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Ronald J. Koenig | Clifford J. Rosen

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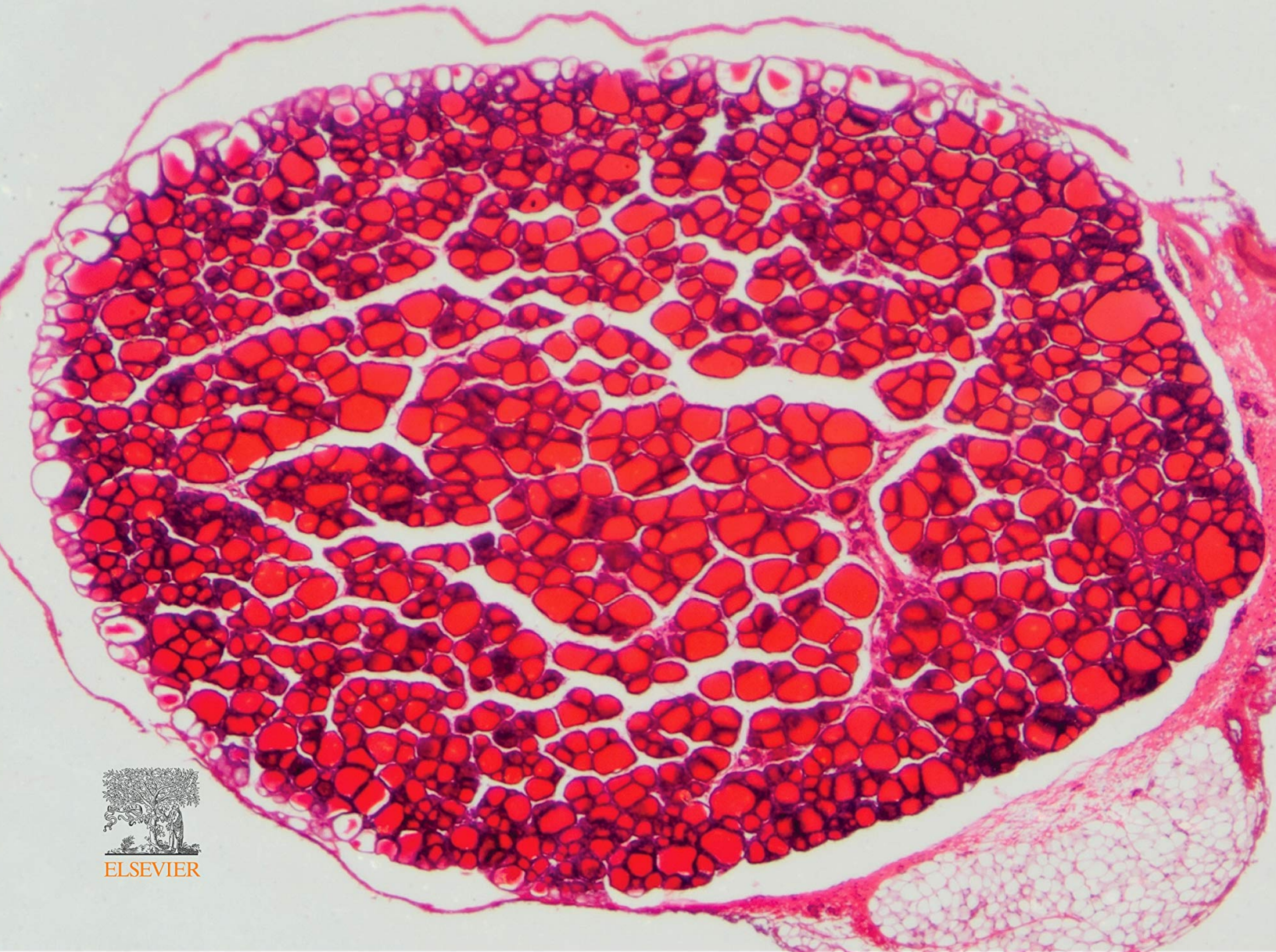
Williams *Textbook of* Endocrinology

14TH EDITION



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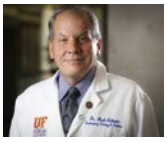

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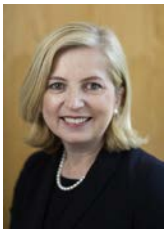

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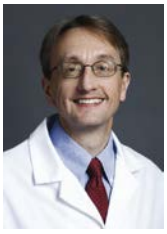

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Preface

The Editors are delighted to welcome you to the 69th anniversary 14th edition of *Williams Textbook of Endocrinology.* In this new edition, we have strived to maintain Robert Williams' original 1950 mandate to publish "a condensed and authoritative discussion of the management of clinical endocrinopathies based upon the application of fundamental information obtained from chemical and physiological investigation." With the passing of the decades, our scholarly goal has been enriched by the addition of genetic, molecular, cellular, and population sciences, together forming the basis of multiple new insights into both the pathogenesis and management of endocrine disorders. Editors of this textbook aim to provide a cogent navigation through the wealth of information emanating from novel medical discoveries that advance the field and bring new therapeutic approaches to patients with endocrine diseases. Our challenge remains to be both concise and didactic, while comprehensively covering relevant translational and clinical endocrine science in an accessible fashion.

With these goals in mind, we have once again assembled a team of outstanding authorities who each contribute their unique expertise to synthesize current knowledge in their respective topic area. For this edition, we have added new chapters on the global burden of endocrine disease and the navigation of the prolific expert endocrine guidelines, as well as chapters devoted to transgender endocrinology, and osteomalacia. The section on diabetes mellitus has been expanded with dedicated chapters on the physiology of insulin secretion, as well as a comprehensive update on therapeutics of type 2 diabetes mellitus. These new contributions reflect the changing emphasis of endocrine practice today and the availability of a wealth of new knowledge and therapeutic options that together affect clinical care. Each section has undergone significant revision and updating to bring the most current information to our readers.

We are deeply appreciative of the valued co-workers in our respective offices, including Shira Berman, and Grace Labrado for their dedicated efforts. We also thank our colleagues at Elsevier—Rae Robertson and Nancy Duffy—for shepherding the production process so professionally. The final product of this exemplary text is due to their skilled navigation of the medical publishing world. We are confident that our combined efforts have succeeded in achieving the high standards set by previous editions that have made Williams the classic "go to" book for all those interested in endocrinology.

1

Principles of Endocrinology

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CHAPTER OUTLINE

KEY POINTS

- Endocrinology is a scientific and medical discipline with a unique focus on hormones that features a multidisciplinary approach to understanding normal and pathologic hormone production and action, as well as diseases related to abnormal hormone signaling.
- Endocrine and paracrine systems differ in important respects that illustrate the evolutionary pressures on these distinct cell-signaling strategies.
- Differentiated hormone-secreting cells are designed to efficiently synthesize hormones and secrete them in a regulated way.
- Hormones in the bloodstream often are associated with binding proteins to enhance their solubility, protect them from degradation and renal excretion, and regulate their stability in the extracellular space.
- Hormones either act on receptors on the plasma membranes of target cells or move into cells to bind to intracellular receptors; in either case, the target cell is not a passive recipient of signals but rather has key roles in regulating hormonal responses.
- Control of hormone secretion involves integrated inputs from multiple distant targets, nervous system inputs, and local paracrine and autocrine factors, all leading to complex patterns of circadian secretion, pulsatile secretion, secretion driven by homeostatic stimuli, or stimuli that lead to secular changes over the lifespan.
- Endocrine diseases fall into broad categories of hormone overproduction or underproduction, altered tissue response to hormones, or tumors arising from endocrine tissue.
- Hormones and synthetic molecules designed to interact with hormone receptors are administered to diagnose and treat diseases.

About a hundred years ago, Starling coined the term *hormone* to describe secretin, a substance secreted by the small intestine into the bloodstream to stimulate pancreatic secretion. In his Croonian Lectures, Starling considered the endocrine and nervous systems as two distinct mechanisms for coordination and control of organ function. Thus, endocrinology found its first home in the discipline of mammalian physiology.

Over the next several decades, biochemists, physiologists, and clinical investigators characterized peptide and steroid hormones secreted into the bloodstream from discrete endocrine glands or other organs. Diseases such as hypothyroidism and diabetes could be treated successfully for the first time by replacing specific hormones. These initial triumphs of discovery formed the foundation of the clinical specialty of endocrinology.

