0. Cell-to-Cell Signaling: Hormones and Receptors

o cell lives in isolation. In all **multicellular organisms**, survival depends on an aborate **intercellular communication network** that coordinates the growth, fferentiation, and metabolism of the multitude of cells in diverse tissues and organs. ells within small groups often communicate by **direct cell-cell contact**. Specialized nctions in the plasma membranes of adjacent cells permit them to exchange small olecules and to coordinate metabolic responses; other junctions between adjacent ells determine the shape and rigidity of many tissues. In addition, the establishment of pecific cell-cell interactions between different types of cells is a necessary step in the evelopment of many tissues. In some cases a particular protein on one cell binds to a eceptor protein on the surface of an adjacent target cell, triggering its differentiation. In is chapter, we examine how cells communicate by means of **extracellular signaling olecules**. These substances are synthesized and **released by *signaling cells*** and roduce a **specific response** only in ***target cells*** that have **receptors** for the signaling olecules. An enormous variety of chemicals, including small molecules (e.g., amino cid derivatives, acetylcholine), peptides, and proteins, are used in this type of cell-to-ell communication. The extracellular products synthesized by signaling cells can diffus way or be transported in the blood, thus providing a means for cells to communicate ver longer distances than is possible by chains of direct cell-cell contacts.

0.1. Overview of Extracellular Signaling

ommunication by extracellular signals usually involves six steps:

1) **synthesis** and

2) **release** of the signaling molecule by the signaling cell;

3) **transport** of the signal to the target cell;

4) **detection** of the signal by a specific receptor protein;

5) **a change (signal transduction pathways)** in cellular metabolism, function, or development triggered by the receptor-signal complex; and

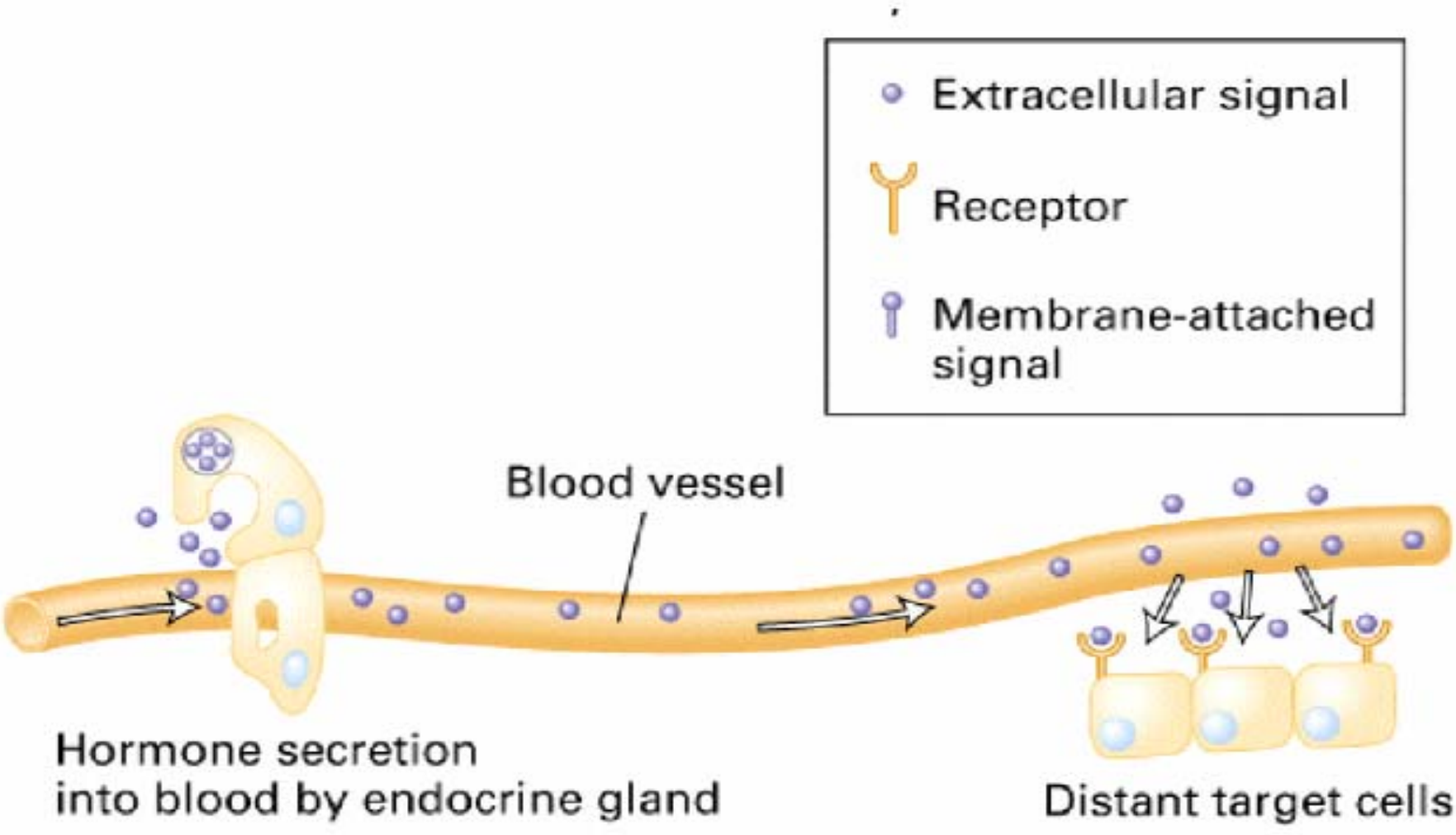
6) **removal of the signal**, which often terminates the cellular response.

hemicals released by one organism that can alter the behavior or gene expression of ther organisms of the same species are called pheromones. Yeast mating-type factors scussed later in this chapter are a well-understood example of pheromone-mediated ell-to-cell signaling. Some algae and animals also release pheromones, usually spersing them into the air or water, to attract members of the opposite sex.

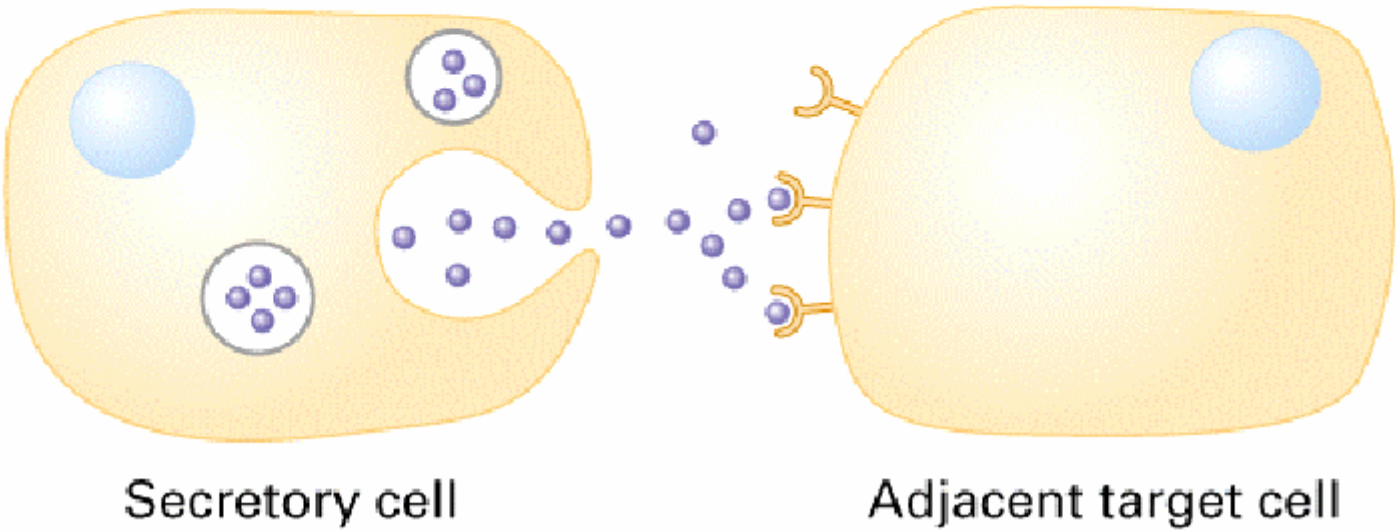
General schemes of intercellular signaling in animals

(A) Endocrine signaling

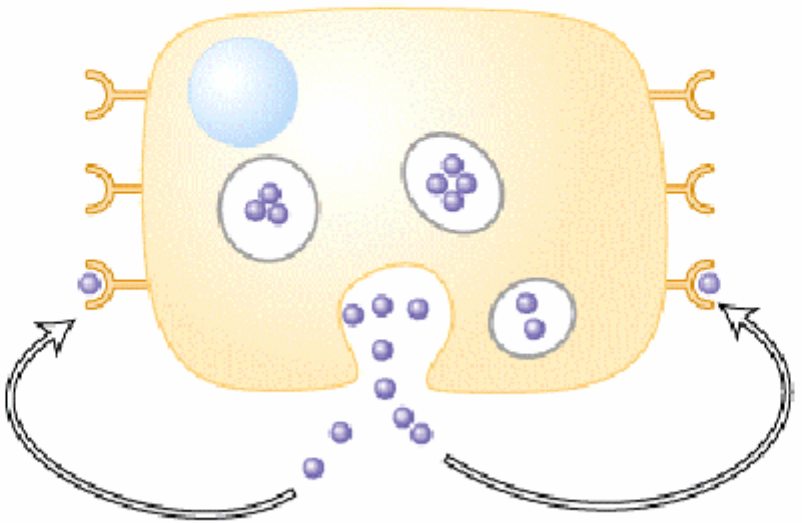
insulin



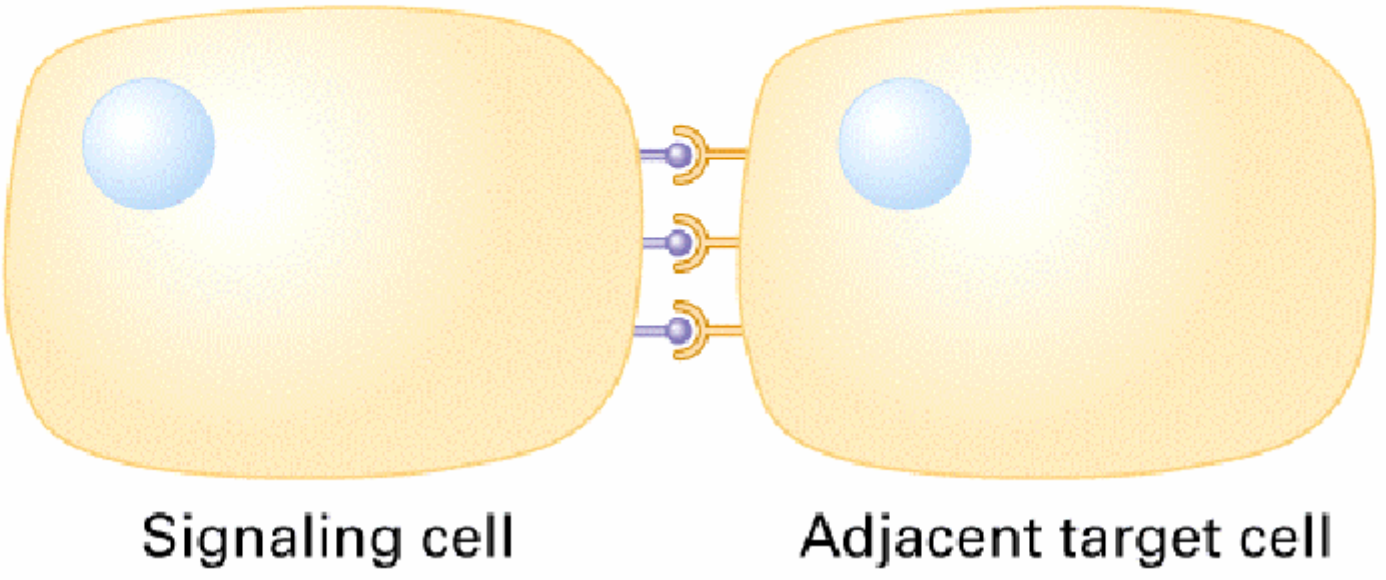
(B) Paracrine signaling
(e.g. at a nerve synapse)



(C) Autocrine signaling
(Usually pathologic, e.g. tumor cells that secrete growth factors that act on the releasing cell)



(D) Signaling by membrane- anchored cell surface proteins



ignaling Molecules Operate over Various Distances in Animals

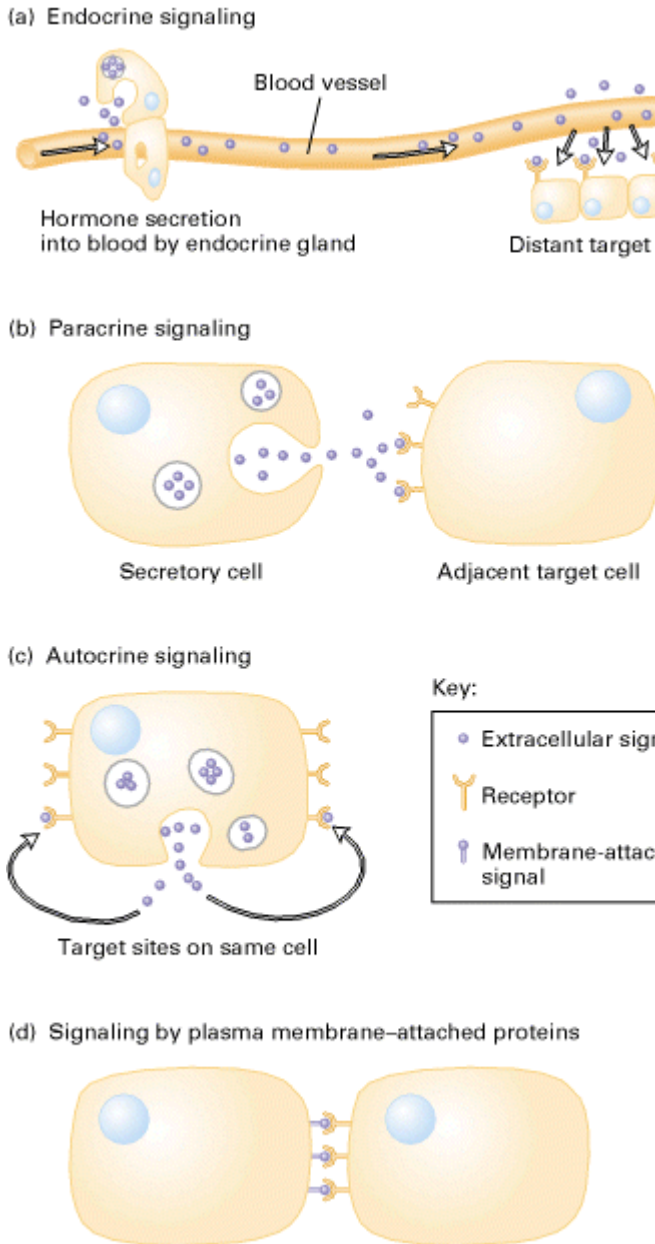
n animals, signaling by extracellular, secreted olecules can be classified into three types ndocrine, paracrine, or autocrine based on the stance over which the signal acts. In addition, ertain membrane-bound proteins on one cell can rectly signal an adjacent cell (Figure 20-1).

