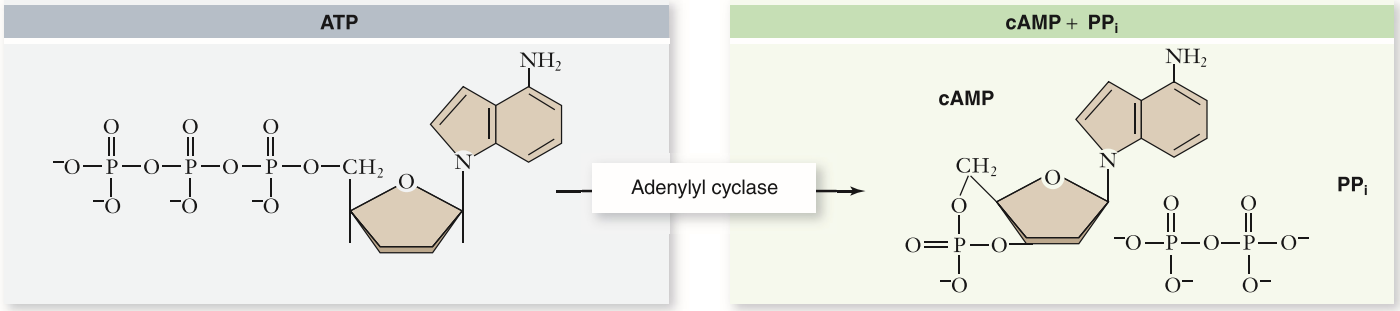
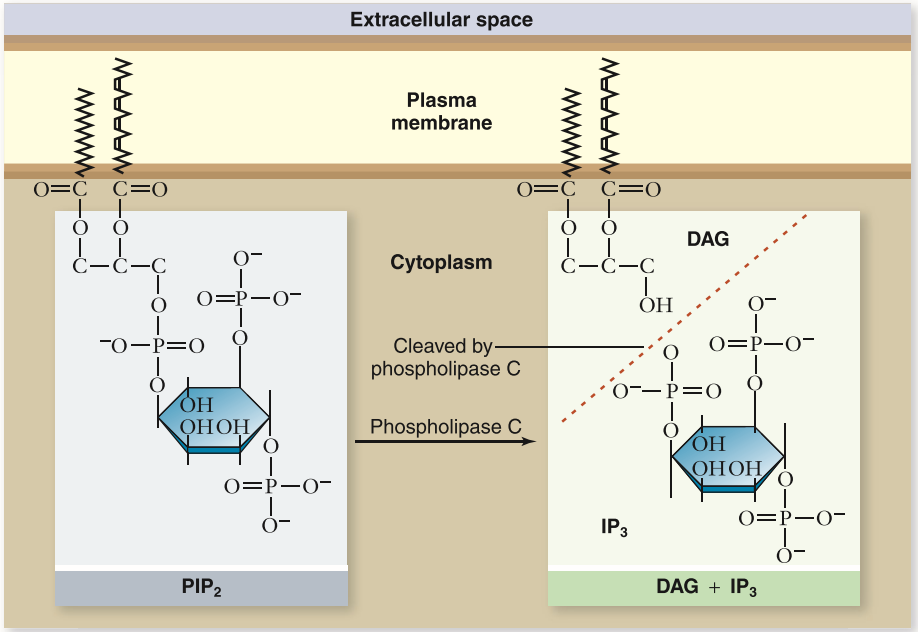


a.



b.

Figure 9.12 Production of second messengers. Second messengers are signaling molecules produced within the cell. ***a.*** The nucleotide ATP is converted by the enzyme adenylyl cyclase into cyclic AMP, or cAMP, and pyrophosphate (PP_i). ***b.*** The inositol phospholipid PIP_2 is composed of two lipids and a phosphate attached to glycerol. The phosphate is also attached to the sugar inositol. This molecule can be cleaved by the enzyme phospholipase C to produce two different second messengers: DAG, made up of the glycerol with the two lipids, and IP_3, inositol triphosphate.

(figure 9.12*a*). The cAMP then binds to and activates the enzyme protein kinase A (PKA), which adds phosphates to specific proteins in the cell (figure 9.13).

