MECHANISMS OF HORMONE ACTION

Introduction

Hormones produce their biologic effects through interaction with high-affinity receptors that are, in turn, linked to one or more effector systems within the cell. These effectors involve many different components of the cell's metabolic machinery, ranging from ion transport at the cell surface to stimulation of the nuclear transcriptional apparatus. Steroids and thyroid hormones exert their effects in the cell nucleus, although regulatory activity in the extranuclear compartment has also been documented. Peptide hormones and neurotransmitters, on the other hand, trigger a spectrum of signaling activity in the cytoplasmic and membrane compartments while at the same time exerting parallel effects on the transcriptional apparatus. The discussion that follows will focus on the primary signaling systems employed by selected hormonal agonists and attempt to identify examples where abnormal signaling results in human disease.

Receptors

The biologic activity of individual hormones is dependent on their interactions with specific high-affinity receptors on the surfaces or in the cytoplasm or nuclei of target cells. The receptors, in turn, are linked to signaling effector systems responsible for generating the observed biologic response. Receptors therefore convey not only specificity of the response (i.e., cells lacking receptors lack responsiveness to the hormone) but also the means for activating the effector mechanism. In general, receptors for the peptide hormones and neurotransmitters are found on the cell surface and those for the steroid hormones, thyroid hormone, and vitamin D are found in the cytoplasmic or nuclear compartments.

Interactions between the hormone ligand and its receptor are governed by the laws of mass action:

$$[H] + [R] \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} [HR]$$

where [H] is the hormone concentration, [R] is the receptor concentration, [HR] is the concentration of the hormone-receptor complex, and k_{+1} and k_{-1} are the rate constants for [HR] formation and dissociation respectively. Thus, at equilibrium,

$$k_{+1}[H][R] = k_{-1}[HR]$$

or

$$\frac{[H][R]}{[HR]} = \frac{k_{-1}}{k_{+1}} = K_D$$

where K_D is the equilibrium dissociation constant that defines the affinity of the hormone-receptor interaction (ie, the lower the dissociation constant, the higher the affinity). Assuming that total receptor concentration $R_o = [HR] + [R]$, this equation can be rearranged to give

$$\frac{[HR]}{[H]} = -\left(\frac{[HR]}{K_D}\right) + \frac{R_o}{K_D}$$

This is the Scatchard equation and states that when bound over free ligand (i.e., [HR]/[H]) is plotted against bound ligand (i.e., [HR]), the slope of the line is defined by $-1/K_D$, the *y*-intercept by R_o/K_D and the *x*-intercept by R_o (Figure 2–1). When $[HR] = R_o/2$, $[H] = K_D$; therefore, the K_D is also the concentration of hormone [H] at which one-half of the available receptors are occupied. Thus, knowledge of bound and free ligand concentrations, which can be determined experimentally, provides information regarding the affinity of the receptor for its ligand and the total concentration of receptor in the preparation.

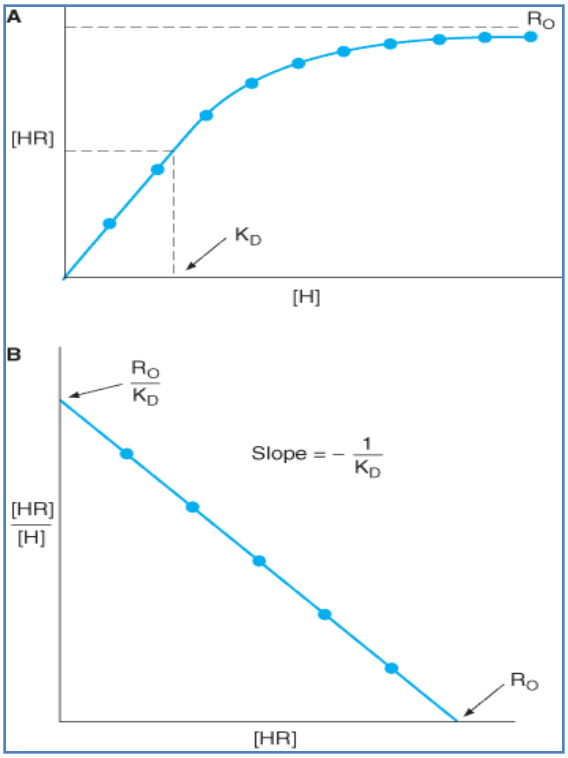


Figure 2-1. Ligand saturation ***(A)*** and Scatchard analysis ***(B)*** of a hypothetical hormone receptor interaction. K_D represents the dissociation constant; R_o the total receptor concentration; [HR] and [H] the bound and free ligand, respectively. Note in ***A*** that the K_D is the concentration [H] at which half of available receptors are occupied.

Agents that bind to receptors with high affinity are classified as either agonists or antagonists based on the functional outcome of this receptor-ligand interaction. Agonists are ligands that trigger the effector mechanisms and produce biologic effects. Antagonists bind to the receptor but do not activate the effector mechanisms. Because they occupy receptor and block association with the agonist, they antagonize the functional activity of the agonist. Partial agonists bind to the receptor but possess limited ability to activate the effector mechanisms.

Neurotransmitter & Peptide Hormone Receptors

Neurotransmitter and peptide hormones interact predominantly with receptors expressed on the plasma membrane at the cell surface. The neurotransmitter and peptide receptors can be divided into four major groups (Table 2–1). The first includes the so-called serpentine or "seven-transmembrane-domain" receptors. These receptors each contain an amino terminal extracellular domain followed by seven hydrophobic amino acid segments, each of which is believed to span the membrane bilayer (Figure 2–2). The seventh of these, in turn, is followed by a hydrophilic carboxyl terminal domain that resides within the cytoplasmic compartment. As a group, they share a dependence on the G protein transducers (see below) to execute many of their biologic effects. A second group includes the single-transmembrane domain receptors that harbor intrinsic tyrosine kinase activity. This includes the insulin, insulin-like growth factor (IGF), and

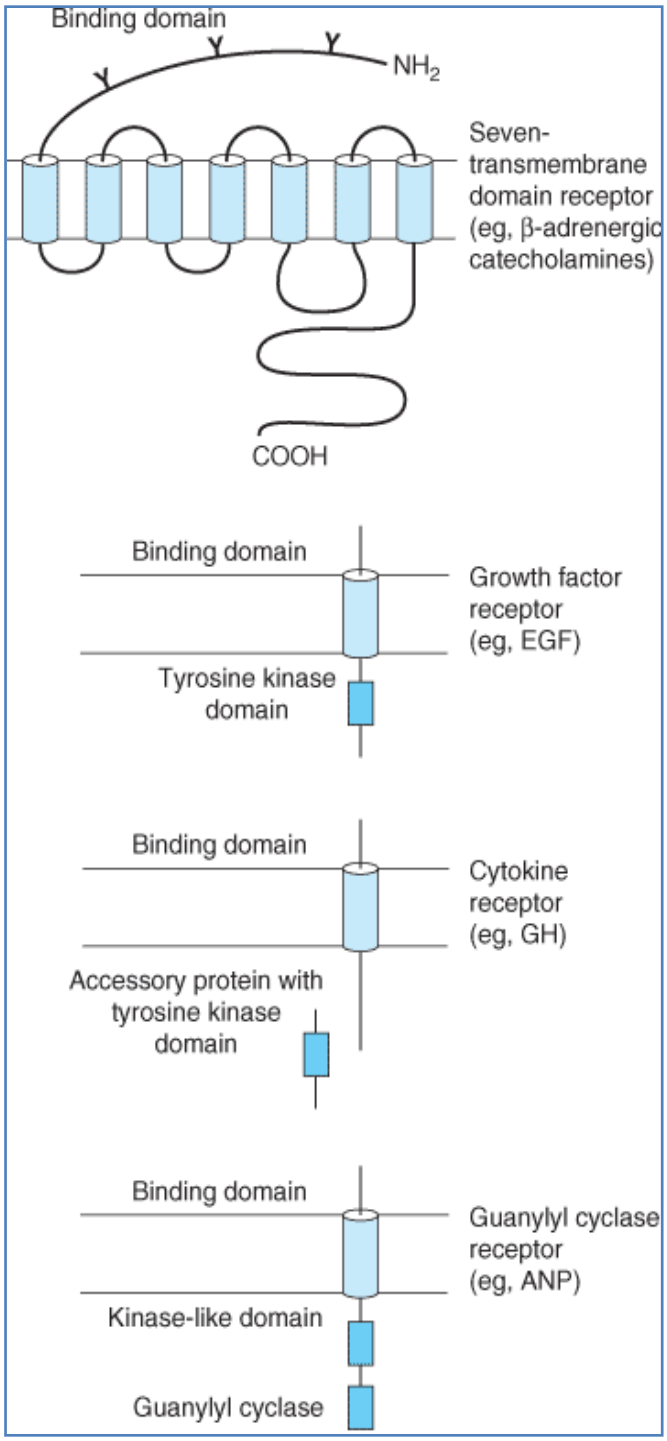
epidermal growth factor (EGF) receptors. A third group, which is functionally similar to the second group, is characterized by a large extracellular binding domain followed by a single membrane-spanning segment and a cytoplasmic tail. These receptors do not possess intrinsic tyrosine kinase activity but appear to function through interaction with soluble transducer molecules which do possess such activity. Prolactin and growth hormone are included in this group. A fourth group, which includes the natriuretic peptide receptors, operates through activation of a particulate guanylyl cyclase and synthesis of cGMP. The cyclase is covalently attached at the carboxyl terminal of the ligand-binding domain and thus represents an intrinsic part of the receptor molecule.

