

Fig. 11.1. General features of chemical messengers.

1 Cell Signaling by Chemical Messengers

Within a complex organism such as the human, different organs, tissues, and individual cell types have developed specialized functions. Yet each cell must contribute in an integrated way as the body grows, differentiates, and adapts to changing conditions. Such integration requires communication that is carried out by chemical messengers traveling from one cell to another or by direct contact of cells with the extracellular matrix or with each other. The eventual goal of such signals is to change actions carried out in target cells by intracellular proteins (metabolic enzymes, gene regulatory proteins, ion channels, or cytoskeletal proteins). In this chapter, we present an overview of signaling by chemical messengers.

Chemical messengers. Chemical messengers (also called signaling molecules) transmit messages between cells. They are secreted from one cell in response to a specific stimulus and travel to a target cell, where they bind to a specific receptor and elicit a response (Fig. 11.1). In the nervous system, these chemical messengers are called neurotransmitters; in the endocrine system, they are hormones, and in the immune system, they are called cytokines. Additional chemical messengers include retinoids, eicosanoids, and growth factors. Depending on the distance between the secreting and target cells, chemical messengers can be classified as endocrine (travel in the blood), paracrine (travel between nearby cells), or autocrine (act on the same cell or on nearby cells of the same type).

Receptors and Signal Transduction. Receptors are proteins containing a binding site specific for a single chemical messenger and another binding site involved in transmitting the message (see Fig. 11.1). The second binding site may interact with another protein or with DNA. They may be either **plasma membrane receptors** (which span the plasma membrane and contain an extracellular binding domain for the messenger) or **intracellular binding proteins** (for messengers able to diffuse into the cell) (see Fig. 11.1). Most plasma membrane receptors fall into the categories of **ion channel receptors**, **tyrosine kinase receptors**, **tyrosine-kinase associated receptors** (**JAK-STAT receptors**), **serine-threonine kinase receptors**, or **heptahelical receptors** (proteins with seven α -helices spanning the membrane). When a chemical messenger binds to a receptor, the signal it is carrying must be converted into an intracellular response. This conversion is called **signal transduction**.

Signal Transduction for Intracellular Receptors. Most intracellular receptors are gene-specific transcription factors, proteins that bind to DNA and regulate the transcription of certain genes (Gene transcription is the process of copying the genetic code from DNA to RNA.).

Signal Transduction for Plasma Membrane Receptors. Mechanisms of signal transduction that follow the binding of signaling molecules to plasma membrane receptors include phosphorylation of receptors at tyrosine residues (receptor tyrosine kinase activity), conformational changes in signal transducer proteins (e.g., proteins with SH2 domains, the monomeric G protein Ras, heterotrimeric G proteins) or increases in the levels of intracellular second messengers. Second messengers are nonprotein molecules generated inside the cell in response to hormone binding that continue transmission of the message. Examples include 3',5'-cyclic AMP (cAMP), inositol trisphosphate (IP₃), and diacylglycerol (DAG).

Signaling often requires a rapid response and rapid termination of the message, which may be achieved by degradation of the messenger or second messenger, the automatic G protein clock, deactivation of signal transduction kinases by phosphatases, or other means.



ТНЕ WAITING ROOM

Mya Sthenia is a 37-year-old woman who complains of increasing muscle fatigue in her lower extremities with walking. If she rests for 5 to 10 minutes, her leg strength returns to normal. She also notes that if she talks on the phone, her ability to form words gradually decreases. By evening, her upper eyelids droop to the point that she has to pull her upper lids back in order to see normally. These symptoms are becoming increasingly severe. When Mya is asked to sustain an upward gaze, her upper eyelids eventually drift downward involuntarily. When she is asked to hold both arms straight out in front of her for as long as she is able, both arms begin to drift downward within minutes. Her physician suspects that Mya Sthenia has myasthenia gravis and orders a test to determine whether she has antibodies in her blood directed against the acetylcholine receptor.



Ann O'Rexia, who suffers from anorexia nervosa, has increased her weight to 102 lb from a low of 85 lb (see Chapter 9). On the advice of her physician, she has been eating more to prevent fatigue during her daily jogging regimen. She runs about 10 miles before breakfast every second day and forces herself to drink a high-energy supplement immediately afterward.



Dennis Veere was hospitalized for dehydration resulting from cholera toxin (see Chapter 10). In his intestinal mucosal cells, cholera A toxin indirectly activated the CFTR channel, resulting in secretion of chloride ion and Na⁺ ion into the intestinal lumen. Ion secretion was followed by loss of water, resulting in vomiting and watery diarrhea. Dennis is being treated for hypovolemic shock.

GENERAL FEATURES OF CHEMICAL MESSENGERS Ι.

Certain universal characteristics to chemical messenger systems are illustrated in Figure 11.1. Signaling generally follows the sequence: (1) the chemical messenger is secreted from a specific cell in response to a stimulus; (2) the messenger diffuses or is transported through blood or other extracellular fluid to the target cell; (3) a receptor in the target cell (a plasma membrane receptor or intracellular receptor) specifically binds the messenger; (4) binding of the messenger to the receptor elicits a response; (5) the signal ceases and is terminated. Chemical messengers elicit their response in the target cell without being metabolized by the cell.

Another general feature of chemical messenger systems is that the specificity of the response is dictated by the type of receptor and its location. Generally, each receptor binds only one specific chemical messenger, and each receptor initiates a characteristic signal transduction pathway that will ultimately activate or inhibit certain processes in the cell. Only certain cells, the target cells, carry receptors for that messenger and are capable of responding to its message.

The means of signal termination is an exceedingly important aspect of cell signaling, and failure to terminate a message contributes to a number of diseases, such as cancer.

Acetylcholine is released by neurons and acts on acetylcholine receptors at neuromuscular junctions to stimulate muscular contraction. Myasthenia gravis is an acquired autoimmune disease in which the patient has developed pathogenic antibodies against these receptors. Mya Sthenia's decreasing ability to form words and her other symptoms of muscle weakness are being caused by the inability of acetylcholine to stimulate repeated muscle contraction when the numbers of effective acetylcholine receptors at neuromuscular junctions are greatly reduced.

Endocrine hormones enable Ann O'Rexia to mobilize fuels from her adipose tissue during her periods of fasting and during jogging. While she fasts overnight, α -cells of her pancreas increase secretion of the polypeptide hormone glucagon. The stress of prolonged fasting and chronic exercise stimulates release of cortisol, a steroid hormone, from her adrenal cortex. The exercise of jogging also increases secretion of the hormones epinephrine and norepinephrine from the adrenal medulla. Each of these hormones is being released in response to a specific signal and causes a characteristic response in a target tissue, enabling her to exercise. However, each of these hormones binds to a different type of receptor and works in a different way.

Ann O'Rexia's fasting is accompanied by high levels of the endocrine hormone glucagon, which is secreted in response to low blood glucose levels. It enters the blood and acts on the liver to stimulate a number of pathways, including the release of glucose from glycogen stores (glycogenolysis) (see Chapter 3). The specificity of its action is determined by the location of receptors. Although liver parenchymal cells have glucagon receptors, skeletal muscle and many other tissues do not. Therefore, glucagon cannot stimulate glycogenolysis in these tissues.

