CHAPTER

42

Hormone Action & Signal Transduction

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OBJECTIVES

After studying this chapter, you should be able to:

- Explain the roles of stimulus, hormone release, signal generation, and effector response in hormone-regulated physiologic processes.
- Describe the role of receptors and guanosine nucleotide-binding G-proteins in hormone signal transduction, particularly with regard to the generation of second messengers.
- Appreciate the complex patterns of signal transduction pathway cross-talk in mediating complicated physiologic outputs.
- Understand the key roles that protein-ligand, protein–protein, protein posttranslational modification, and protein-DNA interactions play in mediating hormone-directed physiologic processes.
- Detail why hormone-modulated receptors, second messengers, and associated signaling molecules represent a rich source of potential drug target development given their key roles in the regulation of physiology.

BIOMEDICAL IMPORTANCE

The homeostatic adaptations an organism makes to a constantly changing environment are in large part accomplished through alterations of the activity and amount of proteins. Hormones provide a major means of facilitating these changes. A hormone-receptor interaction results in generation of an amplified intracellular signal that can either regulate the activity of a select set of genes that alters the amounts of certain proteins in the target cell or affect the activity of specific proteins, including enzymes, transporters, or channel proteins. Signals can influence the location of proteins in the cell, and often affects general processes such as protein synthesis, cell growth, and replication, via their effects on gene expression. Other signaling molecules—including cytokines, interleukins, growth factors, and metabolites—use some of the same general mechanisms and signal transduction pathways. Excessive, deficient, or inappropriate production and release of hormones and the other regulatory signaling molecules are major causes of disease. Many pharmacotherapeutic agents are aimed at correcting or otherwise influencing the pathways discussed in this chapter.

HORMONES TRANSDUCE SIGNALS TO AFFECT HOMEOSTATIC MECHANISMS

The general steps involved in producing a coordinated response to a particular stimulus are illustrated in **Figure 42–1**. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. At the organismic level, this generally involves the nervous system and the special senses (sight, hearing, pain, smell, and touch). At the organ, tissue, or cellular level, recognition involves physicochemical factors such as pH, O_2 tension, temperature, nutrient supply, noxious metabolites, and osmolarity. Appropriate recognition results in the release of one or more hormones that will govern generation of the necessary adaptive response. For purposes of this discussion, the hormones are categorized as described in Table 41–4, that is, based on the location of their specific cellular receptors and the type of signals generated. Group I hormones interact with an intracellular receptor and group II hormones with receptor recognition sites located on the

extracellular surface of the plasma membrane of target cells. The cytokines, interleukins, and growth factors should also be considered in this latter category. These molecules, of critical importance in homeostatic adaptation, are hormones in the sense that they are produced in specific cells, have the equivalent of autocrine, paracrine, and endocrine actions, bind to cell surface receptors, and activate many of the same signal transduction pathways employed by the more traditional group II hormones.

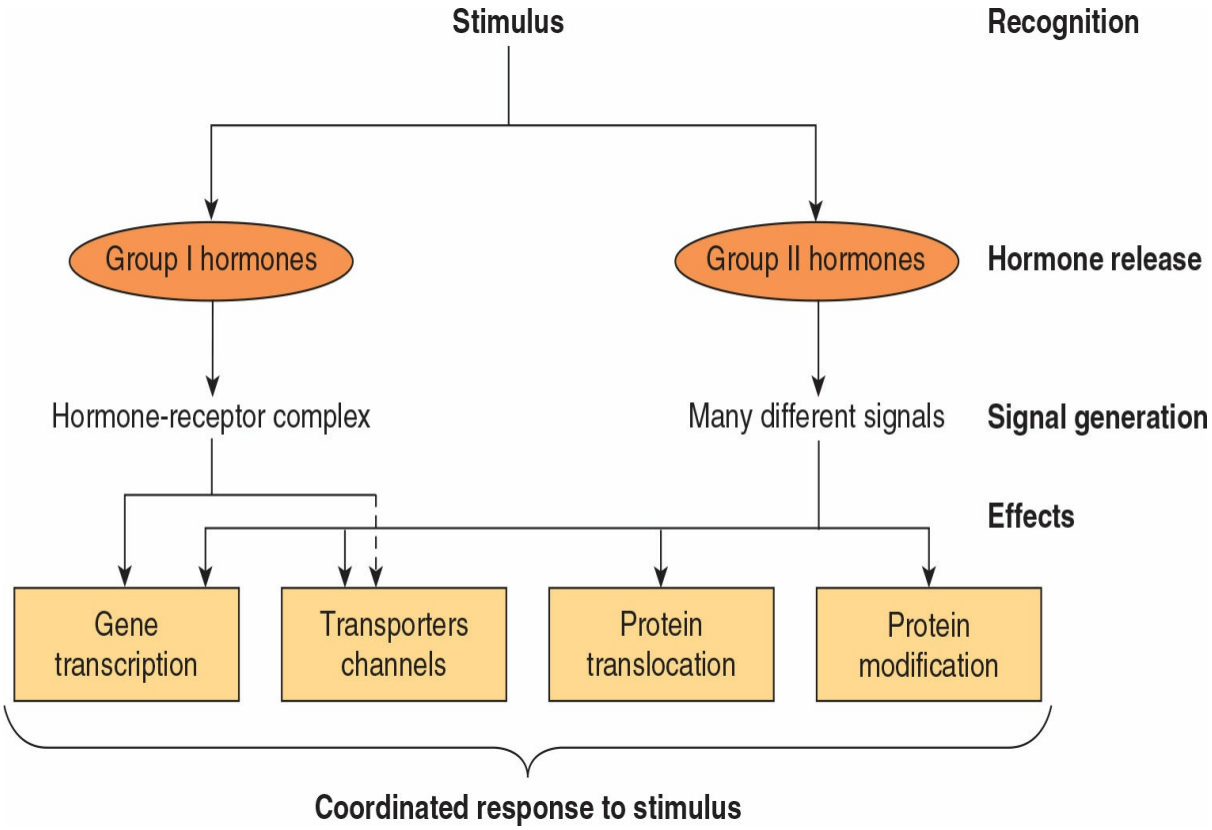

FIGURE 42–1 Hormonal involvement in responses to a stimulus. Physiologic needs, or challenges to the integrity of the organism, elicit a response that includes the release of one or more hormones. These hormones generate signals at or within target cells and these signals regulate a variety of biologic processes that provide for a coordinated response to the stimulus or challenge. See Figure 42–8 for a specific example.

SIGNAL GENERATION

The Ligand-Receptor Complex Is the Signal for Group I Hormones

The lipophilic group I hormones diffuse through the plasma membrane of all cells but only encounter their specific, high-affinity intracellular receptors in target cells. These receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone-receptor complex first undergoes an **activation reaction**. As shown in **Figure 42–2**, receptor activation occurs by at least two mechanisms. For example, glucocorticoids diffuse across the plasma membrane and encounter their cognate receptor in the cytoplasm of target cells. Ligand-receptor binding results in a conformational change in the receptor leading to the dissociation of heat shock protein 90 (hsp90). This step is necessary for subsequent nuclear localization of the glucocorticoid receptor (GR). This receptor also contains a nuclear localization sequence that is now free to assist in the translocation from cytoplasm to nucleus. The activated receptor moves into the nucleus (Figure 42–2) and binds with high affinity to a specific DNA sequence called the **hormone response element (HRE)**. In the case of GR, this is a glucocorticoid response element, or GRE. Consensus sequences for HREs are shown in **Table 42–1**. The DNA-bound, liganded receptor serves as a high-affinity binding target for one or more coactivator proteins, and accelerated gene transcription typically ensues when this occurs. By contrast, certain hormones such as the thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go directly into the nucleus. In this case, the cognate receptor is already bound to the HRE (the thyroid hormone response element [TRE], in this example). However, this DNA-bound receptor fails to activate transcription because it exists in complex with a corepressor. Indeed, this receptor-corepressor complex serves as a tonic repressor of gene transcription. Association of ligand with these receptors results in dissociation of the corepressor(s). The liganded receptor is now capable of binding one or more coactivators with high affinity, resulting in the recruitment of RNA polymerase II and the GTFs and activation of gene transcription as noted above for the GR-GRE complex. The relationship of hormone receptors to other nuclear receptors and to coregulators is discussed in more detail below.