Table 2–1. Major Subdivisions of the Neurotransmitter-Peptide Hormone Receptor Families.[1]

Seven-transmembrane domain
β-Adrenergic
PTH
LH
TSH
GRH
TRH
ACTH
MSH
Glucagon
Dopamine
α_2-Adrenergic (–)
Somatostatin (–)
Single-transmembrane domain
Growth factor receptors
Insulin
IGF
EGF
PDGF
Cytokine receptors
Growth hormone
Prolactin
Erythropoietin
CSF
Guanylyl cyclase-linked receptors
Natriuretic peptides

1Receptors have been subdivided based on shared structural and functional similarities. (–) denotes a negative effect on cyclase activity.

Figure 2-2. Structural schematics of different classes of membrane-associated hormone receptors. Representative ligands are presented in parentheses (EGF, epidermal growth factor; GH, growth hormone; ANP, atrial natriuretic peptide).



G Protein-Coupled Receptors

G protein-coupled receptors constitute a large superfamily of molecules capable of responding to ligands of remarkable structural diversity—ranging from photons to large polypeptide hormones. These receptors share overall structural features, most notably seven membrane-spanning regions connected by intracellular and extracellular loops (Figure 2–2). The receptors are oriented such that the amino terminal domain is extracellular, whereas the carboxyl terminal tail is cytoplasmic. The membrane-spanning segments interact with one another, forming an irregular cylindrical bundle around a central cavity within the molecule. G protein-coupled receptors can assume at least two conformations with differing orientations of the membrane-spanning segments relative to one another. One orientation is favored in the absence of an agonist ligand, and in this orientation the receptor does not activate a G protein (inactive conformation). The second orientation is stabilized by the binding of an appropriate agonist ligand, and in this conformation the receptor activates a G protein (active conformation). All G protein-coupled receptors are thought to undergo a similar conformational switch on agonist binding, producing a structural change in the cytoplasmic domain that promotes G protein activation. Some small agonists, such as catecholamines, are able to enter the cavity formed by the transmembrane segments, thereby directly stabilizing the active receptor conformation. Other agonists, such as large polypeptide hormones, bind primarily to the extracellular domain of their G protein-coupled receptors. This indirectly results in movement of the transmembrane region of the receptor and stabilization of the active receptor conformation.

Until recently, it was thought that G protein-coupled receptors function exclusively as monomers. Many G protein-coupled receptors are now known to dimerize either with themselves (homodimerization) or with other G protein-coupled receptors (heterodimerization). In some cases, dimerization is important for efficient receptor biosynthesis and membrane localization. In other cases, dimerization is important for optimal ligand affinity, specificity, or receptor signaling.

Heritable mutations in a variety of G protein-coupled receptors are known to be associated with disease. Loss-of-function phenotypes result from mutations that eliminate one or both receptor alleles or that result in the synthesis of signaling-defective receptors. Gain-of-function phenotypes generally result from point mutations that produce constitutively active receptors (i.e., stably assume the active receptor conformation even in the absence of an agonist ligand).

G Protein Transducers

G protein-coupled receptors initiate intracellular signaling by activating one (or in some cases multiple) G proteins. G proteins are a family of heterotrimeric proteins that regulate the activity of effector molecules (e.g., enzymes, ion channels) (Table 2–2), resulting ultimately in biological responses. The identity of a G protein is defined by the nature of its α subunit, which is largely responsible for effector activation. The major G proteins involved in hormone action (and their actions on effectors) are G_s (stimulation of adenylyl cyclase), G_i (inhibition of adenylyl cyclase; regulation of calcium and potassium channels), and $G_{q/11}$ (stimulation of phospholipase Cβ). The β and γ subunits of G proteins are tightly associated with one another and function as a dimer. In some cases, the βγ subunit dimer also regulates effector function.

Table 2–2. G Protein Subunits Selectively Interact with Specific Receptor and Effector Mechanisms.

G Protein Subunit	Associated Receptors	Effector
α_s	β-Adrenergic TSH Glucagon	Adenylyl cyclase Ca^{2+} channels K^+ channels
α_i	α_2-Adrenergic Muscarinic (type II)	Adenylyl cyclase Ca^{2+} channels K^+ channels
α_q	α_1-Adrenergic	PLCβ
β/α		Adenylyl cyclase (+ or –) PLC Supports βARK-mediated receptor phosphorylation and desensitization

G proteins are noncovalently attached to the plasma membrane and are thus proximate to their cognate receptors and to their effector targets. The basis for specificity in receptor-G protein interactions has not been fully defined. It is likely that specific structural determinants presented by the cytoplasmic loops of the G protein-coupled receptor determine the identity of the G proteins that are activated. It is the nature of the α subunit of the G protein that is critical for

receptor recognition. There are about a dozen different G protein α subunits and hundreds of distinct G protein-coupled receptors. Thus, it is clear that a particular G protein is activated by a large number of different receptors. For example, G_s is activated by receptors for ligands as diverse as β-adrenergic catecholamines and large polypeptide hormones such as LH. LH is thereby able to stimulate adenylyl cyclase and raise intracellular levels of cAMP in cells that express LH receptors (e.g., Leydig cells of the testis).

Figure 2–3 is a schematic representation of the molecular events associated with activation of G proteins by G protein-coupled receptors. In the basal, inactive state, the G protein is an intact heterotrimer with guanosine diphosphate (GDP) bound to the α subunit. Agonist binding to a G protein-coupled receptor promotes the physical interaction between the receptor and its cognate G protein. This produces a conformational change in the G protein, resulting in the dissociation of GDP. This in turn allows the binding of GTP (which is present at a much higher concentration in cells than is GDP) to the α subunit. Dissociation of the GTP-bound α subunit from the βγ dimer then occurs, allowing these subunits to activate their effector targets. Dissociation of the hormone-receptor complex also occurs. The duration of activation is determined by the intrinsic GTPase activity of the G protein α subunit. Hydrolysis of GTP to GDP terminates the activity and promotes reassociation of the αβγ trimer, returning the system to the basal state.

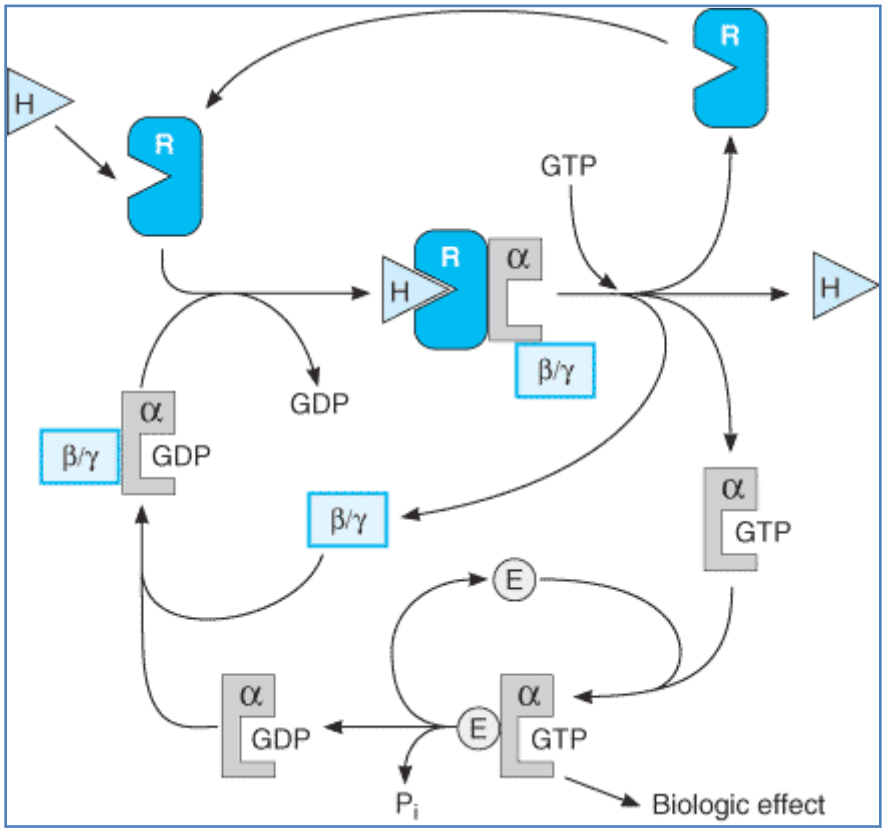


Figure 2-3. G protein-mediated signal transduction. A and β/γ subunits of a representative G protein are depicted. (R, hormone receptor; H, hormonal ligand; E, effector).