Most chemical messengers (including neurotransmitters, cytokines, and endocrine hormones) are contained in vesicles that fuse with a region of the cell membrane when the cell receives a stimulus to release the messenger. Most secretory cells use a similar set of proteins to enable vesicle fusion, and fusion is usually triggered by Ca²⁺ influx, as seen with the release of acetylcholine.



Myasthenia gravis is a disease of autoimmunity caused by the production of an antibody directed against the acetylcholine receptor in skeletal muscle. In this disease, B and T lymphocytes cooperate in producing a variety of antibodies against the nicotinic acetylcholine receptor. The antibodies then bind to various locations in the receptor and cross-link the receptors, forming a multireceptor antibody complex. The complex is endocytosed and incorporated into lysosomes, where it is degraded. Mya Sthenia, therefore, has fewer functional receptors for acetylcholine to activate.

A. General Features of Chemical Messenger Systems Applied to the Nicotinic Acetylcholine Receptor

The individual steps involved in cell signaling by chemical messengers are illustrated with acetylcholine, a neurotransmitter that acts on nicotinic acetylcholine receptors on the plasma membrane of certain muscle cells. This system exhibits the classic features of chemical messenger release and specificity of response.

Neurotransmitters are secreted from neurons in response to an electrical stimulus called the action potential (a voltage difference across the plasma membrane, caused by changes in Na⁺ and K⁺ gradients, that is propagated along a nerve). The neurotransmitters diffuse across a synapse to another excitable cell, where they elicit a response (Fig. 11.2). Acetylcholine is the neurotransmitter at neuromuscular junctions, where it transmits a signal from a motor nerve to a muscle fiber that elicits contraction of the fiber. Before release, acetylcholine is sequestered in vesicles clustered near an active zone in the presynaptic membrane. This membrane also has voltage-gated Ca²⁺ channels that open when the action potential reaches them, resulting in an influx of Ca²⁺. Ca²⁺ triggers fusion of the vesicles with the plasma membrane, and acetylcholine is released into the synaptic cleft. Thus, the chemical messenger is released from a specific cell in response to a specific stimulus.

Acetylcholine diffuses across the synaptic cleft to bind to plasma membrane receptors on the muscle cells called nicotinic acetylcholine receptors (Fig. 11.3). The subunits are assembled around a channel, which has a funnel-shaped opening in the center. As acetylcholine binds to the receptor, a conformational change opens the narrow portion of the channel (the gate), allowing Na⁺ to diffuse in and K⁺ to diffuse out (A uniform property of all receptors is that signal transduction begins with conformational changes in the receptor.). The change in ion concentration



Fig. 11.3. The nicotinic acetylcholine receptor. Each receptor is composed of five subunits, and each subunit has four membranespanning helical regions. The two α subunits are identical and contain binding sites for acetylcholine. When two acetylcholine molecules are bound, the subunits change their conformation so that the channel in the center of the receptor is open, allowing K⁺ ions to diffuse out and Na⁺ ions to diffuse in.



Fig. 11.2. Acetylcholine receptors at the neuromuscular junction. A motor nerve terminates in several branches; each branch terminates in a bulb-shaped structure called the presynaptic bouton. Each bouton synapses with a region of the muscle fiber containing junctional folds. At the crest of each fold, there is a high concentration of acetylcholine receptors, which are gated ion channels.

activates a sequence of events that eventually triggers the cellular response contraction of the fiber.

Once acetylcholine secretion stops, the message is rapidly terminated by acetylcholinesterase, an enzyme located on the postsynaptic membrane that cleaves acetylcholine. It is also terminated by diffusion of acetylcholine away from the synapse. Rapid termination of message is a characteristic of systems requiring a rapid response from the target cell.

B. Endocrine, Paracrine, and Autocrine

The actions of chemical messengers are often classified as endocrine, paracrine, or autocrine (Fig. 11.4). Each endocrine hormone is secreted by a specific cell type



Fig. 11.4. Endocrine, autocrine, and paracrine actions of hormones and other chemical messengers.

Mya Sthenia was tested with an inhibitor of acetylcholinesterase, edrophonium chloride, administered intravenously (see Chapter 8, Fig. 8.18). After this drug inactivates acetylcholinesterase, acetylcholine that is released from the nerve terminal accumulates in the synaptic cleft. Even though Mya expresses fewer acetylcholine receptors on her muscle cells (due to the auto-antibody-induced degradation of receptors), by increasing the local concentration of acetylcholine, these receptors have a higher probability of being occupied and activated. Therefore, acute intravenous administration of this shortacting drug briefly improves muscular weakness in patients with myasthenia gravis.

(generally in an endocrine gland), enters the blood, and exerts its actions on specific target cells, which may be some distance away. In contrast to endocrine hormones, paracrine actions are those performed on nearby cells, and the location of the cells plays a role in specificity of the response. Synaptic transmission by acetylcholine and other neurotransmitters (sometimes called neurocrine signaling) is an example of paracrine signaling. Acetylcholine activates only those acetylcholine receptors located across the synaptic cleft from the signaling nerve and not all muscles with acetylcholine receptors. Paracrine actions are also very important in limiting the immune response to a specific location in the body, a feature that helps prevent the development of autoimmune disease. Autocrine actions involve a messenger acting on the cell from which it is secreted, or on nearby cells that are the same type as the secreting cells.

C. Types of Chemical Messengers

Three major signaling systems in the body employ chemical messengers: the nervous system, the endocrine system, and the immune system. Some messengers are difficult to place in just one such category.

1. THE NERVOUS SYSTEM

The nervous system secretes two types of messengers: small molecule neurotransmitters, often called biogenic amines, and neuropeptides. Small molecule neurotransmitters are nitrogen-containing molecules, which can be amino acids or are derivatives of amino acids (e.g., acetylcholine and γ -aminobutyrate, Fig. 11.5). Neuropeptides are usually small peptides (between 4 and 35 amino acids), secreted by neurons, that act as neurotransmitters at synaptic junctions or are secreted into the blood to act as neurohormones.

THE ENDOCRINE SYSTEM 2.

Endocrine hormones are defined as compounds, secreted from specific endocrine cells in endocrine glands, that reach their target cells by transport through the blood. Insulin, for example, is an endocrine hormone secreted from the β cells of the pancreas. Classic hormones are generally divided into the structural categories of polypeptide hormones (e.g., insulin -see Chapter 6, Fig. 6.15 for the structure of insulin), catecholamines such as epinephrine (which is also a neurotransmitter), steroid hormones (which are derived from cholesterol), and thyroid hormone (which is derived from tyrosine). Many of these endocrine hormones also exert paracrine or autocrine actions. The hormones that regulate metabolism are discussed throughout this chapter and in subsequent chapters of this text.

Some compounds normally considered hormones are more difficult to categorize. For example, retinoids, which are derivatives of vitamin A (also called retinol) and vitamin D (which is also derived from cholesterol) are usually classified as hormones, although they are not synthesized in endocrine cells.

THE IMMUNE SYSTEM 3

The messengers of the immune system, called cytokines, are small proteins with a molecular weight of approximately 20,000 daltons. Cytokines regulate a network of responses designed to kill invading microorganisms. The different classes of cytokines (interleukins, tumor necrosis factors, interferons, and colony-stimulating factors) are secreted by cells of the immune system and usually alter the behavior of other cells in the immune system by activating the transcription of genes for proteins involved in the immune response.



