15 Cell Signaling I: Signal Transduction and Short-Term Cellular Effects

Review the Concepts

1. Extracellular signaling systems usually involve (1) synthesis and (2) release of the signaling molecule by the signaling cell; (3) transport of the signal to the target cell; (4) binding of the signal by a specific receptor protein leading to a conformational change; (5) initiation of one or more intracellular signal-transduction pathways by the activated receptor; (6) specific changes in cellular function, metabolism, or development; (7) feedback regulation usually involving deactivation of the receptor; and (8) removal of the signaling molecule which together terminates the cellular response (see Figure 15-1).

   Extracellular signals are made by signaling cells. Receptor proteins are present in target cells. Binding of extracellular signaling molecules to cell-surface receptors triggers a conformational change in the receptor, which in turn leads to intracellular signal-transduction pathways that ultimately modulate cellular metabolism, function, or gene expression. Intracellular signal transduction pathways are evolutionarily highly conserved.

2. Endocrine, paracrine, and autocrine signaling differ according to the distance over which the signaling molecule acts. In endocrine signaling, signaling molecules are released by a cell and act on target cells at a distance. In animals, the signaling molecule is carried to target cells by the blood or other extracellular fluids. In paracrine signaling, the signaling molecules are released and affect only target cells in close proximity. In autocrine signaling, the cell that releases the signaling molecule is also the target cell. Growth hormone is an example of endocrine signaling because the growth hormone is synthesized in the pituitary, located at the base of the brain, and travels to the liver via the blood.

3. The ligand-receptor complex that shows the lower K_d value has the higher affinity. Because the K_d for receptor 2 (10^{-9} M) is lower than that for receptor 1 (10^{-7} M), the ligand shows greater affinity for receptor 2 than for receptor 1. To calculate the fraction of receptors with bound ligand, $[RL]/R_T$, use Equation 15-2 $[RL]/R_T = 1/(1 + K_d/[L])$. For receptor 1, the K_d is10^{-7} M and the concentration of free ligand [L] is 10^{-8} M. Thus the $[RL]/R_T$ for receptor 1 is 0.091, that is, only 9% of the receptors have bound ligand at a free ligand concentration of 10^{-8} M. In contrast, the $[RL]/R_T$ for receptor 2 is 0.91; 91% of the receptors have bound ligand.

4. To purify a receptor by affinity chromatography, a ligand for the receptor must first be chemically linked to a bead used to form a column. A detergent-solubilized cell membrane extract containing the receptor is then passed through the column. The receptor will bind to the ligand attached to the bead and other proteins will wash out. The receptor can then be eluted from the column with an excess of ligand. A cell surface receptor can be cloned using a functional expression assay. In this approach, cDNAs are synthesized using mRNA extracted from cells expressing the receptor and are inserted into expression vectors. These recombinant plasmids are transfected into cells that do not express the receptor. The rare cells that express the receptor of interest can then be identified by biochemical assays.

5. For trimeric G proteins in the inactive state, $G_{\alpha s}$ is bound to GDP and complexed with $G_{\beta\gamma}$. Upon ligand binding to its receptor, the receptor undergoes a conformational change that affects the associated trimeric G protein. GTP is exchanged for GDP and the $G_{\alpha s}$-GTP complex dissociates from the $G_{\beta\gamma}$ complex. The released $G_{\alpha s}$-GTP or $G_{\beta\gamma}$ complexes then activate downstream effector proteins. Hydrolysis of GTP to GDP by the intrinsic GTPase activity of $G_{\alpha s}$

returns $G_{\alpha s}$ to the inactive state bound to GDP and the $G_{\beta\gamma}$ complex. A mutant G_α subunit with increased GTPase activity would be expected to hydrolyze GTP to GDP at a faster rate and thus reduce the time that the G_α subunit remains in the active state. This, in turn, would lead to reduced activation of the effector protein.

6. Binding of extracellular signaling molecules to cell-surface receptors triggers a conformational change in the receptor, which activates second messenger systems inside the cell. Signal amplification is possible in part because both receptors and G proteins can diffuse rapidly in the plasma membrane. For example, a single epinephrine-GPCR complex causes conversion of up to 100 inactive G proteins to the active form. Each active $G_{\alpha s} \cdot$ GTP, in turn, activates a single adenylyl cyclase molecule (see Figure 15-21), which then catalyzes synthesis of many cAMP molecules during the time $G_{\alpha s} \cdot$ GTP is bound to it.