Effectors

Numerous effectors have been linked to the G protein-coupled receptors. As discussed above, adenylyl cyclase, perhaps the best-studied of the group, is activated by G_s (Figure 2–4). This activation results in a transient increase in intracellular cAMP levels. The cAMP binds to the inhibitory regulatory subunit of inactive protein kinase A (PKA) and promotes its dissociation from the complex, thereby permitting enhanced activity of the catalytic subunit. The latter phosphorylates a variety of cellular substrates, among them the hepatic phosphorylase kinase that initiates the enzymatic cascade that results in enhanced glycogenolysis. It also phosphorylates and activates the cAMP response element binding protein (CREB), which mediates many of the known transcriptional responses to cAMP (and to some extent calcium) in the nuclear compartment. Other transcription factors are also known to be phosphorylated by PKA.

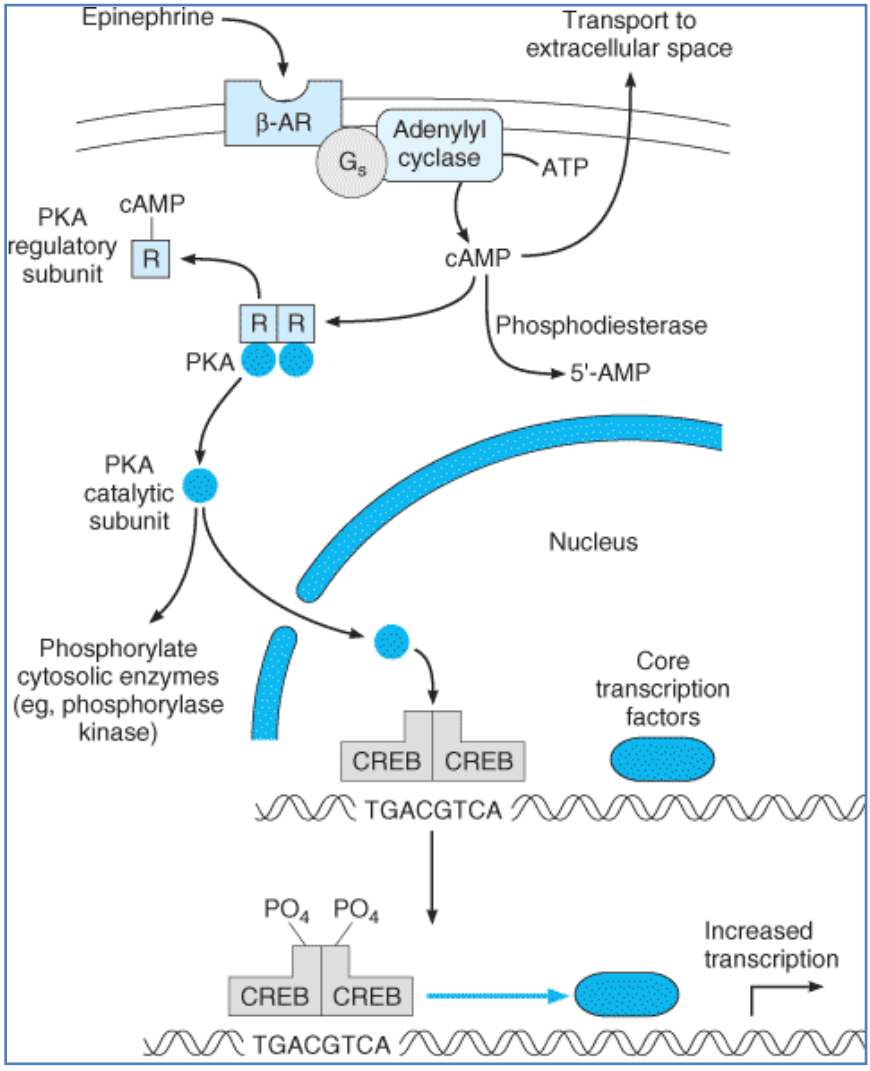


Figure 2-4. β-Adrenergic receptor signaling in the cytoplasmic and nuclear compartments. The cAMP response element binding protein (CREB) is depicted bound to a consensus CRE in the basal state. Phosphorylation of this protein leads to activation of the juxtaposed core transcriptional machinery.

Phospholipase C beta (PLCβ) is a second effector system that has been studied extensively. The enzyme is activated through G_q-mediated transduction of signals generated by a wide array of hormone-receptor complexes, including those for angiotensin II, α-adrenergic agonists, and endothelin. Activation of the enzyme leads to cleavage of phosphoinositol 4,5-bisphosphate in the plasma membrane to generate inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Figure 2–5). The former interacts with a specific receptor present on the endoplasmic reticulum membrane to promote release of Ca^{2+} into the cytoplasmic compartment. The increased calcium, in turn, may activate protein kinases, promote secretion, or promote contractile activity. Depletion of intracellular calcium pools by IP_3 results in enhanced uptake of calcium across the plasma membrane, thereby activating a second indirect, signaling mechanism that serves to increase intracellular calcium levels even further. DAG functions as an activator of protein kinase C (PKC) within the cell. However, not all protein kinase C activity derives from the breakdown of PIP_2 substrate. Metabolism of phosphatidylcholine by PLC_{PC} leads to the generation of phosphocholine and DAG. This latter pathway is believed to be responsible for the more prolonged elevations in PKC activity seen following exposure to agonist.

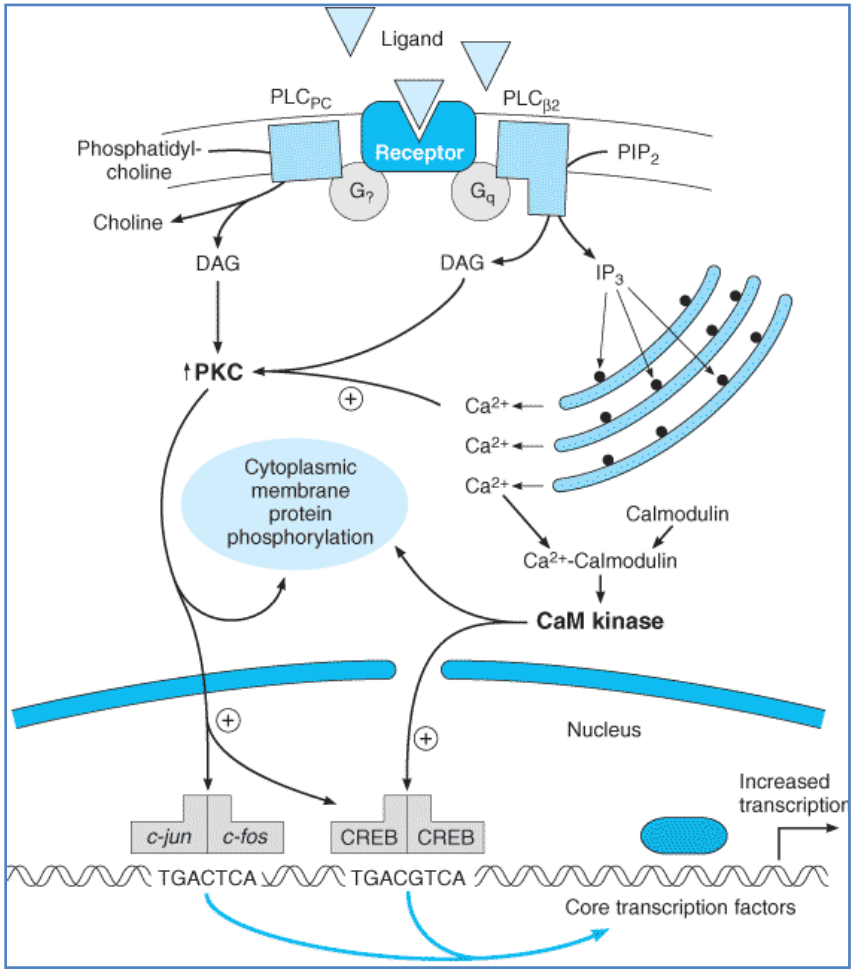


Figure 2-5. PLCβ-coupled receptor signaling in the cytoplasmic and nuclear compartments (PLC, phospholipase; PC, phosphatidylcholine; DAG, diacylglycerol; PKC, protein kinase C).

Other phospholipases may also be important in hormone-dependent signaling. Phospholipase D employs phosphatidylcholine as a substrate to generate choline and phosphatidic acid. The latter may serve as a precursor for subsequent DAG formation. As with PLC_{PC} above, no IP_3 is generated as a consequence of this reaction. Phospholipase A_2 triggers release of arachidonic acid, a precursor of prostaglandins, leukotrienes, and thromboxanes, all are signaling molecules.