Acetylcholine

 $\overline{OOC} - CH_2 - CH_2 - CH_2 - \overline{NH_3}$ γ-aminobutyrate (GABA)

Fig. 11.5. Small molecule neurotransmitters.





Norepinephrine

Selection and Proliferation of B Cells Producing the Desired Antibody. Interleukins, a class of cytokine, illustrate some of the signaling involved in the immune response. Interleukins are polypeptide factors with molecular weights ranging from 15,000 to 25,000 Daltons. They participate in a part of the immune response called humoral immunity, which is carried out by a population of lymphoid B cells producing just one antibody against one particular antigen. The proliferation of cells producing that particular antibody is mediated by receptors and by certain interleukins.



Bacteria phagocytized by macrophages are digested by lysosomes. (1) A partially digested fragment of the bacterial protein (the blue antigen) is presented on the extracellular surface of the macrophage by a membrane protein called an MHC (major histocompatibility complex). (2) Certain lymphoid cells called T-helper cells contain receptors that can bind to the displayed antigen-MHC complex, a process that activates the T-cell (direct cell-to-cell signaling, requiring recognition molecules). (3) The activated T-helper cell then finds and binds to a B cell whose antigen receptor binds a soluble fragment of that same bacterially derived antigen molecule (again, direct cell-to-cell signaling). The bound T cell secretes interleukins, which act on the B cell (a paracrine signal). The interleukins thus stimulate proliferation of only those B cells capable of synthesizing and secreting the desirable antibody. Furthermore, the interleukins determine which class of antibody is produced.

THE EICOSANOIDS 4.

The eicosanoids (including prostaglandins [PG], thromboxanes, and leukotrienes) control cellular function in response to injury (Fig.11.6) These compounds are all derived from arachidonic acid, a 20-carbon polyunsaturated fatty acid that is usually present in cells as part of the membrane lipid phosphatidylcholine (see Chapter 5, Fig. 5.21). Although almost every cell in the body produces an eicosanoid in response to tissue injury, different cells produce different eicosanoids. The eicosanoids act principally in paracrine and autocrine functions, affecting the cells that produce them or their neighboring cells. For example, vascular endothelial cells (cells lining the vessel wall) secrete the prostaglandin PGI₂ (prostacyclin), which acts on nearby smooth muscle cells to cause vasodilation (expansion of the blood vessel).

5. **GROWTH FACTORS**

Growth factors are polypeptides that function through stimulation of cellular proliferation. For example, platelets aggregating at the site of injury to a blood vessel secrete



Lotta Topaigne suffered enormously painful gout attacks affecting her great toe (see Clinical Comments, Chapter 8). The extreme pain was caused by the release of a leukotriene that stimulated pain receptors. The precipitated urate crystals in her big toe stimulated recruited inflammatory cells to release the leukotriene.



Fig. 11.6. Eicosanoids are derived from arachidonic acid and retain its original 20 carbons (thus the name eicosanoids). All prostaglandins, such as prostacyclin, also have an internal ring.

PDGF (platelet-derived growth factor). PDGF stimulates the proliferation of nearby smooth muscle cells, which eventually form a plaque covering the injured site. Some growth factors are considered hormones, and some have been called cytokines.

Each of the hundreds of chemical messengers has its own specific receptor, which will usually bind no other messenger.

II. INTRACELLULAR TRANSCRIPTION FACTOR RECEPTORS

A. Intracellular Versus Plasma Membrane Receptors

The structural properties of a messenger determine, to some extent, the type of receptor it binds. Most receptors fall into two broad categories: intracellular receptors or plasma membrane receptors (Fig. 11.7) Messengers using intracellular receptors must be hydrophobic molecules able to diffuse through the plasma membrane into cells. In contrast, polar molecules such as peptide hormones, cytokines, and catecholamines cannot rapidly cross the plasma membrane and must bind to a plasma membrane receptor.

Most of the intracellular receptors for lipophilic messengers are gene-specific transcription factors. A transcription factor is a protein that binds to a specific site on DNA and regulates the rate of transcription of a gene (i.e., synthesis of the mRNA). External signaling molecules bind to transcription factors that bind to a specific sequence on DNA and regulate the expression of only certain genes; they are called gene-specific or site-specific transcription factors.

B. The Steroid Hormone/Thyroid Hormone Superfamily of Receptors

Lipophilic hormones that use intracellular gene-specific transcription factors include the steroid hormones, thyroid hormone, retinoic acid (active form of vitamin A), and vitamin D (Fig. 11.8). Because these compounds are water-insoluble, they are transported in the blood bound to serum albumin, which has a hydrophobic binding pocket, or to a more specific transport protein, such as steroid hormonebinding globulin (SHBG) and thyroid hormone-binding globulin (TBG). The intracellular receptors for these hormones are structurally similar and are referred to as the steroid hormone/thyroid hormone superfamily of receptors.

The steroid hormone/thyroid hormone superfamily of receptors reside primarily in the nucleus, although some are found in the cytoplasm. The glucocorticoid receptor, for example, exists as cytoplasmic multimeric complexes associated with heat shock proteins. When the hormone cortisol (a glucocorticoid) binds, the receptor undergoes a conformational change and dissociates from the heat shock proteins, exposing a nuclear translocation signal (see Chapter 10, Section VI.) The receptors dimerize, and the complex (including bound hormone) translocates to the nucleus, where it binds to a portion of the DNA called the hormone response element (e.g.,

The steroid hormone cortisol is synthesized and released from the adrenal cortex in response to the polypeptide hormone ACTH (adrenal corticotrophic hormone). Chronic stress (pain, hypoglycemia, hemorrhage, and exercise) signals are passed from the brain cortex to the hypothalamus to the anterior pituitary, which releases ACTH. Cortisol acts on tissues to change enzyme levels and redistribute nutrients in preparation for acute stress. For example, it increases transcription of the genes for regulatory enzymes in the pathway of gluconeogenesis, thereby increasing the content of these enzymes (called gene-specific activation of transcription, or induction of protein synthesis). Induction of gluconeogenic enzymes prepares the liver to respond rapidly to hypoglycemia with increased synthesis of glucose. **Ann O'Rexia**, who has been frequently fasting and exercising, has an increased capacity for gluconeogenesis in her liver.



Fig. 11.7. Intracellular vs. plasma membrane receptors. Plasma membrane receptors have extracellular binding domains. Intracellular receptors bind steroid hormones or other messengers able to diffuse through the plasma membrane. Their receptors may reside in the cytoplasm and translocate to the nucleus, reside in the nucleus bound to DNA, or reside in the nucleus bound to other proteins.



Fig. 11.8. Steroid hormone/thyroid hormone superfamily. A. Cortisol (a glucocorticoid). B. Aldosterone (an androgen). C. Thyroid hormone D. Vitamin D₃. E. Retinoids.

the glucocorticoid receptor binds to the glucocorticoid response element, GRE). Most of the intracellular receptors reside principally in the nucleus, and some of these are constitutively bound, as dimers, to their response element in DNA (e.g., the thyroid hormone receptor). Binding of the hormone changes its activity and its ability to associate with, or disassociate from, DNA. Regulation of gene transcription by these receptors is described in Chapter 16.