Advances in cell biology, molecular biology, and genetics over the ensuing years began to reveal mechanisms underlying endocrine disease pathogenesis, hormone secretion, and action. Even though these advances have embedded endocrinology in the framework of molecular cell biology, they have not changed the essential subject of endocrinology—the signaling that coordinates

and controls functions of multiple organs and processes. Herein we survey general themes and principles that underpin diverse approaches used by clinicians, physiologists, biochemists, cell biologists, and geneticists to understand the endocrine system.

The Evolutionary Perspective

Hormones are broadly defined as chemical signals secreted into the bloodstream that act on distant tissues, usually in a regulatory fashion. Hormonal signaling represents a special case of the more general process of signaling between cells. Even unicellular organisms such as baker's yeast, *Saccharomyces cerevisiae,* secrete short peptide mating factors that act on receptors of other yeast cells to trigger mating between the two cells. These receptors resemble the ubiquitous family of seven membrane-spanning mammalian receptors that respond to diverse ligands such as photons and glycoprotein hormones. Because these yeast receptors trigger activation of heterotrimeric G proteins just as mammalian receptors do, this conserved signaling pathway was likely to have been present in the common ancestor of yeast and humans.

Signals from one cell to adjacent cells, termed *paracrine signals,* often use the same molecular pathways used by hormonal signals. For example, the sevenless receptor controls the differentiation of retinal cells in the *Drosophila* eye by responding to a membrane-anchored signal from an adjacent cell. Sevenless is a membrane-spanning receptor with an intracellular tyrosine kinase domain that closely resembles signaling by hormone receptors such as the insulin receptor tyrosine kinase. As paracrine factors and hormones can share signaling machinery, it is not surprising that hormones can, in some settings, act as paracrine factors. Testosterone, for example, is secreted into the bloodstream but also acts locally in the testes to control spermatogenesis. Insulin-like growth factor 1 (IGF1) is a polypeptide hormone secreted into the bloodstream from the liver and other tissues but is also a paracrine factor produced locally in most tissues to control cell proliferation. Furthermore, one receptor can mediate actions of a hormone, such as parathyroid hormone (PTH), and of a paracrine factor, such as parathyroid hormone–related protein. In some cases, the paracrine actions of "hormones" exhibit functions quite unrelated to the hormonal functions. For example, macrophages synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$), which can then bind to vitamin D receptors in the same cells and stimulate production of antimicrobial peptides.[1] This example illustrates that the vitamin D 1α-hydroxylase (P450 27B1) responsible for activating 25-hydroxyvitamin D is synthesized in tissues in which its function is unrelated to the calcium homeostatic actions of the $1,25(OH)_2D_3$ hormone.

Target cells respond similarly to signals that reach them from the bloodstream (hormones) or from adjacent cells (paracrine factors); the cellular response machinery does not distinguish between sites of origin of hormone signals. The shared final common pathways used by hormonal and paracrine signals should not, however, obscure important differences between hormonal and paracrine signaling systems (Fig. 1.1). Hormone synthesis occurs in specialized cells designed specifically for their production, and the hormone then travels in the bloodstream and diffuses in effective concentrations into tissues. Therefore, hormones must be produced in much larger amounts to act as hormones relative to the amounts needed to act as paracrine factors, which act at specific local locations. Hormones must be able to travel to and be protected from degradation in transit from the site of production to the distant site of action. Therefore, for example, lipophilic hormones bind to soluble proteins that allow them to travel in the aqueous media of blood at relatively high concentrations. The ability of hormones to diffuse through the extracellular space implies local hormone concentrations at target sites will rapidly decrease when glandular secretion of the hormone ceases. As hormones quickly diffuse throughout extracellular fluid, hormonal metabolism can occur in specialized organs, including liver and kidney, in a manner that determines the effective hormone concentration in other tissues.

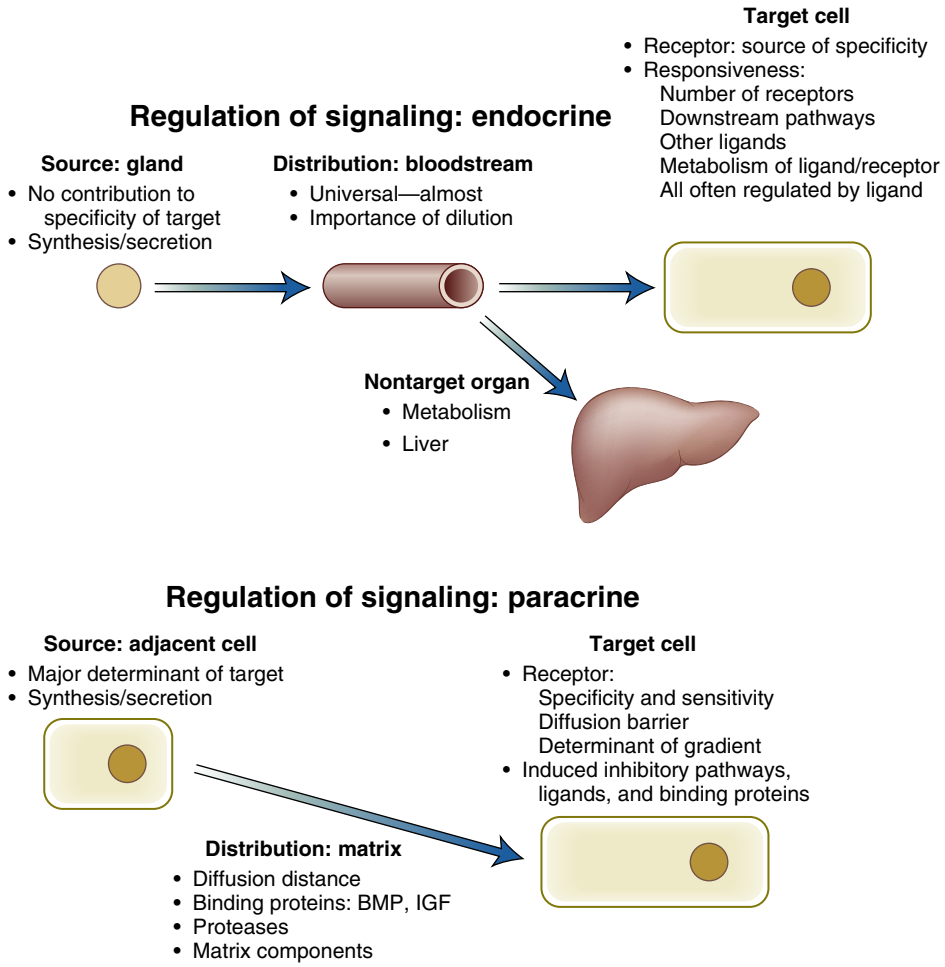
Paracrine factors have rather different constraints. Paracrine signals do not travel very far; consequently, the specific site of origin of a paracrine factor determines where it will act and provides specificity to that action. When the paracrine factor bone morphogenetic protein 4 (BMP4) is secreted by cells in the developing kidney, BMP4 regulates differentiation of renal cells; when the same factor is secreted by cells in bone, it regulates bone formation. Thus the site of origin of BMP4 determines its physiologic role. In contrast, because hormones are secreted into the bloodstream, their sites of origin are often divorced from their functions. Like BMP4, thyroid hormone, for example, acts in many tissues but the site of origin of thyroid hormone in a gland in the neck is not functionally relevant to the sites of action of the hormone.

Because specificity of paracrine factor action is so dependent on its precise site of origin, elaborate mechanisms have evolved to regulate and constrain the diffusion of paracrine factors. Paracrine factors of the hedgehog family, for example, are covalently bound to cholesterol to constrain diffusion of these molecules in the extracellular milieu. Most paracrine factors interact with binding proteins that block their action and control their diffusion. For example, chordin, noggin, and many other distinct proteins bind to various members of the BMP family to regulate their action. Proteases such as tolloid then destroy the binding proteins at specific sites to liberate BMPs so that they can act on appropriate target cells.