The effect of this phosphorylation on cell function depends on the identity of the cell and the proteins that are phosphorylated. In muscle cells, for example, PKA activates an enzyme necessary to break down glycogen and inhibits another enzyme necessary to synthesize glycogen. This leads to an increase in glucose available to the muscle. By contrast, in the kidney the action of PKA leads to the production of water channels that can increase the permeability of tubule cells to water.

Disruption of cAMP signaling can have a variety of effects. The symptoms of the disease cholera are due to altered cAMP levels in cells in the gut. The bacterium *Vibrio cholerae* produces a toxin that binds to a GPCR in the epithelium of the gut, causing it to be locked into an "on" state. This causes a large increase in intracellular cAMP that, in these cells, causes Cl^- ions to be transported out of the cell. Water follows the Cl^-, leading to diarrhea and dehydration characteristic of the disease.

The molecule cAMP is also an extracellular signal. In the slime mold *Dictyostelium discoideum,* secreted cAMP acts as a signal for aggregation under conditions of starvation. Experiments have shown that the receptor for this signal is also a GPCR (figure 9.14).

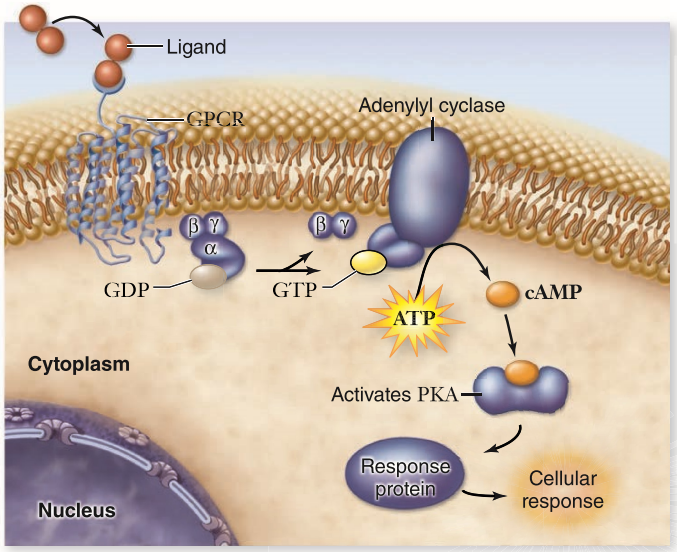


Figure 9.13 cAMP signaling pathway. Extracellular signal binds to a GPCR, activating a G protein. The G protein then activates the effector protein adenylyl cyclase, which catalyzes the conversion of ATP to cAMP. The cAMP then activates protein kinase A (PKA), which phosphorylates target proteins to cause a cellular response.

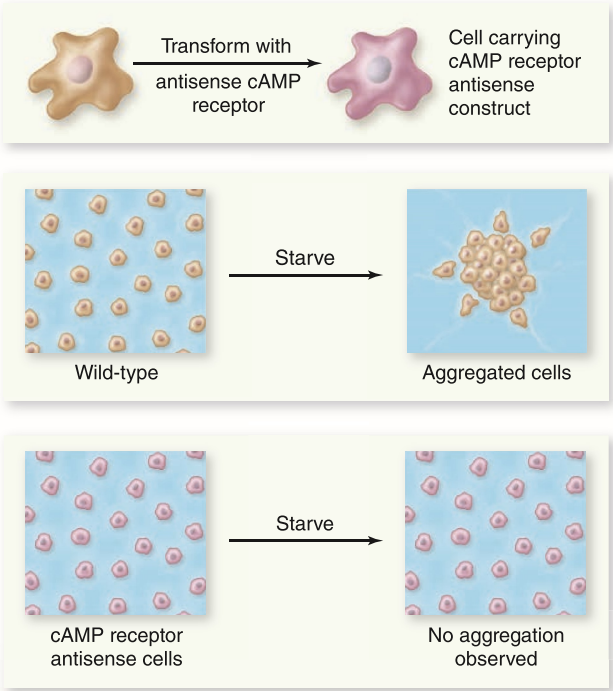
SCIENTIFIC THINKING

Question: *What is the receptor for cAMP?*

Hypothesis: *A previously identified G protein–coupled receptor is the cAMP receptor.*

Prediction: *If the function of the cAMP receptor is removed, then cells will not respond to starvation by aggregating.*

Test: *Use G protein–coupled receptor gene to direct synthesis of antisense RNA complementary to the normal mRNA. This will eliminate gene expression by the cellular copy of the G protein–coupled receptor.*