Recently several nuclear receptors have been identified that play important roles in intermediary metabolism, and they have become the target of lipid-lowering drugs. These include the peroxisome proliferator activated receptors (PPAR α , β and γ), the liver X-activated receptor (LXR), the farnesoid X-activated receptors (FXR), and the pregnane X receptor (PXR). These receptors form heterodimers with the 9-*cis* retinoic acid receptor (RXR) and bind to their appropriate response elements in DNA in an inactive state. When the activating ligand binds to the receptor (oxysterols for LXR, bile salts for FXR, secondary bile salts for PXR, and fatty acids and their derivatives for the PPARs), the complex is activated, and gene expression is altered. Unlike the cortisol receptor, these receptors reside in the nucleus and are activated once their ligands enter the nucleus and bind to them.



Signal transduction pathways, like a river, run in one direction. From a given point in a signal transduction pathway, events closer to the receptor are referred to as "upstream," and events closer to the response are referred to as "downstream."



phate group from ATP to the hydroxyl group of a specific amino acid residue in the protein. Tyrosine kinases transfer the phosphate group to the hydroxyl group of a specific tyrosine residue and serine/threonine protein kinases to the hydroxyl of a specific serine or threonine residue (serine is more often phosphorylated than threonine in target proteins). Different protein kinases have specificity for distinct amino acid sequences (containing a tyrosine, serine, or threonine). Thus, two different protein kinases target distinct sequences (and usually different proteins) for phosphorylation. A protein containing both target sequences could be a substrate for both protein kinases.

III. PLASMA MEMBRANE RECEPTORS AND SIGNAL TRANSDUCTION

All plasma membrane receptors are proteins with certain features in common: an extracellular domain that binds the chemical messenger, one or more membranespanning domains that are α -helices, and an intracellular domain that initiates signal transduction. As the ligand binds to the extracellular domain of its receptor, it causes a conformational change that is communicated to the intracellular domain through the rigid α -helix of the transmembrane domain. The activated intracellular domain initiates a characteristic signal transduction pathway that usually involves the binding of a specific intracellular signal transduction protein.

The pathways of signal transduction for plasma membrane receptors have two major types of effects on the cell: (1) rapid and immediate effects on cellular ion levels or activation/inhibition of enzymes and/or (2) slower changes in the rate of gene expression for a specific set of proteins. Often, a signal transduction pathway will diverge to produce both kinds of effects.

A. Major Classes of Plasma Membrane Receptors

Individual plasma membrane receptors are grouped into the categories of ion channel receptors, receptors that are kinases or bind kinases, and receptors that work through second messengers. This classification is based on the receptor's general structure and means of signal transduction.

1. ION CHANNEL RECEPTORS

The ion channel receptors are similar in structure to the nicotinic acetylcholine receptor (see Fig. 11.3). Signal transduction consists of the conformational change when ligand binds. Most small molecule neurotransmitters and some neuropeptides use ion channel receptors.

2. RECEPTORS THAT ARE KINASES OR BIND KINASES

Several types of receptors that are kinases or bind kinases are illustrated in Figure 11.9. Their common feature is that the intracellular domain of the receptor (or an associated protein) is a kinase that is activated when the messenger binds to the extracellular domain. The receptor kinase phosphorylates an amino acid residue on the receptor (autophosphorylation) or an associated protein. The message is propagated through signal transducer proteins that bind to the activated messenger-receptor complex (e.g., Grb2, STAT, or Smad).



Fig. 11.9. Receptors that are kinases or bind kinases. The kinase domains are shown in blue, and the phosphorylation sites are indicated with blue arrows. A. Tyrosine kinase receptors. B. JAK-STAT receptors. C. Serine/threonine kinase receptors.

3. HEPTAHELICAL RECEPTORS

Heptahelical receptors (which contain 7-membrane spanning α -helices) are the most common type of plasma membrane receptor. They work through second messengers, which are small nonprotein compounds, such as cAMP, generated inside the cell in response to messenger binding to the receptor (Fig. 11.10). They continue intracellular transmission of the message from the hormone/cytokine/neurotransmitter, which is the "first" messenger. Second messengers are present in low concentrations so that their concentration, and hence the message, can be rapidly initiated and terminated.

B. Signal Transduction through Tyrosine Kinase Receptors

The tyrosine kinase receptors are summarized in Figure 11.9A. They generally exist in the membrane as monomers with a single membrane-spanning helix. One molecule of the growth factor generally binds two molecules of the receptor and promotes their dimerization (Fig. 11.11). Once the receptor dimer has formed, the intracellular tyrosine kinase domains of the receptor phosphorylate each other on certain tyrosine residues (autophosphorylation). The phosphotyrosine residues form specific binding sites for signal transducer proteins.

1. RAS AND THE MAP KINASE PATHWAY

One of the domains of the receptor containing a phosphotyrosine residue forms a binding site for intracellular proteins with a specific three-dimensional structure known as the SH2 domain (the Src homology 2 domain, named for the first protein in which it was found, the src protein of the Rous sarcoma virus). The adaptor

Although many different signal transducer proteins have SH2 domains, and many receptors have phosphotyrosine residues, each signal transducer protein is specific for one type of receptor. This specificity of binding results from the fact that each phosphotyrosine residue has a different amino acid sequence around it that forms the binding domain. Likewise, the SH2 domain of the transducer protein is only part of its binding domain.

Heptahelical receptors



Fig. 11.10. Heptahelical Receptors and Second Messengers. The secreted chemical messenger (hormone, cytokine, or neurotransmitter) is the first messenger, which binds to a plasma membrane receptor such as the heptahelical receptors. The activated hormone–receptor complex activates a heterotrimeric G protein and via stimulation of membrane-bound enzymes, different G-proteins lead to generation of one or more intracellular second messengers, such as cAMP, diacylglycerol (DAG), or inositol trisphosphate (IP₃).



Fig. 11.11. Signal transduction by tyrosine kinase receptors. (1) Binding and dimerizaion. (2) Autophosphorylation. (3) Binding of Grb2 and SOS. (4) SOS is a GEF (guanine nucleotide exchange protein) that binds Ras, a monomeric G protein anchored to the plasma membrane. (5) GEF activates the exchange of GTP for bound GDP on Ras. (6) Activated Ras containing GTP binds the target enzyme Raf, thereby activating it.



Fig. 11.12. Major route for generation of the phosphatidyl inositide signal molecules, inositol 1,4,5-trisphosphate (IP_3) and phosphatidylinositol 3,4,5 trisphosphate (PI-3,4,5-trisP). PI 3-kinase phosphorylates PI-4,5-bisP and PI-4P at the 3 position. Prime symbols are sometimes used in these names to denote the inositol ring. DAG is also a second messenger.

protein Grb2, which is bound to a membrane phosphoinositide, is one of the proteins with an SH2 domain that binds to phosphotyrosine residues on growth factor receptors. Binding to the receptor causes a conformational change in Grb2 that activates another binding site called an SH3 domain. These activated SH3 domains bind the protein SOS (SOS is an acronym for "son of sevenless," a name unrelated to the function or structure of the compound). SOS is a guanine nucleotide exchange factor (GEF) for Ras, a monomeric G protein located in the plasma membrane (see Chapter 9, Section III.C.2.) SOS activates exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on Ras, causing a conformational change in Ras that promotes binding of the protein Raf. Raf is a serine protein kinase that is also called MAPKKK (mitogen activated protein kinase kinase kinase.) Raf begins a sequence of successive phosphorylation steps called a phosphorylation cascade (When a kinase in a cascade is phosphorylated, it binds and phosphorylates the next enzyme in the cascade.). The MAP kinase cascade terminates at a gene transcription factor, thereby regulating transcription of certain genes involved in cell survival and proliferation.