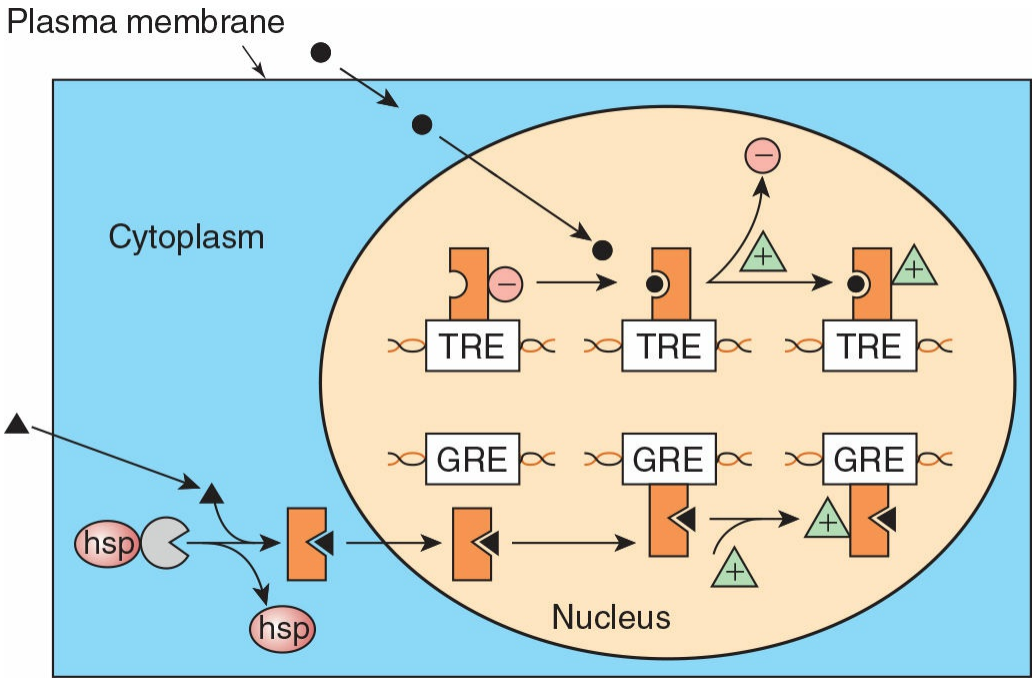

FIGURE 42–2 Regulation of gene expression by two different group I hormones, thyroid hormone and glucocorticoids. The hydrophobic steroid hormones readily gain access to the cytoplasmic compartment of target cells by diffusion through the plasma membrane. Glucocorticoid hormones (solid triangles) encounter their cognate receptor (GR) in the cytoplasm where GR exists in a complex with a chaperone protein, heat shock protein 90 (hsp). Ligand binding causes dissociation of hsp90 and a conformational change of the receptor. The receptor-ligand complex then traverses the nuclear membrane and binds to DNA with specificity and high affinity at a glucocorticoid response element (GRE). This event affects the architecture of a number of transcription coregulators (green triangles), and enhanced transcription ensues. By contrast, thyroid hormones and retinoic acid (black circle) directly enter the nucleus, where their cognate heterodimeric (TR-RXR; see Figure 42–12) receptors are already bound to the appropriate response elements with an associated transcription corepressor complex (red circles). Binding of hormones occurs, which again induces conformational changes in the receptor leading to dissociation of the corepressor complex from the receptor, thereby allowing an activator complex, consisting of the TR-TRE and coactivator, to assemble. The gene is then actively transcribed.

TABLE 42–1 The DNA Sequences of Several Hormone Response Elements (HREs)[a]

Hormone or Effector	HRE	DNA Sequence
Glucocorticoids	GRE	GGTACA NNN TGTTCT ◄— —►
Progestins	PRE	
Mineralocorticoids	MRE	
Androgens	ARE	
Estrogens	ERE	AGGTCA — TGACCT ◄— —►
Thyroid hormone	TRE	AGGTCA $N_{(1-5)}$ AGGTCA —► —►
Retinoic acid	RARE	
Vitamin D	VDRE	
cAMP	CRE	TGACGTCA

[a]Letters indicate nucleotide; N means any one of the four can be used in that position. The arrows pointing in opposite directions illustrate the slightly imperfect inverted palindromes present in many HREs; in some cases, these are called "half binding sites," or half-sites, because each binds one monomer of the receptor. The GRE, PRE, MRE, and ARE consist of the same DNA sequence. Specificity may be conferred by the intracellular concentration of the ligand or hormone receptor, by flanking DNA sequences not included in the consensus, or by other accessory elements. A second group of HREs includes those for thyroid hormones, estrogens, retinoic acid, and vitamin D. These HREs are similar except for the orientation and spacing between the half palindromes. Spacing determines the hormone specificity. VDRE ($N = 3$), TRE ($N = 4$), and RARE ($N = 5$) bind to direct repeats rather than to inverted repeats. Another member of the steroid receptor superfamily, the retinoid X receptor (RXR), forms heterodimers with VDR, TR, and RARE, and these constitute the functional forms of these DNA-binding transacting factors. cAMP affects gene transcription through the CRE.

By selectively affecting gene transcription and the consequent production of appropriate target mRNAs, the amounts of specific proteins are changed and metabolic processes are influenced. The influence of each of these hormones is quite specific; generally, a given hormone directly affects <1% of the genes, mRNA, or proteins in a target cell; sometimes only a few are affected. The nuclear actions of steroid, thyroid, and retinoid hormones are quite well defined. Most evidence suggests that these hormones exert their dominant effect on modulating gene transcription, but they—and many of the hormones in the other classes

discussed below—can act at any step of the "information pathway," as illustrated in **Figure 42–3**, to control specific gene expression and, ultimately, a biologic response. Direct actions of steroids in the cytoplasm and on various organelles and membranes have also been described. Recently, microRNAs and lncRNAs have been implicated in mediating some of the diverse actions of hormones.

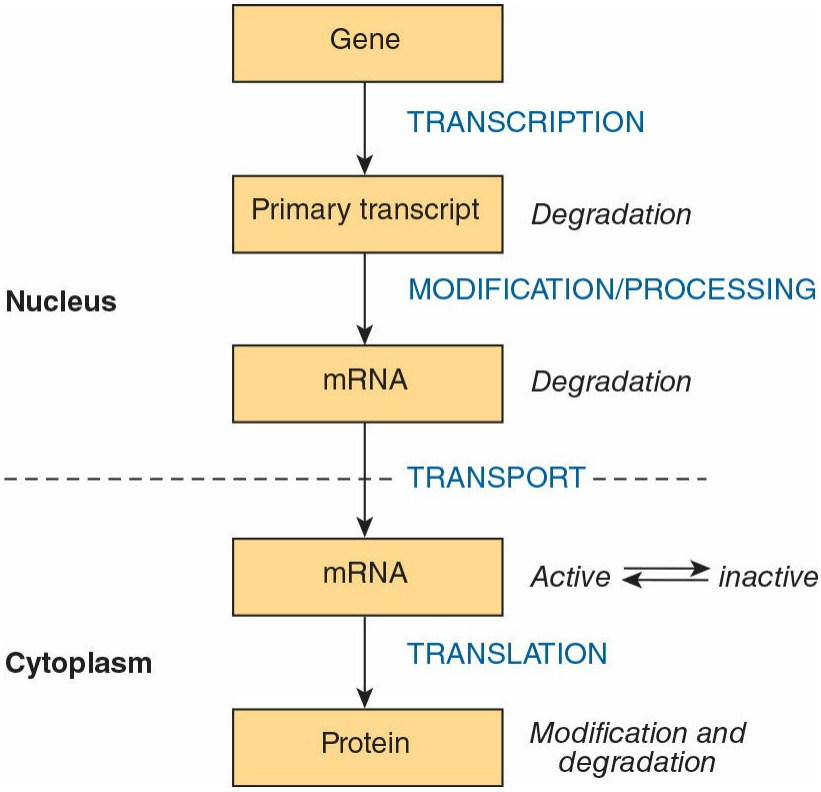

FIGURE 42–3 The "information pathway." Information flows from the gene to the primary transcript to mRNA to protein. Hormones can affect any of the steps involved and can affect the rates of processing, degradation, or modification of the various products.

GROUP II (PEPTIDE & CATECHOLAMINE) HORMONES HAVE MEMBRANE RECEPTORS & USE INTRACELLULAR MESSENGERS

Many hormones are water soluble, have no transport proteins (and therefore have a short plasma half-life), and initiate a response by binding to a receptor located in the plasma membrane (see Tables 41–3 and 41–4). The mechanism of action of this group of hormones can best be discussed in terms of the **intracellular signals** they generate. These signals include **cAMP** (cyclic AMP; 3′,5′-adenylic acid; see Figure 18–5), a nucleotide

derived from ATP through the action of adenylyl cyclase; **cGMP**, a nucleotide formed from GTP by guanylyl cyclase; **Ca^{2+}**; and **phosphatidylinositides**; such small molecules are **termed second messengers** as their synthesis is triggered by the presence of the primary hormone (molecule) binding its receptor. Many of these second messengers affect gene transcription, as described in the previous paragraph; but they also influence a variety of other biologic processes, as shown in Figure 42–3, but see also Figures 42–6 and 42–8.