Result: *Cells transformed with the antisense construct do not aggregate normally.*

Conclusion: *Previously identified G protein–coupled receptor is the cAMP receptor, which controls the aggregation response.*

Further Experiments: *How can this kind of experiment be used to unravel other aspects of this signaling system?*

Figure 9.14 **The receptor for cAMP in *D. discoideum* is a GPCR.**

Inositol phosphates

A common second messenger is produced from the molecules called inositol phospholipids. These are inserted into the plasma membrane by their lipid ends and have the *inositol phosphate* portion protruding into the cytoplasm. The most common inositol phospholipid is phosphatidylinositol-4,5-bisphosphate (PIP_2). This molecule is a substrate of the effector protein phospholipase C, which cleaves PIP_2 to yield **diacylglycerol (DAG)** and **inositol-1,4,5-trisphosphate (IP_3)** (see figure 9.12*b*).

Both of these compounds then act as second messengers with a variety of cellular effects. DAG, like cAMP, can activate a protein kinase, in this case protein kinase C (PKC).

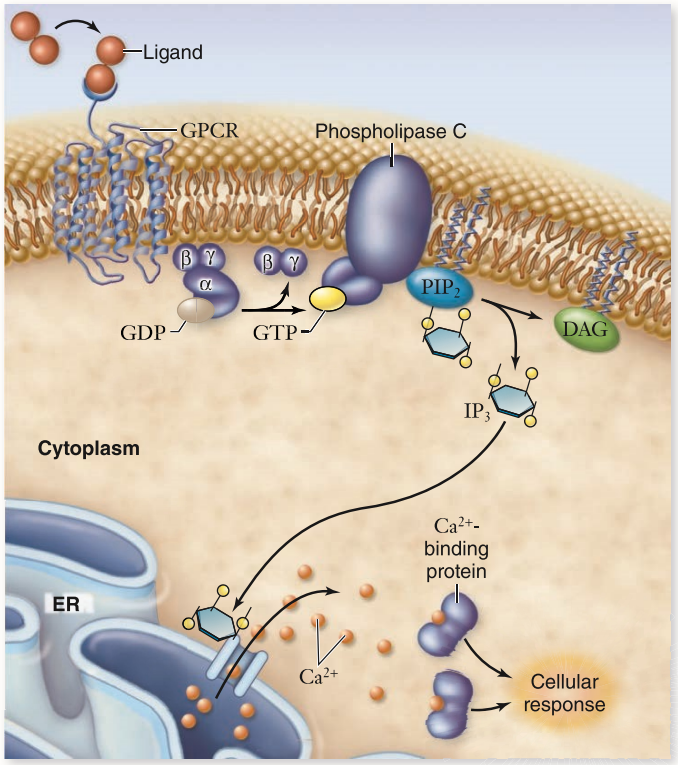


Figure 9.15 **Inositol phospholipid and Ca^{2+} signaling.** Extracellular signal binds to a GPCR activating a G protein. The G protein activates the effector protein phospholipase C, which converts PIP_2 to DAG and IP_3. IP_3 is then bound to a channel-linked receptor on the endoplasmic reticulum (ER) membrane, causing the ER to release stored Ca^{2+} into the cytoplasm. The Ca^{2+} then binds to Ca^{2+}-binding proteins such as calmodulin and PKC to cause a cellular response.

Calcium

Calcium ions (Ca^{2+}) serve widely as second messengers. Ca^{2+} levels inside the cytoplasm are normally very low (less than 10^{-7} M), whereas outside the cell and in the endoplasmic reticulum, Ca^{2+} levels are quite high (about 10^{-3} M). The endoplasmic reticulum has receptor proteins that act as ion channels to release Ca^{2+}. One of the most common of these receptors can bind the second messenger IP_3 to release Ca^{2+}, linking signaling through inositol phosphates with signaling by Ca^{2+} (figure 9.15).

The result of the outflow of Ca^{2+} from the endoplasmic reticulum depends on the cell type. For example, in skeletal muscle cells Ca^{2+} stimulates muscle contraction but in endocrine cells it stimulates the secretion of hormones.