endocrine signaling, signaling molecules, called hormones, act on rget cells distant from their site of synthesis by cells of endocrine organs. In imals, an endocrine hormone usually is carried by the blood from its site of ease to its target.

paracrine signaling, the signaling molecules released by a cell only fect target cells in close proximity to it. The conduction of an electric impulse from e nerve cell to another or from a nerve cell to a muscle cell (inducing or inhibiting uscle contraction) occurs via paracrine signaling. The role of this type of signaling, ediated by neurotransmitters. Many signaling molecules regulating development multicellular organisms also act at short range.

autocrine signaling, cells respond to substances that they themselves ease. Many growth factors act in this fashion, and cultured cells often crete growth factors that stimulate their own growth and proliferation. This type of gnaling is particularly common in tumor cells, many of which overproduce and ease growth factors that stimulate inappropriate, unregulated proliferation of emselves as well as adjacent nontumor cells; this process may lead to formation tumor mass.

ome compounds can act in two or even three types of cell-to-cell signaling. ertain small amino acid derivatives, such as epinephrine, function both as urotransmitters (paracrine signaling) and as systemic hormones (endocrine gnaling). Some protein hormones, such as epidermal growth factor (EGF), are nthesized as the exoplasmic part of a plasma- membrane protein; membrane- und EGF can bind to and signal an adjacent cell by direct contact. Cleavage by a



eceptor Proteins Exhibit Ligand-Binding and Effector Specificity

s noted earlier, the cellular response to a particular extracellular signaling molecule depends on its binding to a specific receptor otein located on the surface of a target cell or in its nucleus or cytosol. The signaling molecule (a hormone, pheromone, or urotransmitter) acts as a ligand, which binds to, or "fits," a site on the receptor. Binding of a ligand to its receptor causes a nformational change in the receptor that initiates a sequence of reactions leading to a **specific cellular response.**

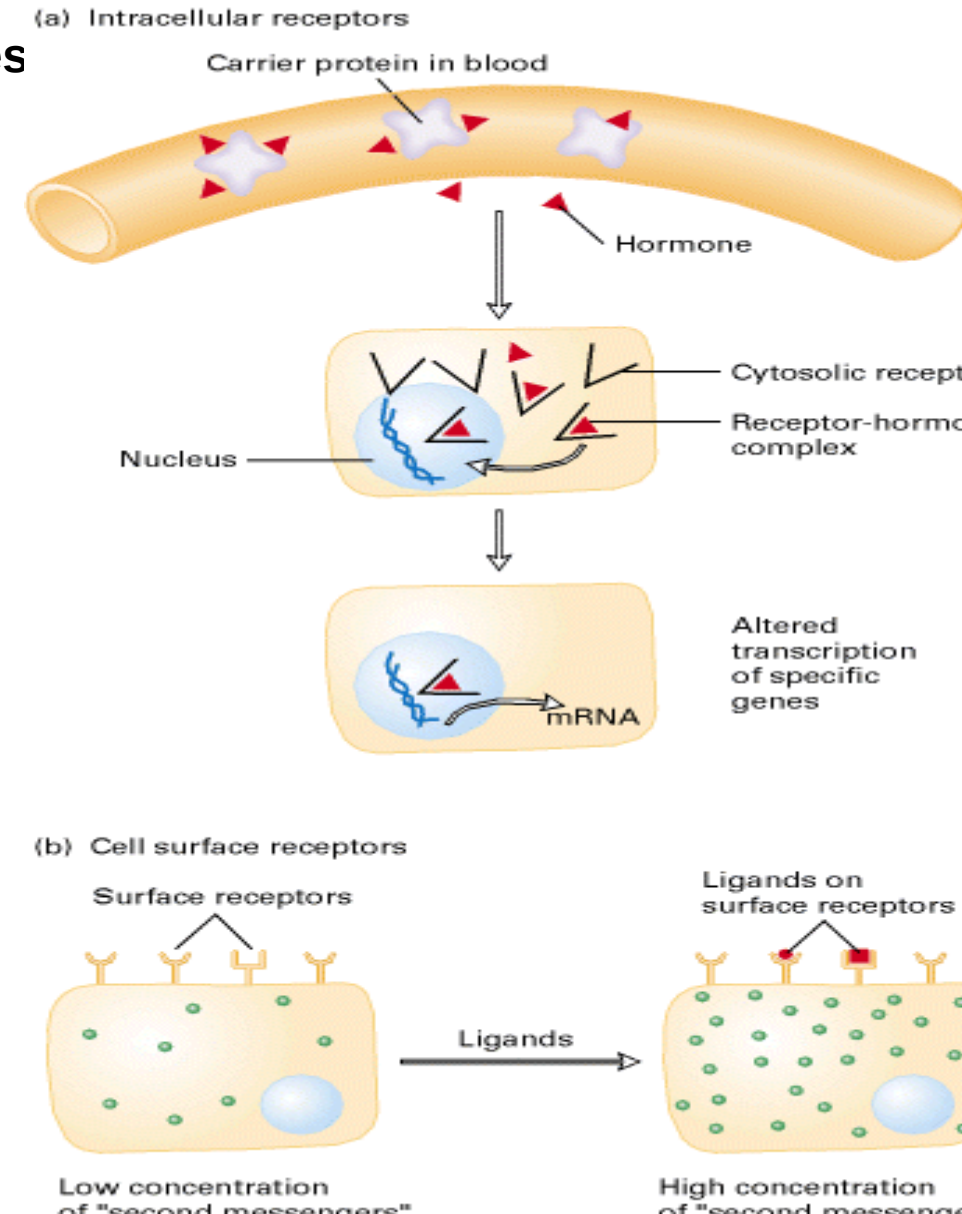
he **response of a cell** or tissue to specific hormones is dictated by the particular hormone receptors it possesses and by e intracellular reactions initiated by the binding of any one hormone to its receptor. **Different cell types may have ifferent sets of receptors for the same ligand**, each of which induces a different response. Or the **same eceptor** may occur on various cell types, and binding of the same ligand may **trigger a different response** in ch type of cell. Clearly, different cells respond in a variety of ways to the same ligand. For instance, **acetylcholine eceptors** are found on the surface of **striated muscle cells, heart muscle cells, and pancreatic cinar cells**. Release of acetylcholine from a neuron adjacent to a **striated muscle cell** triggers contraction, whereas ease adjacent to a **heart muscle** slows the rate of contraction. Release adjacent to a **pancreatic acinar cell** ggers exocytosis of secretory granules that contain digestive enzymes. On the other hand, **different receptor-ligand omplexes** can induce the same cellular response in some cell types. In **liver cells**, for example, the binding of either **lucagon** to its receptors or of **epinephrine** to its receptors can induce degradation of glycogen and release of ucose into the blood.

hese examples show that a receptor protein is characterized by ***binding specificity*** for a particular ligand, and the sulting hormone-ligand complex exhibits ***effector specificity*** (i.e., mediates a specific cellular response). For instance, tivation of either epinephrine or glucagon receptors on liver cells by binding of their respective ligands induces synthesis of **cyclic MP (cAMP),** one of several intracellular signaling molecules, termed second messengers, which regulate various metabolic nctions; as a result, the effects of both receptors on liver-cell metabolism are the same. Thus, the binding specificity of epinephrin d glucagon receptors differ, but their effector specificity is identical.

most receptor-ligand systems, the **ligand appears to have no function** except to bind to the receptor. The and is not metabolized to useful products, is not an intermediate in any cellular activity, and has no enzymatic properties. The onl nction of the ligand appears to be to change the properties of the receptor, which then signals to the cell that a specific product is esent in the environment. Target cells often modify or degrade the ligand and, in so doing, can modify or terminate their response

ormones Can Be Classified Based on Their Solubility and Receptor Location

lost hormones fall into three broad ategories: **(1) small lipophilic molecules** hat diffuse across the plasma membrane nd interact with *intracellular* receptors; nd **(2) hydrophilic or (3) lipophilic olecules that bind to *cell-surface* eceptors** (Figure 20-2). Recently, nitric xide, a gas, has been shown to be a key egulator controlling many cellular esponses.



ipophilic Hormones with Intracellular Receptors

lany lipid-soluble hormones diffuse across the plasma membrane and interact with **eceptors in the cytosol or nucleus**. The resulting hormone-receptor complexes bind o transcription-control regions in DNA thereby affecting expression of specific genes. ormones of this type include the **steroids** (e.g., cortisol, progesterone, estradiol, and estosterone), **thyroxine**, and **retinoic acid**.

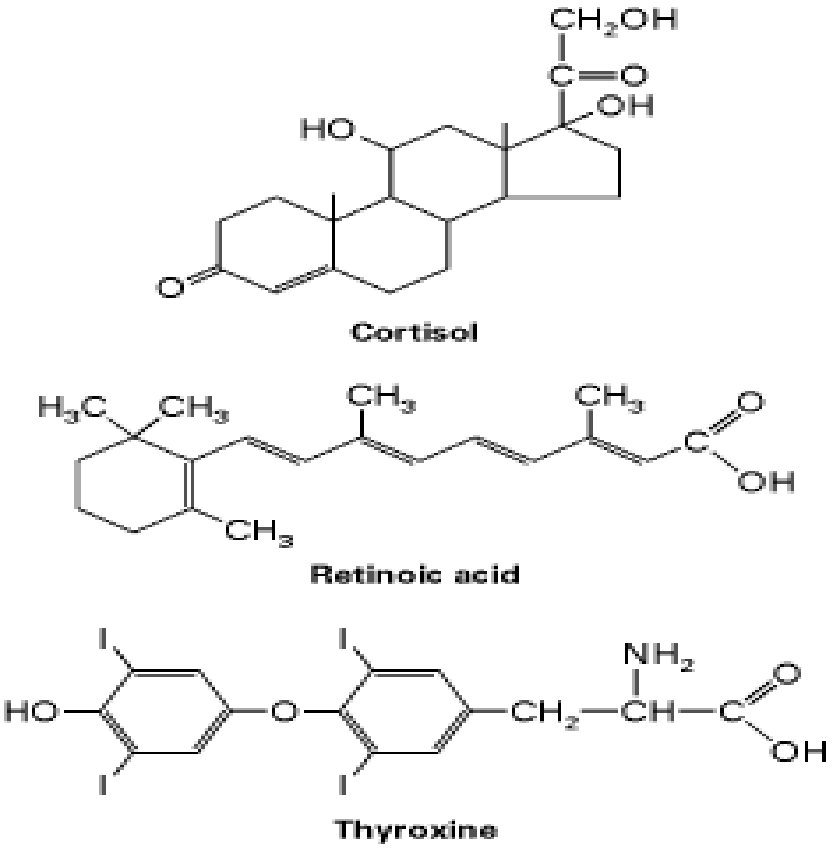
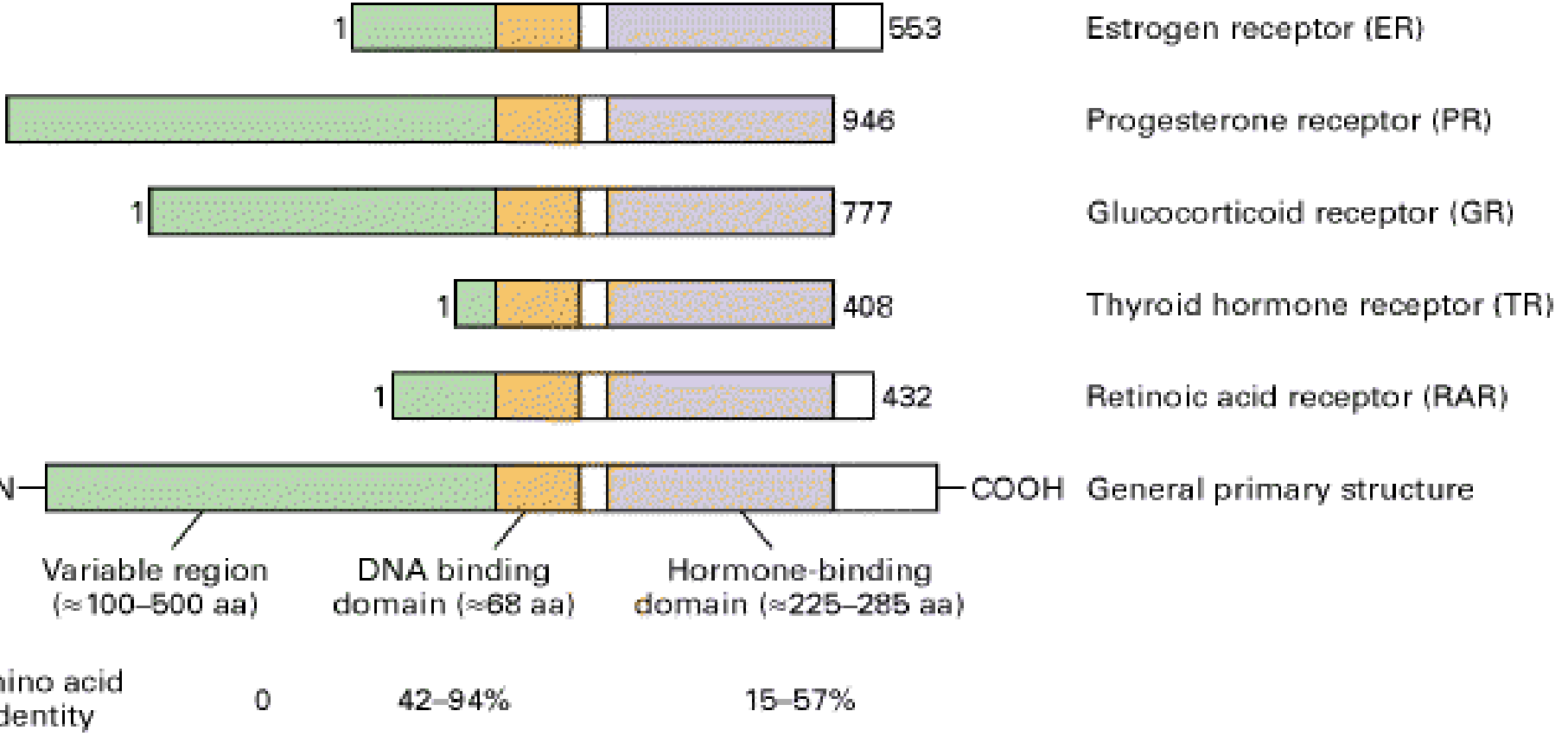