Many tyrosine kinase receptors (as well as heptahelical receptors) also have additional signaling pathways involving phosphatidylinositol phosphates.

2. PHOSPHATIDYLINOSITOL PHOSPHATES IN SIGNAL TRANSDUCTION

Phosphatidylinositol phosphates serve two different functions in signal transduction: (1) Phosphatidylinositol 4',5' bisphosphate (PI-4,5-bisP) can be cleaved to generate the two intracellular second messengers, diacylglycerol (DAG) and inositol trisphosphate (IP₃); and (2) Phosphatidylinositol 3',4',5' trisphosphate (PI-3,4,5-trisP) can serve as a plasma membrane docking site for signal transduction proteins.

Phosphatidyl inositol, which is present in the inner leaflet of the plasma membrane, is converted to PI-4,5-bisP by kinases that phosphorylate the inositol ring at the 4' and 5' positions (Fig. 11.12). PI-4,5-bisP, which has three phosphate groups, is cleaved by a phospholipase C-isozyme to generate IP₃ and DAG. The phospholipase isozyme C_{γ} (PLC_{γ}) is activated by tyrosine kinase growth factor receptors, and phospholipase C β is activated by a heptahelical receptor–G protein signal transduction pathway.

PI-4,5-bisP can also be phosphorylated at the 3' position of inositol by the enzyme phosphatidylinositol 3' kinase (PI 3-kinase) to form PI -3,4,5- trisP (see Fig. 11.12). PI-3,4,5- tris P (and PI -3,4 bis P) form membrane docking sites for proteins containing a certain sequence of amino acids called the pleckstrin homology (PH) domain. PI 3- kinase contains an SH2 domain and is activated by binding to a specific phosphotyrosine site on a tyrosine kinase receptor or receptor-associated protein.

3. THE INSULIN RECEPTOR

The insulin receptor, a member of the tyrosine kinase family of receptors, provides a good example of divergence in the pathway of signal transduction. Unlike other growth factor receptors, the insulin receptor exists in the membrane as a preformed dimer, with each half containing an α and a β subunit (Fig. 11.13). The β subunits

Insulin is a growth factor that is essential for cell viability and growth. It increases general protein synthesis, which strongly affects muscle mass. However, it also regulates immediate nutrient availability and storage, including glucose transport into skeletal muscle and glycogen synthesis. Thus, **Di Abietes** and other patients with type I diabetes mellitus who lack insulin rapidly develop hyperglycemia once insulin levels drop too low. They also exhibit muscle "wasting." To mediate the diverse regulatory roles of insulin, the signal transduction pathway diverges after activation of the receptor and phosphorylation of IRS, which has multiple binding sites for different signal mediator proteins.



Fig. 11.13. Insulin receptor signaling. The insulin receptor is a dimer of two membrane-spanning α - β pairs. The tyrosine kinase domains are shown in blue, and arrows indicate auto-crosspho-sphorylation. The activated receptor binds IRS molecules (insulin receptor substrates) and phosphorylates IRS at multiple sites, thereby forming binding sites for proteins with SH2 domains: Grb2, phospholipase C γ (PLC γ), and PI 3-kinase. These proteins are associated with various phosphatidylinositol phosphates (all designated with PIP) in the plasma membrane.

autophosphorylate each other when insulin binds, thereby activating the receptor. The activated phosphorylated receptor binds a protein called IRS (insulin receptor substrate). The activated receptor kinase phosphorylates IRS at multiple sites, creating multiple binding sites for different proteins with SH2 domains. One of the sites binds Grb2, leading to activation of Ras and the MAP kinase pathway. Grb2 is anchored to PI-3,4,5-trisP in the plasma membrane through its PH (pleckstrin homology) domain. At another phosphotyrosine site, PI 3-kinase binds and is activated. At a third site, phospholipase $C_{\gamma}(PLC_{\gamma})$ binds and is activated. The insulin receptor can also transmit signals through a direct docking with other signal transduction intermediates.

The signal pathway initiated by the insulin receptor complex involving PI 3-kinase leads to activation of protein kinase B, a serine-threonine kinase that mediates many of the downstream effects of insulin (Fig. 11.14). PI 3- kinase binds and phosphorylates PI-4,5- bis P in the membrane to form PI-3,4,5- trisP. Protein kinase Protein kinase B is a serinethreonine kinase, also known as Akt. One of the signal transduction pathways from protein kinase B (Akt) leads to the effects of insulin on glucose metabolism. Other pathways, long associated with Akt, result in the phosphorylation of a host of other proteins that affect cell growth, cell cycle entry, and cell survival. In general, phosphorylation of these proteins by Akt inhibits their action and promotes cell survival.



Fig. 11.14. The insulin receptor–protein kinase B signaling pathway. Abbreviations: Ins, insulin; IRS, insulin receptor substrate; PH domains, pleckstrin homology domains; PDK1, phosphoinositide-dependent protein kinase 1; PKB, protein kinase B. The final phosphorylation step that activates PKB is shown in blue.

B and PDK1 (phosphoinositide-dependent kinase-1) are recruited to the membrane by their PH domains, where PDK1 phosphorylates and activates protein kinase B. Many other signal transducer proteins have PH domains and are docked at the membrane, where they can find and bind each other. Thus, the insulin signal diverges again and again. Insulin is covered in more detail in Chapters 26, 36 and 43.

C. Signal Transduction by JAK-STAT Receptors

Tyrosine kinase-associated receptors called Jak-STAT receptors are often used by cytokines to regulate the proliferation of certain cells involved in the immune response (see Fig. 11.9B). The receptor itself has no intrinsic kinase activity but binds (associates with) the tyrosine kinase Jak (**ja**nus **k**inase). Their signal transducer proteins, called STATs (signal transducer and **a**ctivator of transcription), are themselves gene-specific transcription factors. Thus, Jak-STAT receptors have a more direct route for propagation of the signal to the nucleus than tyrosine kinase receptors.

Each receptor monomer has an extracellular domain, a membrane-spanning region, and an intracellular domain. As the cytokine binds to these receptors, they form dimers (either homodimers or heterodimers, between two distinct receptor molecules) and may cluster (Fig. 11.15). The activated Jaks phosphorylate each other and intracellular tyrosine residues on the receptor, forming phosphotyrosine-binding sites for the SH2 domain of a STAT. STATs are inactive in the cytoplasm until they bind to the receptor complex, where they are also phosphorylated by the bound JAK. Phosphorylation changes the conformation of the STAT, causing it to dissociate from the receptor and dimerize with another phosphorylated STAT, thereby forming an activated transcription factor. The STAT dimer translocates to the nucleus and binds to a response element on DNA, thereby regulating gene transcription.