Thus, hormones and paracrine factors have several distinct strategies regulating biosynthesis, sites of action, transport, and metabolism. These differing strategies may partly explain why a hormone such as IGF1, unlike its close relative insulin, has multiple binding proteins to control its action in tissues. IGF1 exhibits a double life as both a hormone and a paracrine factor. Presumably the IGF1 actions mandate an elaborate binding protein apparatus to enable appropriate hormone signaling.

All the major hormonal signaling programs—G protein–coupled receptors, tyrosine kinase receptors, serine/threonine kinase receptors, ion channels, cytokine receptors, nuclear receptors—are also used by paracrine factors. In contrast, several paracrine signaling programs are used only by paracrine factors and not by hormones. For example, Notch receptors respond to membrane-based ligands to control cell fate, but no known blood-borne ligands use Notch-type signaling. Perhaps the complex intracellular strategy used by Notch, which involves cleavage of the receptor and subsequent nuclear actions of the receptor's cytoplasmic portion, is too inflexible to serve the purposes of hormones.

Analyses of the complete genomes of multiple bacterial species, the yeast *Saccharomyces cerevisiae,* the fruit fly *Drosophila melanogaster,* the worm *Caenorhabditis elegans,* the plant *Arabidopsis thaliana,* humans, and many other species have allowed a comprehensive view of the signaling machinery used by various forms of life. *S. cerevisiae* uses G protein–coupled receptors; this organism,



• **Fig. 1.1** Comparison of determinants of endocrine and paracrine signaling. *BMP,* bone morphogenetic protein; *IGF,* insulin-like growth factor.

however, lacks tyrosine kinases, used in the insulin signaling pathway, and nuclear receptors that resemble the estrogen/thyroid receptor family. In contrast, the worm and fly share with humans the use of each of these signaling pathways, although with substantial variation in numbers of genes committed to each pathway. For example, the *Drosophila* genome encodes 21 nuclear receptors, the *C. elegans* genome encodes about 284, and the human genome encodes 48 such receptors. These patterns suggest ancient multicellular animals must already have established the signaling systems that are the foundation of the endocrine system as we know it in mammals.

Our understanding of endocrine systems and novel physiologic biology continues to expand. Even before the sequencing of the human genome, sequence analyses had made clear that many receptor genes are found in mammalian genomes for which no clear ligand or function was known. Analyses of these "orphan" receptors broadened current understanding of hormonal signaling. For example, the liver X receptor (LXR) was one such orphan receptor found when searching for unknown putative nuclear receptors. Subsequent experiments found oxygenated derivatives of cholesterol are the ligands for LXR, which regulates genes involved in cholesterol and fatty acid metabolism.[2] The examples of LXR and many others raise the question of what constitutes a hormone. The classic view of hormones is that they are synthesized in discrete glands and have no function other than activating receptors on cell membranes or in the nucleus. Cholesterol, which is converted in cells to oxygenated derivatives that activate the LXR receptor, in contrast, uses a hormonal strategy to regulate its own metabolism. Other orphan nuclear receptors similarly respond to ligands such as bile acids and fatty acids. These "hormones" have important metabolic roles quite separate from their signaling properties, although hormone-like signaling permits regulation of the metabolic function. The calcium-sensing receptor is an example from the G protein–coupled receptor family that responds to a nonclassic ligand, ionic calcium. Calcium is released into the bloodstream from bone, kidney, and intestine and acts on the calcium-sensing receptor on parathyroid cells, renal tubular cells, and other cells to coordinate cellular responses to calcium. Thus, many important metabolic factors have hormonal properties as part of a regulatory strategy within complex organisms. Broadened understanding of these new metabolic factors is leading to new therapeutic approaches to treat or prevent human diseases.

Endocrine Glands

Hormone formation may occur either in the endocrine glands, which are localized collections of specific cells, or in cells that have additional roles. Many protein hormones, such as growth hormone (GH), PTH, prolactin (PRL), insulin, and glucagon, are

produced in dedicated cells by standard protein synthetic mechanisms common to all cells. These secretory cells contain specialized secretory granules designed to store large amounts of hormone and to release the hormones in response to specific signals. Hormones made in these glands and specialized cells are considered to be classic endocrine systems. Formation of small hormone molecules initiates with commonly found precursors, usually in specific glands such as the adrenals, gonads, or thyroid. In the case of the steroid hormones, the precursor is cholesterol, which is modified by various cytochrome P450–based hydroxylations and carbon-carbon bond cleavages and by specific oxidoreductases to form the glucocorticoids, androgens, estrogens, and their biologically active derivatives.

However, not all hormones are formed in dedicated and specialized endocrine glands; the adipose, enteroendocrine, and other systems are now also recognized to be complex endocrine systems. Thus with the discovery of novel peptides and amino acid or steroid-based molecules and their regulatory functions, the field of endocrinology and metabolism has recently been greatly expanded. For example, the protein hormone leptin, which regulates appetite and energy expenditure, is formed in adipocytes, providing a specific signal reflecting the nutritional state of the organism to the central nervous system. The enteroendocrine system comprises a unique hormonal system in which peptide hormones that regulate metabolic and other responses to oral nutrients are produced and secreted by specialized endocrine cells scattered throughout the intestinal epithelium. The cholesterol derivative, 7-dehydrocholesterol, the precursor of vitamin D_3, is converted in skin keratinocytes to previtamin D_3 by a photochemical reaction.

Thyroid hormone synthesis occurs via a unique pathway. The thyroid cell synthesizes a 660,000-kDa homodimer, thyroglobulin, which is then iodinated at specific tyrosines. Some iodotyrosines combine enzymatically to form the iodothyronine molecule within thyroglobulin, which is then stored in the lumen of the thyroid follicle. For tyrosine iodination to occur, the thyroid cell must concentrate trace quantities of iodide from the blood and oxidize it via a specific peroxidase. Release of thyroxine (T_4) from thyroglobulin requires phagocytosis and cathepsin-catalyzed digestion by the same cells.

Hormones are synthesized in response to biochemical signals generated by various modulating systems. Many of these systems are specific to the effects of the hormone product; for example, PTH synthesis is regulated by the concentration of ionized calcium, and insulin synthesis is regulated by the concentration of glucose. For others, such as gonadal, adrenal, and thyroid hormones, control of hormone synthesis is achieved by the homeostatic function of the hypothalamic-pituitary axis. Cells in the hypothalamus and pituitary monitor circulating hormone concentrations and secrete trophic hormones, which activate specific pathways for hormone synthesis and release. Typical examples are GH, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH).

These trophic hormones increase rates of hormone synthesis and secretion, and they may induce target cell division, thus causing enlargement of the various target glands. For example, in hypothyroid individuals living in iodine-deficient areas of the world, TSH secretion causes a marked hyperplasia of thyroid cells. In such regions, the thyroid gland may be 20 to 50 times its normal size. Adrenal hyperplasia occurs in patients with genetic deficiencies in cortisol formation. Hypertrophy and hyperplasia of parathyroid cells, initiated by an intrinsic response to the stress of hypocalcemia, occur in patients with renal insufficiency or calcium malabsorption.

Hormones may be fully active when released into the bloodstream (e.g., GH or insulin), or they may require activation in specific cells to produce biologic effects. These activation steps are often highly regulated. For example, T_4 released from the thyroid cell is a prohormone that must undergo specific deiodination to form the active 3,5,3'-triiodothyronine (T_3). This deiodination reaction can occur in target tissues, such as in the central nervous system; in thyrotrophs, where T_3 provides feedback regulation of TSH production; or in hepatic and renal cells, from which it is released into the circulation for uptake by all tissues. A similar postsecretory activation step catalyzed by a 5α-reductase causes tissue-specific activation of testosterone to dihydrotestosterone in target tissues, including the male urogenital tract, prostate, genital skin, and liver. Vitamin D undergoes hydroxylation at the 25 position in the liver and in the 1 position in the kidney. Both hydroxylations must occur to produce the active hormone, 1,25-dihydroxyvitamin D. Activity of 1α-hydroxylase, but not 25-hydroxylase, is stimulated by PTH and hypophosphatemia but is inhibited by calcium, 1,25-dihydroxyvitamin D, and fibroblast growth factor 23 (FGF23).

Most hormones are synthesized as required on a daily, hourly, or minute-to-minute basis with minimal storage, but there are significant exceptions. One is the thyroid gland, which contains enough stored hormone to last for about 2 months. This storage permits a constant supply of thyroid hormone despite significant variations in the availability of iodine. However, if iodine deficiency is prolonged, normal T_4 reservoirs can be depleted.

