There Are Three Major Classes of Cell-Surface Receptor Proteins

Most extracellular signal molecules bind to specific receptor proteins on the surface of the target cells they influence and do not enter the cytosol or nucleus. These cell-surface receptors act as *signal transducers* by converting an extracellular ligand-binding event into intracellular signals that alter the behavior of the target cell.

Most cell-surface receptor proteins belong to one of three classes, defined by their transduction mechanism. **Ion-channel-coupled receptors**, also known as *transmitter-gated ion channels* or *ionotropic receptors,* are involved in rapid synaptic signaling between nerve cells and other electrically excitable target cells such as nerve and muscle cells (**Figure 15–6**A). This type of signaling is mediated by a small number of neurotransmitters that transiently open or close an ion channel formed by the protein to which they bind, briefly changing the ion permeability of the plasma membrane and thereby changing the excitability of the postsynaptic target cell. Most ion-channel-coupled receptors belong to a large family of homologous, multipass transmembrane proteins. Because they are discussed in detail in Chapter 11, we will not consider them further here.

G-protein-coupled receptors act by indirectly regulating the activity of a separate plasma-membrane-bound target protein, which is generally either an enzyme or an ion channel. A *trimeric GTP-binding protein* (*G protein*) mediates the interaction between the activated receptor and this target protein (Figure 15–6B). The activation of the target protein can change the concentration of one or