There are many different STAT proteins, each with a slightly different amino acid sequence. Receptors for different cytokines bind different STATs, which then form heterodimers in various combinations. This microheterogeneity allows different cytokines to target different genes.

D. Receptor Serine/Threonine Kinases

Proteins in the transforming growth factor superfamily use receptors that have serine/threonine kinase activity and associate with proteins from the Smad family, which are gene-specific transcription factors (see Fig. 11.9C). This superfamily includes transforming growth factor β (TGF- β), a cytokine/hormone involved in tissue repair, immune regulation, and cell proliferation, and bone morphogenetic proteins (BMPs), which control proliferation, differentiation, and cell death during development.



Fig. 11.15. Steps in Jak-STAT receptor signaling.

Although Jak is an acronym for **ja**nus kinase, it has been suggested that it stands for "just another kinase". It was named for Janus, a two-headed god of the Romans.



Fig. 11.16. Serine/threonine receptors and Smad proteins. TGF- β (transforming growth factor β), which is composed of two identical subunits, communicates through a receptor dimer of type I and type II subunits that have serine kinase domains. The type I receptor phosphorylates an R-Smad (receptor-specific Smad), which binds a Co-Smad (common Smad, also called Smad 4).

A simplified version of TGF- β 1 binding to its receptor complex and activating Smads is illustrated in Fig. 11.16 The TGF- β receptor complex is composed of two different single membrane-spanning receptor subunits (type I and type II), which have different functions even though they both have serine kinase domains. TGF- β binds to a type II receptor. The activated type II receptor recruits a type I receptor, which it phosphorylates at a serine residue, forming an activated receptor complex. The type I receptor then binds a receptor-specific Smad protein (called R-Smads), which it phosphorylates at serine residues. The phosphorylated R-Smad undergoes a conformational change and dissociates from the receptor. It then forms a complex with another member of the Smad family, Smad 4 (Smad 4 is known as the common Smad, Co-Smad, and is not phosphorylated). The Smad complex, which may contain several Smads, translocates to the nucleus, where it activates or inhibits the transcription of target genes. Receptors for different ligands bind different Smads, which bind to different sites on DNA and regulate the transcription of different genes.

E. Signal Transduction through Heptahelical Receptors

The heptahelical receptors are named for their 7-membrane spanning domains, which are α -helices (see Fig. 11.10; see also Chapter 7, Fig. 7.10). Although hundreds of hormones and neurotransmitters work through heptahelical receptors, the extracellular binding domain of each receptor is specific for just one polypeptide hormone, catecholamine, or neurotransmitter (or its close structural analog). Heptahelical receptors have no intrinsic kinase activity but initiate signal transduction through heterotrimeric G proteins composed of α , β and γ subunits. However, different types of heptahelical receptors bind different G proteins, and different G proteins exert different effects on their target proteins.

1. HETEROTRIMERIC G PROTEINS

The function of heterotrimeric G proteins is illustrated in Figure 11.17 using a hormone that activates adenylyl cyclase (e.g., glucagon or epinephrine). While the α subunit contains bound GDP, it remains associated with the β and γ subunits, either free in the membrane or bound to an unoccupied receptor (see Fig. 11.17, part 1). When the hormone binds, it causes a conformational change in the receptor that activates GDP dissociation and GTP binding. The exchange of GTP for bound GDP causes dissociation of the α subunit from the receptor and from the $\beta\gamma$ subunits (see Fig. 11.17, part 2). The α and β subunits are tethered to the intracellular side of the



Fig. 11.17. Heptahelical receptors and heterotrimeric G proteins. (1) The intracellular domains of the receptor form a binding site for a G protein containing GDP bound to the α -subunit. (2) Hormone binding to the receptor promotes the exchange of GTP for GDP. As a result, the complex disassembles, releasing the G protein α -subunit from the $\beta\gamma$ complex. (3) The Gs α -subunit binds to a target enzyme, thereby changing its activity. The $\beta\gamma$ complex may simultaneously target another protein and change its activity. (4) Over time, bound GTP is hydrolysed to GDP, causing dissociation of the α -subunit from adenylyl cyclase. The GDP- α -subunit reassociates with the $\beta\gamma$ subunit and the receptor.

The importance of signal termination is illustrated by the "internal clock" of G proteins, which is the rate of spontaneous hydrolysis of GTP to GDP. Mutations in ras (the gene encoding Ras) that decrease the rate of GTP hydrolysis are found in about 20 to 30% of all human cancers, including approximately 25% of lung cancers, 50% of colon cancers, and more than 90% of pancreatic cancers. In these mutations of Ras, GTP hydrolysis is decreased and Ras remains locked in the active GTP-bound form, rather than alternating normally between inactive and active state in response to extracellular signals. Consequently, MAP kinase pathways are continuously stimulated and drive cell proliferation, even in the absence of growth factors that would be required for ras activation in normal cells.

plasma membrane through lipid anchors, but can still move around on the membrane surface. The GTP- α subunit binds its target enzyme in the membrane, thereby changing its activity. In this example, the α -subunit binds and activates adenylyl cyclase, thereby increasing synthesis of cAMP (see Fig. 11.17, part 3).

With time, the G α subunit inactivates itself by hydrolyzing its own bound GTP to GDP and P_i. This action is unrelated to the number of cAMP molecules formed. Like the monomeric G proteins, the GDP- α subunit then dissociates from its target protein, adenylyl cyclase (see Fig. 11.16, part 4). It reforms the trimeric G protein complex, which may return to bind the empty hormone receptor. As a result of this GTPase "internal clock," sustained elevations of hormone levels are necessary for continued signal transduction and elevation of cAMP.

A large number of different heterotrimeric G protein complexes are generally categorized according to the activity of the α subunit (Table 11.1). The 20 or more different isoforms of G α fall into four broad categories: G α_s , G $\alpha_{i/0}$, G $\alpha_{q/11}$, and G α 12/13₁₃. G α_s refers to α subunits, which, like the one in Figure 11.17, stimulate adenylyl cyclase (hence the *s*). G α subunits that inhibit adenylyl cyclase are called G α_i . The $\beta\gamma$ subunits likewise exist as different isoforms, which also transmit messages.



Acetylcholine has two types of receptors: nicotinic ion channel receptors, the receptors inhibited by antibodies in myasthenia gravis, and muscarinic receptors, which exist as a variety of subtypes. The M2 muscarinic receptors activate a $G_{\alpha_{i/0}}$ heterotrimeric G protein in which release of the $\beta\gamma$ subunit controls K⁺ channels and pacemaker activity in the heart. Epinephrine has several types and subtypes of heptahelical receptors: β receptors work through a $G\alpha_s$ and stimulate adenylyl cyclase; α_2

receptors in other cells work through a $G\alpha_i$ protein and inhibit adenylyl cyclase; α_1 receptors work through $G\alpha_q$ subunits and activate phospholipase C β . This variety in receptor types allows a messenger to have different actions in different cells.

