bcl-2 family member Bad, so Bad serves as a site of convergence for the PI 3-kinase/Akt and ERK pathways in signaling cell survival. In addition, ERK and RSK phosphorylate transcription factors that affect the expression of genes that regulate apoptosis. Understanding the signals and mechanisms that control cell survival thus remains an active and exciting area of ongoing research, with many questions still to be answered.

KEY TERMS

endocrine signaling, hormone, paracrine signaling, autocrine signaling

steroid hormone, testosterone, estrogen, progesterone, corticosteroid, glucocorticoid, mineralocorticoid, ecdysone, brassinosteroid, thyroid hormone, vitamin D_3, retinoic acid, retinoid, nuclear receptor superfamily

neurotransmitter

peptide hormone, neuropeptide, enkephalin, endorphin, neurohormone, growth factor, nerve growth factor (NGF), nerotrophin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), cytokine, membrane-anchored growth factor

eicosanoid, prostaglandin, prostacyclin, thromboxane, leukotriene

plant hormone, auxin, gibberellin, cytokinin, abscisic acid, ethylene

SUMMARY

SIGNALING MOLECULES AND THEIR RECEPTORS

Modes of Cell-Cell Signaling: Most signaling molecules are secreted by one cell and bind to receptors expressed by a target cell. Cell-cell signaling is divided into three general categories (endocrine, paracrine, and autocrine signaling) based on the distance over which signals are transmitted.

Steroid Hormones and the Nuclear Receptor Superfamily: The steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid are small hydrophobic molecules that diffuse across the plasma membrane of their target cells and bind to intracellular receptors. Members of the nuclear receptor superfamily function as transcription factors to directly regulate gene expression in response to ligand binding.

Nitric Oxide and Carbon Monoxide: The simple gases nitric oxide and carbon monoxide are important paracrine signaling molecules in the nervous system and other cell types.

Neurotransmitters: Neurotransmitters are small hydrophilic molecules that carry signals between neurons or between neurons and other target cells at a synapse. Many neurotransmitters bind to ligand-gated ion channels.

Peptide Hormones and Growth Factors: The widest variety of signaling molecules in animals is peptides, ranging from only a few to more than a hundred amino acids. This group of molecules includes peptide hormones, neuropeptides, and growth factors.

Eicosanoids: The eicosanoids are a class of lipids that function in paracrine and autocrine signaling.

Plant Hormones: Small molecules known as plant hormones regulate plant growth and development.

FUNCTIONS OF CELL SURFACE RECEPTORS

G Protein-Coupled Receptors: The largest family of cell surface receptors, including the receptors for many hormones and neurotransmitters, transmit signals to intracellular targets via the intermediary action of G proteins.

G protein, G protein-coupled receptor, heterotrimeric G protein

Receptor Protein-Tyrosine Kinases: The receptors for most growth factors are protein-tyrosine kinases.

receptor protein-tyrosine kinase, autophosphorylation, SH2 domain, PTB domain

Cytokine Receptors and Nonreceptor Protein-Tyrosine Kinases: The receptors for many cytokines act in association with nonreceptor protein-tyrosine kinases.

cytokine receptor superfamily, nonreceptor protein-tyrosine kinase, Janus kinase (JAK), Src

Receptors Linked to Other Enzymatic Activities: Other kinds of cell surface receptors include protein-tyrosine phosphatases, protein-serine/threonine kinases, and guanylyl cyclases.

protein-tyrosine phosphatase, transforming growth factor β (TGF-β), protein-serine/threonine kinase, guanylyl cyclase

PATHWAYS OF INTRACELLULAR SIGNAL TRANSDUCTION

The cAMP Pathway: Second Messengers and Protein Phosphorylation: Cyclic AMP is an important second messenger in the response of animal cells to a variety of hormones and odorants. Most actions of cAMP are mediated by protein kinase A, which phosphorylates both metabolic enzymes and the transcription factor CREB.

intracellular signal transduction, cyclic AMP (cAMP), second messenger, adenylyl cyclase, cAMP phosphodiesterase, cAMP-dependent protein kinase (protein kinase A), cAMP response element (CRE), CREB

Cyclic GMP: Cyclic GMP is also an important second messenger in animal cells. Its best-characterized role is in visual reception in the vertebrate eye.

cyclic GMP (cGMP), rhodopsin, transducin, cGMP phosphodiesterase

Phospholipids and Ca^{2+}: Phospholipids and Ca^{2+} are common second messengers activated downstream of both G protein-coupled receptors and protein-tyrosine kinases. Hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) yields diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), which activate protein kinase C and mobilize Ca^{2+} from intracellular stores, respectively. Increased levels of cytosolic Ca^{2+} then activate a variety of target proteins, including Ca^{2+}/calmodulin-dependent protein kinases. In electrically excitable cells of nerve and muscle, levels of cytosolic Ca^{2+} are increased by the opening of voltage-gated Ca^{2+} channels in the plasma membrane and ryanodine receptors in the endoplasmic and sarcoplasmic reticula. In addition to being cleaved into diacylglycerol and IP_3, PIP_2 can be phosphorylated to the distinct second messenger PIP_3. This leads to activation of the protein-serine/threonine kinase Akt, which plays a key role in cell survival.

phosphatidylinositol 4,5-bisphosphate (PIP_2), phospholipase C, diacylglycerol, inositol 1,4,5-trisphosphate (IP_3), protein kinase C, phorbol ester, calmodulin, CaM kinase, ryanodine receptor, phosphatidylinositide (PI) 3-kinase, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), Akt

Ras, Raf, and the MAP Kinase Pathway: The MAP kinase pathway is a conserved chain of protein kinases activated downstream of a variety of extracellular signals. In animal cells, the best-characterized forms of MAP kinase are coupled to growth factor receptors by the small GTP-binding protein Ras, which initiates a protein kinase cascade leading to MAP kinase (ERK) activation. ERK then phosphorylates a variety of cytosolic and nuclear proteins, including transcription factors that mediate immediate-early gene induction. Other MAP kinase pathways mediate responses of mammalian cells to inflammation and stress. Components of MAP kinase pathways are organized by scaffold proteins, which play an important role in maintaining the specificity of MAP kinase signaling.