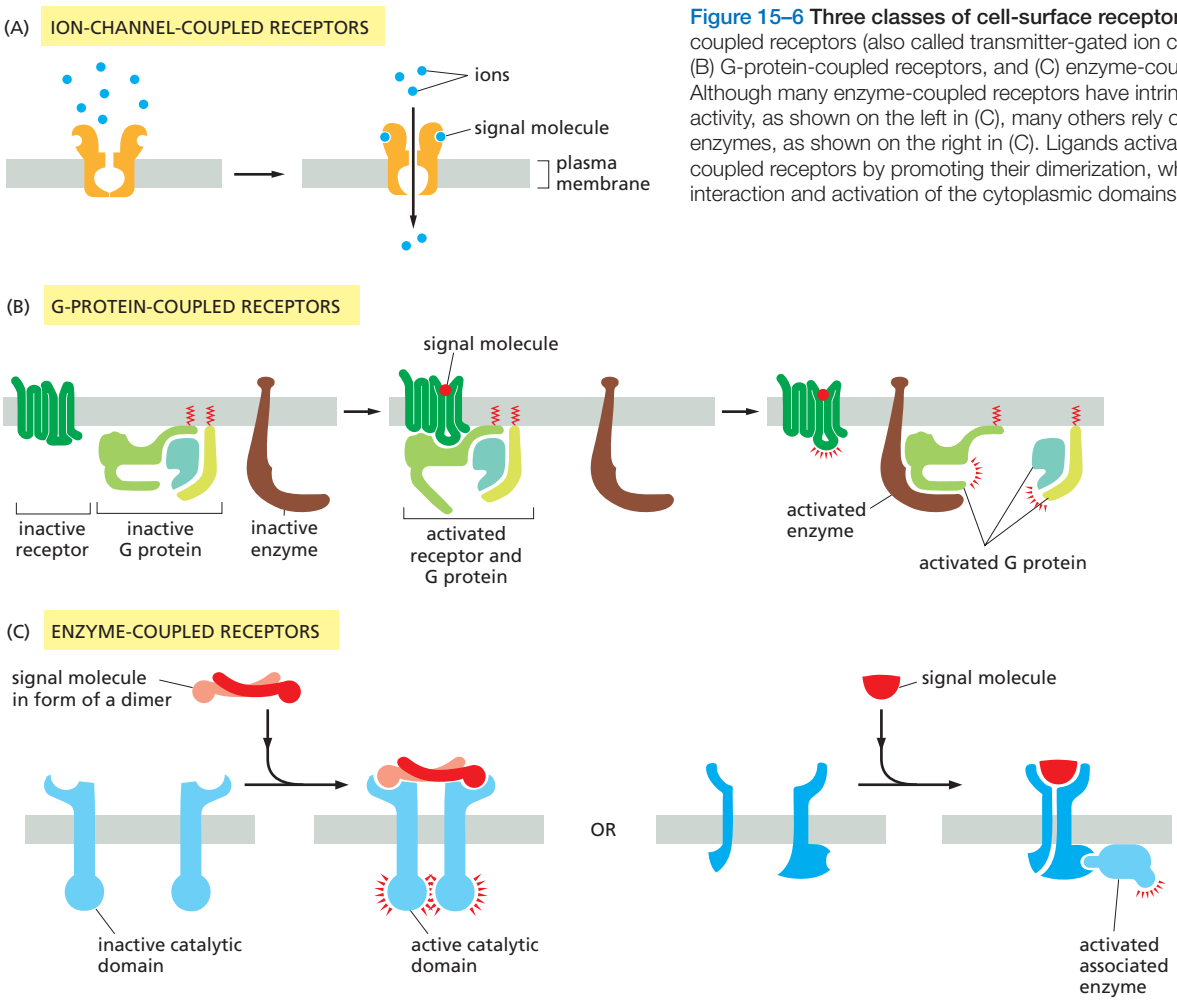

Figure 15–6 Three classes of cell-surface receptors. (A) Ion-channel-coupled receptors (also called transmitter-gated ion channels), (B) G-protein-coupled receptors, and (C) enzyme-coupled receptors. Although many enzyme-coupled receptors have intrinsic enzymatic activity, as shown on the left in (C), many others rely on associated enzymes, as shown on the right in (C). Ligands activate most enzyme-coupled receptors by promoting their dimerization, which results in the interaction and activation of the cytoplasmic domains.

more small intracellular signaling molecules (if the target protein is an enzyme), or it can change the ion permeability of the plasma membrane (if the target protein is an ion channel). The small intracellular signaling molecules act in turn to alter the behavior of yet other signaling proteins in the cell.

Enzyme-coupled receptors either function as enzymes or associate directly with enzymes that they activate (Figure 15–6C). They are usually single-pass transmembrane proteins that have their ligand-binding site outside the cell and their catalytic or enzyme-binding site inside. Enzyme-coupled receptors are heterogeneous in structure compared with the other two classes; the great majority, however, are either protein kinases or associate with protein kinases, which phosphorylate specific sets of proteins in the target cell when activated.

There are also some types of cell-surface receptors that do not fit easily into any of these classes but have important functions in controlling the specialization of different cell types during development and in tissue renewal and repair in adults. We discuss these in a later section, after we explain how G-protein-coupled receptors and enzyme-coupled receptors operate. First, we continue our general discussion of the principles of signaling via cell-surface receptors.

Cell-Surface Receptors Relay Signals Via Intracellular Signaling Molecules

Numerous intracellular signaling molecules relay signals received by cell-surface receptors into the cell interior. The resulting chain of intracellular signaling events ultimately alters effector proteins that are responsible for modifying the behavior of the cell (see Figure 15–1).

Some intracellular signaling molecules are small chemicals, which are often called **second messengers** (the "first messengers" being the extracellular signals). They are generated in large amounts in response to receptor activation and diffuse away from their source, spreading the signal to other parts of the cell. Some, such as *cyclic AMP* and Ca^{2+}, are water-soluble and diffuse in the cytosol, while others, such as *diacylglycerol*, are lipid-soluble and diffuse in the plane of the plasma membrane. In either case, they pass the signal on by binding to and altering the behavior of selected signaling or effector proteins.

Most intracellular signaling molecules are proteins, which help relay the signal into the cell by either generating second messengers or activating the next signaling or effector protein in the pathway. Many of these proteins behave like *molecular switches*. When they receive a signal, they switch from an inactive to an active state, until another process switches them off, returning them to their inactive state. The switching off is just as important as the switching on. If a signaling pathway is to recover after transmitting a signal so that it can be ready to transmit another, every activated molecule in the pathway must return to its original, unactivated state.