Gα subunit	Action	Some Physiologic Uses
α_{s} ; G α (s) *	s timulates adenyl cyclase	Glucagon and epinephrine to regulate metabolic enzymes, regulatory polypeptide hormones to control steroid hor- mone and thyroid hormone synthesis, and by some neurotransmitters (e.g., dopamine) to control ion channels
α _{i/o} ; Gα (i/o) (signal also flows through βγ subunits)	inhibits adenylyl cyclase	Epinephrine, many neurotransmitters including acetylcholine, dopamine, serotonin. Has a role in the transducin pathway, which mediates detection of light in the eye.
α _{q/11} ; Gα (q/11)	activates phospholipase $C_{\boldsymbol{\beta}}$	Epinephrine, acetylcholine, histamine, thyroid-stimulating hormone (TSH), interleukin 8, somatostatin, angiotensin
α _{12/13} ; Gα (12/13)	Physiologic connections are not yet well established	Thromboxane A2, lysophosphatidic acid

Table 11.1 Subunits of Heterotrimeric G Proteins

*There is a growing tendency to designate the heterotrimeric G protein subunits without using subscripts so that they are actually visible to the naked eye.

2. ADENYLYL CYCLASE AND CAMP PHOSPHODIESTERASE

cAMP is referred to as a second messenger because changes in its concentration reflect changes in the concentration of the hormone (the first messenger). When a hormone binds and adenylyl cyclase is activated, it synthesizes cAMP from adenosine triphosphate (ATP). cAMP is hydrolyzed to AMP by cAMP phosphodiesterase, which also resides in the plasma membrane (Fig. 11.18). The concentration of cAMP and other second messengers is kept at very low levels in cells by balancing the activity of these two enzymes so that cAMP levels can change rapidly when hormone levels change. Some hormones change the concentration of cAMP by targeting the phosphodiesterase enzyme rather than adenylyl cyclase. For example, insulin lowers cAMP levels by causing phosphodiesterase activation.

cAMP exerts diverse effects in cells. It is an allosteric activator of protein kinase A (see Chapter 9, section III.B.3), which is a serine/threonine protein kinase that phosphorylates a large number of metabolic enzymes, thereby providing a rapid response to hormones such as glucagon and epinephrine. The catalytic subunits of protein kinase A also enter the nucleus and phosphorylate a gene-specific transcription factor called CREB (cyclic AMP response element-binding protein). Thus, cAMP also activates a slower response pathway, gene transcription. In other cell types, cAMP directly activates ligand-gated channels.

PHOSPHATIDYLINOSITOL SIGNALING BY HEPTAHELICAL RECEPTORS

Certain heptahelical receptors bind the q isoform of the G α subunit (G α_q), which activates the target enzyme phospholipase C $_\beta$ (see Fig.11.12). When activated, phospholipase C $_\beta$ hydrolyzes the membrane lipid phosphatidyl inositol bis phosphate (PI-4,5-bisP) into two second messengers, diacylglycerol (DAG) and 1,4,5-inositol trisphosphate (IP₃). IP₃ has a binding site in the sarcoplasmic reticulum and the endoplasmic reticulum that stimulates the release of Ca²⁺ (Fig. 11.19). Ca²⁺ activates enzymes containing the calcium–calmodulin subunit, including a protein kinase. Diacylglycerol, which remains in the membrane, activates protein kinase C, which then propagates the response by phosphorylating target proteins.

F. Changes in Response to Signals

Tissues vary in their ability to respond to a message through changes in receptor activity or number. Many receptors contain intracellular phosphorylation sites that alter their ability to transmit signals. Receptor number is also varied through

Dennis Veere was hospitalized for dehydration resulting from cholera toxin (see Chapter 10). Cholera A toxin was absorbed into the intestinal mucosal cells, where it was processed and complexed with Arf (ADP-ribosylation factor), a small G protein normally involved in vesicular transport. Cholera A toxin is an NAD-glycohydrolase, which cleaves NAD and transfers the ADP ribose portion to other proteins. It ADP-ribosylates the $G\alpha_s$ subunit of heterotrimeric G proteins, thereby inhibiting their GTPase activity. As a consequence, they remain actively bound to adenylyl cyclase, resulting in increased production of cAMP. The CFTR channel is activated, resulting in secretion of chloride ion and Na⁺ ion into the intestinal lumen. The ion secretion is followed by loss of water, resulting in vomiting and watery diarrhea.

Some signaling pathways cross from the MAP kinase pathway to phosphorylate CREB, and all heterotrimeric G protein pathways diverge to include a route to the MAP kinase pathway. These types of complex interconnections in signaling pathways are sometimes called hormone cross-talk.



Fig. 11.18. Formation and cleavage of the cyclic phosphodiester bond in cAMP. When activated by $G\alpha s$, adenyl cyclase converts ATP to 3',5'cyclic AMP + PPi . cAMP phosphodiesterase hydrolyzes cAMP to AMP.

In myasthenia gravis, increased endocytosis and degradation of acetylcholine receptors lead to a signal transduction pathway that decreases synthesis of new receptors. Thus, downregulation of acetylcholine receptors is part of this disease. down-regulation. After a hormone binds to the receptor, the hormone–receptor complex may be taken into the cell by the process of endocytosis in clathrincoated pits (see Chapter 10, Section III.B.1.) The receptors may be degraded or recycled back to the cell surface. This internalization of receptors decreases the number available on the surface under conditions of constant high hormone levels when more of the receptors are occupied by hormones and results in decreased synthesis of new receptors. Hence, it is called down-regulation.

IV. SIGNAL TERMINATION

Some signals, such as those that modify the metabolic responses of cells or transmit neural impulses, need to turn off rapidly when the hormone is no longer being produced. Other signals, such as those that stimulate proliferation, turn off more slowly. In contrast, signals regulating differentiation may persist throughout our lifetime. Many chronic diseases are caused by failure to terminate a response at the appropriate time.

The first level of termination is the chemical messenger itself (Fig.11.20). When the stimulus is no longer applied to the secreting cell, the messenger is no longer secreted, and existing messenger is catabolized. For example, many polypeptide hormones such as insulin are taken up into the liver and degraded. Termination of the acetylcholine signal by acetylcholinesterase has already been mentioned.

Within each pathway of signal transduction, the signal may be turned off at specific steps. The receptor might be desensitized to the messenger by phosphorylation. G proteins, both monomeric and heterotrimeric, automatically terminate messages as they hydrolyze GTP. Termination also can be achieved through degradation of the second messenger (e.g., phosphodiesterase cleavage of cAMP). Each of these terminating processes is also highly regulated.

Another important pathway for reversing the message is through protein phosphatases, enzymes that reverse the action of kinases by removing phosphate groups



Fig. 11.19. IP₃ signaling calcium release from the endoplasmic reticulum.

from proteins. Specific tyrosine or serine/threonine phosphatases (enzymes that remove the phosphate group from specific proteins) exist for all of the sites phosphorylated by signal transduction kinases. Some receptors are even protein phosphatases.

CLINICAL COMMENTS

Mya Sthenia. Mya Sthenia has myasthenia gravis, an autoimmune disease caused by the production of antibodies directed against the nicotinic acetylcholine receptor in skeletal muscles. The diagnosis is made by history (presence of typical muscular symptoms), physical examination (presence of inability to do specific repetitive muscular activity over time), and tests such as the inhibition of acetylcholinesterase activity. The diagnosis can be further confirmed with an electromyogram (EMG) showing a partial blockade of ion flux across muscular membranes and a diagnostic procedure involving repetitive electrical nerve stimulation.