Activation of effectors by G protein-coupled receptors is subject to regulatory mechanisms that prevent overstimulation of cells by an agonist ligand. At the level of the receptor, two regulatory events are known to occur. One is desensitization, wherein initial stimulation of a receptor by its agonists leads to a loss of the ability of the receptor to subsequently elicit G protein activation. This is shown schematically in Figure 2–6 for the β-adrenergic receptor. A similar regulatory mechanism exists for many G protein-coupled receptors. Agonist binding to the receptor produces G protein activation and results also in activation of a kinase that phosphorylates the cytoplasmic domain of the receptor. Due to this phosphorylation, the receptor acquires high affinity for a member of the arrestin family of proteins. The name "arrestin" derives from the observation that the receptor is no longer capable of interacting with a G protein when arrestin is bound. Thus, the phosphorylated receptor becomes uncoupled from its G protein, preventing signaling to the effector. The receptor remains inactive until a phosphatase acts to restore the receptor to its unphosphorylated state.

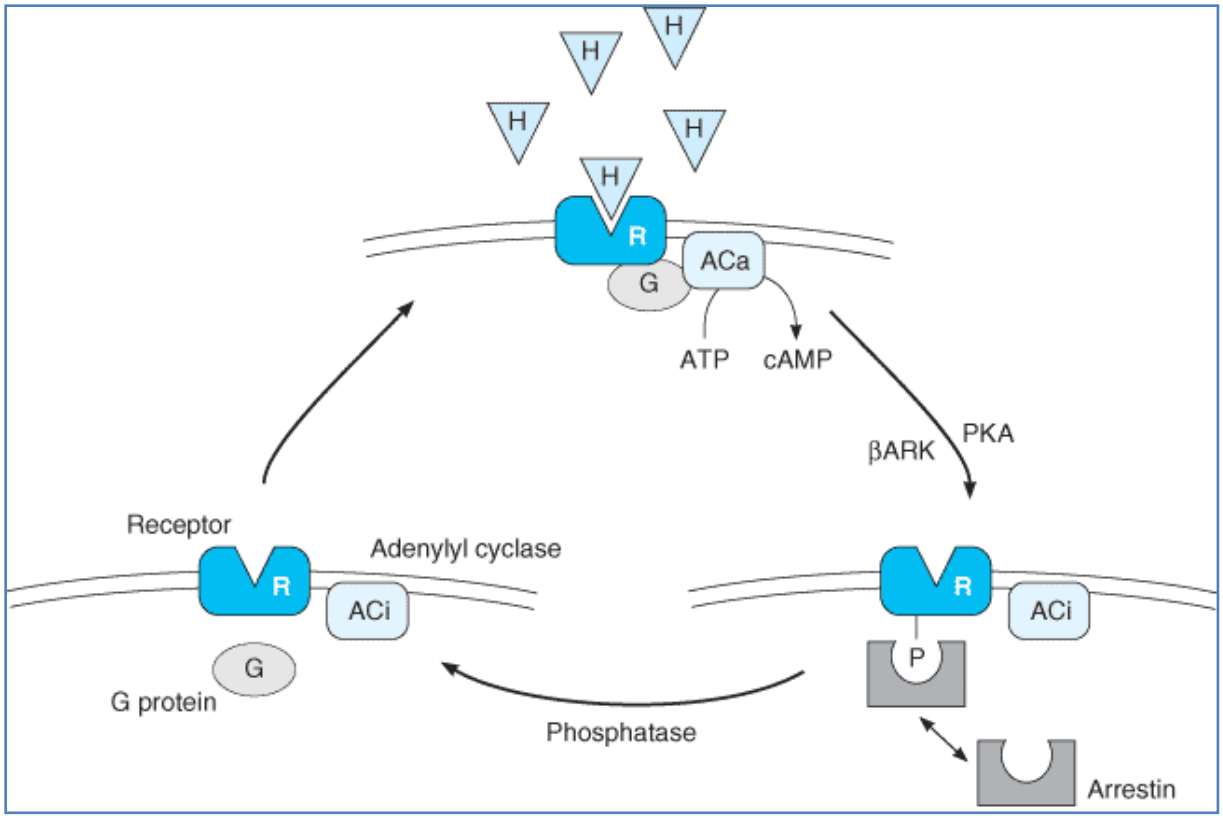


Figure 2-6. **Kinase-dependent desensitization of the ligand-receptor complex**. Schema shown is that for the β-adrenergic receptor, but similar systems probably exist for other types of G protein-linked receptors (βARK identifies the β-adrenergic receptor kinase; PKA, protein kinase A; ACa, active adenylyl cyclase; ACi, inactive adenylyl cyclase).

Many G protein-coupled receptors are also susceptible to agonist-induced down-regulation, resulting in a reduced level of cell surface receptors following exposure of cells to an agonist. This can result from agonist-induced internalization of receptors, followed by trafficking of receptors to lysosomes where degradation occurs. In addition, chronic exposure of cells to an agonist may result in signaling events that suppress the biosynthesis of new receptors, thereby lowering steady state receptor levels. Together, these regulatory events ensure that the cell is protected from excessive stimulation in the presence of sustained high levels of an agonist.

Disorders of G Proteins and G Protein-Coupled Receptors

Two bacterial toxins are capable of covalently modifying specific G protein α subunits, thereby altering their functional activity. Cholera toxin is a protein that binds to receptors present on all cells, resulting in the internalization of the enzymatic subunit of the toxin. The toxin enzyme is an ADP-ribosyl transferase that transfers ADP-ribose from NAD to an acceptor site (Arg^{201}) on the α subunit of G_s. This covalent modification greatly inhibits the GTPase activity of α_s, enhancing the activation of adenylyl cyclase by extending the duration of the active GTP-bound form of the G protein. Even in the absence of an active G protein-coupled receptor, GDP dissociates (albeit very slowly) from the G protein. Thus, cholera toxin will eventually activate adenylyl cyclase activity even without agonist binding to a G protein-coupled receptor. The result is a large and sustained activation of adenylyl cyclase. When this occurs in intestinal epithelial cells, the massive increase in cAMP results in the increased water and salt secretion characteristic of cholera.

Pertussis toxin is also an ADP-ribosyl transferase. However, in this case, the substrates are the α subunits of different G proteins, most notably G_i and G_o. Once ADP-ribosylated by pertussis toxin, these G proteins are no longer able to interact with activated receptors and are thus stuck in an inactive (GDP-bound) conformation. Inhibition of receptor-mediated activation of G_i and G_o accounts for many of the clinical manifestations of pertussis infection.

Genetic mutations in G protein α subunits are seen in a number of human diseases. Acquired, activating mutations in α_s can produce a variety of phenotypes depending on the site of expression of the mutant protein. In McCune-Albright syndrome, the mutation occurs in a subset of neural crest cells during embryogenesis. In cells where cAMP is linked to cell proliferation (eg, thyrotropes, somatotropes), a subset of patients with benign tumors has been shown to have acquired activating mutations in α_s. Activating mutations in one of the G_i proteins that is coupled to cell proliferation, α_{i2}, have been reported in a subset of adrenal and ovarian tumors.

Mutations in the genes encoding G protein-coupled receptors are being increasingly recognized as important in the pathogenesis of endocrine disorders. Loss-of-function mutations generally need to be homozygous (or compound heterozygous) in order to result in a significant disease phenotype. This is probably due to the fact that most cells have a complement of receptors which exceeds what is needed for maximal cellular response ("spare receptors"). Thus, a 50% reduction in the amount of a cell surface receptor may have little influence on the ability of a target cell to respond.

Mutations that render G protein-coupled receptors constitutively active (in the absence of an agonist ligand) are seen in a number of endocrine disorders. Generally speaking, such mutations produce a disease phenotype resembling that seen with excessive levels of the corresponding hormone agonist. Thus, activating mutations in the TSH receptor produce neonatal thyrotoxicosis. Activating mutations in the PTH receptor result in hypercalcemia and increased bone resorption (mimicking the effects of excess PTH on bone) and delayed cartilage differentiation (mimicking the effects of excess PTH-related protein on cartilage).

Growth Factor Receptors

The growth factor receptors differ from those described above both structurally and functionally. Unlike the G protein-associated receptors, these proteins span the membrane only once and acquire their signaling ability, at least in part, through activation of tyrosine kinase activity, which is intrinsic to the individual receptor molecules. The insulin and IGF receptors fall within this group as do those for the autocrine or paracrine regulators platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and EGF. Signaling is initiated by the association of ligand (e.g., insulin) with the receptor's extracellular domain (Figure 2–7) and subsequent receptor dimerization. This results in phosphorylation of tyrosines both on the receptor itself as well as on nonreceptor substrates. It is assumed that phosphorylation of these substrates results in a cascade of activation events, similar to those described for the G protein-coupled systems, which contribute to perturbations in intracellular pathways. The autophosphorylation of the receptor molecules themselves has been studied extensively and provided some intriguing insights into the mechanisms that underlie signal transduction by this group of proteins.