Feedback signaling systems exemplified earlier enable the hormonal *homeostasis* characteristic of virtually all endocrine systems. Regulation may include the central nervous system or local signal recogniton mechanisms in the glandular cells, such as the calcium-sensing receptor of the parathyroid cell. Disruption of hormonal homeostasis due to glandular or central regulatory system dysfunction has both clinical and laboratory consequences. Recognition and correction of disorders of these systems are the essence of clinical endocrinology.

Transport of Hormones in Blood

Protein hormones and some small molecules, such as catecholamines, are water soluble and readily transported via the circulatory system. Others are nearly insoluble in water (e.g., steroid and thyroid hormones), and their distribution presents special problems. Such molecules are tightly bound to 50-kDa to 60-kDa carrier plasma glycoproteins such as thyroxine-binding globulin (TBG), sex hormone–binding globulin (SHBG), and corticosteroid-binding globulin (CBG) or weakly bound to abundant albumin. Ligand-protein complexes serve as hormone reservoirs, ensure ubiquitous distribution of water-insoluble ligands, and protect small molecules from rapid inactivation or excretion in urine or bile. Protein-bound hormones exist in equilibria with the often-minute quantities of hormone in the aqueous plasma, with the "free" fraction of the circulating hormone taken up by the target cell. For example, if tracer thyroid hormone is injected into the portal vein in a protein-free solution, it binds to hepatocytes at the periphery of the hepatic sinusoid. When the same experiment is repeated with a protein-containing solution, uniform distribution of the tracer hormone occurs throughout the hepatic lobule.[3] Despite the very high affinity of some binding proteins for their

respective ligands, one specific protein may not be essential for hormone distribution. For example, in humans with congenital TBG deficiency, other proteins—transthyretin (TTR) and albumin—subsume its role. As the affinity of these secondary thyroid hormone transport proteins is several orders of magnitude lower than that of TBG, it is possible for the hypothalamic-pituitary feedback system to maintain free thyroid hormone in the normal range at a much lower total hormone concentration. The fact that the free hormone concentration is normal in individuals with TBG deficiency indicates that the hypothalamic-pituitary axis defends the free, active hormone.[4]

Availability of gene-targeting techniques allows specific assessments of the physiologic roles of hormone-binding proteins. For example, mice with targeted inactivation of the vitamin D–binding protein (DBP) have been generated.[5] Although the absence of DBP markedly reduces circulating concentrations of vitamin D, the mice are otherwise normal. However, they have enhanced susceptibility to a vitamin D–deficient diet due to the reduced reservoir of this sterol. In addition, the absence of DBP markedly reduces the half-life of 25-hydroxyvitamin D by accelerating its hepatic uptake, making the mice less susceptible to vitamin D intoxication.

Protein hormones and some small ligands (e.g., catecholamines) produce their effects by interacting with cell surface receptors. Others, such as steroid and thyroid hormones, must enter the cell to bind to cytosolic or nuclear receptors. In the past, it has been thought that much of the transmembrane transport of hormones was passive. Evidence now demonstrates specific transporters involved in cellular uptake of thyroid and some steroid hormones,[7] providing yet another mechanism for regulating the distribution of a hormone to its site of action. Studies in mice devoid of megalin, a large, cell surface protein in the low-density lipoprotein (LDL) receptor family, suggest estrogen and testosterone bound to SHBG use megalin to enter peripheral tissues while still bound to SHBG.[8] In this scenario, the hormone bound to SHBG, rather than "free" hormone, is the active moiety that enters cells. It is unclear how frequently this apparent exception to the "free hormone" hypothesis occurs.

MicroRNAs (miRNAs) have recently also been shown to elicit remote metabolic actions. For example, exosomal miRNA derived from adipose tissue regulates distant tissue gene expression, glucose tolerance, and circulating fibroblast growth factor 21 (FGF21) levels. MiRNAs may thus function as circulating adipokines.[9] Other small lipid signaling molecules are being discovered, especially for their role in activating or suppressing what were previously designated as orphan receptors.

Target Cells as Active Participants

Hormones determine cellular target actions by binding with high specificity to receptor proteins. Whether a peripheral cell is hormonally responsive depends to a large extent on the presence and function of specific and selective hormone receptors and the downstream signaling pathway molecules. Thus receptor expression and intracellular effector pathways activated by the hormone signal are key determinants for which cells will respond, and how. Receptor proteins may be localized to the cell membrane, cytoplasm, or nucleus. Broadly, polypeptide hormone receptors are cell membrane associated, but steroid hormones selectively bind soluble intracellular proteins (Fig. 1.2).

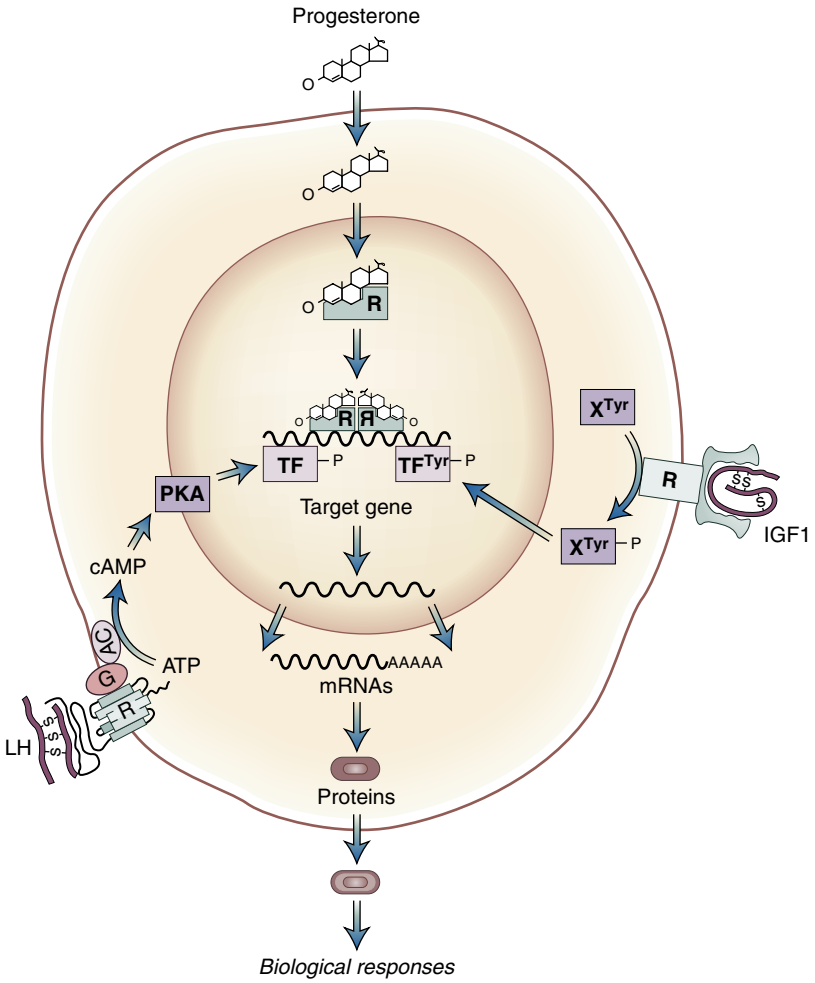
Membrane-associated receptor proteins usually consist of extracellular sequences that recognize and bind ligand, transmembrane-anchoring hydrophobic sequences, and intracellular sequences, which initiate intracellular signaling. Intracellular signaling is mediated by covalent modification and activation of intracellular signaling molecules (e.g., signal transducers and activators of transcription [STAT] proteins) or by generation of small molecule second messengers (e.g., cyclic adenosine monophosphate) through activation of heterotrimeric G proteins. Subunits of these G proteins (α-subunits, β-subunits, and γ-subunits) activate or suppress effector enzymes and ion channels that generate the second messengers. Some of these receptors (e.g., those for somatostatin) may in fact exhibit low constitutive activity and have been shown to signal in the absence of added ligand.