The largest class of molecular switches consists of proteins that are activated or inactivated by **phosphorylation** (discussed in Chapter 3). For these proteins, the switch is thrown in one direction by a **protein kinase**, which covalently adds one or more phosphate groups to specific amino acids on the signaling protein, and in the other direction by a **protein phosphatase**, which removes the phosphate groups (**Figure 15–7**A). The activity of any protein regulated by phosphorylation depends on the balance between the activities of the kinases that phosphorylate it and of the phosphatases that dephosphorylate it. About 30–50% of human proteins contain covalently attached phosphate, and the human genome encodes about 520 protein kinases and about 150 protein phosphatases. A typical mammalian cell makes use of hundreds of distinct types of protein kinases at any moment.

Protein kinases attach phosphate to the hydroxyl group of specific amino acids on the target protein. There are two main types of protein kinase. The great majority are **serine/threonine kinases**, which phosphorylate the hydroxyl groups of serines and threonines in their targets. Others are **tyrosine kinases**, which

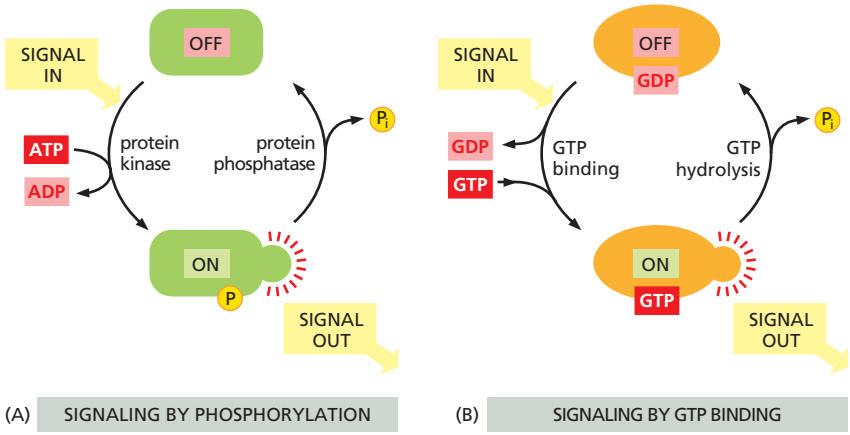

Figure 15–7 Two types of intracellular signaling proteins that act as molecular switches. (A) A protein kinase covalently adds a phosphate from ATP to the signaling protein, and a protein phosphatase removes the phosphate. Although not shown, many signaling proteins are activated by dephosphorylation rather than by phosphorylation. (B) A GTP-binding protein is induced to exchange its bound GDP for GTP, which activates the protein; the protein then inactivates itself by hydrolyzing its bound GTP to GDP.

phosphorylate proteins on tyrosines. The two types of protein kinase are closely related members of a large family, differing primarily in the structure of their protein substrate binding sites.

Many intracellular signaling proteins controlled by phosphorylation are themselves protein kinases, and these are often organized into **kinase cascades**. In such a cascade, one protein kinase, activated by phosphorylation, phosphorylates the next protein kinase in the sequence, and so on, relaying the signal onward and, in some cases, amplifying it or spreading it to other signaling pathways.

The other important class of molecular switches consists of **GTP-binding proteins** (discussed in Chapter 3). These proteins switch between an "on" (actively signaling) state when GTP is bound and an "off" state when GDP is bound. In the "on" state, they usually have intrinsic GTPase activity and shut themselves off by hydrolyzing their bound GTP to GDP (Figure 15–7B). There are two major types of GTP-binding proteins. Large, *trimeric GTP-binding proteins* (also called *G proteins*) help relay signals from G-protein-coupled receptors that activate them (see Figure 15–6B). Small **monomeric GTPases** (also called *monomeric GTP-binding proteins*) help relay signals from many classes of cell-surface receptors.

Specific regulatory proteins control both types of GTP-binding proteins. **GTPase-activating proteins** (**GAPs**) drive the proteins into an "off" state by increasing the rate of hydrolysis of bound GTP. Conversely, **guanine nucleotide exchange factors** (**GEFs**) activate GTP-binding proteins by promoting the release of bound GDP, which allows a new GTP to bind. In the case of trimeric G proteins, the activated receptor serves as the GEF. **Figure 15–8** illustrates the regulation of monomeric GTPases.