Ca^{2+} initiates some cellular responses by binding to *calmodulin,* a 148-amino-acid cytoplasmic protein that contains four binding sites for Ca^{2+} (figure 9.16). When four Ca^{2+} ions are bound to calmodulin, the calmodulin/Ca^{2+} complex is able to bind to other proteins to activate them. These proteins include protein kinases, ion channels, receptor proteins, and cyclic nucleotide phosphodiesterases. These many uses of Ca^{2+} make it one of the most versatile second messengers in cells.

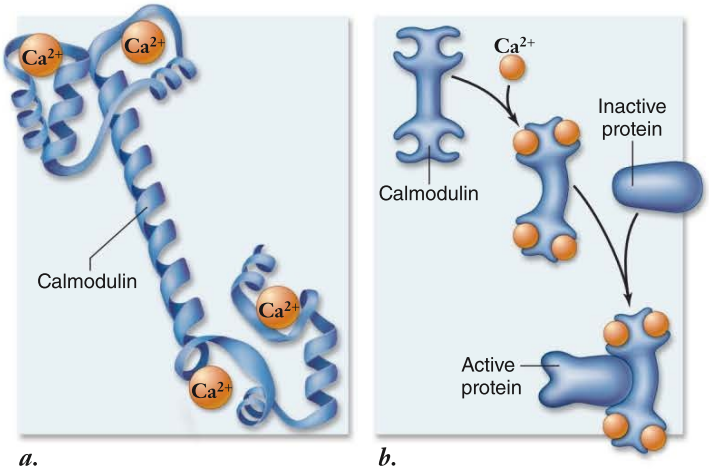


Figure 9.16 Calmodulin. *a.* Calmodulin is a protein containing 148 amino acid residues that mediates Ca^{2+} function. *b.* When four Ca^{2+} are bound to the calmodulin molecule, it undergoes a conformational change that allows it to bind to other cytoplasmic proteins and effect cellular responses.

Different receptors can produce the same second messengers

As mentioned in section 9.1, the two hormones glucagon and epinephrine can both stimulate liver cells to mobilize glucose. The reason that these different signals have the same effect is that they both act by the same signal transduction pathway to stimulate the breakdown and inhibit the synthesis of glycogen.

The binding of either hormone to its receptor activates a G protein that simulates adenylyl cyclase. The production of cAMP leads to the activation of PKA, which in turn activates another protein kinase called phosphorylase kinase. Activated phosphorylase kinase then activates glycogen phosphorylase, which cleaves off units of glucose 6-phosphate from the glycogen polymer (figure 9.17). The action of multiple kinases again leads to amplification such that a few signaling molecules result in a large number of glucose molecules being released.

At the same time, PKA also phosphorylates the enzyme glycogen synthase, but in this case it inhibits the enzyme, thus preventing the synthesis of glycogen. In addition, PKA phosphorylates other proteins that activate the expression of genes encoding the enzymes needed to synthesize glucose. This convergence of signal transduction pathways from different receptors leads to the same result—glucose is mobilized.

Receptor subtypes can lead to different effects in different cells

We also saw in section 9.1, how a single signaling molecule, epinephrine, can have different effects in different cells. One way this happens is through the existence of multiple forms of the same receptor. The receptor for epinephrine actually has nine different subtypes, or isoforms. These are encoded by different genes and are actually different receptor molecules. The sequences of these proteins are very similar, especially in the ligand-binding domain, which allows them to bind epinephrine. They differ mainly in their cytoplasmic domains, which interact with G proteins. This leads to different isoforms activating different G proteins, thereby leading to different signal transduction pathways.

Thus, in the heart, muscle cells have one isoform of the receptor that, when bound to epinephrine, activates a G protein that activates adenylyl cyclase, leading to increased cAMP. This increases the rate and force of contraction. In the intestine, smooth muscle cells have a different isoform of the receptor that, when bound to epinephrine, activates a different G protein that inhibits adenylyl cyclase, which decreases cAMP. This has the result of relaxing the muscle.