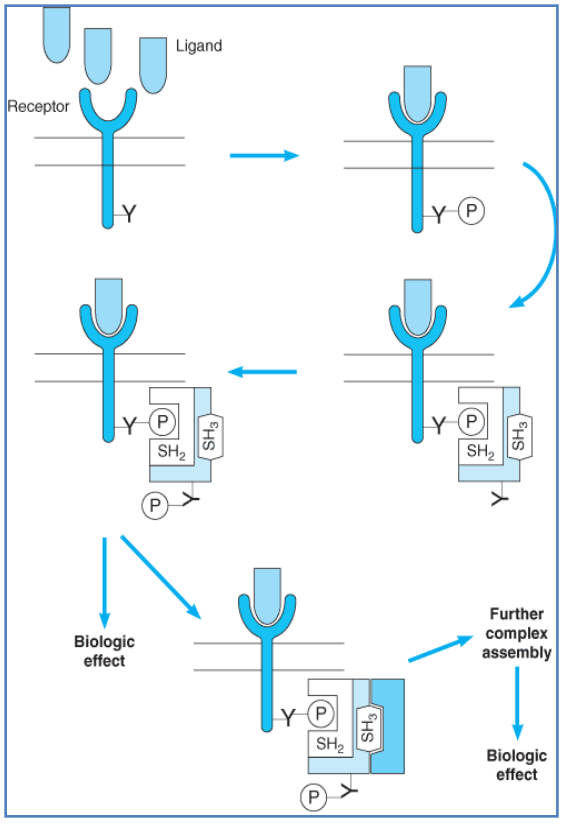


Figure 2-7. Signaling by tyrosine kinase-containing growth factor receptor. Receptors depicted here as monomers for simplicity; typically dimerization of receptors follows association with ligand. Autophosphorylation of one or more critically positioned tyrosine residues in the receptor leads to association with accessory proteins or effectors through SH2 domains present on the latter. In some cases an SH3 domain present on the same protein leads to recruitment of yet other proteins leading to further complex assembly.

Tyrosine phosphorylation takes place at specific locations in the receptor molecule. Once phosphorylated, these sites associate, in highly specific fashion, with a variety of accessory proteins that possess independent signaling capability. These include phospholipase Cγ (PLCγ), phosphoinositol (PI) 3' kinase, GTPase-activating protein (GAP), growth factor receptor-bound protein-2 (GRB2), and the Src family non-receptor tyrosine kinases. These interactions are fostered by the presence of highly conserved type 2 *src* homology (based on sequence homology to the *src* proto-oncogene) domains (SH2) in each of the accessory molecules.

Some of these associations trigger immediate signaling events, but others (e.g., GRB2) may act largely to provide the scaffolding needed to construct a more complex signaling apparatus (Figure 2–8). In the case of GRB2, another accessory protein (SOS) associates with the receptor-GRB2 complex through a type 3 *src* homology (SH3) domain present in the latter. SOS, in turn, facilitates assembly of the Ras-Raf complex, which permits activation of downstream effectors such as mitogen-activated protein kinase (MAPK) kinase (MEK). This latter kinase, which possesses both serine-threonine and tyrosine kinase activity, activates the p42 and p44 MAPKs (also called extracellular signal-regulated kinases; ERKs). ERK acts on a variety of substrates within the cell, including the RSK kinases, which, in turn, phosphorylate the ribosomal S6 protein and thereby stimulates protein synthesis.

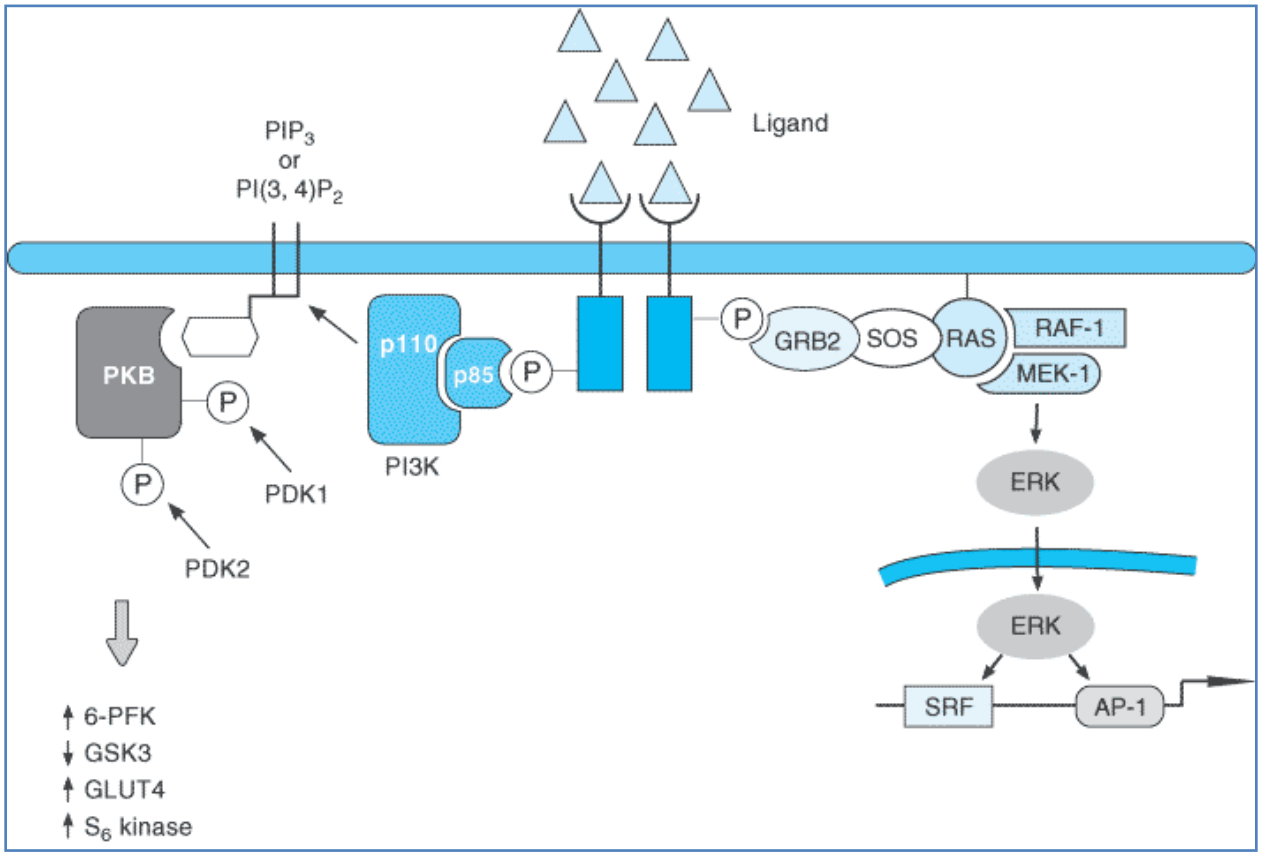


Figure 2-8. Growth factor-dependent pathway. Assembly of the components involved in the *ras/raf*/MEK/MAPK and PI-3K/PKB signaling mechanisms.

The liganded growth factor receptors, including the insulin receptor, may also signal through the phosphoinositide 3-OH kinase (PI-3K). SH2 domains of the p85 regulatory subunit of PI-3K associate with the growth factor receptor through specific phosphotyrosine residues in a manner similar to that described above for GRB2 (Figure 2–8). This leads to activation of the p110 catalytic subunit of PI-3K and increased production of phosphatidylinositol-3,4,5-trisphosphate (PIP_3) and phosphatidylinositol-3,4-bisphosphate ($PI[3,4]P_2$). These latter molecules sequester protein kinase B (also known as Akt) at the cell membrane. This in turn leads to phosphorylation of PKB by PIP_3-dependent kinases (PDK1 and PDK2). These phosphorylations result in activation of PKB. In the case of insulin-sensitive target cells, downstream targets of activated PKB (e.g., following insulin stimulation) include 6-phosphofructo-2-kinase (increased activity), glycogen synthase kinase-3 (decreased activity), the insulin-responsive glucose transporter GLUT 4 (translocation and increased activity) and p70 S6 kinase (increased activity). This leads to increased glycolysis, increased glycogen synthesis, increased glucose transport, and increased protein synthesis, respectively. There is also a growing body of evidence suggesting that PKB may protect cells from programmed cell death through phosphorylation of key proteins in the apoptotic pathway.

It has been reported that G protein-coupled receptors may also activate the Raf-MEK-ERK cascade. The details of the mechanism are incompletely understood, but it appears to require the participation of β-arrestin.