Not all molecular switches in signaling systems depend on phosphorylation or GTP binding. We see later that some signaling proteins are switched on or off by the binding of another signaling protein or a second messenger such as cyclic AMP or Ca^{2+}, or by covalent modifications other than phosphorylation or dephosphorylation, such as ubiquitylation (discussed in Chapter 3).

For simplicity, we often portray a signaling pathway as a series of activation steps (see Figure 15–1). It is important to note, however, that most signaling pathways contain inhibitory steps, and a sequence of two inhibitory steps can have the same effect as one activating step (**Figure 15–9**). This *double-negative* activation is very common in signaling systems, as we will see when we describe specific pathways later in this chapter.

Intracellular Signals Must Be Specific and Precise in a Noisy Cytoplasm

Ideally, an activated intracellular signaling molecule should interact only with the appropriate downstream targets, and, likewise, the targets should only be activated by the appropriate upstream signal. In reality, however, intracellular

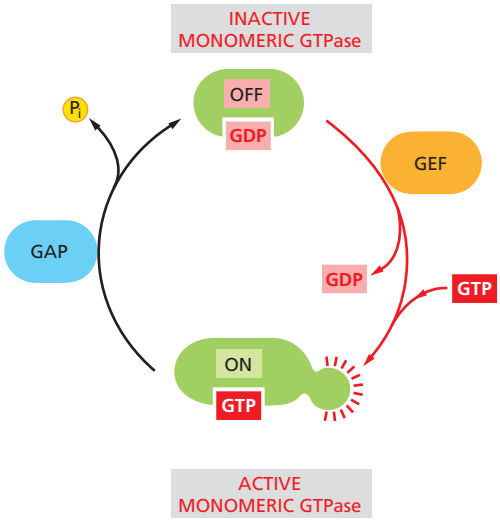

Figure 15–8 The regulation of a monomeric GTPase. GTPase-activating proteins (GAPs) inactivate the protein by stimulating it to hydrolyze its bound GTP to GDP, which remains tightly bound to the inactivated GTPase. Guanine nucleotide exchange factors (GEFs) activate the inactive protein by stimulating it to release its GDP; because the concentration of GTP in the cytosol is 10 times greater than the concentration of GDP, the protein rapidly binds GTP and is thereby activated.

signaling molecules share the cytoplasm with a crowd of closely related signaling molecules that control a diverse array of cellular processes. It is inevitable that an occasional signaling molecule will bind or modify the wrong partner, potentially creating unwanted cross-talk and interference between signaling systems. How does a signal remain strong, precise, and specific under these noisy conditions?

The first line of defense comes from the high affinity and specificity of the interactions between signaling molecules and their correct partners compared to the relatively low affinity of the interactions between inappropriate partners. The binding of a signaling molecule to the correct target is determined by precise and complex interactions between complementary surfaces on the two molecules. Protein kinases, for example, contain active sites that recognize a specific amino acid sequence around the phosphorylation site on the correct target protein, and they often contain additional *docking sites* that promote a specific, high-affinity interaction with the target. These and related mechanisms help provide a strong and persistent interaction between the correct partners, reducing the likelihood of inappropriate interactions with other proteins.

Another important way that cells avoid responses to unwanted background signals depends on the ability of many downstream target proteins to simply ignore such signals. These proteins respond only when the upstream signal reaches a high concentration or activity level. Consider a signaling pathway in which a protein kinase activates some downstream target protein by phosphorylation. If a response is triggered only when more than half of the target proteins are phosphorylated, then there will be little harm done if a small number of them are occasionally phosphorylated by some inappropriate protein kinase. Furthermore, constitutively active protein phosphatases will further reduce the impact of background phosphorylation by rapidly removing much of it. In these and other ways, intracellular signaling systems filter out noise, generating little or no response to low levels of stimuli.