   Second messengers such as Ca^{2+} and cAMP rapidly diffuse through the cytosol and bind to intracellular targets. Activation of a *single* cell-surface receptor molecule can result in an increase in thousands of cAMP molecules or Ca^{2+} ions in the cytosol. Further signal amplification is possible because second messengers can initiate a cascade of protein activation or inhibition. Second messengers activate target proteins (PK-A or calmodulin, respectively), which in turn can affect the activity of multiple downstream proteins. The amplification that occurs in such a cascade depends on the number of steps in it.

7. Cholera toxin can penetrate the plasma membrane of cells. In the cytosol it catalyzes a chemical modification of G_α proteins that prevents hydrolysis of bound GTP to GDP. As a result, G_α remains in the active state. This causes continuous activation of adenylyl cyclase even in the absence of hormonal stimulation. The resulting excessive rise in intracellular cAMP leads to the loss of electrolytes and water into the intestinal lumen, producing the watery diarrhea and dramatic fluid loss characteristic of cholera infections.

8. Epinephrine binding to the β-adrenergic receptor causes an activation of adenylyl cyclase through the activation of $G_{\alpha s}$, a stimulatory G protein. In contrast, epinephrine binding to the α-adrenergic receptor causes an inhibition of adenylyl cyclase through the activation of $G_{\alpha i}$, an inhibitory G protein. An agonist acts like the normal hormone, which in this case would be epinephrine. Thus agonist binding to a β-adrenergic receptor would result in activation of adenylyl cyclase. In contrast, an antagonist binds to the receptor but does not activate the receptor. Thus antagonist binding to a β-adrenergic receptor would have no effect on adenylyl cyclase activity. In fact, it would reduce a normal epinephrine stimulated response because it would prevent epinephrine from binding to the receptor.

9. Activation of muscarinic acetylcholine receptors in cardiac muscle slows the rate of heart muscle contraction. These receptors are coupled to an inhibitory G protein. Activation of this system causes a decrease in cAMP in the cell that leads to opening of K^+ channels on the cell membrane. The muscle cell becomes hyperpolarized, which reduces the frequency of muscle contraction.

   Rhodopsin is a G protein–coupled receptor that is activated by light. Rhodopsin contains a light absorbing pigment, 11-*cis*-retinal, that is covalently linked to opsin. In the presence of light, 11-*cis*-retinal is converted to all-*trans*-retinal. This activated opsin then interacts and activates transducin, an associated G protein. The activated G_α-GTP complex binds to the inhibitory subunit of a phosphodiesterase. The released catalytic subunits of the phosphodiesterase hydrolyzes cGMP to 5′-GMP. As a result, the cGMP level declines, leading to the closing of a nucleotide-gated ion channel. As with the cardiac muscle system, signal activation ultimately results in hyperpolarization of the photoreceptor cells.

10. Epinephrine binds to its receptor and activates the $G_{\alpha s}$ subunit of the trimeric G protein. The activated $G_{\alpha s}$-GTP complex then binds to and activates adenylyl cyclase, leading to an increase in the levels of cAMP. The resultant rise in cAMP activates protein kinase A. To attenuate the signal, cAMP is hydrolyzed to 5′-AMP by the

action of cAMP phosphodiesterase. In addition, the intrinsic GTPase activity of the $G_{\alpha s}$ subunit hydrolyzes GTP to GDP, which converts the $G_{\alpha s}$ subunit to its inactive form bound to $G_{\beta\gamma}$. Thus, adenylyl cyclase is inactivated, blocking the further synthesis of cAMP. When camp levels drop, protein kinase A is inactivated.

11. Receptor desensitization can involve phosphorylation of the receptor itself. The increase in cAMP levels as a result of ligand binding to the receptor leads to an activation of protein kinase A. Protein kinase A can phosphorylate target proteins as well as cytosolic serine and threonine residues in the receptor itself. Phosphorylated receptor can bind ligand but is reduced in its ability to activate adenylyl cyclase. Thus the receptor is desensitized to the effect of ligand binding. Phosphorylated receptors are resensitized by the removal of phosphates by phosphatases. A mutant receptor that lacked serine or threonine phosphorylation sites could be resistant to desensitization by phosphorylation and thus would continuously activate adenylyl cyclase in the presence of ligand.