G protein–coupled receptors and receptor tyrosine kinases can activate the same pathways

Different receptor types can affect the same signaling module. For example, RTKs were shown to activate the MAP kinase cascade, but GPCRs can also activate this same cascade. Similarly,

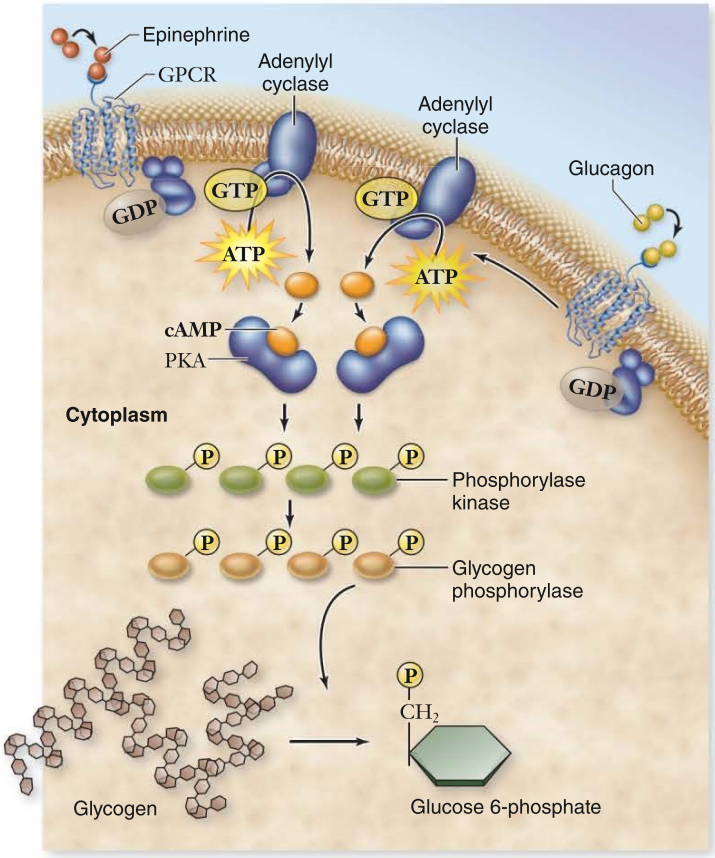


Figure 9.17 Different receptors can activate the same signaling pathway. The hormones glucagon and epinephrine both act through GPCRs. Each of these receptors acts via a G protein that activates adenylyl cyclase, producing cAMP. The activation of PKA begins a kinase cascade that leads to the breakdown of glycogen.

the activation of phospholipase C was mentioned earlier in this section in the context of GPCR signaling, but it can also be activated by RTKs.

This cross-reactivity may appear to introduce complications into cell function, but in fact it provides the cell with an incredible amount of flexibility. Cells have a large, but limited number of intracellular signaling modules, which can be turned on and off by different kinds of membrane receptors. This leads to signaling networks that interconnect possible cellular effectors with multiple incoming signals.

The Internet represents an example of a network in which many different kinds of computers are connected globally. This network can be broken down into subnetworks that are connected to the overall network. Because of the nature of the connections, when you send an e-mail message across the Internet, it can reach its destination through many different pathways. Likewise, the cell has interconnected networks of signaling pathways in which many different signals, receptors, and response proteins are interconnected. Specific pathways like the MAP kinase cascade, or signaling through second messengers like cAMP and Ca^{2+}, represent subnetworks within the global signaling network. A specific signal can activate different pathways in different cells, or different signals can activate the same pathway. We do not yet understand the cell at this level, but the field of systems biology is moving toward such global understanding of cell function.

Learning Outcomes Review 9.5

Signaling through GPCRs uses a three-part system—a receptor, a G protein, and an effector protein. G proteins are active when bound to GTP and inactive when bound to GDP. A ligand binding to the receptor activates the G protein, which then activates the effector protein. Effector proteins include adenylyl cyclase, which produces the second messenger cAMP. Another effector protein, phospholipase C, cleaves the inositol phosphates and results in the release of Ca^{2+} from the ER.

- ***There are far more GPCRs than any other receptor type. What is a possible explanation for this?***

Chapter Review

9.1 Overview of Cell Communication (figure 9.1)

Cell communication requires signal molecules, called ligands, binding to specific receptor proteins producing a cellular response.

Signaling is defined by the distance from source to receptor (figure 9.2).

Direct contact—molecules on the plasma membrane of one cell contact the receptor molecules on an adjacent cell.

Paracrine signaling—short-lived signal molecules are released into the extracellular fluid and influence neighboring cells.

Endocrine signaling—long-lived hormones enter the circulatory system and are carried to target cells some distance away.

Synaptic signaling—short-lived neurotransmitters are released by neurons into the gap, called a synapse, between nerves and target cells.