Cytokine Receptors

These include the receptors for a variety of cytokines, erythropoietin, colony-stimulating factor, GH, and prolactin. These receptors have a single internal hydrophobic stretch of amino acids, suggesting that they span the membrane but once (Figure 2–9). Interestingly, alternative splicing of the GH receptor gene primary transcript results in a shortened "receptor" that lacks the membrane anchor and carboxyl terminal domain of the protein. This "receptor" is secreted and serves to bind GH in the extracellular space (e.g., circulating plasma). Unlike the growth factor receptors described above, GH receptors lack a tyrosine kinase domain. Different domains of a single GH molecule associate with homologous regions of two independent GH receptors, promoting dimerization of the receptors and subsequent association with and activation of Janus kinase (JAK) 2. JAK2 undergoes autophosphorylation and concurrently tyrosine phosphorylates the GH receptors. The latter provide a docking site for the signal transducer and activator of transcription (STAT) factors; STAT 5a and 5b appear to be particularly relevant to GH and prolactin action. The STATs are phosphorylated, and they dissociate from the GH receptor, migrate to the nucleus, and bind to specific STAT-binding DNA regulatory elements (SIE/ISRE/GAS) responsible for transcriptional control of GH target genes such as IGF-1. The latter further amplifies GH effects through activation of its own tyrosine kinase receptor and subsequent stimulation of downstream signaling pathways such as the MAPK and PI-3K pathways.

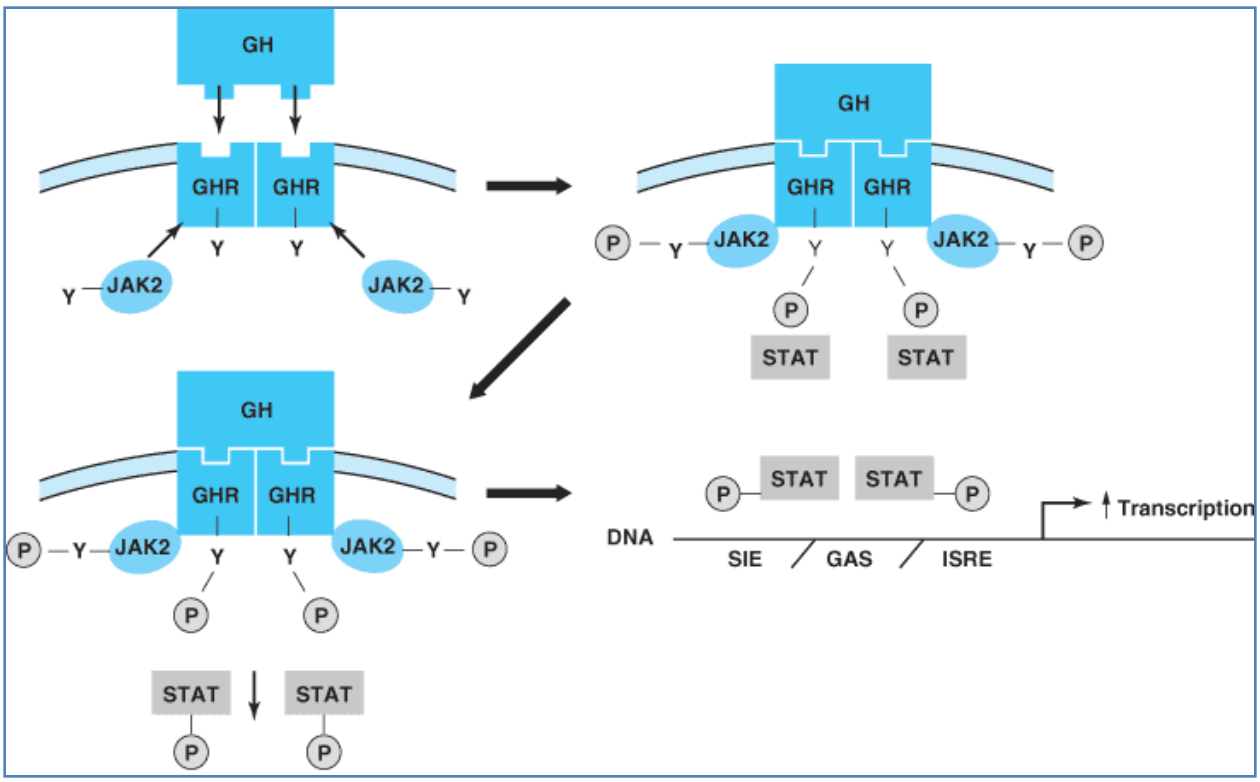


Figure 2-9. **Signaling by the growth hormone receptor**. Different portions of a single growth hormone molecule associate with homologous regions of two independent growth hormone receptor (GHR) molecules. This is believed to lead to the recruitment of Janus kinase 2 (JAK2), which phosphorylates the GHR, providing a docking site for STAT. The latter is phosphorylated, dissociates from the liganded receptor complex, and migrates to the nucleus, where it associates with binding elements of target genes and regulates transcription.

Guanylyl Cyclase-Linked Receptors

Activation of guanylyl cyclase-dependent signaling cascades can occur through two independent mechanisms. The first involves activation of the soluble guanylyl cyclase, a heme-containing enzyme that is activated by the gas nitric oxide (NO) generated in the same or neighboring cells. NO is produced by the enzyme nitric oxide synthase. NO synthase exists as three different isozymes in selected body tissues. Constitutive forms of NO synthase are produced in endothelial (NOS-3) and neuronal (NOS-1) cells. Agents such as bradykinin and acetylcholine, which interact with receptors on the surface of endothelial cells and increase intracellular calcium levels, trigger an increase in constitutive NO synthase activity with consequent generation of NO and activation of soluble guanylyl cyclase activity in neighboring vascular smooth muscle cells (Figure 2–10). Thus, in this instance, the cGMP-dependent vasodilatory activity of acetylcholine requires sequential waves of signaling activity in two different cell types to realize the ultimate physiologic effect.

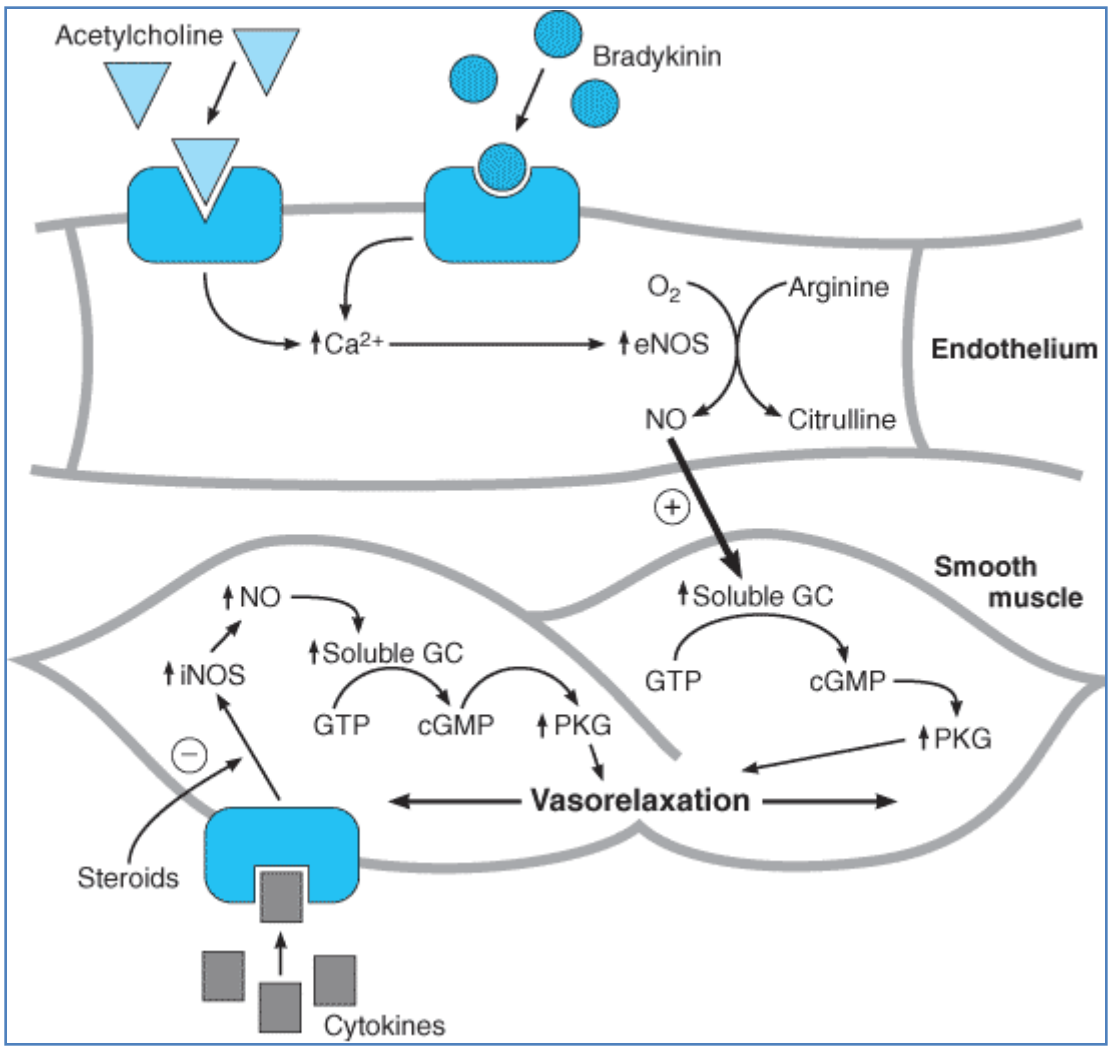


Figure 2-10. Signaling through the endothelial (e) and inducible (i) nitric oxide synthases (NOS) in the vascular wall. Activation of eNOS in the endothelial cell or iNOS in the vascular smooth muscle cell leads to an increase in NO and stimulation of soluble guanylyl cyclase (GC) activity. Subsequent elevations in cGMP activate cGMP-dependent protein kinase (PKG) and promote vasorelaxation.