Several growth factors and hormone receptors (e.g., for insulin) behave as intrinsic tyrosine kinases or activate intracellular protein tyrosine kinases. Ligand activation may cause receptor dimerization (e.g., GH) or heterodimerization (e.g., interleukin 6), followed by activation of intracellular phosphorylation cascades. These activated proteins ultimately determine specific nuclear gene expression.

Both the number of receptors expressed per cell and their responses are regulated, thus providing a further level of control for hormone action. Several mechanisms account for altered receptor function. Receptor endocytosis causes internalization of cell surface receptors; the hormone-receptor complex is subsequently dissociated, resulting in abrogation of the hormone signal. Receptor trafficking may then result in recycling back to the cell surface (e.g., as for the insulin receptor) or the internalized receptor may undergo lysosomal degradation. Both these mechanisms triggered by activation of receptors effectively lead to impaired hormone signaling downregulation of the receptors. The hormone signaling pathway may also be downregulated by receptor desensitization (e.g., as for epinephrine); ligand-mediated receptor phosphorylation leads to a reversible deactivation of the receptor. Desensitization mechanisms can be activated by a receptor's ligand (homologous desensitization) or by another signal (heterologous desensitization), thereby attenuating receptor signaling in the continued presence of ligand. Receptor function may also be limited by action of specific phosphatases (e.g., Src homology phosphatase [SHP]) or by intracellular negative regulation of the signaling cascade (e.g., suppressor of cytokine signaling [SOCS] proteins inhibiting Janus kinase/signal transducers and activators of transcription [JAK-STAT] signaling). Certain ligand-receptor complexes may also translocate to the nucleus.

Mutational changes in receptor structure can also determine hormone action. Constitutive receptor activation may be induced by activating mutations (e.g., TSH receptor) leading to endocrine organ hyperfunction, even in the absence of ligand. Conversely, inactivating receptor mutations may lead to endocrine hypofunction (e.g., testosterone or vasopressin receptors). These syndromes are well characterized (Table 1.1) and are well described in subsequent chapters.

The functional diversity of receptor signaling results in overlapping or redundant intracellular pathways. For example, GH, PRL, and cytokines each activate JAK-STAT signaling, whereas the distal effects of these stimuli clearly differ. Thus, despite common upstream signaling pathways, hormones can elicit highly specific cellular effects. Tissue-type or cell-type genetic programs or receptor-receptor interactions at the cell surface (e.g., hetero-oligomerization of dopamine D2 with somatostatin receptor, or insulin with IGF1 receptor) may also confer a specific cellular response to a hormone and provide an additive cellular effect.[10] In addition, effector protein expression may differ in select cells to modulate



• **Fig. 1.2** Hormonal signaling by cell surface and intracellular receptors. The receptors for the water-soluble polypeptide hormones, luteinizing hormone (LH), and insulin-like growth factor 1 (IGF1) are integral membrane proteins located at the cell surface. They bind the hormone-utilizing extracellular sequences and transduce a signal by the generation of second messengers: cyclic adenosine monophosphate (cAMP) for the LH receptor and tyrosine-phosphorylated substrates for the IGF1 receptor. Although effects on gene expression are indicated, direct effects on cellular proteins (e.g., ion channels) are also observed. In contrast, the receptor for the lipophilic steroid hormone progesterone resides in the cell nucleus. It binds the hormone and becomes activated and capable of directly modulating target gene transcription. *AC,* adenylyl cyclase; *ATP,* adenosine triphosphate; *G,* heterotrimeric G protein; *mRNAs,* messenger RNAs; *PKA,* protein kinase A; *R,* receptor molecule; *TF,* transcription factor; *Tyr,* tyrosine found in protein X; *X,* unknown protein substrate. (From Mayo K. Receptors: molecular mediators of hormone action. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press; 1997:11.)

hormonal response. For example, the glucose transporter-4 protein, which leads to insulin-mediated glucose uptake, is most abundantly expressed in muscle, hepatic and adipose tissues, causing these tissues to be the most sensitive tissues for insulin-mediated glucose disposal.

A final mechanism of nuclear receptor modulation is prereceptor regulation via intracellular enzymes that convert the circulating molecules to more or less potent hormones. In addition to the activation of T_4 and testosterone described earlier, selective hormone inactivation occurs in some cells. In the distal nephron, the enzyme 11β-hydroxysteroid dehydrogenase type 2 converts the mineralocorticoid-receptor ligand cortisol to inactive cortisone, thus preventing receptor activation. This mechanism allows aldosterone, which is not a substrate for the enzyme, to regulate mineralocorticoid activity in the kidney despite circulating aldosterone concentrations 1000 times lower than those of cortisol.

Control of Hormone Secretion

Anatomically distinct endocrine glands are composed of highly differentiated cells that synthesize, store, and secrete hormones. Circulating hormone concentrations are a function of glandular secretory patterns and hormone clearance rates. Hormone secretion is tightly regulated to attain circulating levels that are most conducive to elicit the appropriate target tissue response. For example, longitudinal bone growth is initiated and maintained by exquisitely regulated levels of circulating GH, yet mild GH hypersecretion results in gigantism, and GH deficiency causes growth retardation. Ambient circulating hormone concentrations are not uniform, and secretion patterns determine appropriate physiologic function. Thus, insulin secretion occurs in short pulses elicited by nutrient and other signals; gonadotropin secretion is episodic, determined by a hypothalamic pulse generator; and PRL

TABLE 1.1 Diseases Caused by Mutations in G Protein–Coupled Receptors.

Condition[a]	Receptor	Inheritance	ΔFunction[b]
Retinitis pigmentosa	Rhodopsin	AD/AR	Loss
Nephrogenic diabetes insipidus	Vasopressin V2	X-linked	Loss
Familial glucocorticoid deficiency	ACTH	AR	Loss
Color blindness	Red/green opsins	X-linked	Loss
Familial precocious puberty	LH	AD (male)	Gain
Familial hypercalcemia	Ca^{2+} sensing	AD	Loss
Neonatal severe parathyroidism	Ca^{2+} sensing	AR	Loss
Dominant form hypocalcemia	Ca^{2+} sensing	AD	Gain
Congenital hyperthyroidism	TSH	AD	Gain
Hyperfunctioning thyroid adenoma	TSH	Somatic	Gain
Metaphyseal chondrodysplasia	PTH-PTHrP	Somatic	Gain
Hirschsprung disease	Endothelin-B	Multigenic	Loss
Coat color alteration (*E* locus, mice)	MSH	AD/AR	Loss and gain
Dwarfism (*little* locus, mice)	GHRH	AR	Loss

[a]All are human conditions with the exception of the final two entries, which refer to the mouse.

[b]Loss of function refers to inactivating mutations of the receptor, and gain of function to activating mutations.

ACTH, Adrenocorticotropic hormone; *AD,* autosomal dominant inheritance; *AR,* autosomal recessive inheritance; *FSH,* follicle-stimulating hormone; *GHRH,* growth hormone–releasing hormone; *LH,* luteinizing hormone; *MSH,* melanocyte-stimulating hormone; *PTH-PTHrP,* parathyroid hormone and parathyroid hormone–related peptide; *TSH,* thyroid-stimulating hormone.