Signal transduction pathways lead to cellular responses.

Intracellular events initiated by a signaling event are called signal transduction.

Phosphorylation is key in control of protein function.

Proteins can be controlled by phosphate added by kinase and removed by phosphatase enzymes.

9.2 Receptor Types (figure 9.4)

Receptors are defined by location.

Receptors are broadly defined as intracellular or cell-surface receptors (membrane receptors).

Membrane receptors are transmembrane proteins that transfer information across the membrane, but not the signal molecule.

Membrane receptors include three subclasses.

Channel-linked receptors are chemically gated ion channels that allow specific ions to pass through a central pore.

Enzymatic receptors are enzymes activated by binding a ligand; these enzymes are usually protein kinases.

G protein–coupled receptors interact with G proteins that control the function of effector proteins: enzymes or ion channels.

Membrane receptors can generate second messengers.

Some enzymatic and most G protein–coupled receptors produce second messengers, to relay messages in the cytoplasm.

9.3 Intracellular Receptors (figure 9.5)

Many cell signals are lipid-soluble and readily pass through the plasma membrane and bind to receptors in the cytoplasm or nucleus.

Steroid hormone receptors affect gene expression.

Steroid hormones bind cytoplasmic receptors, then are transported to the nucleus. Thus, they are called nuclear receptors. These can directly affect gene expression, usually activating transcription of the genes they control.

Nuclear receptors have three functional domains: hormone-binding, DNA-binding, and transcription-activating domains.

Ligand binding changes receptor shape, releasing an inhibitor occupying the DNA-binding site.

A cell's response to a lipid-soluble signal depends on the hormone–receptor complex and the other protein coactivators present.

Other intracellular receptors act as enzymes.

9.4 Signal Transduction Through Receptor Kinases

Receptor kinases in plants and animals recognize hydrophilic ligands and influence the cell cycle, cell migration, cell metabolism, and cell proliferation.

Because they are involved in growth control, alterations of receptor kinases and their signaling pathways can lead to cancer.

RTKs are activated by autophosphorylation.

The activated receptor can also phosphorylate other intracellular proteins.

Phosphotyrosine domains mediate protein–protein interactions.

Adapter proteins can bind to phosphotyrosine and act as links between the receptors and downstream signaling events.

Protein kinase cascades can amplify a signal.

Scaffold proteins organize kinase cascades.

Scaffold proteins and protein kinases form a single complex where the enzymes act sequentially and are optimally functional.

Internalized receptors are degraded or recycled.

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

Small G proteins act as molecular switches linking external signals to signal transduction pathways.

RTKs are inactivated by internalization.

9.5 Signal Transduction Through G Protein–Coupled Receptors (figure 9.11)

G protein–coupled receptors function through activation of G proteins.

G proteins link receptors with effector proteins.

G proteins are active bound to GTP and inactive bound to GDP. Receptors promote exchange of GDP for GTP.

The activated G protein dissociates into two parts, G_α and $G_{\beta\gamma}$, each of which can act on effector proteins.

G_α also hydrolyzes GTP to GDP to inactivate the G protein.

Effector proteins produce multiple second messengers.

Two common effector proteins are adenylyl cyclase and phospholipase C, which produce second messengers known as cAMP, and DAG and IP_3, respectively.

Ca^{2+} is also a second messenger. Ca^{2+} release is triggered by IP_3 binding to channel-linked receptors in the ER.

Ca^{2+} can bind to a cytoplasmic protein calmodulin, which in turn activates other proteins, producing a variety of responses.

Different receptors can produce the same second messengers.

Different GPCR receptors can converge to activate the same effector enzyme and thus produce the same second messenger.

Receptor subtypes can lead to different effects in different cells.

Epinephrine causes increased contraction in heart muscle but relaxation in smooth muscle.

G protein–coupled receptors and receptor tyrosine kinases can activate the same pathways.

Both RTKs and GPCRs can activate MAP kinase cascades.