Figure 10-63. Examples of lipid-soluble hormones that bind to members of the nuclear-receptor superfamily of transcription factors. Cortisol is a steroid hormone that binds to the glucocorticoid receptor (GR). Like other **steroid hormone** it is synthesized from cholesterol. **Retinoic acid** is a metabolic derivative of **vitamin A** that has powerful effects on limb bud development in embryos and skin renewal in adult mammals. It is the ligand for the **retinoic acid A receptor (RAR). Thyroxine** is synthesized from tyrosine residues in the protein thyroglobulin in the thyroid gland. It is a ligand for the **thyroid hormone receptor (TR)**.

omain Structure of Nuclear Receptors

ll the nuclear receptors have a unique N-terminal region of variable length (100-500 mino acids) containing regions that function as **transcription-activation domains**. he DNA-binding domain maps near the center of the primary sequence and has the C **inc-finger motif**. The hormone-binding domain lies near the C-terminal end of these eceptors and contains a **hormone-dependent activation domain**. In some cases the ormone-binding domain functions as a **repression domain in the absence of ligand**



uclear-Receptor Response Elements

he characteristic nucleotide sequences of the DNA sites, called *response elements,* hat bind several major nuclear receptors have been determined. The sequences of the onsensus response elements for the glucocorticoid and estrogen receptors are 6-bp **nverted repeats** separated by any three base pairs (Figure 10-65a, b). This finding uggested that these steroid hormone receptors would bind to DNA as **symmetrical imers**

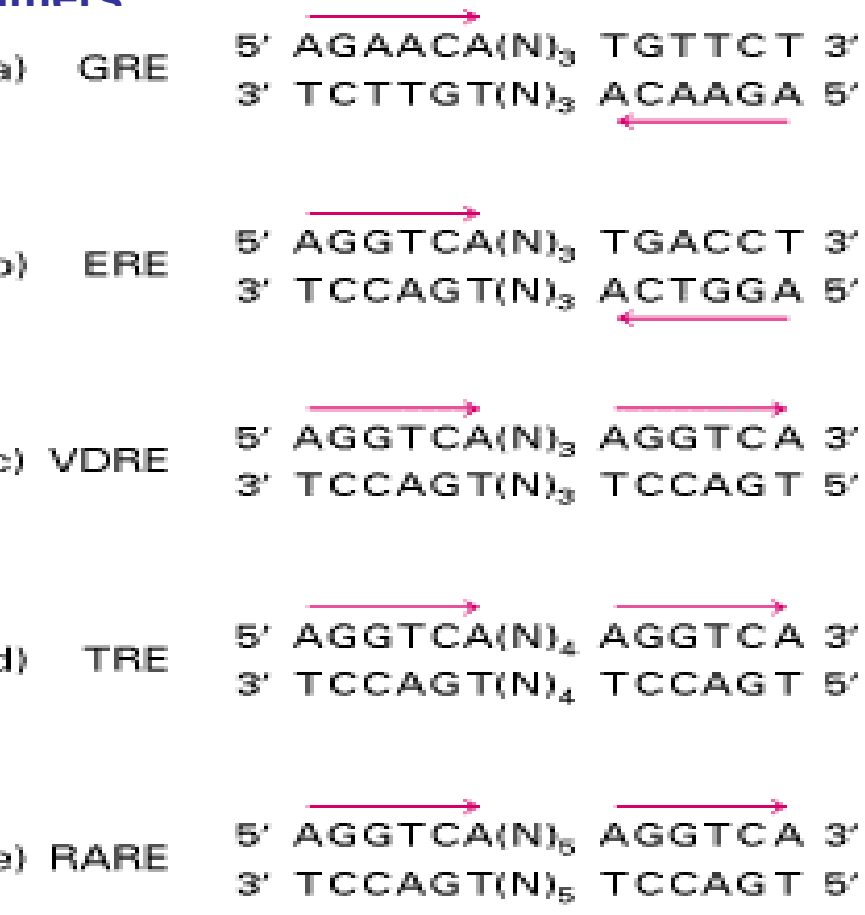


Figure 10-65. Consensus sequences of DNA sites, called response elements, that bind the glucocorticoid receptor (GRE), estrogen receptor (ERE), vitamin D3 receptor (VDRE), thyroid hormone receptor (TRE), and retinoic acid receptor (RARE). The inverted repeats in GRE and ERE and direct repeats in VDRE TRE, and RARE are indicated by red arrows.

The receptors that bind to such direct-repeat response elemen do so as heterodimers with a common **nuclear-receptor monom** called RXR. The vitamin D3 response element, for example, is bou by the RXR-VDR heterodimer, and the retinoic acid response element is bound by RXR-RAR. The monomers composing these heterodimers interact with each other in such a way that the two DNA-binding domains lie in the same rather than inverted orientatio allowing the RXR heterodimers to bind to direct repeats of the binding site for each monomer. In contrast, the monomers in homodimeric nuclear receptors (e.g., GRE and ERE) have an

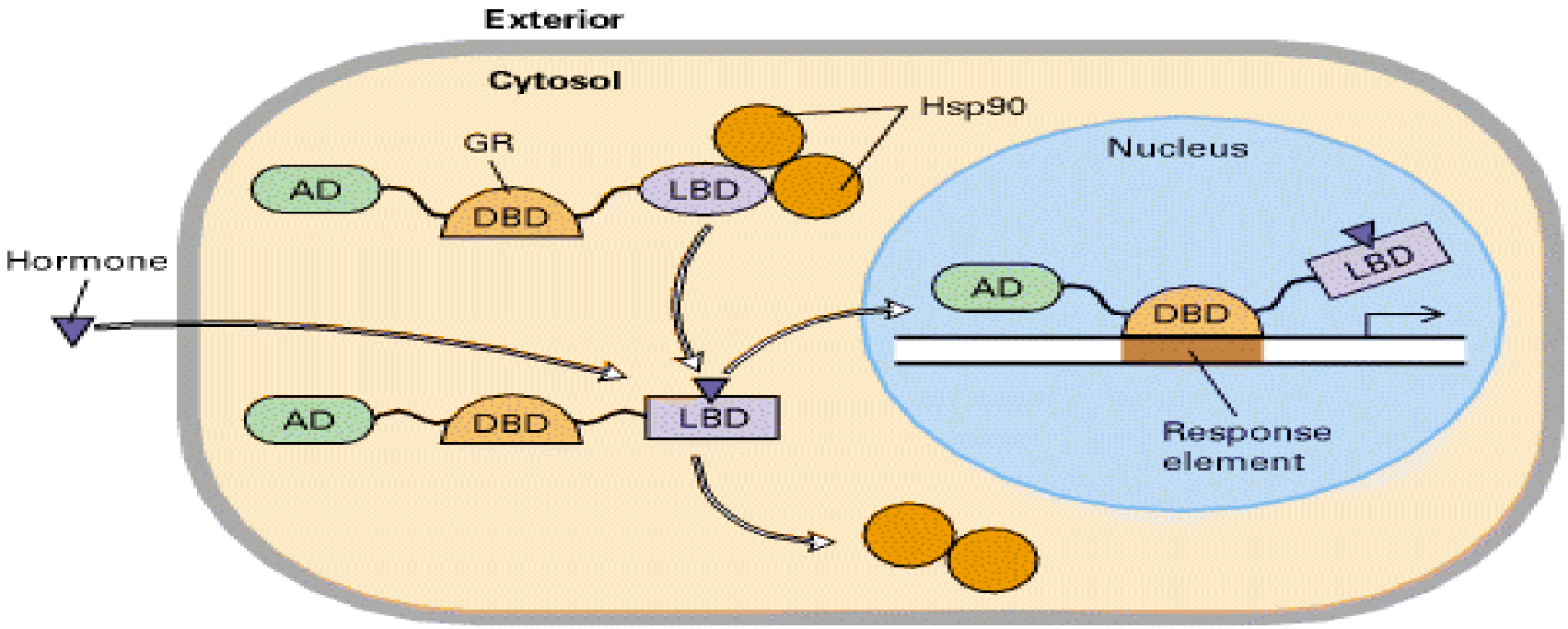
Mechanisms of Hormonal Control of Nuclear-Receptor Activity

Hormone binding to a nuclear receptor regulates its activity as a transcription factor. This regulation differs in some respects for **heterodimeric and homodimeric nuclear receptors**.

When **heterodimeric nuclear receptors** (e.g., RXR-VDR, RXR-TR, and RXR-RAR) are bound to their cognate sites in DNA, they act as **repressors or activators** of transcription depending on whether **hormone occupies the ligand-binding** site. In the absence of hormone, these nuclear receptors direct histone deacetylation at nearby nucleosomes. In the ligand-bound conformation, these nuclear receptors can direct hyperacetylation of histones in nearby nucleosomes, thereby reversing the repressing effects of the free ligand-binding domain. The N-terminal activation domain in these nuclear receptors then probably interacts with additional factors, stimulating the cooperative assembly of an initiation complex.

n contrast to **heterodimeric nuclear receptors, which are located exclusively in the ucleus**, **homodimeric receptors are found both in the cytoplasm and nucleus**, nd their activity is regulated by controlling their transport from the cytoplasm to the ucleus. The **hormone-dependent translocation** of the homodimeric glucocorticoid eceptor (GR) was demonstrated in the transfection experiments shown in Figure 10-66 he GR hormone-binding domain alone mediates this transport.

igure 10-66. Experimental demonstration that hormone-binding domain of the lucocorticoid receptor (GR) mediates translocation to the nucleus in the resence of hormone. Cultured animal cells were transfected with expression vectors ncoding the proteins diagrammed at the bottom. Immunofluorescence with a labeled ntibody specific for b-galactosidase was used to detect the expressed proteins in ansfected cells. (a) When cells were transfected with b-galactosidase alone, the xpressed enzyme was localized to the cytoplasm in the presence and absence of the ucocorticoid hormone **dexamethasone (Dex**). (b) When a fusion protein consisting of -galactosidase and the entire 794-aa rat glucocorticoid receptor (GR) was expressed i he cultured cells, it was present in the cytoplasm in the absence of hormone but was ansported to the nucleus in the presence of hormone. (c) A fusion protein composed o 382-aa region of GR including the **ligand-binding domain** (light purple) and b-



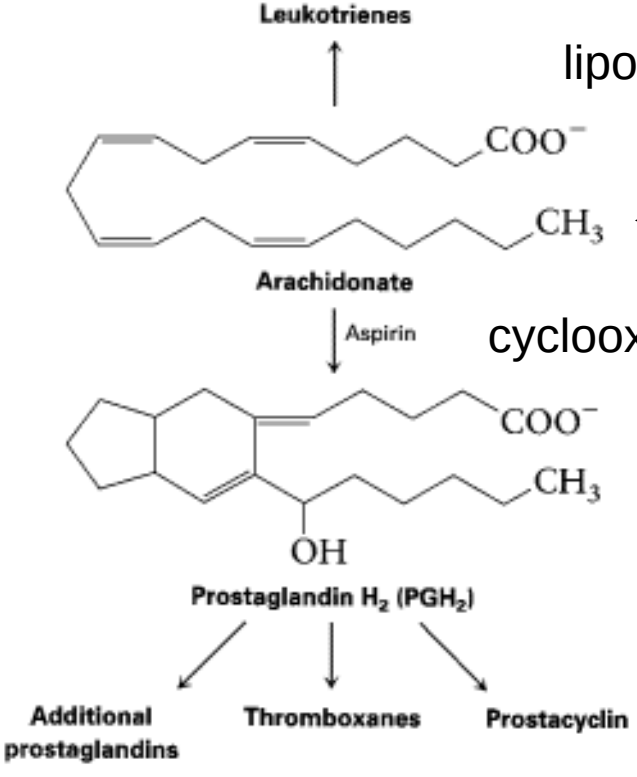
igure 10-67. Model of hormone-dependent gene activation by the glucocorticoid eceptor (GR). In the absence of hormone, GR is bound in a complex with Hsp90 in the ytoplasm via its ligand-binding domain (light purple). When hormone is present, it ffuses through the plasma membrane and binds to the GR ligand-binding domain, ausing a conformational change in the ligand-binding domain that releases the recepto om Hsp90. The receptor with bound ligand is then translocated into the nucleus where s DNA-binding domain (orange) binds to response elements, allowing the activation omain (green) to stimulate transcription of target genes.

rphan Receptors

he ligands for the hormone-binding domains in many members of the nuclear-receptor uperfamily are as-yet unknown.

ipophilic Hormones with Cell-Surface Receptors

he primary lipid-soluble hormones that bind to cell-surface receptors are the **rostaglandins**. There are at least 16 different prostaglandins in nine different chemica asses, designated PGA PGI. Prostaglandins are part of an even larger family of 20 arbon containing hormones called eicosanoid hormones. In addition to prostaglandins, ey include prostacyclins, thromboxanes, and leukotrienes. Eicosonoid hormones are ynthesized from a common precursor, arachidonic acid. Arachidonic acid is generated om phospholipids and diacylglycerol.