G-Protein–Coupled Receptors

Many of the group II hormones bind to receptors that couple to effectors through a **guanine-binding protein (G-proteins)** intermediary. These receptors typically have seven α-helical hydrophobic plasma membrane-spanning domains, here illustrated by the seven interconnected cylinders extending through the lipid bilayer in **Figure 42–4**. Receptors of this class, which signal through G-proteins, are known as **G-protein–coupled receptors (GPCRs)**. To date, hundreds of *GPCR* genes have been identified, and represent the largest family of cell surface receptors in humans. Not surprisingly, a wide variety of responses are mediated by the GPCRs.

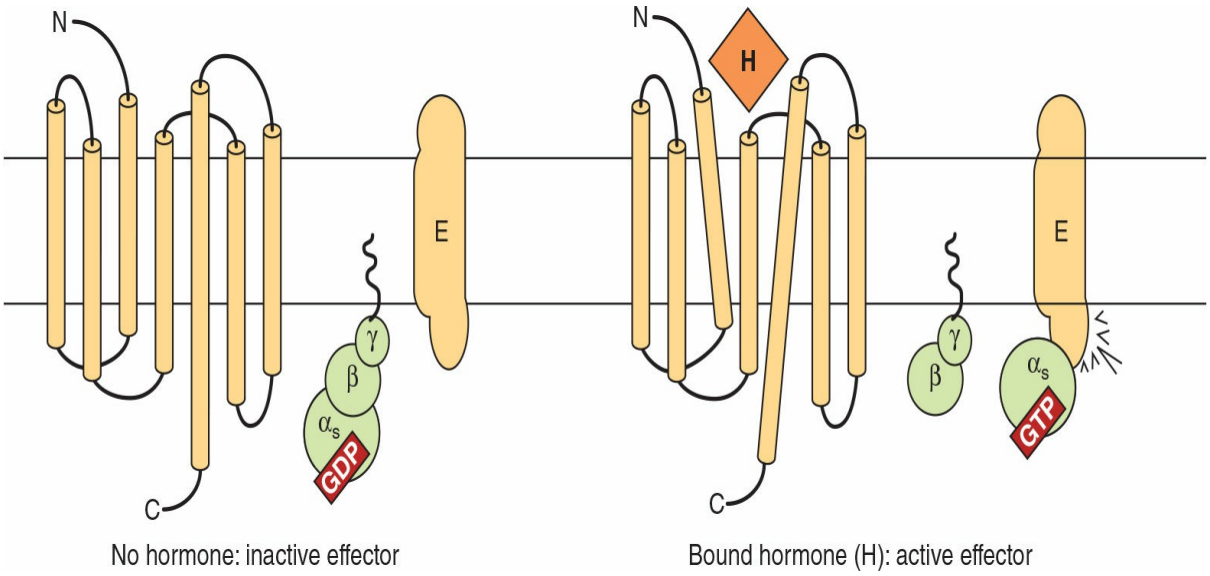

FIGURE 42–4 Components of the hormone receptor–G-protein effector system. Receptors that couple to effectors through G-proteins, the G-protein–coupled receptors (GPCRs), typically have seven α-helical membrane-spanning domains (here shown as long cylinders). In the absence of hormone (**left**), the heterotrimeric G-protein complex (α,β,γ) is

in an inactive guanosine diphosphate (GDP)-bound form and is probably not associated with the receptor. This complex is anchored to the plasma membrane through prenylated groups on the βγ subunits (**wavy lines**) and perhaps by myristoylated groups on α subunits (not shown). On binding of hormone (H) to the receptor, there are conformational changes within the receptor (as indicated by the tilted membrane-spanning domains) and association of the G-protein complex with the rearranged receptor—this the activates the G-protein complex. This activation results from the exchange of GDP with guanosine triphosphate (GTP) on the α subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E). E can be adenylyl cyclase, Ca^{2+}, Na^{+}, or Cl^{-} channels (α_s), or it could be a K^{+} channel (α_i), phospholipase Cβ (α_q), or cGMP phosphodiesterase (α_t); see Table 42–3. The βγ subunit can also have direct actions on E. (Modified and reproduced, with permission, from Granner DK. In: *Principles and Practice of Endocrinology and Metabolism*, 2nd ed. Becker KL (editor). Lippincott, 1995.)

cAMP Is the Intracellular Signal for Many Responses

Cyclic AMP was the first intracellular second messenger signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP (**Table 42–2**).

TABLE 42–2 Subclassification of Group II.A Hormones

Hormones That Stimulate Adenylyl Cyclase (H_s)	Hormones That Inhibit Adenylyl Cyclase (H_i)
ACTH	Acetylcholine
ADH	α_2-Adrenergics
β-Adrenergics	Angiotensin II
Calcitonin	Somatostatin
CRH	
FSH	
Glucagon	
hCG	
LH	
LPH	
MSH	
PTH	
TSH	

Abbreviations: ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; LPH, lipotropin; MSH, melanocyte-stimulating hormone; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone.

Adenylyl Cyclase

Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cyclase through the action of the G-proteins. G-proteins are encoded by at least 10 different genes (**Table 42–3**). Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a catalytic molecule (C). Each consists of a receptor, R_s or R_i, and a regulatory G-protein complex termed G_s and G_i. Both G_s and G_i are **heterotrimeric G-proteins composed of α, β, and γ subunits**. Since the α subunit in G_s differs from that in G_i, the proteins, which are distinct gene products, are designated α_s and α_i. The α subunits bind guanine nucleotides. The β and γ subunits are likely always associated (βγ) and appear to function predominantly as a heterodimer. The binding of a

hormone to R_s or R_i results in a receptor-mediated activation of G-protein, which entails the exchange of GDP by GTP on α and the concomitant dissociation of βγ from α.

TABLE 42–3 Classes and Functions of Selected G-Proteins[a]

Class or Type	Stimulus	Effector	Effect
G_s			
α_s	Glucagon, β-adrenergics	↑Adenylyl cyclase	Glyconeogenesis, lipolysis, glycogenolysis
		↑Cardiac Ca^{2+}, Cl^-, and Na^+ channels	Olfaction
α_{olf}	Odorant	↑Adenylyl cyclase	
G_i			
$\alpha_{i-1,2,3}$	Acetylcholine, α_2-adrenergics	↓Adenylyl cyclase	Slowed heart rate
		↑Potassium channels	
	M_2 cholinergics	↓Calcium channels	
α_o	Opioids, endorphins	↑Potassium channels	Neuronal electrical activity
α_t	Light	↑cGMP phosphodiesterase	Vision
G_q			
α_q	M_1 cholinergics		
	α_1-Adrenergics	↑Phospholipase C-β1	↓Muscle contraction
α_{11}	α_1-Adrenergics	↑Phospholipase C-β2	↓Blood pressure
G_{12}			
α_{12}	Thrombin	Rho	Cell shape changes

[a]The four major classes or families of mammalian G-proteins (G_s, G_i, G_q, and G_{12}) are based on protein sequence conservation. Representative members of each are shown, along with known stimuli, effectors, and well-defined biologic effects. Nine isoforms of adenylyl cyclase have been identified (isoforms I-IX). All isoforms are stimulated by α_s; α_i isoforms inhibit types V and VI, and α_o inhibits types I and V. At least 16 different α subunits have been identified.
Source: Modified and reproduced, with permission, from Granner DK: In: *Principles and Practice of Endocrinology and Metabolism*, 2nd ed. Becker KL (editor). Lippincott, 1995.

The α_s protein has intrinsic GTPase activity. The active form, α_s-GTP, is inactivated on hydrolysis of the GTP to GDP; the **trimeric Gs complex (αβγ)** is then reformed and is ready for another cycle of activation. **Cholera** and **pertussis toxins** catalyze the **ADP ribosylation** of α_s and α_{i-2} (Table 42–3), respectively. In the case of α_s, this modification disrupts the intrinsic GTPase activity; thus, α_s cannot reassociate with βγ and is therefore irreversibly activated. ADP ribosylation of α_{i-2} prevents the

dissociation of α_{i-2} from βγ, and free α_{i-2} thus cannot be formed. α_s activity in such cells is therefore unopposed.

There is a large family of G-proteins, and these are part of the superfamily of GTPases. The G-protein family is classified according to sequence homology into four subfamilies, as illustrated in Table 42–3. There are 21 α, 5 β, and 8 γ subunit genes. Various combinations of these subunits provide a large number of possible αβγ complexes.