The inducible (i) form of NO synthase (NOS-2) is found predominantly in inflammatory cells of the immune system, although it has also been reported to be present in smooth muscle cells of the vascular wall. Unlike the endothelial form of NO synthase, expression of iNO synthase is low in the basal state. Treatment of cells with a variety of cytokines triggers an increase in new iNO synthase synthesis (hence, the inducible component of iNO synthase activity). Thus, hormones, cytokines, or growth factors with the capacity for induction of iNO synthase activity may direct at least a portion of their signaling activity through a cGMP-dependent pathway.

A third mechanism for increasing cGMP levels within target cells involves the activation of particulate guanylyl cyclases. From an endocrine standpoint, this involves predominantly the natriuretic peptide receptors (NPR). NPR-A is a single-transmembrane-domain receptor (about

130 kDa) with a large extracellular domain that provides ligand recognition and binding. This is followed by a hydrophobic transmembrane domain and a large intracellular domain that harbors the signaling function. The amino terminal portion of this intracellular region contains a kinase-like ATP-binding domain that is involved in regulating cyclase activity, whereas the carboxyl terminal domain contains the catalytic core of the particulate guanylyl cyclase. It is believed that association of ligand with the extracellular domain leads to a conformational change in the receptor that causes activation of guanylyl cyclase activity. NPR-B, the product of a separate gene, has a similar topology and a relatively high level of sequence homology to the NPR-A gene product; however, while NPR-A responds predominantly to the cardiac atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), NPR-B is activated by the C-type NP (CNP), a peptide found in the central nervous system, endothelium, and reproductive tissues but not in the heart. Thus, segregated expression of the ligand and its cognate receptor convey a high level of response specificity to these two systems despite the fact that they share a common final effector mechanism.

Nuclear Action of Peptide Hormones

Although the initial targets of peptide hormone receptor signaling appear to be confined to the cytoplasm, it is clear that these receptors can also have profound effects on nuclear transcriptional activity. They accomplish this through the same mechanisms they use to regulate enzymatic activity in the cytoplasmic compartment (e.g., through activation of kinases and phosphatases). In this case, however, the ultimate targets are transcription factors that govern the expression of target genes. Examples include hormonal activation of c-*jun* and c-*fos,* nuclear transcription factors that make up the heterodimeric AP-1 complex. This complex has been shown to alter the expression of a wide variety of eukaryotic. The cAMP-dependent activation of protein kinase A results in the phosphorylation of a nuclear protein CREB (cAMP response element binding protein), an event that results in enhanced transcriptional activity of closely positioned promoters. The latter requires the participation of an intermediate CREB-binding protein (CBP). CBP is a coactivator molecule that functionally tethers CREB to proteins of the core transcriptional machinery. Interestingly, CBP may also play a similar role in nuclear receptor (NR) signaling. GH is known to induce the phosphorylation of an 84-kDa and a 97-kDa protein in target cells. These proteins have been shown to play a role in signaling cytokine activity.

Nuclear Receptors

The nuclear receptors (NRs), which include those for the glucocorticoids, mineralocorticoids, androgens, progesterone, estrogens, thyroid hormone, and vitamin D, differ from the receptors of the surface membrane described above in that they are soluble receptors with a proclivity for using transcriptional regulation as a means of promoting their biologic effects. Thus, although some receptors are compartmentalized in the cytoplasm (e.g., glucocorticoid receptor), whereas others are confined to the nucleus (e.g., thyroid hormone receptor), they all operate within the nuclear chromatin to initiate the signaling cascade. These receptors can be grouped into two major subtypes based on shared structural and functional properties. The first, the steroid receptor family, includes the glucocorticoid receptor (GR) and the receptors for mineralocorticoids (MR), androgens (AR), and progesterone (PR). The second, the thyroid

receptor family, includes the thyroid hormone receptor (TR) and the receptors for estrogen (ER), retinoic acid (RAR and RXR), and vitamin D (VDR) as well as the peroxisome proliferator-activated receptor (PPAR). In addition, there are more than 100 so-called orphan receptors that bear structural homology to members of the extended NR family. For most of these the "ligand" is unknown, and their functional roles in the regulation of gene expression have yet to be determined.

Steroid Receptor Family

Steroid receptors (i.e., GR, MR, AR, and PR), under basal conditions, exist as cytoplasmic, multimeric complexes that include the heat shock proteins HSP 90, HSP 70, and HSP 56. ER, although demonstrating similar association with HSP, is largely confined to the nuclear compartment. Association of the steroid ligand with the receptor results in dissociation of the HSP. This in turn exposes a nuclear translocation signal previously buried in the receptor structure and initiates transport of the receptor to the nucleus, where it associates with the hormone response element (Figure 2–11).

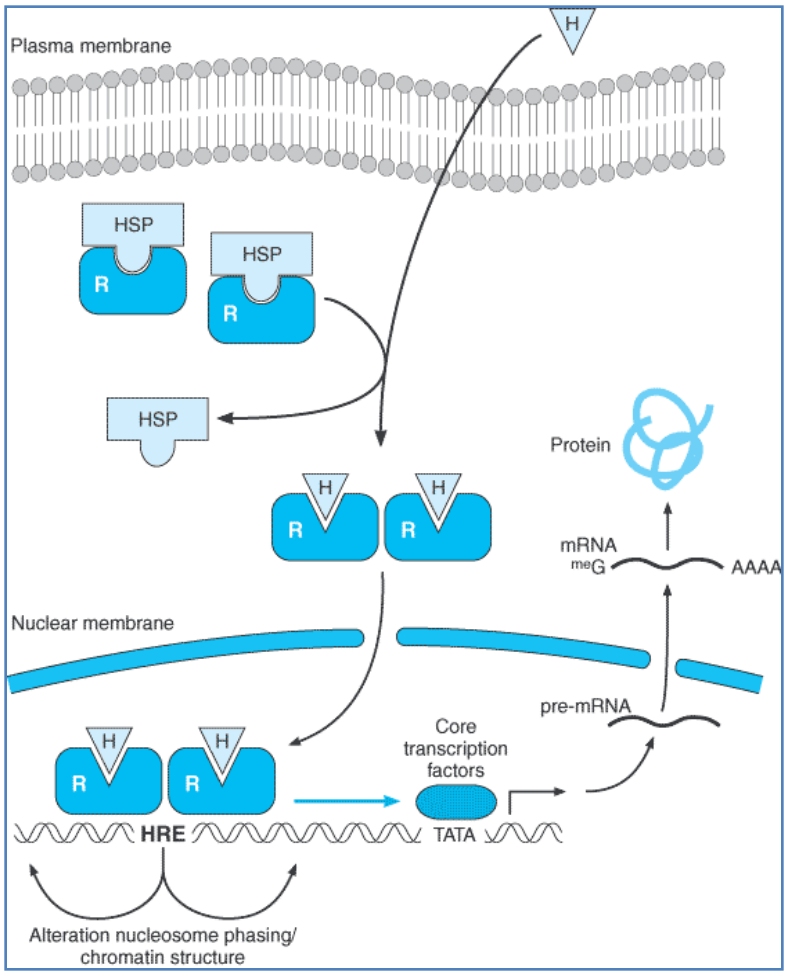


Figure 2-11. Signaling through the steroid receptor complex. Similar mechanisms are employed by members of the TR gene family, though most of the latter are concentrated in the nuclear compartment and are not associated with the heat shock protein complex prior to binding ligand.