Phospholipase A2

PPAR transcription factors

his large class of compounds is composed of two groups: **(1) peptide hormones**, suc
s insulin, growth factors, and glucagon, which range in size from a few amino acids to
rotein-size compounds, and **(2) small charged molecules**, such as epinephrine and
stamine (see Figure 21-28), that are derived from amino acids and function as
ormones and neurotransmitters.

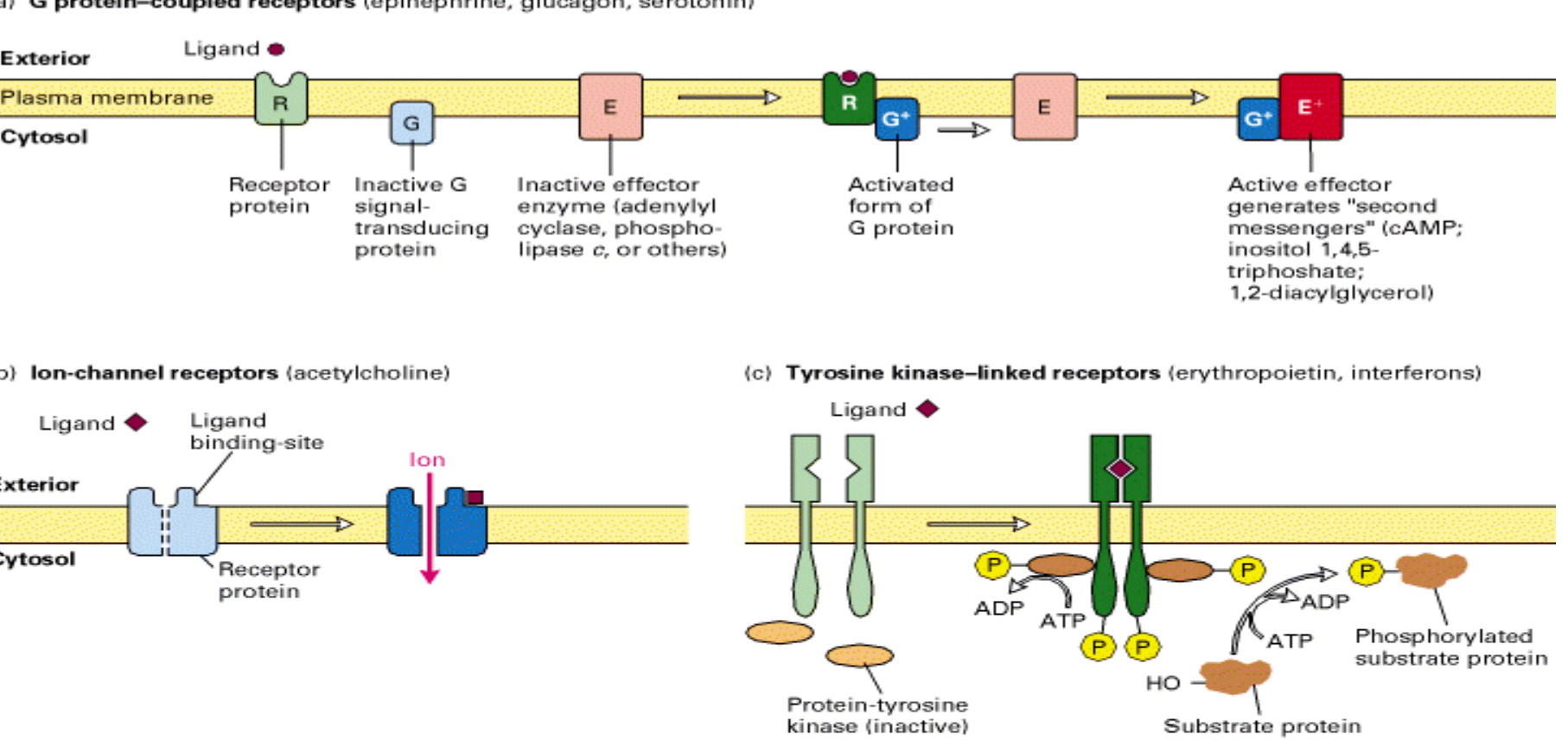
ell-Surface Receptors Belong to Four Major Classes

G protein-coupled receptors (**GPCR**, see Figure 20-3a): Ligand binding activates a G protein, which in turn
ctivates or inhibits an enzyme that generates a specific second messenger or modulates an ion channel, causin
change in membrane potential. The receptors for epinephrine, serotonin, and glucagon are examples.

on-channel receptors (see Figure 20-3b): Ligand binding changes the conformation of the receptor so that
ecific ions flow through it; the resultant ion movements alter the electric potential across the cell membrane. Th
cetylcholine receptor at the nerve-muscle junction is an example.

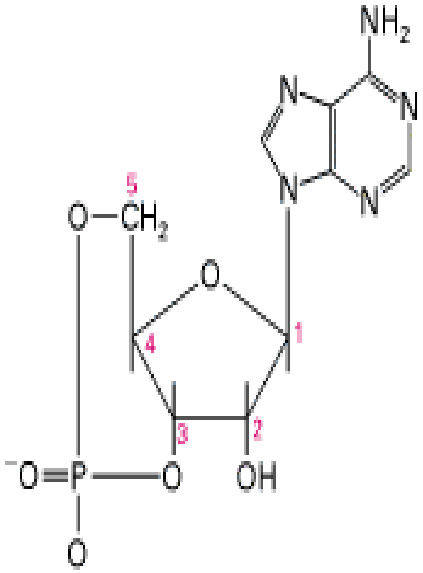
Tyrosine kinase linked receptors (see Figure 20-3c): These receptors lack intrinsic catalytic activity, but
gand binding stimulates formation of a dimeric receptor, which then interacts with and activates one or more
tosolic protein-tyrosine kinases. The receptors for many cytokines, the interferons, and human growth factor ar
this type. These tyrosine kinase linked receptors sometimes are referred to as the *cytokine-receptor*
perfamily.

Receptors with intrinsic enzymatic activity (see Figure 20-3d): Several types of receptors have intrinsic
talytic activity, which is activated by binding of ligand. For instance, some activated receptors catalyze
nversion of GTP to cGMP; others act as protein phosphatases, removing phosphate groups from
osphotyrosine residues in substrate proteins, thereby modifying their activity. The receptors for insulin and
any growth factors are ligand-triggered protein kinases; in most cases, the ligand binds as a dimer, leading to
merization of the receptor and activation of its kinase activity. These receptors often referred to as receptor
rine/threonine kinases or receptor tyrosine kinases autophosphorylate residues in their own cytosolic domain

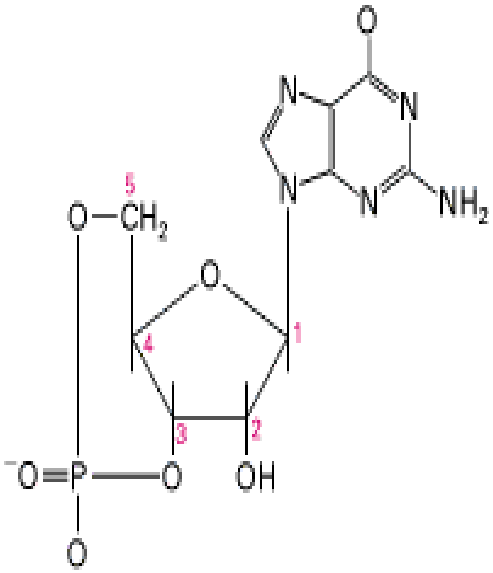

Exterior
Ligand
Plasma membrane
Cytosol
R
G
E
R
G*
E
G*
E*
Receptor protein
Inactive G signal-transducing protein
Inactive effector enzyme (adenylyl cyclase, phospho-lipase c, or others)
Activated form of G protein
Active effector generates "second messengers" (cAMP; inositol 1,4,5-triphoshate; 1,2-diacylglycerol)
Ion-channel receptors (acetylcholine)
Ligand
Ligand binding-site
Ion
Exterior
Cytosol
Receptor protein
(c) Tyrosine kinase–linked receptors (erythropoietin, interferons)
Ligand
P
ADP
ATP
Phosphorylated substrate protein
HO
Protein-tyrosine kinase (inactive)
Substrate protein
Receptors with intrinsic enzymatic activity
(transforming-growth factor β)
Ligand
Exterior
Cytosol
GTP
3',5'-cyclic GMP
PPi
Guanylate cyclase activity (atrial naturetic factor)
Ligand
Tyrosine kinase activity (nerve growth factor, platelet-derived growth factor)
ATP
ADP
P

ffects of Many Hormones Are Mediated by Second Messengers

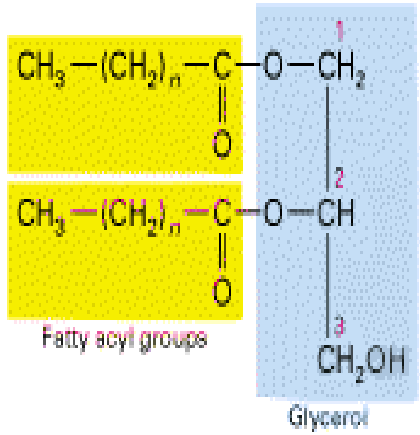
he binding of ligands to many cell-surface receptors leads to a short-lived increase (or ecrease) in the concentration of the intracellular signaling molecules termed second nessengers. These **low-molecular-weight signaling molecules** include 3 ,5 -cyclic MP (cAMP); 3 ,5 -cyclic GMP (cGMP); 1,2-diacylglycerol (DAG); inositol 1,4,5-isphosphate (IP3); various inositol phospholipids (phosphoinositides); and **Ca2+** Figure 20-4).



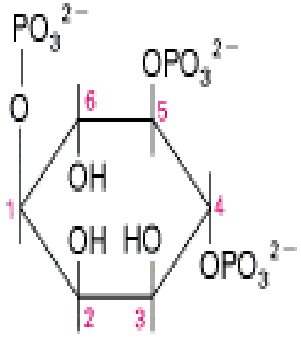
3′,5′-Cyclic AMP
(cAMP)



3′,5′-Cyclic GMP
(cGMP)



1,2-Diacylglycerol
(DAG)



Inositol
1,4,5-trisphosphate
(IP_3)

↓

cAMP-dependent kinase

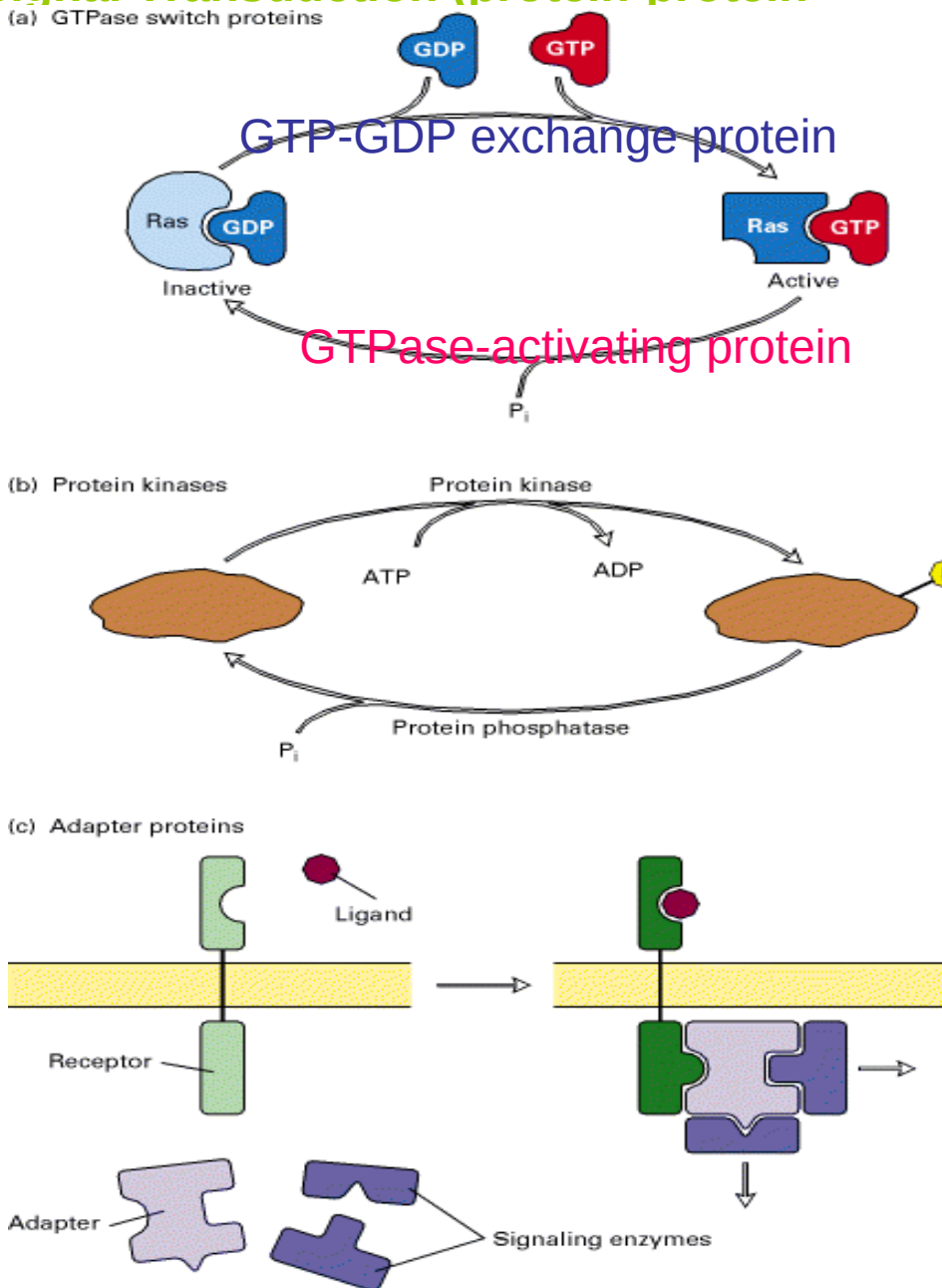
Protein kinase C

Ca++

Ca-Calmodulin kinas

ther Conserved Proteins Function in Signal Transduction (protein-protein nteraction)

igure 20-5. Common intracellular ignaling proteins. (a) GTP-binding roteins with GTPase activity function s molecular switches. When bound to TP they are active; when bound to GDP, ey are inactive. They fall into two ategories, **trimeric G proteins and as-like proteins**. **(b) Protein kinases odulate the activity or the binding roperties of substrate proteins** by osphorylating **serine, threonine, or rosine residues**. The phosphorylated rm of some proteins is active, whereas e dephosphorylated form of other roteins is active. The combined action of **inases and phosphatases**, which ephosphorylate specific substrates, can ycle proteins between active and active states. **(c) Adapter proteins** ontain various protein-binding motifs that romote the formation of multiprotein