MAP kinase, ERK, Ras, Raf, MEK, guanine nucleotide exchange factor, GTPase-activating protein, small GTP-binding protein, immediate-early gene, serum response element (SRE), serum response factor (SRF), Elk-1, scaffold proteins

JAK/STAT pathway, STAT protein

The JAK/STAT Pathway: STAT proteins are transcription factors that contain SH2 domains and are activated directly by protein-tyrosine kinases associated with cytokine and growth factor receptors.

SIGNAL TRANSDUCTION AND THE CYTOSKELETON

FAK

Integrins and Signal Transduction: Binding of integrins to the extracellular matrix stimulates the FAK and Src nonreceptor protein-tyrosine kinases, leading to activation of phospholipase C, PI 3-kinase, and Ras/Raf/ERK signaling pathways.

Rho, Rac, Cdc42

Regulation of the Actin Cytoskeleton: Growth factors induce alterations in cell movement and cell shape by remodeling the actin cytoskeleton. These cytoskeletal alterations are mediated by members of the Rho subfamily of small GTP-binding proteins.

SIGNALING IN DEVELOPMENT AND DIFFERENTIATION

***The Receptor Tyrosine Kinase/Ras/Raf/ERK Pathway in* Drosophila *and* C. elegans:** The role of the Ras/Raf/ERK pathway in development has been elucidated by studies of the differentiation of photoreceptor neurons in *Drosophila* and vulval induction in *C. elegans*.

Hedgehog, Wnt

Hedgehog and Wnt: The Hedgehog and Wnt signaling pathways play key roles in determination of cell fate and patterning during development of both invertebrate and vertebrate embryos.

Notch

Notch Signaling: The Notch pathway controls cell fate by direct cell-cell interactions during animal development.

REGULATION OF PROGRAMMED CELL DEATH

programmed cell death, apoptosis, caspase, Bcl-2, IAP, apoptosome

Caspases and Apoptosis: Programmed cell death plays a key role both in the maintenance of adult tissues and embryonic development. In contrast to the accidental death of cells from an acute injury, programmed cell death takes place by the active process of apoptosis. Genes responsible for the regulation and execution of apoptosis are conserved from *C. elegans* to humans. These components of the cell death machinery include a family of proteases (caspases) that are the effectors of apoptosis as well as proteins that regulate caspase activation. In mammalian cells, release of cytochrome *c* from mitochondria plays a key role in initiating caspase activation.

tumor necrosis factor (TNF)

Cell Death Receptors and Caspase Activation: Some secreted polypeptides induce programmed cell death by activating receptors that are directly linked to caspases.

Signaling Cell Survival: Many cells are dependent on survival signals from secreted factors or cell-cell contacts that suppress apoptosis. The PI 3-kinase/Akt pathway is a major signaling pathway responsible for promoting cell survival.

Questions

1. How is paracrine signaling different from endocrine signaling?

2. Why do most cell types not use autocrine signaling to stimulate cell proliferation?

3. How does signaling by hydrophobic signal molecules like steroid hormones differ from signaling by hydrophilic signal molecules like protein hormones?

4. How does aspirin act to reduce inflammation and blood clotting?

5. The proliferation of thyroid cells is stimulated by hormones that activate a receptor coupled to G_s. How would inhibitors of cAMP phosphodiesterase affect the proliferation of these cells?

6. The epinephrine receptor is coupled to G_s, whereas the acetylcholine receptor (on heart muscle cells) is coupled to G_i. Suppose you were to construct a recombinant molecule containing the extracellular sequences of the epinephrine receptor joined to the cytosolic sequences of the acetylcholine receptor. What effect would epinephrine have on cAMP levels in cells expressing such a recombinant receptor? What would be the effect of acetylcholine?

7. Platelet-derived growth factor (PDGF) is a dimer of two polypeptide chains. What would be the predicted effect of PDGF monomers on signaling from the PDGF receptor?

8. In an attempt to block growth of tumor cells that are expressing a growth-factor-independent receptor tyrosine kinase (one whose intracellular activity is constantly on, even without external growth factor bound), you create a truncated version of the receptor that lacks the tyrosine kinase domain but contains all the other parts of the receptor. You find that not only is your truncated receptor inactive when expressed in normal cells, but when expressed in the tumor cells, it has a dominant negative effect and stops the proliferation of those cells. Explain these results.

9. Explain how increased cAMP in cells can activate genes.

10. How would overexpression of protein phosphatase 1 affect the induction of cAMP-inducible genes in response to hormone stimulation of appropriate target cells? Would protein phosphatase 1 affect the function of cAMP-gated ion channels involved in odorant reception?

11. In the multicellular organism, what advantage is gained by having cells die by apoptosis rather than by necrosis or acute injury?

12. What would be the effect on cells of injecting a complex that forms a pore in mitochondrial outer membranes that was large enough for proteins to pass across the membranes?

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