The α subunits and the βγ complex have actions independent of those on adenylyl cyclase (see Figure 42–4 and Table 42–3). Some forms of α_i stimulate K^+ channels and inhibit Ca^{2+} channels, and some α_s molecules have the opposite effects. Members of the G_q family activate the phospholipase C group of enzymes. The βγ complexes have been associated with K^+ channel stimulation and phospholipase C activation. G proteins are involved in many important biologic processes in addition to hormone action. Notable examples include olfaction (α_{OLF}) and vision (α_t). Some examples are listed in Table 42–3. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

Protein Kinase

As discussed in Chapter 38, in prokaryotic cells, cAMP binds to a specific protein called cAMP activator protein (CAP) that binds directly to DNA and influences gene expression. By contrast, in eukaryotic cells, cAMP binds to a protein kinase called **protein kinase A (PKA)**, a heterotetrameric molecule consisting of two regulatory subunits (R) that inhibit the activity of the two catalytic subunits (C) when bound as a tetrameric complex. cAMP binding to the R_2C_2 tetramer results in the following reaction:

$$4\text{cAMP} + R_2C_2 \rightleftarrows R_2\text{-}4\text{cAMP} + 2C$$

The R_2C_2 complex has no enzymatic activity, but the binding of cAMP to the R subunit induces dissociation of the R-C complex, thereby activating the latter (**Figure 42–5**). The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The consensus PKA phosphorylation sites are -ArgArg/Lys-X-Ser/Thr- and -Arg-Lys-X-X-Ser-, where X can be any amino acid.

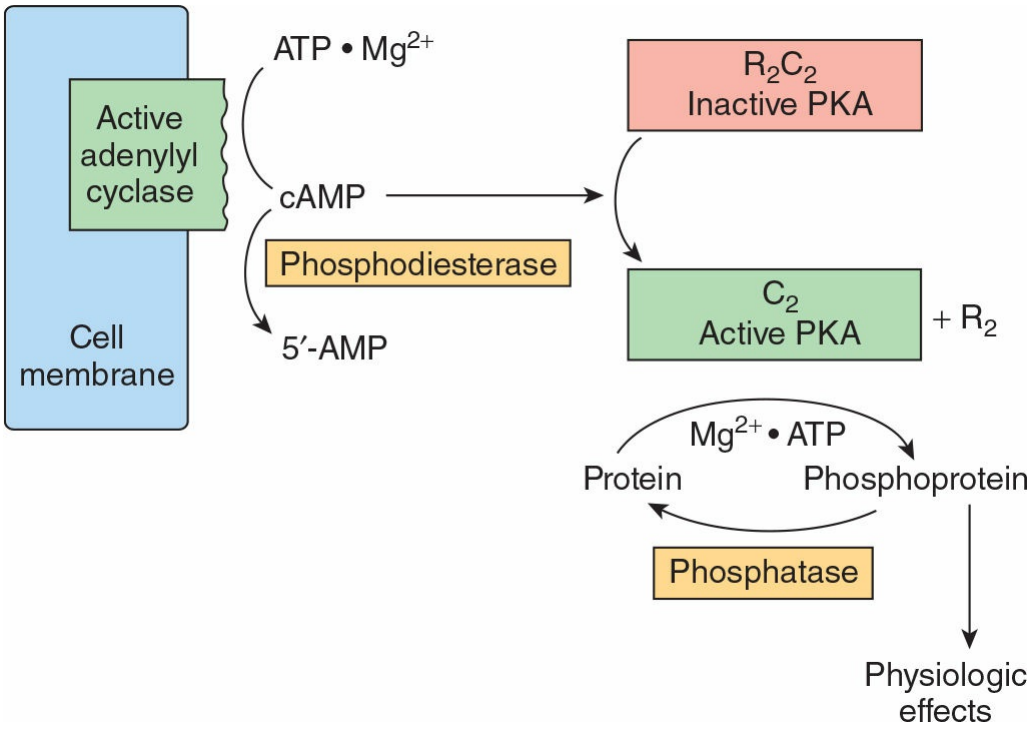

FIGURE 42–5 Hormonal regulation of cellular processes through cAMP-dependent protein kinase (PKA). PKA exists in an inactive form as an R_2C_2 heterotetramer consisting of two regulatory (R) and two catalytic (C) subunits. The cAMP generated by the action of adenylyl cyclase (activated as shown in Figure 42–4) binds to the regulatory subunit of PKA. This results in dissociation of the regulatory and catalytic subunits and activation of the latter. The active catalytic subunits phosphorylate a number of target proteins on serine and threonine residues. Phosphatases remove phosphate from these residues and thus terminate the physiologic response. A phosphodiesterase can also terminate the response by converting cAMP to 5′-AMP.

Historically protein kinase activities were described as being "cAMP-dependent" or "cAMP-independent." This classification has changed, as protein phosphorylation is now recognized as being a major and ubiquitous regulatory mechanism. Several hundred protein kinases have now been described. These kinases are related in sequence and structure within the catalytic domain, but each is a unique molecule with considerable variability with respect to subunit composition, molecular weight, autophosphorylation, K_m for ATP, and substrate specificity. Both kinase and protein phosphatase activities can be targeted by interaction with specific kinase-binding proteins. In the case of PKA, such targeting proteins are termed A kinase anchoring proteins, or **AKAPs**. AKAPs serve

as scaffolds, which localize PKA near to substrates thereby focusing PKA activity toward physiologic substrates and facilitating spatiotemporal biologic regulation while also allowing for common, shared proteins to elicit specific physiologic responses. Multiple AKAPs have been described and importantly they can bind PKA and other kinases as well as phosphatases, phosphodiesterases (which hydrolyze cAMP), and protein kinase substrates. The multifunctionality of AKAPs facilitates signaling localization, rate (production and destruction of signals), specificity, and dynamics.

Phosphoproteins

The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation. The array of specific substrates contributes critically to defining a target tissue, and are involved in defining the extent of a particular response within a given cell. For example, the effects of cAMP on gene transcription are mediated by the cyclic AMP response element binding protein (**CREB)**. When CREB binds to a cAMP responsive DNA enhancer element (**CRE**) (see Table 42–1) in its nonphosphorylated state, it is a weak activator of transcription. However, when phosphorylated by PKA at key amino acids, CREB binds the coactivator **CREB-binding protein CBP/p300** (see below) and as a result is a much more potent transcriptional activator. CBP and the related p300 contain histone acetyltransferase activities (HATs), and hence serve as chromatin-active transcriptional coregulators (see Chapters 36, 38). Interestingly, CBP/p300 can also acetylate certain transcription factors thereby stimulating their ability to bind DNA and modulate transcription.

Phosphodiesterases

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases (see Figure 42–5). The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once the hormonal

stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes. These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin. Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal.

Phosphoprotein Phosphatases

Given the importance of protein phosphorylation, it is not surprising that regulation of the protein dephosphorylation reaction is another important control mechanism (see Figure 42–5). The phosphoprotein phosphatases are themselves subject to regulation by phosphorylation-dephosphorylation reactions and by a variety of other mechanisms, such as protein–protein interactions. In fact, the substrate specificity of the phosphoserine-phosphothreonine phosphatases may be dictated by distinct regulatory subunits whose binding is regulated hormonally. One of the best-studied roles of regulation by the dephosphorylation of proteins is that of glycogen metabolism in muscle (see Figures 18–6 to 18–8). Two major types of phosphoserine-phosphothreonine phosphatases have been described. Type I preferentially dephosphorylates the β subunit of phosphorylase kinase, whereas type II dephosphorylates the α subunit. Type I phosphatase is implicated in the regulation of glycogen synthase, phosphorylase, and phosphorylase kinase. This phosphatase is itself regulated by phosphorylation of certain of its subunits, and these reactions are reversed by the action of one of the type II phosphatases. In addition, two heat-stable protein inhibitors regulate type I phosphatase activity. Inhibitor-1 is phosphorylated and activated by cAMP-dependent protein kinases, and inhibitor-2, which may be a subunit of the inactive phosphatase, is also phosphorylated, possibly by glycogen synthase kinase-3. Phosphatases that target phosphotyrosine are also important in signal transduction (see Figure 42–8).

cGMP Is Also an Intracellular Signal

Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms. Each of these enzyme forms has unique physiologic properties. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. These peptides (eg, atrial

natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above. Other evidence links cGMP to vasodilation. A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle relaxation and are potent vasodilators. These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (eg, the drug sildenafil [Viagra]) enhance and prolong these responses. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. Presumably, this is involved in relaxation of smooth muscle and vasodilation.

Several Hormones Act Through Calcium or Phosphatidylinositols

Ionized calcium, Ca^{2+}, is an important regulator of a variety of cellular processes, including muscle contraction, stimulus-secretion coupling, the blood clotting cascade, enzyme activity, and membrane excitability. Ca^{2+} is also an intracellular messenger of hormone action.