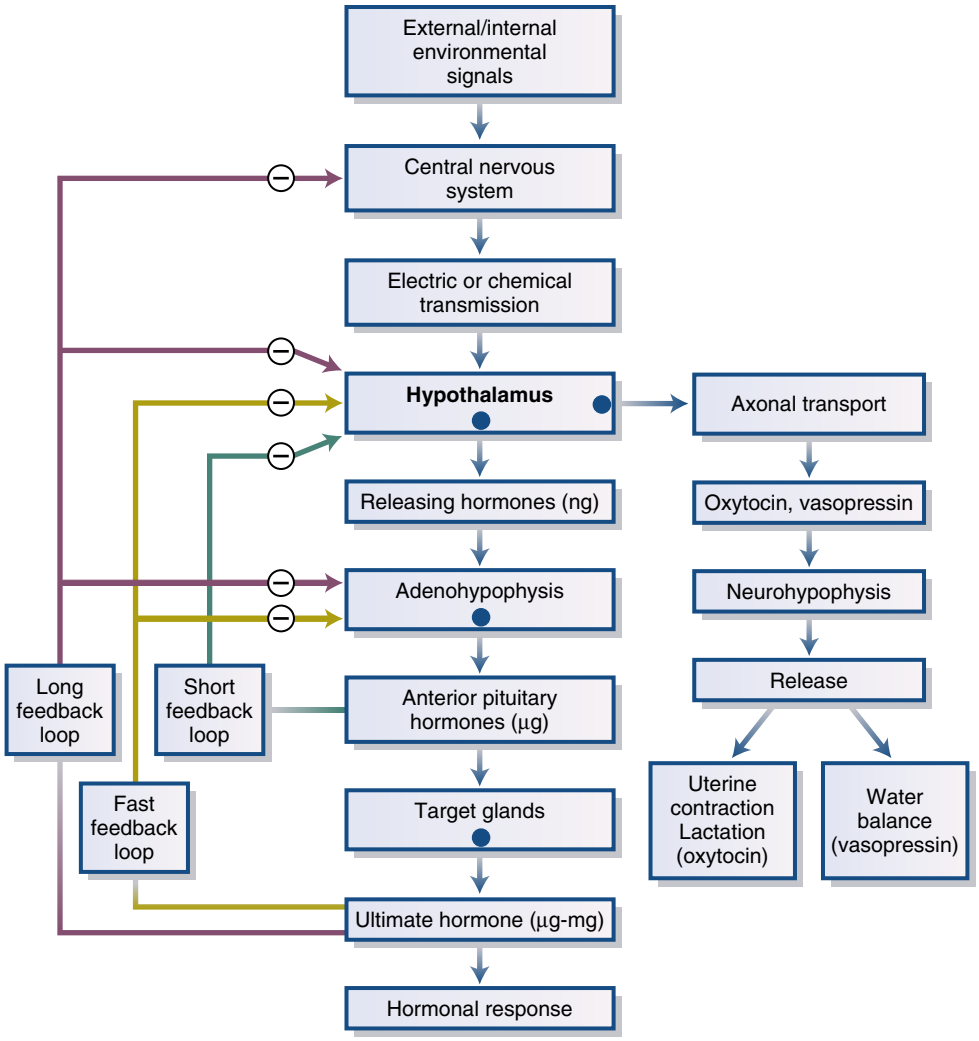
From Mayo K. Receptors: molecular mediators of hormone action. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press; 1997:27.

secretion appears to be relatively continuous, with secretory peaks elicited during suckling.

Hormone secretion also adheres to rhythmic patterns. Circadian rhythms serve as adaptive responses to environmental signals and are controlled by a circadian timing mechanism.[11] Light is the major environmental cue adjusting the endogenous clock. The retinohypothalamic tract entrains circadian pulse generators situated within hypothalamic suprachiasmatic nuclei. These signals subserve timing mechanisms for the sleep-wake cycle and determine patterns of hormone secretion and action. Disturbed circadian timing results in hormonal dysfunction and may also be reflective of entrainment or pulse generator lesions. For example, adult GH deficiency due to a damaged hypothalamus or pituitary is associated with elevations in integrated 24-hour leptin concentrations and decreased leptin pulsatility, yet preserved circadian rhythm of leptin. GH replacement restores leptin pulsatility, promoting loss of body fat mass.[12] Sleep is an important cue regulating hormone pulsatility. About 70% of overall GH secretion occurs during slow-wave sleep, and increasing age is associated with declining slow-wave sleep and concomitant decline in GH and elevation of cortisol secretion.[13] Most pituitary hormones are secreted in a circadian (day-night) rhythm, best exemplified by ACTH peaks before 9 AM, whereas ovarian steroids follow a 28-day menstrual rhythm. Disrupted episodic rhythms are often a hallmark of endocrine dysfunction. For example, loss of circadian ACTH secretion with high midnight cortisol levels is a feature of Cushing disease.

Hormone secretion is induced by multiple specific biochemical and neural signals. Integration of these stimuli results in the net temporal and quantitative secretion of the hormone (Fig. 1.3). Signals elicited by hypothalamic hormones (growth hormone–releasing hormone [GHRH], somatostatin), peripheral hormones (IGF1, sex steroids, thyroid hormone), nutrients, adrenergic pathways, stress, and other neuropeptides all converge on the somatotroph cell, resulting in the ultimate pattern and quantity of GH secretion. Networks of reciprocal interactions allow for dynamic adaptation and shifts in environmental signals. These regulatory systems involve the hypothalamic, pituitary, and target endocrine glands, as well as the adipocytes and lymphocytes. Peripheral inflammation and stress elicit cytokine signals that interface with the neuroendocrine system, resulting in hypothalamic-pituitary axis activation. Parathyroid and pancreatic secreting cells are less tightly controlled by the hypothalamus, but their functions are tightly regulated by the distal effects they elicit. For example, PTH secretion is induced when serum calcium levels fall and the signal for sustained PTH secretion is abrogated by rising calcium levels, whereas insulin secretion is induced when blood glucose rises but suppressed when glucose concentrations fall.

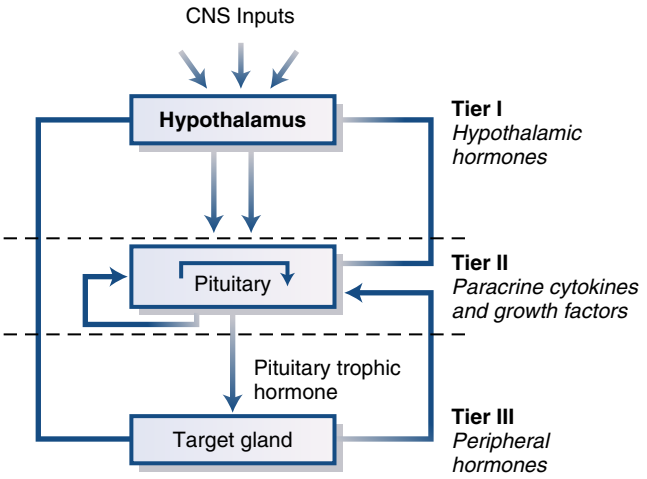
Several tiers of control subserve the ultimate net glandular secretion. First, central nervous system signals, including afferent stimuli, neuropeptides, and stress, signal the synthesis and secretion of hypothalamic hormones and neuropeptides (Fig. 1.4). Four hypothalamic-releasing hormones (GHRH, corticotropin-releasing hormone [CRH], thyrotropin-releasing hormone [TRH], and gonadotropin-releasing hormone [GnRH]) traverse the hypothalamic portal vessels and impinge upon their respective transmembrane trophic hormone-secreting cell receptors. These distinct cells express GH, ACTH, TSH, and gonadotropins, respectively. In contrast, hypothalamic somatostatin and dopamine suppress GH or PRL and TSH secretion, respectively. Trophic hormones



• **Fig. 1.3** Peripheral feedback mechanism and a millionfold amplifying cascade of hormonal signals. Environmental signals are transmitted to the central nervous system, which innervates the hypothalamus, which responds by secreting nanogram amounts of a specific releasing hormone. These are transported down a closed portal system, pass the blood-brain barrier at either end through fenestrations, and bind to specific anterior pituitary cell membrane receptors to elicit secretion of micrograms of specific anterior pituitary hormones. These hormones enter the venous circulation through fenestrated local capillaries, bind to specific target gland receptors, trigger release of micrograms to milligrams of daily hormone amounts, and elicit responses by binding to receptors in distal target tissues. Peripheral hormone receptors enable widespread cell signaling by a single initiating environmental signal, thus facilitating intimate homeostatic association with the external environment. Arrows with a large dot at their origin indicate a secretory process. (From Normal AW, Litwack G. *Hormones.* 2nd ed. New York: Academic Press; 1997:14.)

maintain the structural and functional integrity of endocrine organs, including the thyroid and adrenal glands and the gonads. Target hormones, in turn, serve as powerful negative feedback regulators of their respective trophic hormone, often also suppressing secretion of hypothalamic-releasing hormones. In certain circumstances (e.g., during puberty), peripheral sex steroids may positively induce the hypothalamic-pituitary-target gland axis. For example, LH induces ovarian estrogen secretion, which feeds back positively to induce further LH release. Pituitary hormones themselves, in a short feedback loop, also regulate their own respective hypothalamic-controlling hormone. Hypothalamic-releasing hormones are secreted in nanogram amounts and have short half-lives of a few minutes. Anterior pituitary hormones are produced in microgram amounts and have longer half-lives, but peripheral hormones can be produced in up to milligram amounts daily, with much longer half-lives.