12. During receptor desensitization, the β-adrenergic receptor is phosphorylated by protein kinase A and also by the β-adrenergic receptor kinase (BARK). BARK phosphorylates only activated receptor, i.e., receptor bound to its ligand. The phosphorylated receptor is reduced in its ability to activate downstream effector proteins. During visual adaptation, activated opsin is phosphorylated by rhodopsin kinase. Phosphorylated opsin is reduced in its ability to activate its associated G protein, $G_{\alpha\tau}$. In both cases, receptor resensitization is mediated by dephosphorylation of the receptor by a phosphatase.

13. AKAP15, a member of a family of anchoring proteins, localizes PKA near a particular type of gated Ca^{2+} channel in certain heart muscle cells, thereby reducing the time that otherwise would be required for diffusion of PKA catalytic subunits from their sites of generation to their Ca^{2+}-channel substrates. Another AKAP in heart muscle anchors both PKA and cAMP phosphodiesterase (PDE) to the outer nuclear membrane. Because of the close proximity of PDE to PKA, negative feedback provides tight local control of the cAMP concentration and hence local PKA activity.

14. Cleavage of PIP_2 by phospholipase C generates IP_3 and DAG. IP_3 interacts with and opens Ca^{2+} channels in the endoplasmic reticulum (ER) membrane, resulting in release of Ca^{2+} from the ER. Ca^{2+}-ATPase pumps located in the ER membrane pump cytosolic Ca^{2+} back into the ER lumen. Because Ca^{2+} is also pumped from the cytosol to the exterior of the cell by Ca^{2+}-ATPase pumps in the plasma membrane, Ca^{2+} must be transported back into the cell. The IP_3-gated Ca^{2+} channels bind to and open store-operated Ca^{2+} channels in the plasma membrane, allowing an influx of Ca^{2+}. The principal function of DAG is to activate protein kinase C, which then phosphorylates specific target proteins.

15. Orexins, also known as hypocretins, are peptide signaling proteins produced by neurons in the lateral hypothalmus. Recent research has shown that orexins are involved in appetite stimulation, regulation of sleep/wakefulness, and possibly other aspects of regulation of energy expenditure. Orexin receptors are G protein–coupled receptor proteins. When rats are given orexins, they dramatically increase their food intake. Orexin deficiency can lead to narcolepsy.

Analyze the Data

a. For the wild-type G protein, the activity of adenylyl cyclase is what you would expect. In the presence of GTP there is a basal level of adenylyl cyclase activity, which can be greatly stimulated by the addition of isoproterenol. Isoproterenol binds to the β_2-adrenergic receptor and causes activation of adenylyl cyclase. In comparing adenylyl cyclase activity in the presence of GTP or $GTP_{\gamma S}$, again the expected result is seen. The addition of $GTP_{\gamma S}$ leads to an increase in adenylyl cyclase activity because $GTP_{\gamma S}$ is non-hydrolyzable. Thus the $G_{\alpha s}$ subunit remains active, leading to prolonged activation of adenylyl cyclase. In the case of the mutant, again the addition of isoproterenol results in an increase in adenylyl cyclase activity as expected. The adenylyl cyclase activity, however, is not different

in the presence of GTP or $GTP_{\gamma S}$. Thus the mutation causes an increase in the basal activity of adenylyl cyclase, likely due to a change in the GTPase activity.

b. In cells transfected with the mutant G protein, higher levels of adenylyl cyclase would be present relative to cells transfected with wild-type G protein. Thus mutant-transfected cells would have higher cAMP levels, which would result in higher levels of active protein kinase A. The higher protein kinase A levels would result in more extensive phosphorylation of target proteins, which would affect normal cell development and proliferation.

c. From the GTPase results, it is clear that the mutation affects the intrinsic GTPase activity of the $G_{\alpha s}$ subunit. These results are consistent with the adenylyl cyclase results. For the mutant G protein, binding GTP or $GTP_{\gamma S}$ to the $G_{\alpha s}$ subunit leads to the same level of adenylyl cyclase activation because the $G_{\alpha s}$ subunit has greatly reduced ability to cleave GTP.