0.2. Identification and Purification of Cell-Surface Receptors

s noted earlier, **hormone receptors bind ligands with great specificity and high affinity**. Binding of a hormone to a receptor volves the same types of weak interactions ionic and van der Waals bonds and hydrophobic interactions that characterize the ecific binding of a substrate to an enzyme (Section 2.2). The *specificity* of a receptor refers to its ability to distinguish closely ated substances.

ormone binding usually can be viewed as a simple reversible reaction,

$$R + H \longrightarrow RH$$

hich can be described by the equation

$$K_D = \frac{[R][H]}{[RH]} \qquad (20\text{-}1)$$

here [R] and [H] are the concentrations of free receptor and hormone (ligand), espectively, and [RH] is the concentration of the receptor-hormone complex. *K*D, the ssociation constant of the receptor-ligand complex, measures the *affinity* of the eceptor for the ligand. This binding equation can be rewritten as

$$\frac{[RH]}{R_T} = \frac{1}{1 + K_D/[H]} \qquad (20\text{-}2)$$

here *R*T is the sum of free and bound receptors: [R] + [RH]. Equation 20-2 is similar in orm to the Michaelis-Menten equation used to analyze enzymatic reactions (Section .3).

he lower the *K*D value, the higher the affinity of a receptor for its ligand. The *K*D value equivalent to the concentration of ligand at which one-half of the receptors contain ound ligand. If [H] = *K*D, then from Equation 20-2 we can see that [RH] = 0.5 *R*T.

ormone Receptors Are Detected by Binding Assays

ormone receptors are **difficult to identify and purify**, mainly because they are resent in such minute amounts. The surface of a typical cell bears 10,000-20,000 eceptors for a particular hormone, but this quantity is only ≈10-6 of the total protein in he cell, or ≈10-4 of the plasma-membrane protein. Purification is also difficult because hese integral membrane proteins first must be solubilized with a nonionic detergent (se igure 3-38).

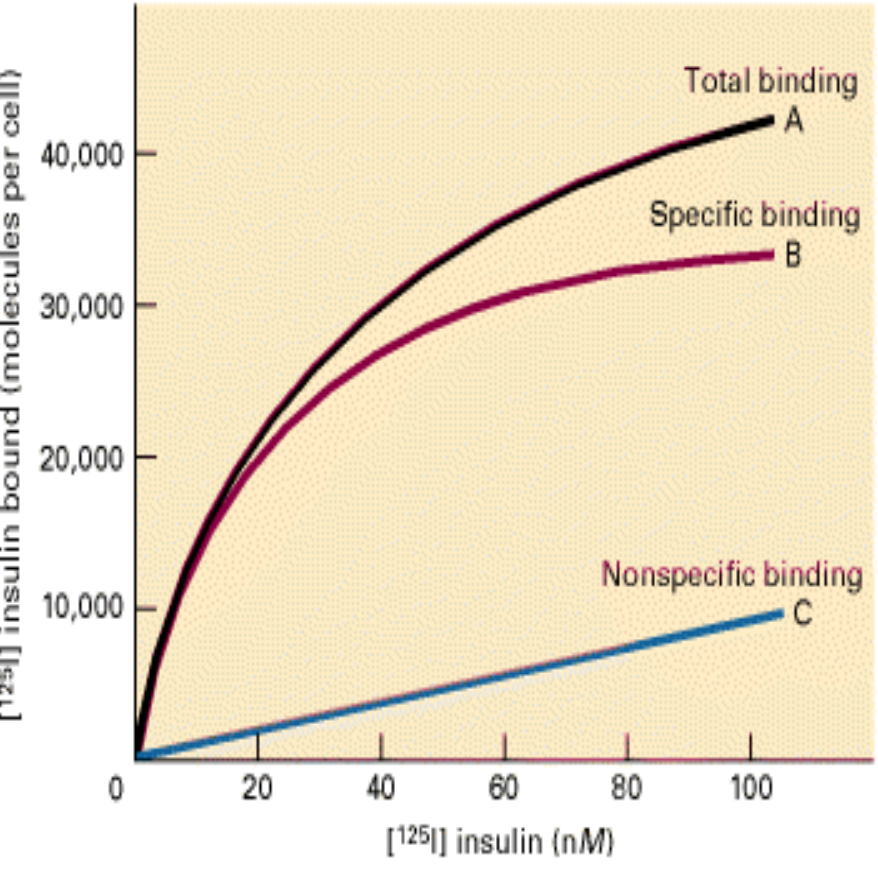


Figure 20-7. Identification of insulin-specific receptors on the surface of cells by their binding of radioactive insulin. A suspension of cells is incubated for **1 hour at 4° C** with increasing concentrations of 125I-labeled insulin; the low temperature is used to prevent endocytosis of the cell-surface receptors. The total binding curve A represents insulin specifically bound to high-affinity receptors as well as insulin nonspecifically bound with low affinity to other molecules on the cell surface. The contribution of nonspecific binding to total binding is determined by repeating the binding assay in the presence of a 100-fold excess of unlabeled insulin, which saturates all the specific high-affinity sites. In this case, all the labeled insulin binds to nonspecific sites, yielding curve C. The specific binding curve B, which fits Equation 20-2, is calculated as the difference between curves A and C. For this insulin receptor, ***K*D is ≈20 nM** (2ᵡ10 8 M), and the number of receptor molecules per cell, ***R*T, is ≈30,000.**

or many hormone receptors, the ligand concentration needed to induce a maximal ellular response is lower than that needed to saturate all the receptor molecules on a ell. Likewise, **the ligand concentration that induces a 50-percent maximal esponse is less than the *K*D value for binding.**

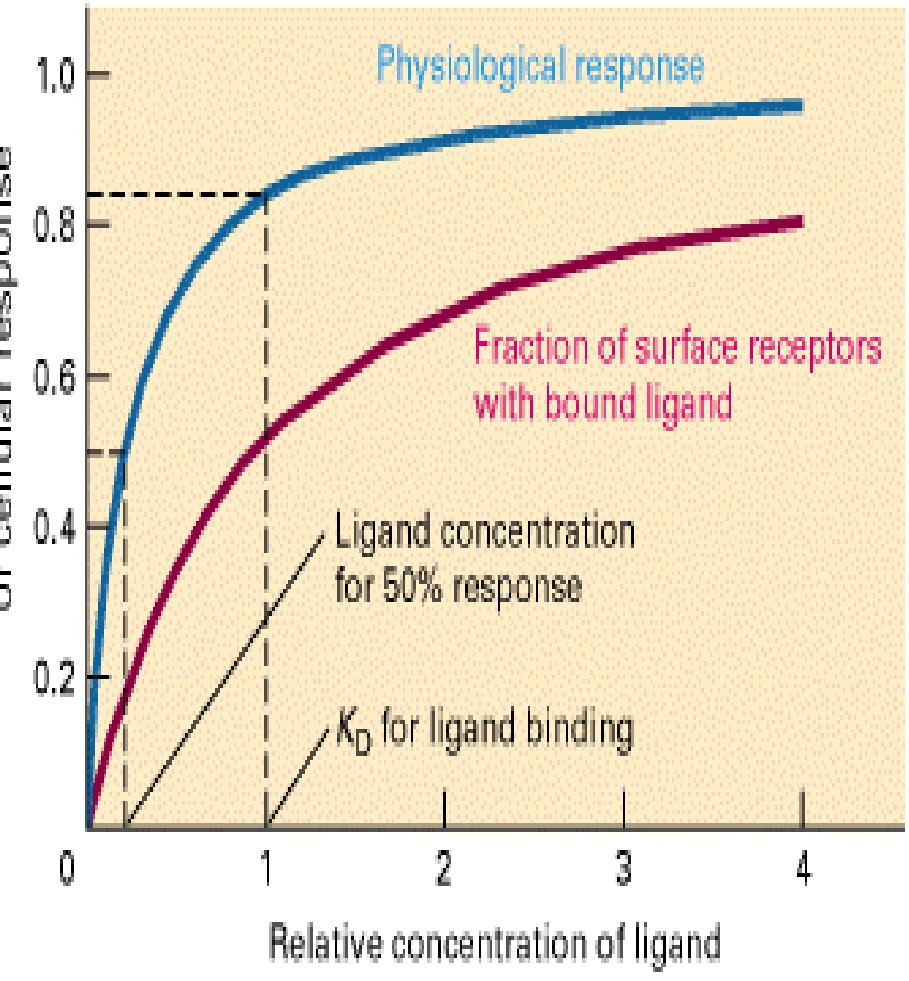


Figure 20-8. Comparison of binding curve and response curve for a cell-surface receptor and its ligand. As illustrated here, the maximal physiological response to many hormones occurs when only a fraction of the cell receptors are occupied by ligand. In this example, 50 percent of the maximal response is induced at a ligand concentration at which only 18 percent of the receptors are occupied. Likewise, 80 percent of the maximal response is induced when the ligand concentration equals the *K*D value, at which 50 percent of the receptors are occupied.

ffinity Techniques Permit Purification of Receptor Proteins

ell-surface hormone receptors often can be identified and followed through isolation rocedures by ***affinity labeling***. In this technique, cells are mixed with an excess of a adiolabeled hormone to saturate the hormone-binding sites on its specific receptor. fter unbound hormone is washed away, the mixture is treated with a chemical agent hat **covalently cross-links the bound labeled** hormone to the receptor. Most cross-nking agents contain two groups that react with free amino groups; by reacting with an mino group in the receptor and with one in the bound ligand, the cross-linking agent ovalently joins the receptor and ligand. A radiolabeled ligand that is cross-linked to its eceptor remains bound even in the presence of detergents and other denaturing agent hat are used to solubilize receptor proteins from the cell membrane. Another technique ften used in purifying cell-surface receptors that retain their hormone-binding ability hen solubilized is ***affinity chromatography***. In this technique, a ligand of the recepto f interest is chemically linked to polystyrene beads. A crude, detergent-solubilized reparation of membrane proteins is passed through a column containing these beads. nly the receptor binds to the beads; the other proteins are washed through the colum y excess fluid. When an excess of the ligand is passed through the column, the bound eceptor is displaced from the beads and eluted from the column. This technique is milar in principle to antibody- affinity chromatography (see Figure 3-43c), except that a ormone ligand rather than an antibody is attached to the column beads. In some cases hormone receptor can be purified as much as 100,000-fold in a single affinity hromatographic step.