Calcium Metabolism

The extracellular Ca^{2+} concentration is ~5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca^{2+}) is very low: 0.05 to 10 μmol/L. In spite of this large concentration gradient and a favorable transmembrane electrical gradient, Ca^{2+} is restrained from entering the cell. A considerable amount of energy is expended to ensure that the intracellular Ca^{2+} is controlled, as a prolonged elevation of Ca^{2+} in the cell is very toxic. A Na^{+}/Ca^{2+} exchange mechanism that has a high-capacity but low-affinity pumps Ca^{2+} out of cells. There also is a Ca^{2+}/proton ATPase-dependent pump that extrudes Ca^{2+} in exchange for H^{+}. This has a high affinity for Ca^{2+} but a low capacity and is probably responsible for fine-tuning cytosolic Ca^{2+}. Furthermore, Ca^{2+}-ATPases pump Ca^{2+} from the cytosol to the lumen of the endoplasmic reticulum. There are three ways of changing cytosolic Ca^{2+} levels: (1) Certain

hormones (class II.C, Table 41–3) by binding to receptors that are themselves Ca^{2+} channels, enhance membrane permeability to Ca^{2+}, and thereby increase Ca^{2+} influx. (2) Hormones also indirectly promote Ca^{2+} influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated Ca^{2+} channels and allows for Ca^{2+} influx. (3) Ca^{2+} can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

An important observation linking Ca^{2+} to hormone action involved the definition of the intracellular targets of Ca^{2+} action. The discovery of a Ca^{2+}-dependent regulator of phosphodiesterase activity provided the basis for a broad understanding of how Ca^{2+} and cAMP interact within cells.

Calmodulin

The calcium-dependent regulatory protein is calmodulin, a 17-kDa protein that is homologous to the muscle protein troponin C in structure and function. Calmodulin has four Ca^{2+}-binding sites, and full occupancy of these sites leads to a marked conformational change, which allows calmodulin to activate enzymes and ion channels. The interaction of Ca^{2+} with calmodulin (with the resultant change of activity of the latter) is conceptually similar to the binding of cAMP to PKA and the subsequent activation of this molecule. Calmodulin can be one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. A partial list of the enzymes regulated directly or indirectly by Ca^{2+}, probably through calmodulin, is presented in **Table 42–4**.

TABLE 42–4 Some Enzymes and Proteins Regulated by Calcium or Calmodulin

- Adenylyl cyclase
- Ca^{2+}-dependent protein kinases
- Ca^{2+}-Mg^{2+}-ATPase
- Ca^{2+}-phospholipid–dependent protein kinase
- Cyclic nucleotide phosphodiesterase
- Some cytoskeletal proteins
- Some ion channels (eg, l-type calcium channels)
- Nitric oxide synthase
- Phosphorylase kinase
- Phosphoprotein phosphatase 2B
- Some receptors (eg, NMDA-type glutamate receptor)

Abbreviations: NDMA, N-methyl-D-aspartate receptor.

In addition to its effects on enzymes and ion transport, Ca^{2+}/calmodulin regulates the activity of many structural elements in cells. These include the actin-myosin complex of smooth muscle, which is under β-adrenergic control, and various microfilament-mediated processes in noncontractile cells, including cell motility, cell conformation changes, mitosis, granule release, and endocytosis.

Calcium Is a Mediator of Hormone Action

A role for Ca^{2+} in hormone action is suggested by the observations that the effect of many hormones is (1) blunted by Ca^{2+}-free media or when intracellular calcium is depleted; (2) can be mimicked by agents that increase cytosolic Ca^{2+}, such as the Ca^{2+} ionophore A23187; and (3) influences cellular calcium flux. Again, the regulation of glycogen metabolism in liver (by vasopressin and β-adrenergic catecholamines; see Figures 18–6 and 18–7).

A number of critical metabolic enzymes are regulated by Ca^{2+}, phosphorylation, or both. These include glycogen synthase, pyruvate kinase, pyruvate carboxylase, glycerol-3-phosphate dehydrogenase, and pyruvate dehydrogenase among others (see Figure 19–1).

Phosphatidylinositide Metabolism Affects Ca^{2+}-Dependent Hormone Action

Some signal must provide communication between the hormone receptor on the plasma membrane and the intracellular Ca^{2+} reservoirs. This is accomplished by products of phosphatidylinositol metabolism. Cell surface receptors such as those for acetylcholine, antidiuretic hormone,

and α_1-type catecholamines are, when occupied by their respective ligands, potent activators of phospholipase C. Receptor binding and activation of phospholipase C are coupled by the G_q isoforms (Table 42–3 and **Figure 42–6**). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate (IP_3) and 1,2-diacylglycerol (**Figure 42–7**). Diacylglycerol **(DAG)** is itself capable of activating **protein kinase C (PKC)**, the activity of which also depends on Ca^{2+} (see Chapter 21 and Figures, 24–1, 24–2, and 55–1). IP_3, by interacting with a specific intracellular receptor, is an effective releaser of Ca^{2+} from intracellular storage sites in the endoplasmic reticulum. Thus, the hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to activation of PKC and promotes an increase of cytoplasmic Ca^{2+}. As shown in Figure 42–4, the activation of G-proteins can also have a direct action on Ca^{2+} channels. The resulting elevations of cytosolic Ca^{2+} activate Ca^{2+}-calmodulin–dependent kinases and many other Ca^{2+}-calmodulin–dependent enzymes.

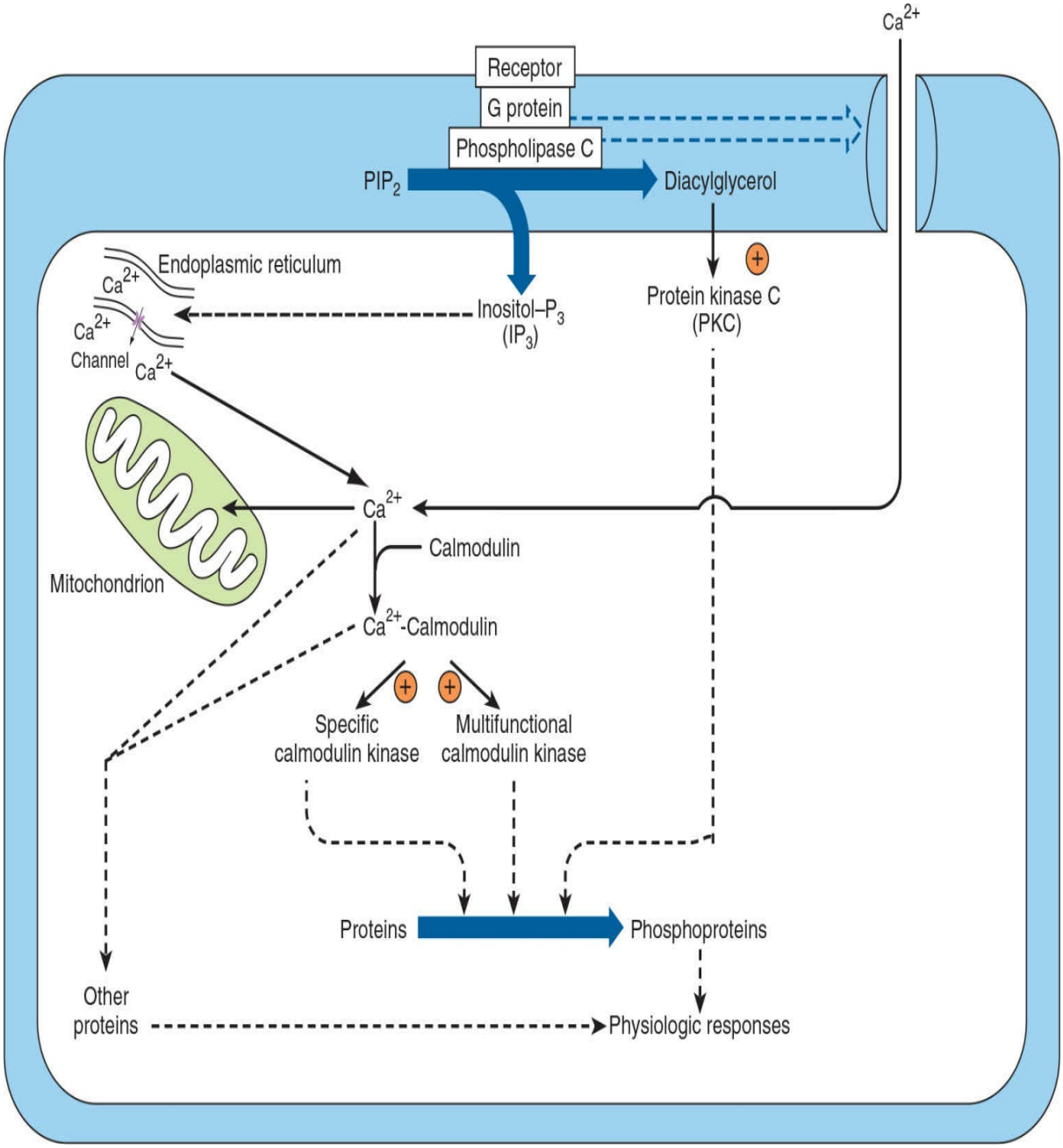

FIGURE 42–6 Certain hormone-receptor interactions result in the activation of phospholipase C (PLC). PLC activation appears to involve a specific G-protein, which also may activate a calcium channel. Phospholipase C generates inositol trisphosphate (IP_3) from PIP_2 (phosphoinositol 4,5-bisphosphate; see Figure 42–7), which liberates stored intracellular Ca^{2+}, and diacylglycerol (DAG), a potent activator of protein kinase C (PKC). In this scheme, the activated PKC phosphorylates specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases, two of which are shown here. These actions result in phosphorylation of substrates, and this

leads to altered physiologic responses. This figure also shows that Ca^{2+} can enter cells through voltage- or ligand-gated Ca^{2+} channels. The intracellular Ca^{2+} is also regulated through storage and release by the mitochondria and endoplasmic reticulum. (Reprinted with permission from JH Exton.)