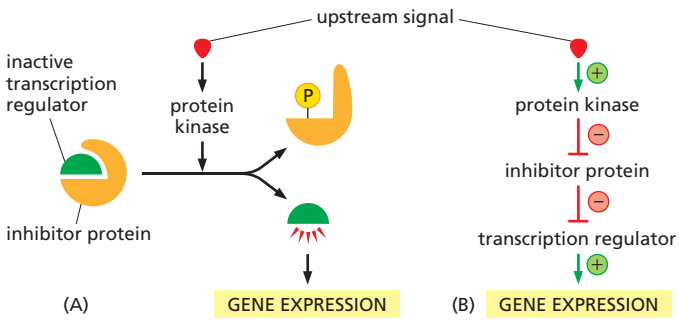

Figure 15–9 A sequence of two inhibitory signals produces a positive signal. (A) In this simple signaling system, a transcription regulator is kept in an inactive state by a bound inhibitor protein. In response to some upstream signal, a protein kinase is activated and phosphorylates the inhibitor, causing its dissociation from the transcription regulator and thereby activating gene expression. (B) This signaling pathway can be diagrammed as a sequence of four steps, including two sequential inhibitory steps that are equivalent to a single activating step.

Cells in a population often exhibit random variation in the concentration or activity of their intracellular signaling molecules. Similarly, individual molecules in a large population of molecules vary in their activity or interactions with other molecules. This *signal variability* introduces another form of noise that can interfere with the precision and efficiency of signaling. Most signaling systems, however, are built to generate remarkably robust and precise responses even when upstream signals are variable or even when some components of the system are disabled. In many cases, this *robustness* depends on the presence of backup mechanisms: for example, a signal might employ two parallel pathways to activate a single common downstream target protein, allowing the response to occur even if one pathway is crippled.

Intracellular Signaling Complexes Form at Activated Receptors

One simple and effective strategy for enhancing the specificity of interactions between signaling molecules is to localize them in the same part of the cell or even within large protein complexes, thereby ensuring that they interact only with each other and not with inappropriate partners. Such mechanisms often involve **scaffold proteins**, which bring together groups of interacting signaling proteins into *signaling complexes*, often before a signal has been received (**Figure 15–10**A). Because the scaffold holds the proteins in close proximity, they can interact at high local concentrations and be sequentially activated rapidly, efficiently, and selectively in response to an appropriate extracellular signal, avoiding unwanted cross-talk with other signaling pathways.

In other cases, such signaling complexes form only transiently in response to an extracellular signal and rapidly disassemble when the signal is gone. They often assemble around a receptor after an extracellular signal molecule has activated it. In many of these cases, the cytoplasmic tail of the activated receptor is phosphorylated during the activation process, and the phosphorylated amino acids then serve as docking sites for the assembly of other signaling proteins (Figure 15–10B). In yet other cases, receptor activation leads to the production of modified phospholipid molecules (called phosphoinositides) in the adjacent plasma membrane, which then recruit specific intracellular signaling proteins to this region of membrane, where they are activated (Figure 15–10C).

Modular Interaction Domains Mediate Interactions Between Intracellular Signaling Proteins

Simply bringing intracellular signaling proteins together into close proximity is sometimes sufficient to activate them. Thus, *induced proximity*, where a signal triggers assembly of a signaling complex, is commonly used to relay signals from protein to protein along a signaling pathway. The assembly of such signaling complexes depends on various highly conserved, small **interaction domains**, which are found in many intracellular signaling proteins. Each of these compact protein modules binds to a particular structural motif in another protein or lipid. The recognized motif in the interacting protein can be a short peptide sequence, a covalent modification (such as a phosphorylated amino acid), or another protein domain. The use of modular interaction domains presumably facilitated the evolution of new signaling pathways; because it can be inserted at many locations in a protein without disturbing the protein's folding or function, a new interaction domain added to an existing signaling protein could connect the protein to additional signaling pathways.