lany Receptors Can Be Cloned without Prior Purification

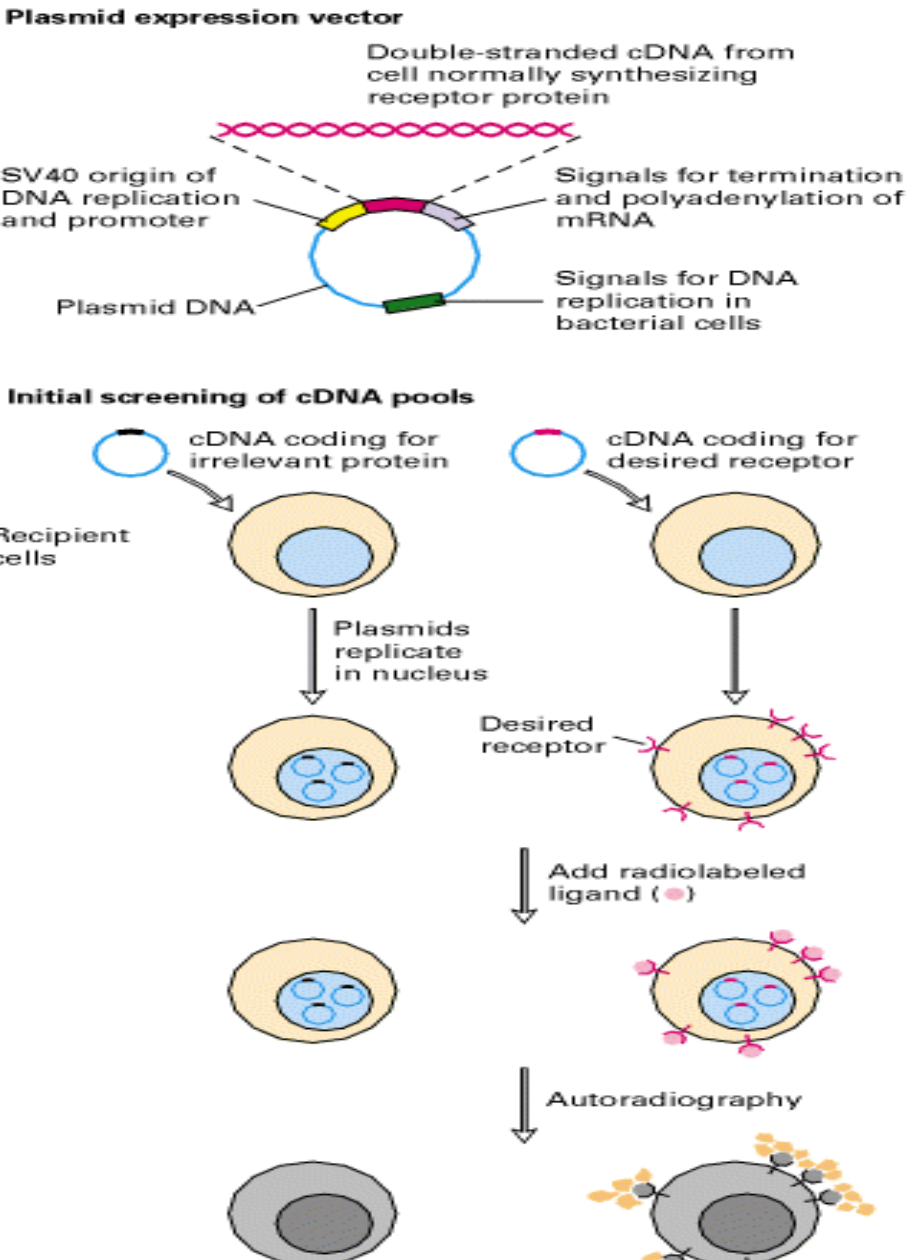


Figure 20-9. Identification and isolation of a cDNA encoding a desired cell-surface receptor by plasmid expression cloning. All mRNA is extracted from cells that normally express the receptor and reverse-transcribed into double-stranded cDNA. (a) The entire population of cDNAs is inserted into plasmid expression vectors in between a strong promoter and a terminator of transcription. The plasmids are transfected into bacterial cells that do not normally express the receptor of interest. The resulting cDNA library is divided into pools, each containing about 1000 different cDNAs. (b) Plasmids in each pool are transfected into a population of cultured cells (e.g., CO cells) that lack the receptor of interest. Only transfected cells th contain the cDNA encoding the desired receptor synthesize it; other transfected cells produce irrelevant proteins. To detect th few cells producing the desired receptor, a radiolabeled ligand specific for the receptor is added to the culture dishes containin the transfected cells; the cells are fixed and subjected to autoradiography. Positive cells synthesizing the specific recepto will be covered with many grains. Alternatively, **transfected cells can be treated with a fluorescent-labeled ligand** and passed through fluorescence-activated cell sorter (see Figure 5-21). Cells expressing the receptor will bind the fluorescent label and be separated from those that do not. Plasmid cDNA pools giving rise to a positive signal are maintained in bacteria and subdivid into smaller pools, each of which is rescreened by transfection into cultured cells. After several cycles of screening and subdividing positive cDNA pools, a pure cDNA clone encoding

0.3. G Protein-Coupled Receptors and Their Effectors

Many different mammalian cell-surface receptors are coupled to a trimeric signal-transducing G protein. As noted earlier, ligand binding to these receptors activates their associated G protein, which then activates an *effector enzyme* to generate an intracellular second messenger. **All G protein-coupled receptors (GPCRs) contain seven membrane-spanning regions with their N-terminal segment on the exoplasmic face and their C-terminal segment on the cytosolic face of the plasma membrane (Figure 20-10).** This large receptor family includes light-activated receptors (rhodopsins) in the eye and literally thousands of odorant receptors in the mammalian nose (Section 21.6), as well as numerous receptors for various hormones and neurotransmitters (Section 21.5). Although these receptors are activated by different ligands and may mediate different cellular responses, they all mediate a similar signaling pathway (see Figure 20-6).

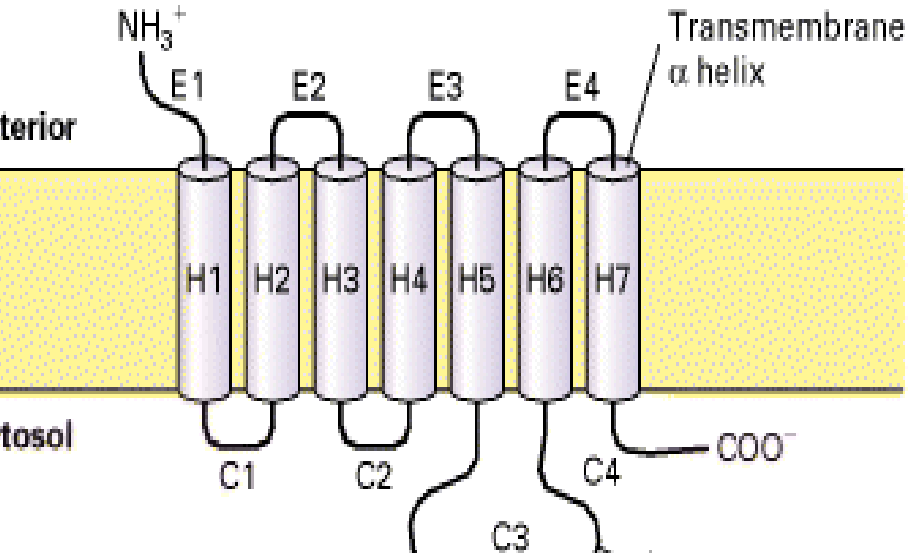
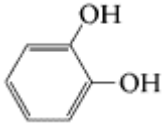


Figure 20-10. Schematic diagram of the general structure of G protein-linked receptors. All receptors of this type contain seven transmembrane a-helical regions. The loop between a helices 5 and 6, and in some cases the loop between helices 3 and 4, which face the cytosol, are important for interactions with the coupled G protein. E1 E4 = extracellular loops; H1 H7 = transmembrane domains; C1 C4 = cytosolic loops

inding of Epinephrine to Adrenergic Receptors Induces Tissue-Specific esponses

pinephrine and norepinephrine were originally recognized as products of the *edulla,* or core, of the **adrenal gland** and are also known as *adrenaline* and *oradrenaline.* Embryologically, nerve cells derive from the same tissue as adrenal edulla cells, and norepinephrine is also secreted by differentiated nerve cells. Both ormones are charged compounds that belong to the catecholamines, active amines ontaining a *catechol* moiety:



pinephrine, which binds to two types of GPCRs, is particularly important in **mediating he body's response to stress**, such as fright or heavy exercise, when all tissues have n increased need for glucose and fatty acids. These principal metabolic fuels can be upplied to the blood in seconds by the **rapid breakdown of glycogen** in the liver *glycogenolysis)* and of triacylglycerol in the **adipose storage cells** *(lipolysis).*

n mammals, the liberation of glucose and fatty acids can be triggered by binding of pinephrine (or norepinephrine) to **β-*adrenergic receptors*** on the surface of **hepatic iver) and adipose cells**. Epinephrine bound to similar **β-adrenergic receptors on eart muscle cells increases the contraction rate**, which increases the blood supply o the tissues. Epinephrine bound to b-adrenergic receptors on **smooth muscle cells o he intestine** causes them to relax. Another type of epinephrine receptor, the a **α2-*drenergic receptor*, is found on smooth muscle cells lining the blood vessels in he intestinal tract, skin, and kidneys**. Epinephrine bound to a α2 receptors causes he **arteries to constrict**, cutting off circulation to these peripheral organs. These verse effects of epinephrine are directed to a common end: **supplying energy for the apid movement of major locomotor muscles in response to bodily stress**.

s discussed in more detail later, **β- and α-adrenergic receptors are coupled to ifferent G proteins**. Both **β1- and β2-adrenergic receptors** are coupled to **G roteins (Gs),** which activate adenylyl cyclase. In contrast, **α1 and α2 receptors** are oupled to two other G proteins, **Gq and Gi**, respectively. Gi inhibits adenylyl cyclase, nd Gq stimulates phospholipase C to generate IP3 and DAG as second messengers.

Principles of intracellular signaling by cell surface receptors

1. Each receptor generally binds only a single hormone

2. But there are often multiple types of receptors that bind the same hormone (e.g. the β_2- and α_2-adrenergic receptors both bind adrenaline)

3. Such different types of receptors that bind the same hormone often induce different cellular responses even in the same cell

4. Different receptors of the same class that bind different hormones often induce the same cellular responses in a cell

> **(e.g. In liver epinephrine, glucagon, and ACTH bind to different 7- spanning receptors, but all of these receptors activate the same G_s protein and induce the same cellular response of causing glycogen breakdown)**

5. Typical body cells contain on their surface multiple types of receptors that bind different hormones. Cells must integrate the responses induced by hormones acting via different types of receptors.

timulation of β-Adrenergic Receptors Leads to a Rise in cAMP

lany of the very different tissue-specific responses induced by binding of epinephrine t
-adrenergic receptors are mediated by a *rise* in the intracellular level of cAMP, resultin
om activation of **adenylyl cyclase**. As a second messenger, cAMP acts to modify the
ates of different enzyme-catalyzed reactions in specific tissues generating various
etabolic responses. Binding of numerous other hormones to their receptors also leads
a rise in intracellular cAMP and characteristic tissue-specific metabolic responses
see later section).

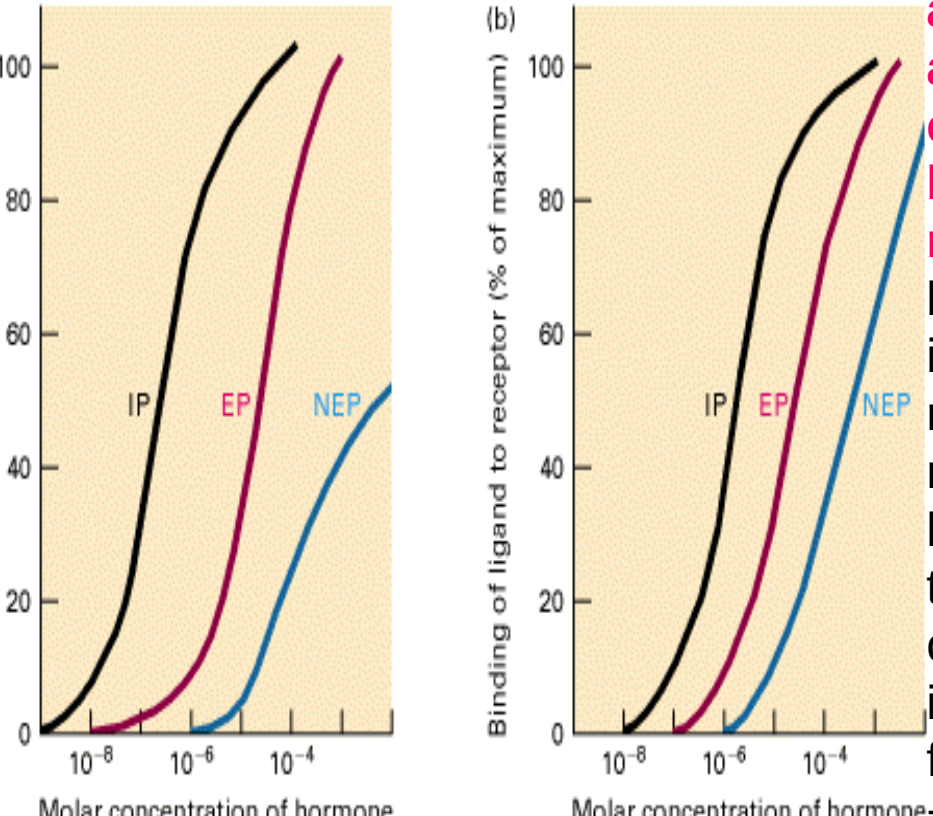


Figure 20-11. Comparison of the abilities of three catecholamines to activate adenylyl cyclase, which catalyzes synthesis of cAMP, and to bind to cell-surface b-adrenergic receptors. The curves show that each ligand induces adenylyl cyclase activity (a) in proportion to its ability to bind to the receptor (b). Moreover, the concentration required for half-maximal binding of each ligand to the receptor is about the same as that required for activation of adenylyl cyclase. Note that the ligand concentration is plotted on a logarithmic scale ranging from 10-9 to 10-2 M. IP = isoproterenol; E = epinephrine; NEP = norepinephrine.