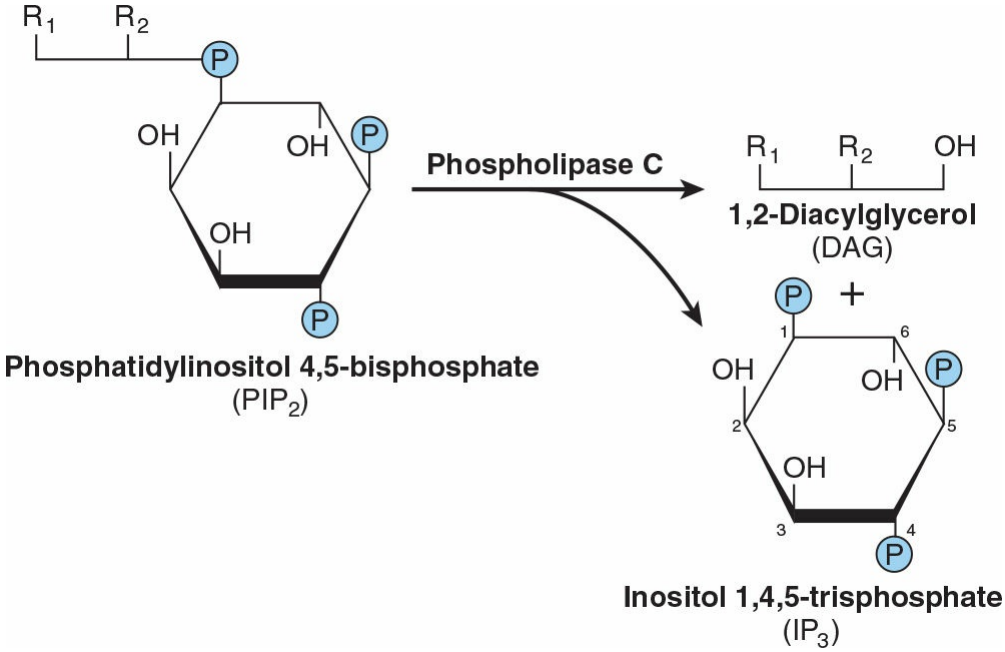

FIGURE 42–7 Phospholipase C cleaves PIP_2 into diacylglycerol and inositol trisphosphate. R_1 generally is stearate, and R_2 is usually arachidonate. IP_3 can be dephosphorylated (to the inactive I-1,4-P_2) or phosphorylated (to the potentially active I-1,3,4,5-P_4).

Steroidogenic agents—including ACTH and cAMP in the adrenal cortex; angiotensin II, K^+, serotonin, ACTH, and cAMP in the zona glomerulosa of the adrenal; LH in the ovary; and LH and cAMP in the Leydig cells of the testes—have been associated with increased amounts of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides (see Chapter 21) in the respective target tissues. Several other examples could be cited.

The roles that Ca^{2+} and polyphosphoinositide breakdown products might play in hormone action are presented in Figure 42–6. In this scheme, the activated protein kinase C can phosphorylate specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases. These then modify substrates and thereby

alter physiologic responses.

Some Hormones Act Through a Protein Kinase Cascade

Single protein kinases such as PKA, PKC, and Ca^{2+}-calmodulin (CaM) kinases, which result in the phosphorylation of serine and threonine residues in target proteins, play a very important role in hormone action. The discovery that the epidermal growth factor (EGF) receptor contains an intrinsic tyrosine kinase activity that is activated by the binding of the ligand EGF was an important breakthrough. The insulin and insulin-like growth factor 1 (IGF-1) receptors also contain intrinsic ligand-activated tyrosine kinase activity. Several receptors—generally those involved in binding ligands involved in growth control, differentiation, and the inflammatory response—either have intrinsic tyrosine kinase activity or are tightly associated with proteins that are tyrosine kinases. Another distinguishing feature of this class of hormone action is that these kinases preferentially phosphorylate tyrosine residues, and tyrosine phosphorylation is infrequent (<0.03% of total amino acid phosphorylation) in mammalian cells. A third distinguishing feature is that the ligand-receptor interaction that results in a tyrosine phosphorylation event initiates a cascade that may involve several protein kinases, phosphatases, and other regulatory proteins.

Insulin Transmits Signals by Several Kinase Cascades

The **insulin, EGF**, and **IGF-1 receptors** have intrinsic protein tyrosine kinase activities located in their cytoplasmic domains. These activities are stimulated when their ligands bind to the cognate receptor. The receptors are then autophosphorylated on tyrosine residues, and this phosphorylation initiates a complex series of events (summarized in simplified fashion in **Figure 42–8**). The phosphorylated insulin receptor next phosphorylates **insulin receptor substrates** (there are at least four of these molecules, called **IRS 1-4**) on tyrosine residues. Phosphorylated IRS binds to the **Src homology 2 (SH2)** domains of a variety of proteins that are directly involved in mediating different effects of insulin. One of these proteins, PI-3 kinase, links insulin receptor activation to insulin action through activation of a number of molecules, including the kinase phosphoinositide-dependent kinase 1 (**PDK1)**. This enzyme propagates the signal through several other kinases, including **PKB** (also known as

AKT), **SKG**, and **aPKC** (see legend to Figure 42–8 for definitions and expanded abbreviations). An alternative pathway downstream from PDK1 involves **p70S6K** and perhaps other as yet unidentified kinases. A second major pathway involves **mTOR**. This enzyme is directly regulated by amino acid levels and insulin and is essential for p70S6K activity. The mTOR-signaling system provides a distinction between the PKB and p70S6K branches downstream from PKD1. These pathways are involved in protein translocation, enzyme activation, and the regulation, by insulin, of genes involved in metabolism (Figure 42–8). Another SH2 domain–containing protein is **GRB2**, which binds to IRS-1 and links tyrosine phosphorylation to several proteins, the result of which is activation of a cascade of threonine and serine kinases. A pathway showing how this insulin-receptor interaction activates the mitogen-activated protein kinase (**MAPK**) pathway and the anabolic effects of insulin is illustrated in Figure 42–8. The exact roles of many of these docking proteins, kinases, and phosphatases are actively being studied.

