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RECEPTORS, HORMONES, AND CELL SIGNALING

REVIEW THE CONCEPTS

1. The following common features are shared by most cell-signaling systems: 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.Inparacrine sig-naling, the signaling molecules are released and affect only target cells in close prox-imity. 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 affi nity. 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 is 10^{-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 affi nity chromatography , a ligand for the receptor is chemically linked to beads 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 beads and other proteins will wash out. The receptor can then be eluted from the column with an excess of ligand. Analyzing its molecular weight by SDS-PAGE may be suffi cient to identify the recovered receptor or provide protein for sequencing.
   A similar column chromatography approach or an alternative affi nity approach known as a pull-down assay can be used to isolate active G protein. To isolate active G with a pull-down assay, the adenylyl cyclase (AC) effector enzyme to which only the active form of G binds is linked to beads either directly by chemical linkage or indirectly through an anti-AC antibody bound to protein A beads. Mixing the AC-coupled beads with a cell extract and then pel-leting the beads by centrifugation pulls down only active G because inactive G (the GDP-bound form) fails to interact with the effector enzyme. The amount of G pulled down can be quantifi ed under different signaling conditions.

5. Binding of the extracellular signal or, in the case of rhodopsin, absorption of a photon by retinal, causes a conformational change in the orientation of the transmembrane helices that changes the conformation of the C3 loop and C4 segment (and in some cases the C2 loop) on the cytosolic side of the receptor. This increases the affi nity of the receptor cytosolic region for binding and activating a G protein.

6. For trimeric G proteins in the inactive state, G_α 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_α-GTP or $G_{\beta\gamma}$ complexes then activate downstream effector proteins. Hydrolysis of GTP to GDP by the intrinsic GTPase activity of G_α returns G_α 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.

7. As in Figure 15-17, the $G_{\alpha s}$ protein could be expressed as a CFP-fusion protein and adenylyl cyclase, instead of $G_{\beta\gamma}$, could be expressed as a YFP-fusion protein. In this case, association of $G_{\alpha s}$ with adenylyl cyclase would yield an increase in energy transfer and fl uorescence at 527 nm when excited with 440 nm light.

8. Steps at which a single active component activates multiple targets amplify the signal. Each active receptor activates multiple $G_{\alpha s}$ proteins, and each active PKA initiates a short kinase cascade by phosphorylating multiple GPKs, each of which phosphorylates multiple glycogen phosphorylase enzymes to continue the signal amplification. In contrast, each active $G_{\alpha s}$ protein activates only one AC, and each cAMP participates in the activation of only one PKA, neither of which directly

amplifies the signal response. An increase in the number of receptors would have a greater effect on amplification because proportionally more $G_{\alpha s}$ proteins would be activated for the same level of epinephrine, whereas if only the number of $G_{\alpha s}$ proteins were increased, the signal response would still depend on the number of receptors activated.

9. Cholera toxin can penetrate the plasma membrane of cells. In the cytosol it catalyzes a chemical modifi cation 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 fl uid loss characteristic of cholera infections.

10. 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 nucleo-tide-gated ion channel. As with the cardiac muscle system, signal activation ultimately results in hyperpolarization of the photoreceptor cells.

11. The epinephrine-cAMP signaling pathway—from binding of epinephrine to the receptor to activation of PKA—is essentially the same in all the cells. The downstream biochemical pathway activated is specifi ed by the substrate(s) phosphorylated by PKA.

12. 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.

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14. Cleavage of PIP_2 by phospholipase C generates IP_3 and DAG. IP_3 opens IP_3-gated Ca^{2+} channels in the endoplasmic reticulum (ER) membrane, resulting in release of Ca^{2+} from the ER. When ER stores of Ca^{2+} are depleted, 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+}. To restore resting levels of cytosolic Ca^{2+} ($<10^{-7}$ M), Ca^{2+}-ATPase pumps located in the ER membrane and in the plasma membrane pump cytosolic Ca^{2+} back into the ER lumen and out of the cell. The principal function of DAG is to activate protein kinase C, which then phosphorylates specifi c target proteins.

15. Prior to stimulation of the IP_3-DAG signaling pathway, Ca^{2+}-ATPases establish a resting level of Ca^{2+} in the cytosol of $<10^{-7}$ M, at which level few calmodulin sites have Ca^{2+} bound and the calmodulin is inactive. IP_3 stimulates a rise in cytosolic Ca^{2+} concentration to $>10^{-5}$ M, at which level most calmodulin sites have Ca^{2+} bound and calmodulin is active. The Ca^{2+} binding affinity of calmodulin is exquisitely tuned to bind and release Ca^{2+} in response to the physiological changes of Ca^{2+} concentration in the cytoplasm used for signaling.

16. In rod cells, cGMP opens cation channels, whereas the primary activity of cGMP in smooth muscle cells, like cAMP, is to activate a kinase.