Ann O'Rexia. Anorexia nervosa presents as a distorted visual selfimage often associated with compulsive exercise. Although Ann has been gaining weight, she is still relatively low on stored fuels needed to sustain the metabolic requirements of exercise. Her prolonged starvation has resulted in release of the steroid hormone cortisol and the polypeptide hormone glucagon, whereas levels of the polypeptide hormone insulin have decreased. Cortisol activates transcription of genes for some of the enzymes of gluconeogenesis (the synthesis of glucose from amino acids and other precursors; see Chapter 3.) Glucagon binds to heptahelical receptors in liver and adipose tissue and, working through cAMP and protein kinase A, activates many enzymes involved in fasting fuel metabolism. Insulin, which is released when she drinks her high-energy supplement, works through a specialized tyrosine kinase receptor to promote fuel storage. Epinephrine, a catecholamine released when she exercises, promotes fuel mobilization.

Dennis Veere. In the emergency room, Dennis received intravenous rehydration therapy (normal saline [0.9% NaCl]) and oral hydration therapy with a glucose-electrolyte solution to increase his glucose-dependent Na⁺ uptake from the intestinal lumen (see Chapter 10). Dennis quickly recovered from his bout of cholera. Cholera is self-limiting, possibly because the bacteria remain in the intestine, where they are washed out of the system by the diffuse watery diarrhea. Over the past three years, **Percy Veere** has persevered through the death of his wife and the subsequent calamities of his grandson Dennis "the Menace" Veere, including salicylate poisoning, suspected malathion poisoning, and now cholera. Mr. Veere decided to send his grandson home for the remainder of the summer.

BIOCHEMICAL COMMENTS

Death domain receptors. The cytokine TNF (tumor necrosis factor) uses a type of receptor called the death domain receptor (Fig. 11.21). These receptors function as a trimer when they bind TNF (which is also a trimer). On TNF binding, an inhibitory protein called the "silencer of death" is released from the receptor. The receptor then binds and activates several adaptor proteins. One adaptor protein, FADD (fas-associated death domain), recruits and activates the zymogen form of a proteolytic enzyme called caspase. Caspases



Fig. 11.20. Sites of signal termination. Processes that terminate signals are shown in blue.



Fig. 11.21. Death domain receptors. The portion of the receptor shown in blue is called the death domain because it binds adaptor proteins that initiate different signaling pathways leading to cell death. The adaptor protein FADD forms a scaffold on which proteolytic procaspases cleave each other, thereby initiating a death pathway. The adaptor protein TRADD binds a protein that binds a serine-threonine kinase (Ser-T kinase) that initiates another signaling pathway leading to activation of the transcription factor NF- κ B.

initiate a signal transduction pathway leading to apoptosis (programmed cell death) (see Chapter 18). Another adaptor protein, TRADD (TNF receptor-associated death domain), initiates signaling pathways that lead to activation of the gene-specific transcription factors Jun and NF- κ B (nuclear factor- κ B). Through these pathways, TNF mediates cell-specific responses, such as cell growth and death, the inflammatory response, and immune function.

Guanylyl cyclase receptors. Guanylyl cyclase receptors convert GTP to the second messenger 3',5' cyclic GMP (cGMP), which is analogous to cAMP (Fig. 11.22). Like cAMP, cGMP is degraded by a membrane-bound phosphodiesterase. Elevated cGMP activates protein kinase G, which then phosphorylates target proteins to propagate the response.

One type of guanylyl cyclase exists in the cytoplasm and is a receptor for nitric oxide (NO), a neurotransmitter/neurohormone. NO is a lipophilic gas that is able to diffuse into the cell. This receptor thus is an exception to the rule that intracellular receptors are gene transcription factors. The other type of guanyl cyclase receptor is a membrane-spanning receptor in the plasma membrane with an external binding domain for a signal molecule (e.g., natriuretic peptide).

Suggested References

Signaling Pathways. The May 31, 2002 issue of Science, Vol 296, provides a 2–3page synopsis of many signaling pathways. The articles most relevant to the pathways discussed in this chapter are:

- Aaronson DA, Horvath CM. A road map for those who don't know JAK-STAT. Science 2002;296:1653–1655.
- Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002;296:1655-1657.
- Attisano L, Wrana, JL. Signal transduction by the TGF-β superfamily. Science 2002;296:1646-1647.
- Neves SR, Ram PT, Iyengar R. G protein pathways. Science 2002;296:1636-1639.
- Chen Q, Goeddel DV. TNF-R1 signaling: A beautiful pathway. Science 2002;296:1634–1635.

Caspases are proteolytic enzymes that have a critical role in programmed cell death (also called apoptosis) (see Chapter 16). Caspases are present as latent zymogens until their autoproteolysis (self-cleavage) is activated by "death signals" to the receptor complex. Once activated, they work systematically to dismantle a cell through degrading a wide variety of proteins, such as DNA repair enzymes and cellular structural proteins.



Fig. 11.22. Structure of 3',5'-cyclic GMP (cGMP).

REVIEW QUESTIONS-CHAPTER 11

- 1. Which of the following is a general characteristic of all chemical messengers?
 - (A) They are secreted by one cell, enter the blood, and act on a distant target cell.
 - (B) To achieve a coordinated response, each messenger is secreted by several types of cells.
 - (C) Each messenger binds to a specific protein receptor in a target cell.
 - (D) Chemical messengers must enter cells to transmit their message.
 - (E) Chemical messengers are metabolized to intracellular second messengers to transmit their message.
- 2. Which of the following is a characteristic of chemical messengers that bind to intracellular transcription factor receptors?
 - (A) They are usually cytokines or polypeptide hormones.
 - (B) They are usually small molecule neurotransmitters.
 - (C) They exert rapid actions in cells.
 - (D) They are transported through the blood bound to proteins.
 - (E) They are always present in high concentrations in the blood.

Use the following case history for questions 3 and 4. To answer this question, you do not need to know more about parathyroid hormone or pseudophypoparathyroidism than the information given.

Pseudohypoparathyroidism is a heritable disorder caused by target organ unresponsiveness to parathyroid hormone (a polypeptide hormone secreted by the parathyroid gland). One of the mutations causing this disease occurs in the gene encoding $G_s \alpha$ in certain cells.

- 3. The receptor for parathyroid hormone is most likely
 - (A) an intracellular transcription factor.
 - (B) a cytoplasmic guanylyl cyclase.
 - (C) a receptor that must be endocytosed in clathrin-coated pits to transmit its signal.
 - (D) a heptahelical receptor.
 - (E) a tyrosine kinase receptor.
- 4. This mutation most likely
 - (A) is a gain-of-function mutation.
 - (B) decreases the GTPase activity of the $G_{\alpha s}$ subunit.
 - (C) decreases synthesis of cAMP in response to parathyroid hormone.
 - (D) decreases generation of IP_3 in response to parathyroid hormone.
 - (E) decreases synthesis of phosphatidylinositol 3,4,5-trisphosphate in response to parathyroid hormone.
- 5. SH2 domains on proteins are specific for which of the following sites?
 - (A) Certain sequences of amino acids containing a phosphotyrosine residue
 - (B) PI-3,4,5 trisphosphate in the membrane
 - (C) GTP-activated Ras
 - (D) Ca²⁺-calmodulin
 - (E) Receptor domains containing phosphoserine residues