There are many types of interaction domains in signaling proteins. *Src homology 2* (*SH2*) *domains* and *phosphotyrosine-binding* (*PTB*) *domains*, for example, bind to phosphorylated tyrosines in a particular peptide sequence on activated receptors or intracellular signaling proteins. *Src homology 3* (*SH3*) domains bind to short, proline-rich amino acid sequences. Some *pleckstrin homology* (*PH*) domains bind to the charged head groups of specific phosphoinositides that are produced in the plasma membrane in response to an extracellular signal; they

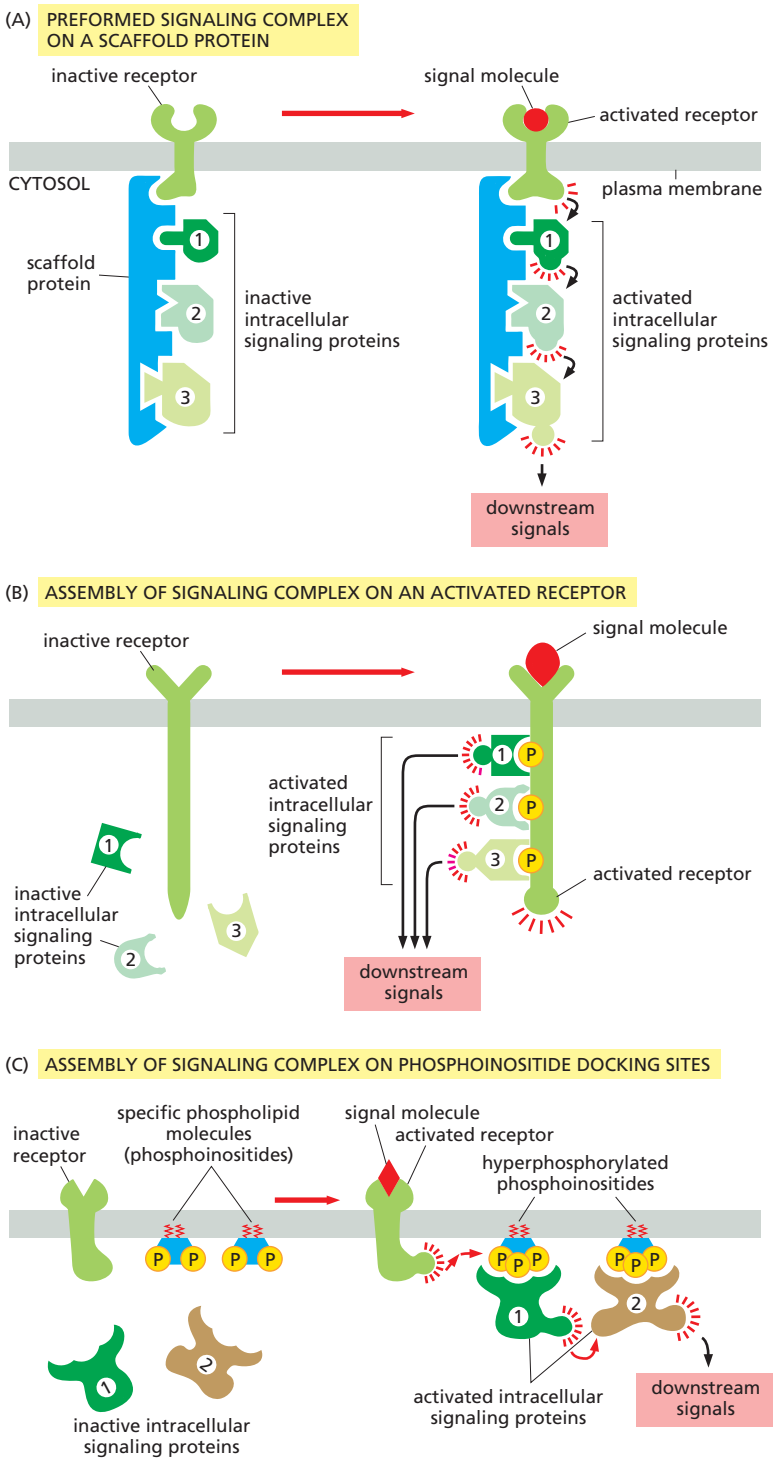

Figure 15–10 Three types of intracellular signaling complexes. (A) A receptor and some of the intracellular signaling proteins it activates in sequence are preassembled into a signaling complex on the inactive receptor by a large scaffold protein. (B) A signaling complex assembles transiently on a receptor only after the binding of an extracellular signal molecule has activated the receptor; here, the activated receptor phosphorylates itself at multiple sites, which then act as docking sites for intracellular signaling proteins. (C) Activation of a receptor leads to the increased phosphorylation of specific phospholipids (phosphoinositides) in the adjacent plasma membrane; these then serve as docking sites for specific intracellular signaling proteins, which can now interact with each other.