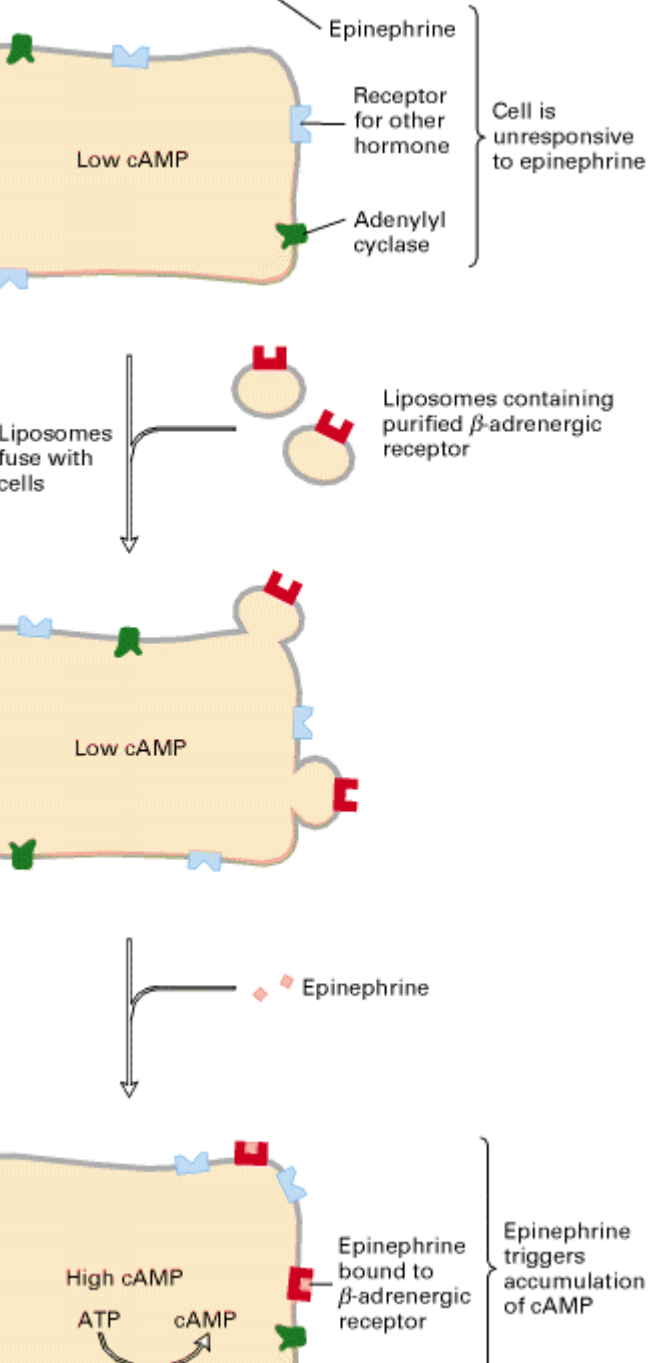


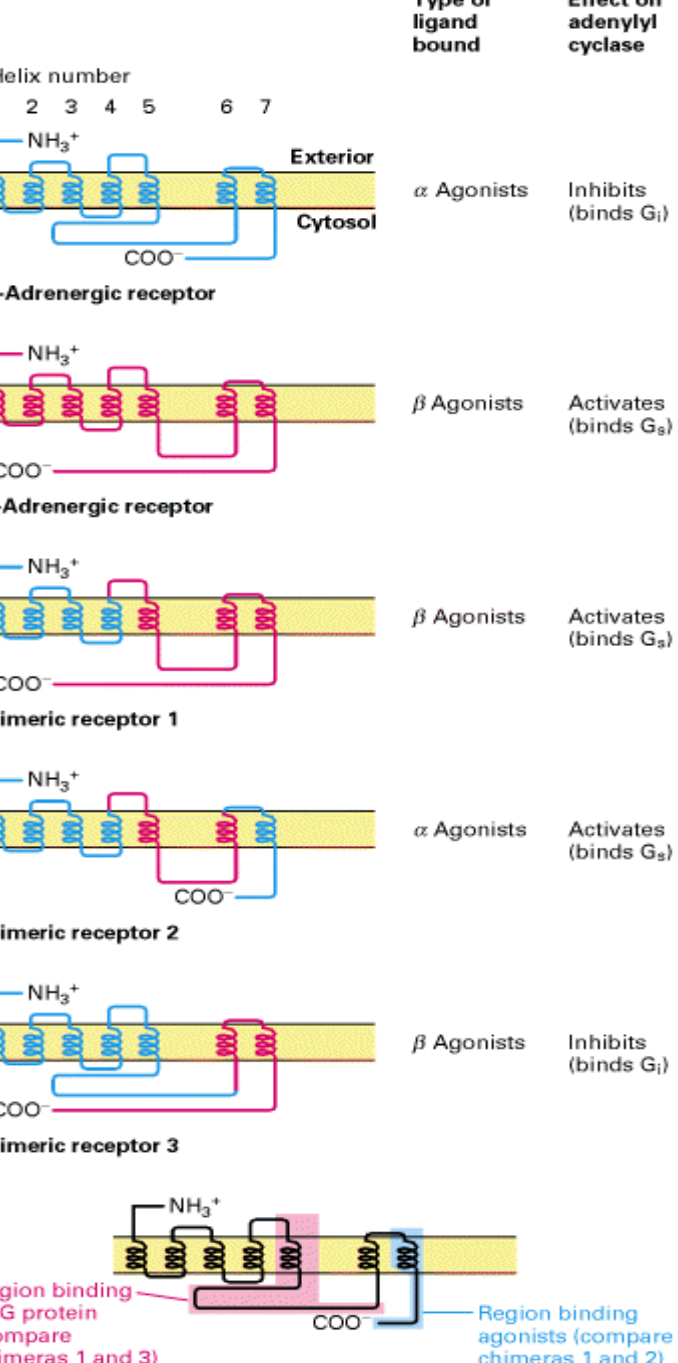
Figure 20-12. Experimental demonstration that β-adrenergic receptors mediate the induction of epinephrine-initiated cAMP synthesis. Target cells **lacking any receptors** for epinephrine but **expressing adenylyl cyclase** and the appropriate signal-transducing **G proteins** were incubated with liposomes containing b-adrenergic receptors purified by affinity chromatography. Cells that fused with the liposomes became responsive to epinephrine, producing high levels of cAMP when the hormone was added to the medium. See Figure 15-4 for formation of liposomes containing membrane protein

Assay model system
G proteins

ritical Features of Catecholamines and Their Receptors Have Been Identified

variety of experimental approaches have provided information about which parts of atecholamine molecules and their receptors are essential for ligand binding and the ubsequent activation of adenylyl cyclase. In many of these studies, **chemically ynthesized analogs of epinephrine** have proved useful. These analogs fall into two asses: **agonists, which mimic the function of a hormone by binding to its eceptor and causing the normal response**, and ***antagonists*, which bind to the eceptor but do not activate hormone-induced effects**. An antagonist acts as an hibitor of the natural hormone (or agonist) by competing for binding sites on the eceptor, thereby blocking the physiological activity of the hormone.

umans possess **two types of b-adrenergic receptors** that are located on different ce ypes and differ in their relative affinities for various catecholamines. **Cardiac muscle ells possess β1 *receptors*,** which promote increased heart rate and contractility by nding catecholamines with the rank order of affinities isoproterenol > norepinephrine > pinephrine. Drugs such as **practolol**, which are used to slow heart contractions in the eatment of cardiac arrhythmia and angina, are **β1-selective antagonists** (see Table 0-2). These so-called **beta blockers** usually have little effect on b-adrenergic receptor n other cell types. The smooth muscle cells lining the **bronchial passages possess 2 *receptors*,** which mediate relaxation by binding catecholamines with the rank order f affinities isoproterenol >> epinephrine = norepinephrine. **Agonists selective for β2 eceptors**, such as terbutaline, are used in the treatment of **asthma** because they ecifically mediate opening of the bronchioles, the small airways in the lungs.



Although all GPCRs are thought to span the membrane seven times and hence to have similar three-dimensional structures, their amino acid sequences generally are **quite dissimilar**. For example, the sequences of the closely related b1- and b2-adrenergic receptors are only 50 percen identical; the sequences of the a- and b-adrenergic receptors exhibit even less homology. The specific amino acid sequence of each receptor determines which ligands it binds and which G proteins interact with it.

Figure 20-14. Demonstration of functional domains in G protein coupled receptors by experiments with chimeric proteins containing portions of the b 2- and a 2-adrenergic receptors. *Xenopus* oocytes microinjected with mRNA encoding the wild-type receptors or chimeric a-b receptors expressed the corresponding receptor protein on cell surfaces. Although *Xenopus* oocytes do not express adrenergic receptors, they do express G proteins, which can couple to the foreign receptors. Binding assays were conducted using agonists known to bind selectively to a or b receptors to determine the ligand-binding specificity of the chimeric receptors. The effects of the agonists on adenylyl cyclase activity were taken as a measure of whether the receptor protein bound to the stimulatory (Gs) or inhibitory (Gi) type of oocyte G protein. A comparison of chimeric receptor 1, which interacts with Gs, and chimeric receptor 3, which interacts with Gi, shows that the G protei specificity is determined primarily by the source of the cytosol-facing loop between a helices 5 and 6. A comparison of chimeras 1 and 2 indicates that a helix 7 plays a role in determining the ligand-binding specificity.

rimeric Gs Protein Links β-Adrenergic Receptors and Adenylyl Cyclase

s noted above, the initial response following binding of epinephrine to b-adrenergic eceptors is an elevation in the intracellular level of cAMP. The increase in cAMP results om activation of adenylyl cyclase, which converts ATP to cAMP and pyrophosphate PPi). This membrane-bound enzyme has two catalytic domains on the cytosolic face o he plasma membrane that can bind ATP in the cytosol (Figure 20-15). The link betwee ormone binding to an exterior domain of the receptor and activation of adenylyl cyclas provided by **stimulatory G protein** Gs, which functions as a signal transducer.

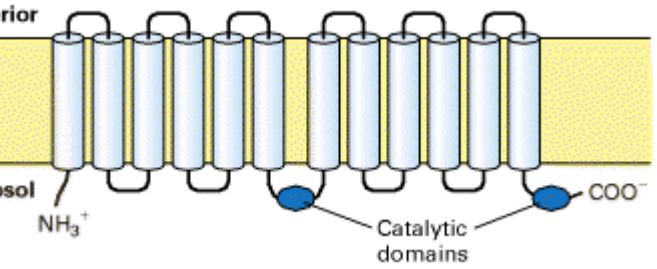
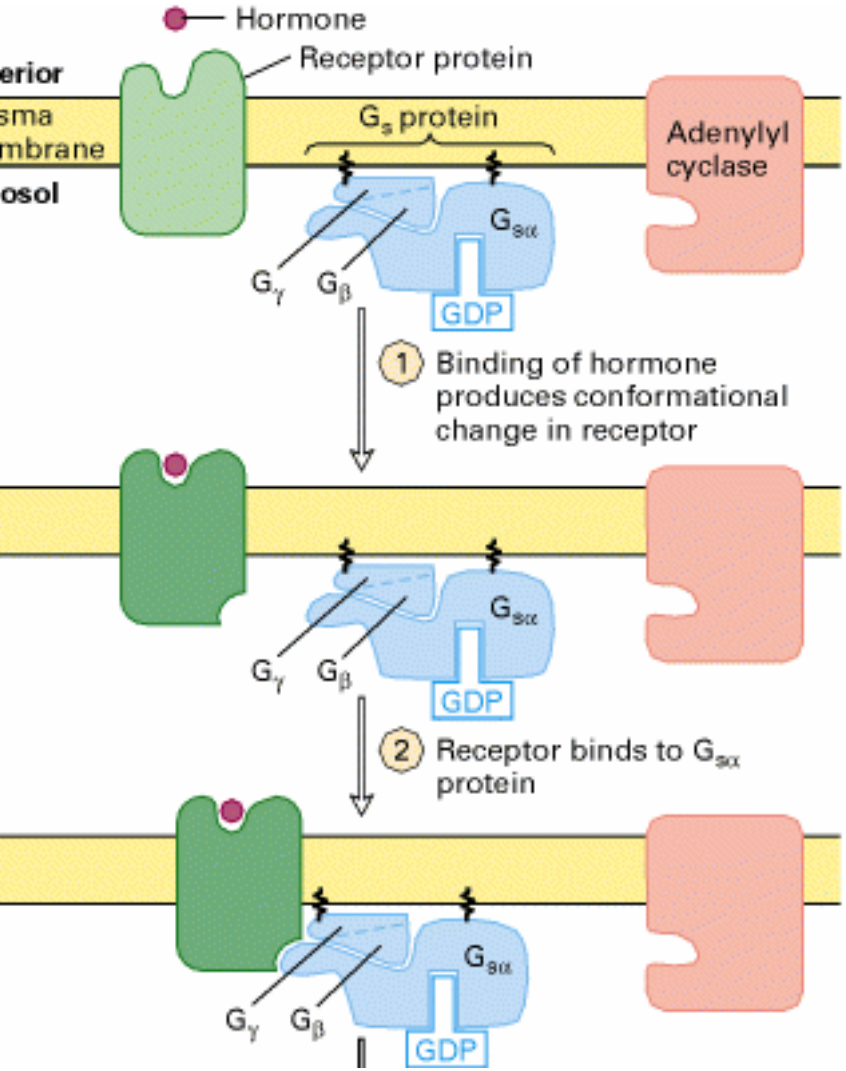
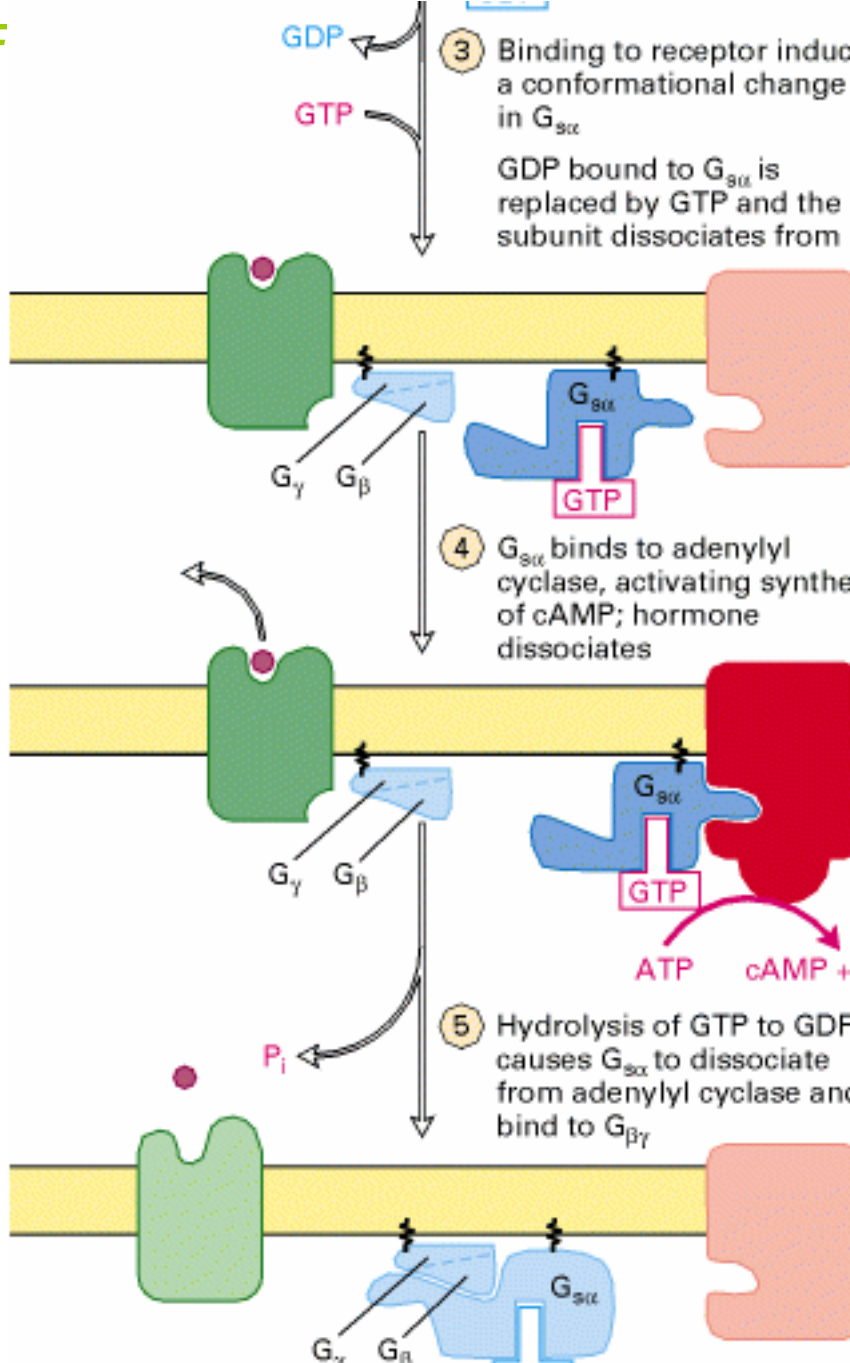


Figure 20-15. Schematic diagram of mammalian adenylyl cyclases. The membrane-bound enzyme contains two similar catalytic domains on the cytosolic face of the membrane and two integral membrane domains, each of which is thought to contain six transmembrane a helices. The **six adenylyl cyclase isoform** present in mammals are activated or inhibited by transducing G proteins following hormone binding to an appropriate receptor. One isoform found mainly in the brain also is activated by Ca2+ ions complexed to the protein calmodulin. [See W. -J. Tang and A G. Gilman, 1992, *Cell* **70:**869.]