Review Questions

UNDERSTAND

1. Paracrine signaling is characterized by ligands that are
   a. produced by the cell itself.
   b. secreted by neighboring cells.
   c. present on the plasma membrane of neighboring cells.
   d. secreted by distant cells.
2. Signal transduction pathways
   a. are necessary for signals to cross the membrane.
   b. include the intracellular events stimulated by an extracellular signal.
   c. include the extracellular events stimulated by an intracellular signal.
   d. are only found in cases where the signal can cross the membrane.
3. The function of a _____ is to add phosphates to proteins, whereas a _____ functions to remove the phosphates.
   a. tyrosine; serine
   b. protein phosphatase; protein dephosphatase
   c. protein kinase; protein phosphatase
   d. receptor; ligand
4. Which of the following receptor types is NOT a membrane receptor?
   a. Channel-linked receptor
   b. Enzymatic receptor
   c. G protein–coupled receptor
   d. Steroid hormone receptors
5. How does the function of an intracellular receptor differ from that of a membrane receptor?
   a. The intracellular receptor binds a ligand.
   b. The intracellular receptor binds DNA.
   c. The intracellular receptor activates a kinase.
   d. The intracellular receptor functions as a second messenger.
6. Signaling through receptor tyrosine kinases often
   a. leads to the production of the second messenger cAMP.
   b. leads to the production of the second messenger IP_3.
   c. stimulates gene expression directly.
   d. leads to the activation of a cascade of kinase enzymes.

7. What is the function of Ras during tyrosine kinase cell signaling?
    a. It activates the opening of channel-linked receptors.
    b. It is an enzyme that synthesizes second messengers.
    c. It links the receptor protein to the MAP kinase pathway.
    d. It phosphorylates other enzymes as part of a pathway.
8. Which of the following best describes the immediate effect of ligand binding to a G protein–coupled receptor?
    a. The G protein trimer releases a GDP and binds a GTP.
    b. The G protein trimer dissociates from the receptor.
    c. The G protein trimer interacts with an effector protein.
    d. The α subunit of the G protein becomes phosphorylated.

APPLY

1. The action of steroid hormones is often longer-lived than that of peptide hormones. This is because they
    a. enter the cell and act like enzymes for a longer period of time.
    b. they turn on gene expression to produce proteins that persist in the cell.
    c. result in the production of second messengers that act directly on cellular processes.
    d. stimulate G proteins that act directly on cellular processes.
2. The ion Ca^{2+} can act as a second messenger because it is
    a. produced by the enzyme calcium synthase.
    b. normally at a high level in the cytoplasm.
    c. normally at a low level in the cytoplasm.
    d. stored in the cytoplasm.
3. Different receptors can have the same effect on a cell. One reason for this is that
    a. most receptors produce the same second messenger.
    b. different isoforms of receptors bind different ligands, but stimulate the same signaling pathway.
    c. signal transduction pathways intersect—the same pathway can be stimulated by different receptors.
    d. all receptors converge on the same signal transduction pathways.
4. In comparing small G proteins like Ras and GPCR proteins, we can say that
    a. both proteins have intrinsic GTPase activity that stops signaling.
    b. both proteins are active bound to GTP.
    c. Ras is active bound to GDP and GPCRs are active bound to GTP.
    d. both a and b are true.
5. The same signal can have different effects in different cells because there
    a. are different receptor subtypes that initiate different signal transduction pathways.
    b. may be different coactivators in different cells.
    c. may be different target proteins in different cells' signal transduction pathways.
    d. All of the choices are correct.
6. The receptors for steroid hormones and peptide hormones are fundamentally different because
    a. of the great difference in size of the molecule.
    b. peptides are one of the four major polymers and steroids are simple ringed structures.
    c. peptides are hydrophilic and steroids are hydrophobic.
    d. peptides are hydrophobic and steroids are hydrophilic.

SYNTHESIZE

1. Describe the common features found in all examples of cellular signaling discussed in this chapter. Provide examples to illustrate your answer.
2. The sheet of cells that form the gut epithelium folds into peaks called villi and valleys called crypts. The cells within the crypt region secrete a protein, Netrin-1, that becomes concentrated within the crypts. Netrin-1 is the ligand for a receptor protein that is found on the surface of all gut epithelial cells. Netrin-1 binding triggers a signal pathway that promotes cell growth. Gut epithelial cells undergo apoptosis (cell death) in the absence of Netrin-1 ligand binding.
    a. How would you characterize the type of signaling (autocrine, paracrine, endocrine) found in this system?
    b. Predict where the greatest amount of cell growth and cell death would occur in the epithelium.
    c. The loss of the Netrin-1 receptor is associated with some types of colon cancer. Suggest an explanation for the link between this signaling pathway and tumor formation.