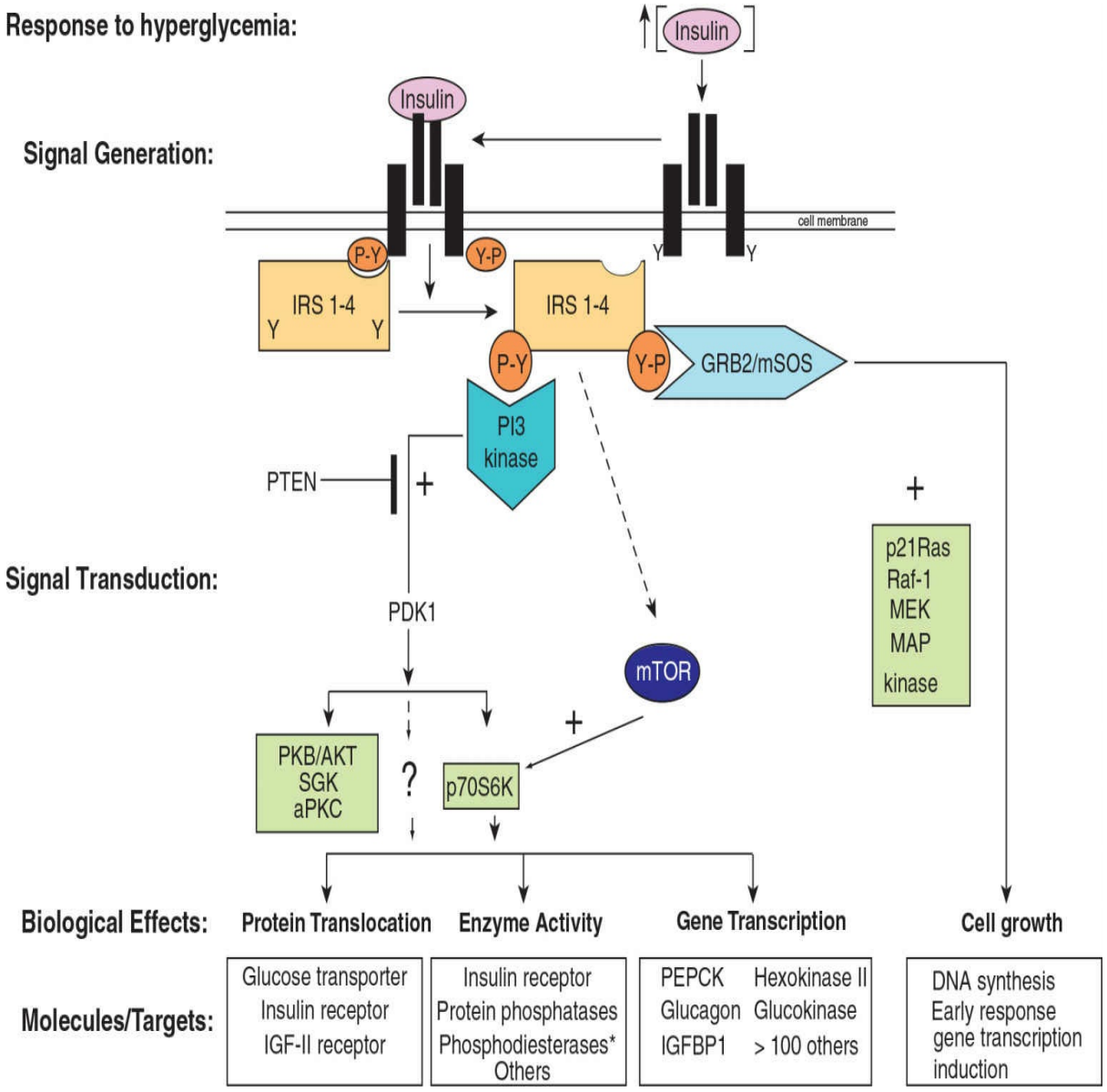

FIGURE 42–8 Insulin signaling pathways. The insulin signaling pathways provide an excellent example of the "recognition → hormone release → signal generation → effects" paradigm outlined in Figure 42–1. Insulin is released into the bloodstream from pancreatic β cells in response to hyperglycemia. Binding of insulin to a target cell-specific plasma membrane heterotetrameric insulin receptor (IR) results in a cascade of intracellular events. First, the intrinsic tyrosine kinase activity of the insulin receptor is activated, and marks the initial event. Receptor activation results in increased tyrosine phosphorylation (conversion of specific Y residues → Y-P) within the receptor. One or more of the insulin receptor substrate (IRS) molecules (IRS 1-4) then bind to the tyrosine-phosphorylated receptor and themselves are specifically tyrosine phosphorylated. IRS proteins interact with the activated IR via N-terminal

PH (pleckstrin homology) and phosphotyrosine binding (PTB) domains. IR-docked IRS proteins are tyrosine phosphorylated and the resulting P-Y residues form the docking sites for several additional signaling proteins (ie, PI-3 kinase, GRB2, and mTOR). GRB2 and PI-3K bind to IRS P-Y residues via their SH (*Src* homology) domains, binding to IRS-Y-P residues leads to activation of the activity of many intracellular signaling molecules such as GTPases, protein kinases, and lipid kinases, all of which play key roles in certain metabolic actions of insulin. The two best-described pathways are shown. In detail, phosphorylation of an IRS molecule (probably IRS-2) results in docking and activation of the lipid kinase, PI-3 kinase; PI-3K generates novel inositol lipids that act as "second messenger" molecules. These, in turn, activate PDK1 and then a variety of downstream signaling molecules, including protein kinase B (PKB/AKT), SGK, and aPKC. An alternative pathway involves the activation of p70S6K and perhaps other as yet unidentified kinases. Next, phosphorylation of IRS (probably IRS-1) results in docking of GRB2/mSOS and activation of the small GTPase, p21Ras, which initiates a protein kinase cascade that activates Raf-1, MEK, and the p42/p44 MAP kinase isoforms. These protein kinases are important in the regulation of proliferation and differentiation of many cell types. The mTOR pathway provides an alternative way of activating p70S6K and is involved in nutrient signaling as well as insulin action. Each of these cascades may influence different biologic processes, as shown (protein translocation, protein/enzyme activity, gene transcription, cell growth). All of the phosphorylation events are reversible through the action of specific phosphatases. As an example, the lipid phosphatase PTEN dephosphorylates the product of the PI-3 kinase reaction, thereby antagonizing the pathway and terminating the signal. Representative effects of major actions of insulin are shown in each of the boxes (bottom). The asterisk after phosphodiesterase indicates that insulin indirectly affects the activity of many enzymes by activating phosphodiesterases and reducing intracellular cAMP levels. (aPKC, atypical protein kinase C; GRB2, growth factor receptor binding protein 2; IGFBP, insulin-like growth factor binding protein; IRS 1-4, insulin receptor substrate isoforms 1-4; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase and ERK kinase; mSOS, mammalian son of sevenless; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal protein S6 kinase; PDK1, phosphoinositide-dependent kinase; PI-3 kinase, phosphatidylinositol 3-kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SGK, serum and

glucocorticoid-regulated kinase.

The Jak/STAT Pathway Is Used by Hormones and Cytokines

Tyrosine kinase activation can also initiate a phosphorylation and dephosphorylation cascade that involves the action of several other protein kinases and the counterbalancing actions of phosphatases. Two mechanisms are employed to initiate this cascade. Some hormones, such as growth hormone, prolactin, erythropoietin, and the cytokines, initiate their action by activating a tyrosine kinase, but this activity is not an integral part of the hormone receptor. The hormone-receptor interaction promotes binding and activation of **cytoplasmic protein tyrosine kinases**, such as **JAK1**, or **JAK2** or **TYK**.

These kinases phosphorylate one or more cytoplasmic proteins, which then associate with other docking proteins through binding to SH2 domains. One such interaction results in the activation of a family of cytosolic proteins called **STATs**, or **signal transducers and activators of transcription**. The phosphorylated STAT protein dimerizes and translocates into the nucleus, binds to a specific DNA element such as the interferon response element (IRE), and activates transcription. This is illustrated in **Figure 42–9**. Other SH2 docking events may result in the activation of PI-3 kinase, the MAP kinase pathway (through SHC or GRB2), or G-protein–mediated activation of phospholipase C (PLCγ) with the attendant production of diacylglycerol and activation of protein kinase C. It is apparent that there is a potential for “cross-talk” when different hormones activate these various signal transduction pathways.

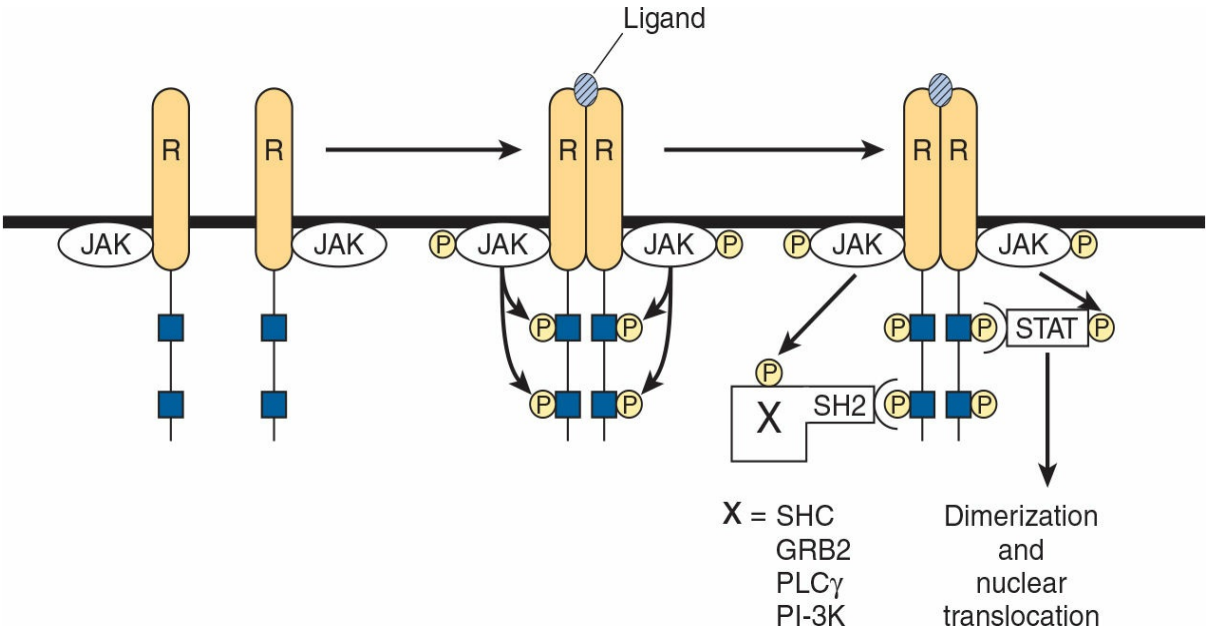

FIGURE 42–9 Initiation of signal transduction by receptors linked to Jak kinases. The receptors (R) that bind prolactin, growth hormone, interferons, and cytokines lack endogenous tyrosine kinase. Upon ligand binding, these receptors dimerize and an associated, though inactive protein kinase (JAK1, JAK2, or TYK) is phosphorylated. Phospho-JAK is now activated, and proceeds to phosphorylate the receptor on tyrosine residues. The STAT proteins associate with the phosphorylated receptor and then are themselves phosphorylated by JAK-P. The phosphorylated STAT protein, STAT Ⓟ dimerizes, translocates to the nucleus, binds to specific DNA elements, and regulates transcription. The phosphotyrosine residues of the receptor also bind to several SH2 domain-containing proteins (X-SH2), which result in activation of the MAP kinase pathway (through SHC or GRB2), PLCγ, or PI-3 kinase.