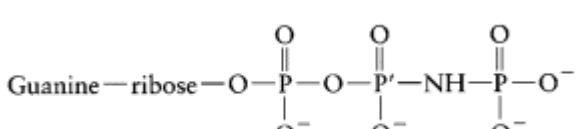
ycling of Gs between Active and Inactive F





he G proteins that transduce signals from the b-adrenergic receptor and other GPCRs ontain three subunits designated a, b, and r. As explained earlier, these GTPase switc roteins alternate between an "on" state with bound GTP and an "off" state with bound DP (see Figure 20-5a). For example, when no ligand is bound to a b-adrenergic eceptor, the a subunit of Gs protein (Gsa) is bound to GDP and complexed with the b nd r subunits (Figure 20-16). Binding of a hormone or agonist to the receptor changes s conformation, causing it to bind to the trimeric Gs protein in such a way that GDP is splaced from Gsa and GTP is bound. The Gsa ·GTP complex, which dissociates from he Gbr complex, then binds to and activates adenylyl cyclase. This activation is short- ved, however, because GTP bound to Gsa hydrolyzes to GDP in seconds, leading to he association of Gsa with Gbr and inactivation of adenylyl cyclase. **The Gsa subunit hus relays the conformational change in the receptor triggered by hormone inding to adenylyl cyclase**.

mportant evidence supporting this model has come from studies with a **onhydrolyzable analog of GTP called GMPPNP**, in which a P-NH-P replaces the erminal phosphodiester bond in GTP:



lthough this analog cannot be hydrolyzed, it binds to Gsa as well as GTP does. The addition of MPPNP and an agonist to an erythrocyte membrane preparation results in a much larger and nger-lived activation of adenylyl cyclase than occurs with an agonist and GTP. Once the GDP ound to Gsa is displaced by GMPPNP, it remains permanently bound to Gsa . Because the Gsa MPPNP complex is as functional as the normal Gsa · GTP complex in activating adenylyl cyclase

mplification of Hormone Signal

he cellular responses triggered by cAMP may require tens of thousands or even illions of cAMP molecules per cell. Thus the hormone signal must be amplified in orde o generate sufficient second messenger from the few thousand b-adrenergic receptors resent on a cell. *Amplification* is possible because both receptors and Gs proteins can ffuse rapidly in the plasma membrane. A single receptor-hormone complex causes onversion of up to 100 inactive Gs molecules to the active form. Each active Gsa · GT turn, probably activates a single adenylyl cyclase molecule, which then catalyzes ynthesis of many cAMP molecules during the time Gs · GTP is bound to it.

ermination of Cellular Response

ermination of the response to hormones recognized by b-adrenergic receptors is acilitated by a **decrease in the affinity** of the receptor that occurs when Gs is onverted from the inactive to active form. When the GDP bound to Gsa is replaced wit GTP following hormone binding, the *K*D of the receptor-hormone complex increases, hifting the **equilibrium toward dissociation**. The GTP bound to Gsa is quickly ydrolyzed, reversing the activation of adenylyl cyclase and terminating the cellular esponse unless the concentration of hormone remains high enough to form new eceptor-hormone complexes. Thus, the continuous presence of hormone is required fo ontinuous activation of adenylyl cyclase.

ome Bacterial Toxins Irreversibly Modify G Proteins

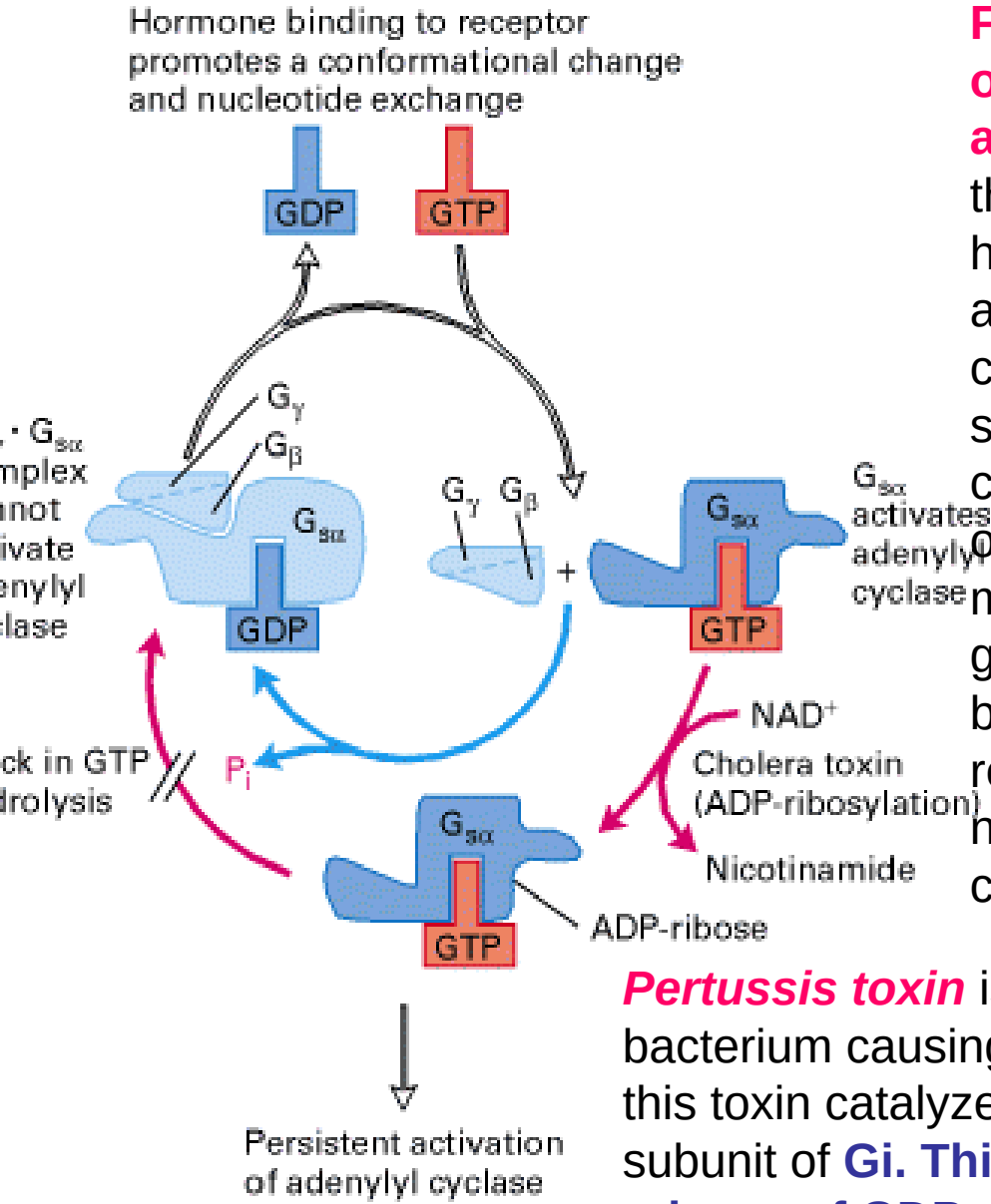
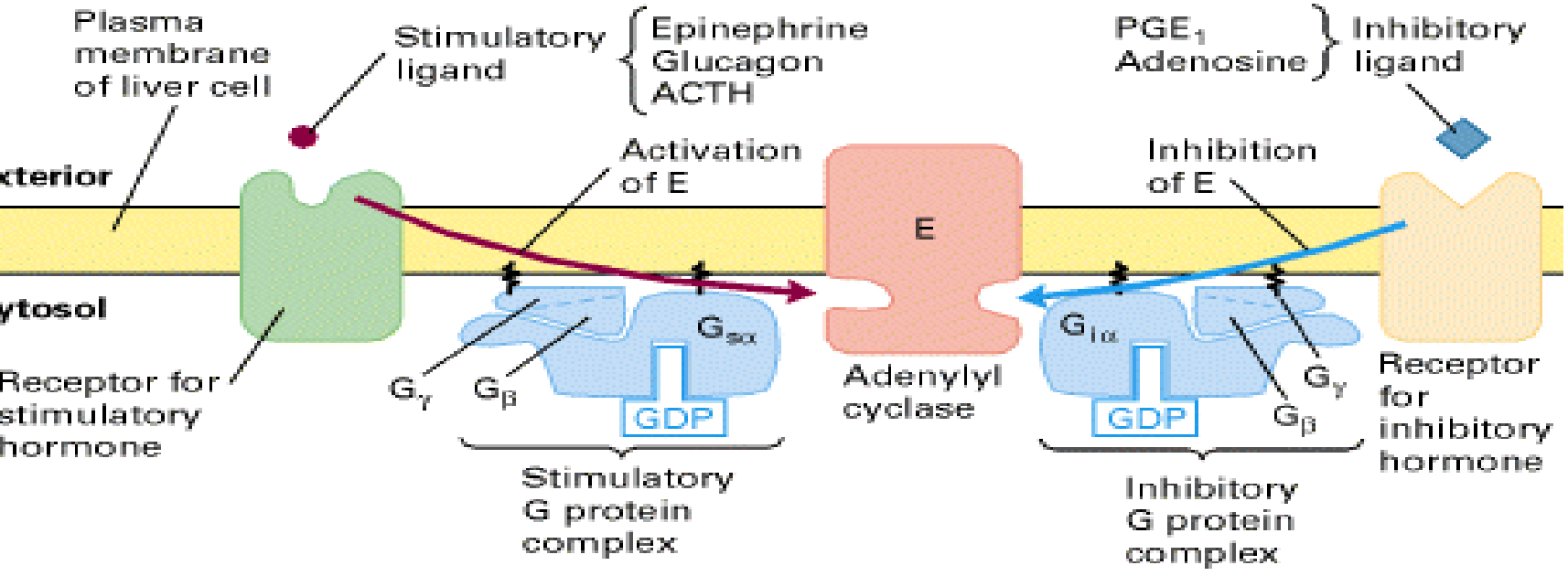


Figure 20-17. Effect of cholera toxin on cycling of Gsa between the active and inactive forms. Normally, GTP in the active Gsa · GTP is rapidly hydrolyzed (blue arrow), so that the activation of adenylyl cyclase and rise in cAMP persist only as long as hormone stimulation. Hydrolysis of GTP to GDP is catalyzed by Gsa itself. In the presence of cholera toxin, Gsa is irreversibly modified by addition of an ADP-ribosyl group; the modified Gsa can bind GTP but cannot hydrolyze it (red arrows). As a result, there is an excessive, nonregulated rise in the intracellular cAMP level.

Pertussis toxin is secreted by *Bordetella pertussis,* the bacterium causing whooping cough. The S1 subunit of this toxin catalyzes addition of ADP-ribose to the a subunit of **Gi. This irreversible modification prevents release of GDP**, locking Gia in the GDP-bound state.

denylyl Cyclase Is Stimulated and Inhibited by Different Receptor-Ligand



igure 20-18. Hormone-induced activation and inhibition of adenylyl cyclase is ediated by Gsa (blue) and Gia (brown), respectively. Binding of Gsa · GTP to denylyl cyclase activates the enzyme (see Figure 20-16), whereas binding of Gia hibits adenylyl cyclase. The G b g subunit in both stimulatory and inhibitory G proteins identical; the G a subunits and the receptors differ. Some isoforms of adenylyl cyclase re directly inhibited by binding to G b g . Others require coincident binding of ssociated Gsa =GTP and G b g subunits (see Section 20.7).

egradation of cAMP Also Is Regulated

he level of cAMP usually is controlled by the hormone-induced activation of adenylyl yclase. Another point of regulation is the hydrolysis of cAMP to 5 -AMP by ***cAMP hosphodiesterase***. This hydrolysis terminates the effect of hormone stimulation. As scussed later, the activity of many cAMP phosphodiesterases is stimulated by an crease in cytosolic Ca2+ (another intracellular second messenger), which often is duced by neuron or hormone stimulation. Some cells also modulate the level of cAMP y secreting it into the extracellular medium.

he synthesis and degradation of cAMP are both subject to complex regulation by ultiple hormones, which allows the cell to integrate responses to many types of hanges in its internal and external environments.