A further level of secretion control occurs within the gland itself. Intraglandular paracrine or autocrine growth peptides serve to autoregulate pituitary hormone secretion, as exemplified by epidermal growth factor (EGF) control of PRL or IGF1 control of GH secretion. Molecules within the endocrine cell may also subserve an intracellular feedback loop. For example, corticotrope SOCS-3 induction by gp130-linked cytokines serves to abrogate the ligand-induced JAK-STAT cascade and block proopiomelanocortin (POMC) transcription and ACTH secretion. This rapid on-off regulation of ACTH secretion provides a plasticity for the endocrine response to changes in environmental signaling and serves to maintain homeostatic integrity.[14]



• **Fig. 1.4** Model for regulation of anterior pituitary hormone secretion by three tiers of control. Hypothalamic hormones impinge directly on their respective target cells. Intrapituitary cytokines and growth factors regulate trophic cell function by paracrine (and autocrine) control. Peripheral hormones exert negative feedback inhibition of respective pituitary trophic hormone synthesis and secretion. *CNS,* central nervous system. (From Ray D, Melmed S. Pituitary cytokine and growth factor expression and action. *Endocr Rev.* 1997;18:206–228.)

In addition to the central nervous system–neuroendocrine interface mediated by hypothalamic chemical signal transduction, the central nervous system directly controls several hormonal secretory processes. Posterior pituitary hormone secretion occurs as direct efferent neural extensions. Postganglionic sympathetic nerves also regulate rapid changes in renin, insulin, and glucagon secretion, while preganglionic sympathetic nerves signal to adrenal medullary cells eliciting epinephrine release.

Hormone Measurement

Endocrine function can be assessed by measuring levels of basal circulating hormone, evoked or suppressed hormone, or hormone-binding proteins. Alternatively, hormone function can be assessed. When a feedback loop exists between the hypothalamic-pituitary axis and a target gland, the circulating level of the pituitary trophic hormone, such as TSH or ACTH, is typically an exquisitely sensitive index of deficient or excessive function of the thyroid or the adrenal cortex, respectively. Meaningful strategies for timing hormonal measurements vary from system to system. In some cases, circulating hormone concentrations can be measured in randomly collected serum samples. This measurement, when standardized for fasting, environmental stress, age, and gender, is reflective of true hormone concentrations only when levels do not fluctuate appreciably. For example, thyroid hormone, PRL, and IGF1 levels can be accurately assessed in fasting morning serum samples. On the other hand, when hormone secretion is clearly episodic, timed samples may be required over a defined time course to reflect hormone bioavailability. Thus, early morning and late evening cortisol measurements are most appropriate. A 24-hour sampling for GH measurements, with samples collected every 2, 10, or 20 minutes, is expensive and cumbersome, yet may yield valuable diagnostic information. Random sampling may also reflect secretion peaks or nadirs, thus confounding adequate interpretation of results.

In general, confirmation of failed glandular function is made by attempting to evoke hormone secretion by recognized stimuli. Testing of pituitary hormone reserve may be accomplished by injecting appropriate hypothalamic-releasing hormones. Injection of trophic hormones, including TSH and ACTH, evokes specific target gland hormone secretion. Pharmacologic stimuli (e.g., metoclopramide for induction of PRL secretion) may also be useful tests of hormone reserve. In contrast, hormone hypersecretion can best be diagnosed by suppressing glandular function. Failure to appropriately suppress GH levels after a standardized glucose load implies inappropriate GH hypersecretion. Failure to suppress insulin secretion during hypoglycemia indicates inappropriate hypersecretion of insulin and should prompt a search for the cause, such as an insulin-secreting tumor.

Radioimmunoassays use highly specific antibodies that uniquely recognize the hormone, or a hormone fragment, to quantify hormone levels. Enzyme-linked immunosorbent assays (ELISAs) use enzyme-conjugated antibodies, and enzyme activity is reflective of hormone concentration. Immunometric assays use two antibodies directed to different epitopes of a polypeptide hormone: one "capture" antibody that isolates the hormone to a solid support and one "signal" antibody coupled to a signal-generating molecule such as acridinium ester or an enzyme. These sensitive techniques have allowed ultrasensitive measurements of physiologic hormone concentrations. Hormone-specific receptors may be used in place of the antibody in a radioreceptor assay. However, all antibody-based assays may be subject to artifacts, which should be kept in mind especially when the assay results are discordant with the clinical picture.

Endocrine Diseases

Endocrine diseases fall into four broad categories: (1) hormone overproduction, (2) hormone underproduction, (3) altered tissue responses to hormones, and (4) tumors of endocrine glands. An additional albeit atypical fifth category is exemplified by one kind of hypothyroidism in which overexpression of a hormone-inactivating enzyme in a tumor leads to thyroid hormone deficiency. Other disorders of inadequate hormone inactivation include apparent mineralocorticoid excess, vitamin D 24-hydroxylase deficiency, and X-linked hypophosphatemic rickets (PHEX deficiency).

Hormone Overproduction

Occasionally, hormones are secreted in increased amounts because of genetic abnormalities that cause abnormal regulation of hormone synthesis or release. For example, in glucocorticoid-remediable hyperaldosteronism, an abnormal chromosomal crossover event creates a fusion gene that encodes a protein with aldosterone synthase activity under the control of the ACTH-regulated 11β-hydroxylase promoter. More often, diseases of hormone overproduction are associated with an increase in the total number of hormone-producing cells. For example, hyperthyroidism associated with Graves disease, in which antibodies mimic TSH and activate the TSH receptors on thyroid cells, is accompanied by dramatic increase in thyroid cell proliferation and increased synthesis and release of thyroid hormone from each thyroid cell. In this example, the increase in thyroid cell number represents a polyclonal expansion of thyroid cells, in which large numbers of thyroid cells proliferate in response to an abnormal stimulus. However, most endocrine tumors are not polyclonal expansions but instead represent monoclonal expansions of a single mutated cell. Pituitary and parathyroid tumors, for example, are usually monoclonal expansions in which somatic mutations in multiple

tumor suppressor genes and proto-oncogenes occur. These mutations lead to an increase in proliferation or survival of the mutant cells. Sometimes this proliferation is associated with abnormal secretion of hormone from each tumor cell. For example, mutant $G_s\alpha$ proteins in somatotrophs can lead to both increased cellular proliferation and increased secretion of GH from each tumor cell.

Hormone Underproduction

Underproduction of hormone can result from a wide variety of processes, ranging from surgical removal of parathyroid glands during neck surgery, to tuberculous destruction of adrenal glands, to iron deposition in pancreatic beta cells of islets in hemochromatosis. A frequent cause of destruction of hormone-producing cells is autoimmunity. Autoimmune destruction of beta cells in type 1 diabetes mellitus or of thyroid cells in chronic lymphocytic (Hashimoto) thyroiditis are two of the most common disorders treated by endocrinologists. Recently a direct passage of insulin fragments by exocytosis from pancreatic islets to lymphoid tissue has been shown to trigger autoimmune diabetes in mice.[15] Multiple genetic abnormalities can also lead to decreased hormone production. These disorders can result from abnormal development of hormone-producing cells (e.g., hypogonadotropic hypogonadism caused by *KAL* gene mutations), from abnormal synthesis of hormones (e.g., deletion of the GH gene), or from abnormal regulation of hormone secretion (e.g., the hypoparathyroidism associated with activating mutations of the parathyroid cell's calcium-sensing receptor). Drugs are important causes of endocrine gland dysfunction as exemplified by immune checkpoint inhibitors leading to multiple endocrinopathies.

Altered Tissue Responses to Hormones

Resistance to hormones can be caused by a variety of genetic disorders. Examples include mutations in the GH receptor in Laron dwarfism and mutations in the $G_s\alpha$ gene in the hypocalcemia of pseudohypoparathyroidism type 1A. Insulin resistance in muscle and liver central to the cause of type 2 diabetes mellitus is complex in origin, resulting from inherited variations in many genes, as well as from theoretically reversible physiologic stresses. Type 2 diabetes is also an example of a disease in which end-organ insensitivity is worsened by signals from other organs, in this case by signals originating in fat cells. In other cases, the target organ of hormone action is more directly abnormal, as in PTH resistance occurring with renal failure.