The NF-κ B Pathway Is Regulated by Glucocorticoids

The DNA-binding transcription factor **NF-κB** is a heterodimeric complex typically composed of two subunits termed **p50** and **p65** (**Figure 42–10**). Normally NF-κβ is sequestered in the cytoplasm in a transcriptionally inactive form by members of the **IκB** (inhibitor of NF-κβ) family of proteins. Extracellular stimuli such as proinflammatory cytokines, reactive oxygen species, and mitogens lead to activation of the **IKK** (IκB kinase) **complex**, which is a heterohexameric structure consisting of α, β, and γ subunits. IKK phosphorylates IκB on two serine residues. This phosphorylation targets IκB for polyubiquitylation and subsequent degradation by the proteasome. Following IκB degradation, free NF-κB translocates to the nucleus, where it binds to a number of gene enhancers and activates transcription, particularly of genes involved in the **inflammatory response**. Transcriptional regulation by NF-κB is mediated by a variety of coactivators such as CREB-binding protein (CBP), as described below (Figure 42–13).

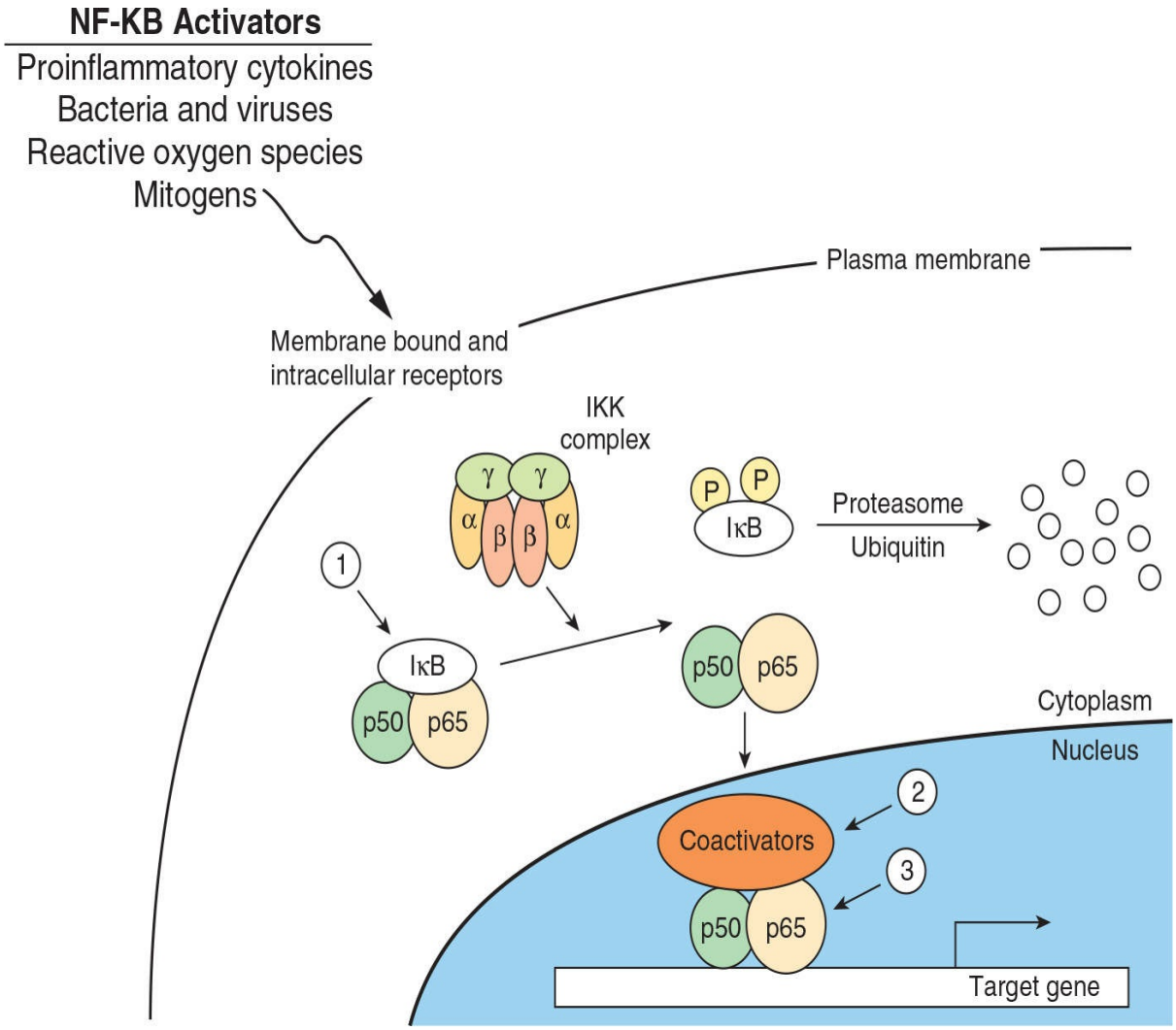

FIGURE 42–10 Regulation of the NF-κβ pathway. NF-κβ consists of two subunits, p50 and p65, which when present in the nucleus regulates transcription of the multitude of genes important for the inflammatory response. NF-κB is restricted from entering the nucleus by IκB, an inhibitor of NF-κB. IκB binds to—and masks—the nuclear localization signal of NF-κB. This cytoplasmic protein is phosphorylated by an IKK complex which is activated by cytokines, reactive oxygen species, and mitogens. Phosphorylated IκB can be ubiquitylated and degraded, thus releasing its hold on NF-κB, and allowing for nuclear translocation. Glucocorticoids, potent anti-inflammatory agents, are thought to affect at least three steps in this process (1, 2, 3), as described in the text.

Glucocorticoid hormones are therapeutically useful agents for the treatment of a variety of inflammatory and immune diseases. Their anti-inflammatory and immunomodulatory actions are explained in part by the inhibition of NF-κB and its subsequent actions. Evidence for three

mechanisms for the inhibition of NF-κB by glucocorticoids has been described: (1) glucocorticoids increase IκB mRNA, which leads to an increase of IκB protein and more efficient sequestration of NF-κB in the cytoplasm. (2) The glucocorticoid receptor competes with NF-κB for binding to coactivators. (3) The glucocorticoid receptor directly binds to the p65 subunit of NF-κB and inhibits its activation (Figure 42–10).

HORMONES CAN INFLUENCE SPECIFIC BIOLOGIC EFFECTS BY MODULATING TRANSCRIPTION

The signals generated as described above have to be translated into an action that allows the cell to effectively adapt to a challenge (Figure 42–1). Much of this adaptation is accomplished through alterations in the rates of transcription of specific genes. Many different observations have led to the current view of how hormones affect transcription. Some of these are as follows: (1) actively transcribed genes are in regions of "open" chromatin (experimentally defined as relative susceptibility to the enzyme DNase I, and containing certain histone PTMs or "marks"), which allows for the access of transcription factors to DNA. (2) Genes have regulatory regions, and transcription factors bind to these to modulate the frequency of transcription initiation. (3) The hormone-receptor complex can be one of these transcription factors. The DNA sequence to which receptors bind is called a **HRE** (see Table 42–1 for examples). (4) Alternatively, other hormone-generated signals can modify the location, amount, or activity of transcription factors and thereby influence binding to the regulatory or response element. (5) Members of a large superfamily of nuclear receptors act with—or in a manner analogous to—the hormone receptors described above. (6) These nuclear receptors interact with another large group of coregulatory molecules to effect changes in the transcription of specific genes.

SUMMARY

- Hormones, cytokines, interleukins, and growth factors use a variety of signaling mechanisms to facilitate cellular adaptive responses.
- The ligand-receptor complex serves as the initial signal for members of the nuclear receptor family.
- Class II peptide/protein and catecholamine hormones, which bind to cell surface receptors, generate a variety of intracellular signals. These include cAMP, cGMP, Ca^{2+}, phosphatidylinositides, and protein kinase cascades.
- Many hormone responses are accomplished through alterations in the rate of transcription of specific genes.
- The nuclear receptor superfamily of proteins plays a central role in the regulation of gene transcription.
- DNA-binding nuclear receptors, which may have hormones, metabolites, or drugs as ligands, bind to specific HREs as homodimers or as heterodimers with RXR.
- Another large family of coregulator proteins remodel chromatin, modify other transcription factors, and bridge the nuclear receptors to the basal transcription apparatus.