The sturucture and function of the steroid receptor family is shown in Figure 2-12. Each of the family members has an extended amino terminal domain of varying length and limited sequence homology to other family members. In at least some receptors, this region, which has been termed AF-1, is believed to participate in the transactivation function through which the individual receptors promote increased gene transcription. The amino terminal is followed by a basic region that has a high degree of sequence homology in both the steroid and thyroid receptor gene families. This basic region encodes two zinc finger motifs (Figure 2–13) that have been shown to establish contacts in the major groove of the cognate DNA recognition element. The amino acid sequence lying between the first and

second fingers (i.e., recognition helix) is responsible for establishing specific contacts with the DNA. The second finger provides the stabilizing contacts that increase the affinity of the receptor for DNA. The DNA-binding region also harbors amino acid residues that contribute to the dimerization of monomers contiguously arrayed on the DNA recognition element. Following the basic region is the carboxyl terminal domain of the protein. This domain is responsible for binding of the relevant ligand, receptor dimerization or heterodimerization, and association with the heat shock proteins. It also contributes to the ligand-dependent transactivation function that drives transcriptional activity. Interestingly, in selected cases, nonligands have been shown to be capable of activating steroid receptors. Dopamine activates the progesterone receptor and increases PR-dependent transcriptional activity, probably through a phosphorylation event, which elicits a conformational change similar to that produced by the association of the receptor with progesterone.

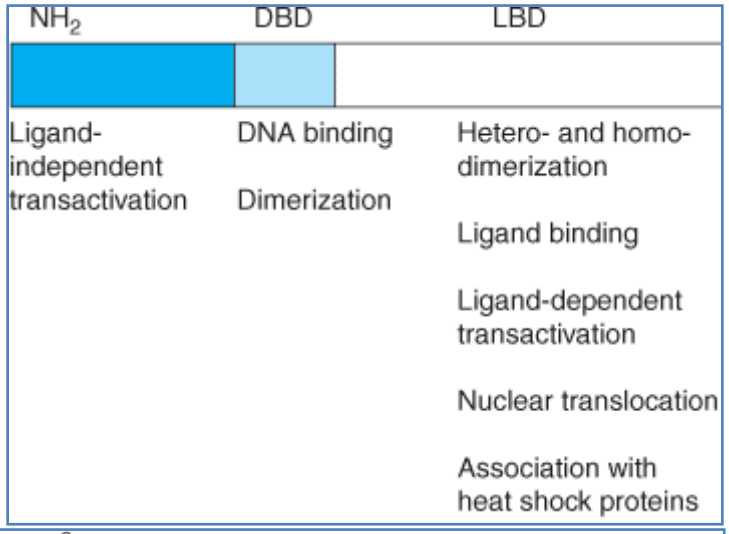


Figure 2-12. Structural schematic of a representative steroid receptor molecule. Separate designations are given to the amino terminal (NH_2), DNA-binding (DBD), and ligand-binding (LBD) domains. Functional activity associated with each of these individual domains, as determined by mutagenesis studies, are indicated below.

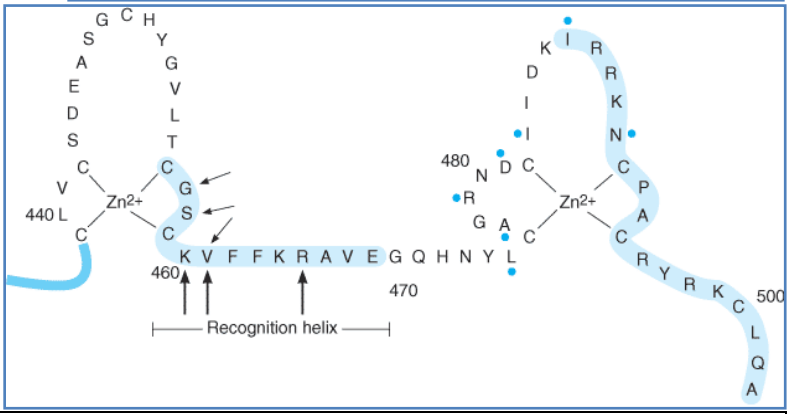


Figure 2-13. Schema of the two zinc fingers, together with coordinated zinc ion, which make up the DNA-binding domain of the glucocorticoid receptor (amino acids are numbered relative to the full length receptor). Shaded regions denote two alpha helical structures which are oriented perpendicularly to one another in the receptor molecule. The first of these, the recognition helix, makes contact with bases in the major groove of the DNA. Large arrows identify amino acids which contact specific bases in the glucocorticoid response element (GRE). Lighter arrows identify amino acids that confer specificity for the GRE; selective substitutions at these positions can shift receptor specificity to other response elements. Dots identify amino acids making specific contacts with the phosphate backbone of DNA.

Thyroid Receptor Family

Included in this group are the TR, RAR, RXR, ER, PPAR, and VDR. They share a high degree of homology to the proto-oncogene c-*erb*A and high affinity for a common DNA recognition site. With the exception of the ER, they do not associate with the HSPs, and they are constitutively bound to chromatin in the cell nucleus.

The ER binds to its RE as a homodimer, whereas the VDR, RAR, RXR, and TR prefer binding as heterodimers. The most prevalent TR-associated partners appear to be the retinoid X receptors. Heterodimerization with RXR amplifies both the DNA binding and the functional activity of these other receptors. Thus, the ability to form such heterodimeric complexes may add significantly to the flexibility and potency of these hormone receptor systems in regulating gene expression.

Nongenomic Effects of the Steroid Hormones

Although steroids exert most of their biologic activity through direct genomic effects, there are several lines of evidence suggesting that this does not provide a complete picture of steroid hormone action. There are several examples that, for kinetic or experimental reasons, do not fit the classic paradigm associated with a transcriptional regulatory mechanism. Included within this group are the rapid suppression of ACTH secretion following steroid administration, the modulation of oocyte maturation and neuronal excitability by progesterone, the stimulation of endothelial nitric oxide synthase (through interaction of ERα with the p85 subunit of PI-3K) by estrogen, the inhibition of type II deiodinase and stimulation of mitochondrial oxygen consumption by thyroid hormone, and regulation of calcium channel function by 1,25-$(OH)_2$ vitamin D. Recent studies have demonstrated the presence of conventional estrogen receptors on the plasma membrane of target cells. The relationship of these receptors to their nuclear counterparts and their role in signaling estrogen-dependent activity (genomic versus nongenomic) is being actively investigated. The PR displays a novel ability to interact with the SH3 domains of the Src family tyrosine kinases, thereby accessing the Ras/Raf/MEK 1/ERK signaling pathway.

Neurosteroids represent another class of nontraditional hormonal agonists with unique biologic activity. Some of these are native steroids (e.g., progesterone), whereas others are conjugated derivatives or metabolites of the native steroids (e.g., dihydroprogesterone). These agonists have been identified in the central nervous system and in some instances shown to have potent biologic activity. It is believed that they operate through interaction with the receptor for γ-aminobutyric acid, a molecule that increases neuronal membrane conductance to chloride ion. This has the net effect of hyperpolarizing the cellular membrane and suppressing neuronal excitability. Interactions that promote receptor activity would be predicted to produce sedative-hypnotic effects in the whole animal, whereas inhibitory interactions would be expected to lead to a state of central nervous system excitation.

Steroid & Thyroid Hormone Receptor Resistance Syndromes

Heritable defects in these receptors have been linked to the pathophysiology of a number of hormone resistance syndromes. These syndromes are characterized by a clinical phenotype suggesting hormone deficiency, by elevated levels of the circulating hormone ligand, and increased (or inappropriately detectable) levels of the relevant trophic regulatory hormone (e.g., ACTH, TSH, FSH, or LH). Point mutations in the zinc fingers of the DNA-binding domain as well the ligand-binding domain of the vitamin D receptor leads to a form of vitamin D-dependent rickets (type II). It is inherited as an autosomal recessive disorder. Molecular defects scattered along the full length of the androgen receptor, although concentrated in the ligand-binding domain, have been linked to syndromes characterized by varying degrees of androgen resistance ranging from infertility to testicular feminization syndrome. Clinical severity, in this case, is thought to be related to the severity of the functional impairment that the mutation imposes on the receptor. Because the androgen receptor is located on the X chromosome, these disorders are inherited in an X-linked fashion. Defects in the glucocorticoid receptor are less common, perhaps reflecting the life-threatening nature of derangements in this system. However, mutations have been identified that impact negatively on receptor function. Clinical presentations in these cases have been dominated by signs and symptoms referable to glucocorticoid deficiency and adrenal androgen and mineralocorticoid overproduction. This presumably results from defective steroid-mediated suppression of ACTH secretion and adrenal. Resistance to thyroid hormone has been linked to a large number of mutations scattered along the full length of the β form of the receptor. Different target tissues harboring the mutant receptors display variable sensitivity to thyroid hormone, with some tissues (e.g., pituitary) displaying profound resistance and others (e.g., heart) responding in a fashion suggesting hyperstimulation with thyroid hormone (i.e., thyrotoxicosis). The latter effects (e.g., tachycardia) may reflect the predominance of the normal α isoform, as opposed to defective β TR isoform, in the target tissue (e.g., heart).