enable the protein they are part of to dock on the membrane and interact with other similarly recruited signaling proteins (see Figure 15–10C). Some signaling proteins consist solely of two or more interaction domains and function only as **adaptors** to link two other proteins together in a signaling pathway.

Interaction domains enable signaling proteins to bind to one another in multiple specific combinations. Like Lego® bricks, the proteins can form linear or branching chains or three-dimensional networks, which determine the route followed by the signaling pathway. As an example, **Figure 15–11** illustrates how some interaction domains mediate the formation of a large signaling complex around the receptor for the hormone *insulin*.

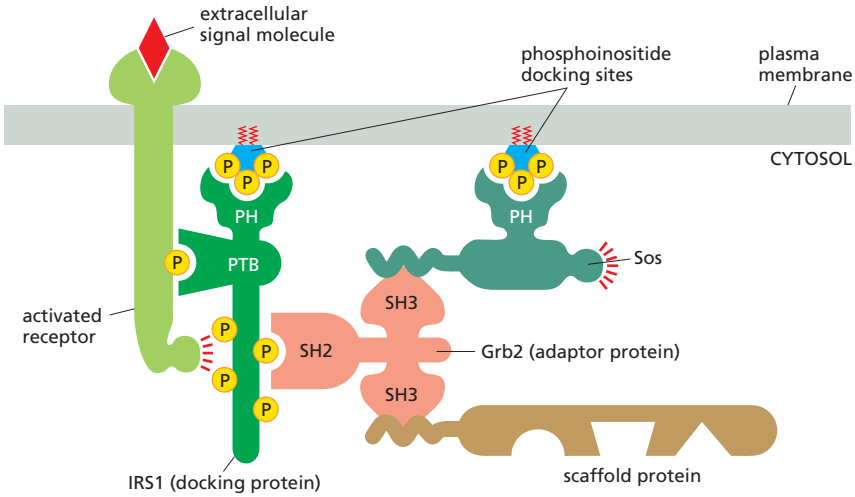

Figure 15–11 A specific signaling complex formed using modular interaction domains. This example is based on the insulin receptor, which is an enzyme-coupled receptor (a receptor tyrosine kinase, discussed later). First, the activated receptor phosphorylates itself on tyrosines, and one of the phosphotyrosines then recruits a docking protein called insulin receptor substrate-1 (IRS1) via a PTB domain of IRS1; the PH domain of IRS1 also binds to specific phosphoinositides on the inner surface of the plasma membrane. Then, the activated receptor phosphorylates IRS1 on tyrosines, and one of these phosphotyrosines binds the SH2 domain of the adaptor protein Grb2. Next, Grb2 uses one of its two SH3 domains to bind to a proline-rich region of a protein called Sos, which relays the signal downstream by acting as a GEF (see Figure 15–8) to activate a monomeric GTPase called Ras (not shown). Sos also binds to phosphoinositides in the plasma membrane via its PH domain. Grb2 uses its other SH3 domain to bind to a proline-rich sequence in a scaffold protein. The scaffold protein binds several other signaling proteins, and the other phosphorylated tyrosines on IRS1 recruit additional signaling proteins that have SH2 domains (not shown).

Another way of bringing receptors and intracellular signaling proteins together is to concentrate them in a specific region of the cell. An important example is the **primary cilium** that projects like an antenna from the surface of most vertebrate cells (discussed in Chapter 16). It is usually short and nonmotile and has microtubules in its core, and a number of surface receptors and signaling proteins are concentrated there. We shall see later that light and smell receptors are also highly concentrated in specialized cilia.