Increased end-organ function can be caused by mutations in signal reception and propagation. For example, activating mutations in TSH, LH, and PTH receptors can cause increased activity of thyroid cells, Leydig cells, and osteoblasts, even in the absence of ligand. Similarly, activating mutations in the $G_s\alpha$ protein can cause precocious puberty, hyperthyroidism, and acromegaly in McCune-Albright syndrome.

Tumors of Endocrine Glands

Tumors of endocrine glands often result in hormone overproduction. Some endocrine gland tumors produce little if any hormone but cause disease by local, compressive symptoms or by metastatic spread. Examples include so-called nonfunctioning pituitary tumors, which are usually benign but can cause a variety of symptoms due to compression of adjacent structures, and thyroid cancer, which can metastasize without causing hyperthyroidism.

Excessive Hormone Inactivation or Destruction

Although most enzymes important for endocrine systems activate a prohormone or precursor protein, there are also those whose function is to inactivate the hormone in a physiologically regulated fashion. An example is the type 3 iodothyronine deiodinase (D3), which inactivates T_3 and T_4 by removing an inner ring iodine atom from the iodothyronine, blocking its nuclear receptor binding. Large infantile hepatic hemangiomas express high D3 levels, causing "consumptive hypothyroidism," because thyroid hormone is inactivated at a more rapid rate than it can be produced.[16,17] Furthermore, D3 may also be induced in other tumors by tyrosine kinase inhibitors. In theory, accelerated destruction of other hormones could occur from similar processes as yet to be determined.

Diagnostic and Therapeutic Uses of Hormones

In general, hormones are used pharmacologically for their replacement or suppressive effects. Hormones may also be used for diagnostic stimulatory effects (e.g., hypothalamic hormones) to evoke target organ responses or to diagnose endocrine hyperfunction by suppressing hormone hypersecretion (e.g., T_3). Ablation of endocrine gland function due to genetic or acquired causes can be restored by hormone replacement therapy. Thyroid hormones and some steroids can be replaced orally, whereas peptide hormones and analogues (e.g., insulin, PTH, GH) are administered parenterally or absorbed through mucous membranes (inhaled insulin, intranasal desmopressin). Gastrointestinal absorption and first-pass kinetics determine oral hormone dosage and availability. Physiologic replacement can achieve both appropriate hormone levels (e.g., thyroid) and approximate hormone secretory patterns (e.g., GnRH delivered intermittently via a pump). Hormones can also be used to treat diseases associated with glandular hyperfunction. Long-acting depot preparations of somatostatin receptor ligands suppress GH hypersecretion in acromegaly and hypersecretion of diarrhea-causing mediators from neuroendocrine tumors of the pancreas and small intestine. Estrogen receptor antagonists (e.g., tamoxifen) are useful for some patients with breast cancer, and GnRH analogues may downregulate the gonadotropin axis and benefit patients with prostate cancer.

Novel formulations of receptor-specific hormone ligands are now being clinically developed (e.g., estrogen agonists/antagonists, somatostatin receptor subtype-specific ligands, or peroxisome proliferator-activated receptor alpha [PPARα] ligands), resulting in more selective therapeutic targeting. Modes of hormone injection (e.g., for PTH) may also determine therapeutic specificity and efficacy. Improved hormone delivery systems, including computerized minipumps, intranasal sprays (e.g., for desmopressin), pulmonary inhalers, depot intramuscular injections, and orally bioavailable peptide formulations, will also enhance patient compliance and improve ease of administration. Cell-based therapies using the reprogramming of human cells to perform differentiated functions, either through differentiation of induced pluripotent stem cells or directed differentiation of one somatic cell type into another, are under active investigation.[18] Novel technologies offer promise of marked prolongation in the half-life of peptide hormones, thereby requiring infrequent administration. For example, a once weekly preparation of glucagon-like peptide-1 (GLP-1) analogues is used in the treatment of type 2 diabetes.

Important progress has been made in the therapeutic use of hormones. Although delivery of insulin usually still relies on frequent administration by injection and close monitoring by the patient, purity of the insulin preparations, as well as novel delivery devices, has enhanced patient compliance and quality of life. Preparations with differing pharmacokinetics allow the normal physiology of insulin secretion to be more closely mimicked. Continuous administration via subcutaneous pump infusion enhances therapeutic effectiveness in carefully selected patients. These include closed-loop systems, in which the dose of insulin is automatically adjusted depending on continuously monitored interstitial glucose concentrations. Implementation of such systems has the potential to substantially reduce the burden of this disease. However, hormones are biologically powerful molecules that exert therapeutic benefit and effectively replace pathologic deficits. They should not be prescribed without clear-cut indications and should not be administered without careful evaluation by an appropriately qualified medical practitioner.

Future Perspectives

An introduction to the principles underlying endocrinology should end by emphasizing the rapidly changing dynamics of discovery in this field and attempting to foresee what remains to be discovered. New hormones are continually being discovered, from the recent focus on major regulators of metabolism and phosphate homeostasis (FGF19, FGF21, and FGF23) to the continued quest to identify ligands for orphan nuclear and G protein–coupled receptors.[19] Presumably other equally important hormones remain to be discovered. The observation that nuclear receptors, like most transcription factors, bind to thousands of specific sites within the cell's nucleus stresses how little we understand about hormone action. Even the name "nuclear receptors" may be viewed in the future as misleading, since there is an increasing appreciation of extranuclear, rapid actions of nuclear receptors. Many of our diagnostic tests are severely limited by both technology and our inability to foresee novel diagnostic targets. For example, the "disappearance" of isolated GH deficiency when many children with that diagnosis achieve adulthood means either that we have little understanding of the etiology/pathogenesis of that childhood deficiency or that our diagnostic tools today yield many false-positive results. Although endocrinologists pride themselves with having logical treatments for many diseases, these treatments seldom address their underlying causes. We have no satisfactory tools for preventing autoimmune endocrine deficiencies or for preventing the benign tumors that underlie many diseases characterized by hormone excess. Treatments for diseases such as type 1 diabetes, although highly effective, are still very obtrusive in the lives of patients with this disease.

This new edition communicates major advances that have been made in our field over the past 5 years, yet gaps in our knowledge about endocrinology remain. Importantly, debilitating chronic endocrine illnesses with significant morbidity (e.g., diabetes and Cushing disease) still pose significant diagnostic and therapeutic challenges.

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2

Principles of Hormone Action

EVAN D. ROSEN AND CHRISTIN CARTER-SU

CHAPTER OUTLINE

KEY POINTS

- Hormones signal to target cells via receptors on the cell surface or in the cell nucleus.
- Polypeptide hormones act at the cell surface and trigger a cascade of events in the cytoplasm as well as in the nucleus that alter the function of their target cells.
- In addition to polypeptide hormones, many nonpolypeptide hormones such as catecholamines signal via cell surface receptors.
- There are multiple classes of cell surface receptors, including ligand-gated ion channel receptors, G protein–coupled receptors, receptors with intrinsic enzymatic activity, and receptors that associate with enzymes.
- Some of the cell surface receptors have intrinsic catalytic activity, whereas others depend on interaction with other signaling proteins to exert their actions.
- Ligand binding to the extracellular domain of cell surface receptors causes conformational changes in the receptors that activate enzymatic activity and recruitment of cytoplasmic signaling proteins.
- Steroid and thyroid hormones signal via nuclear receptors.
- Some nuclear receptors transduce signals from vitamins, metabolites, and drugs acting as ligands to regulate reproduction, growth, and metabolism.
- Nuclear receptors work directly in the cell nucleus to regulate gene transcription, acting at the genome and recruiting coregulator proteins called corepressors and coactivators.
- Hormone binding to nuclear receptors causes a conformational change in the receptor that favors the recruitment of coactivators to the specific genes that are regulated.
- Some nuclear receptors may work through additional pathways that involve nongenomic mechanisms.

Introduction to Hormone Signaling

The evolution of multicellularity enabled specialization of organs and tissues. As organs took on distinct functions, mechanisms were required to allow communication between tissues; this is the fundamental purpose of hormones. Hormones encode information about environmental or developmental conditions in one location and transmit that information to a separate location. This process ultimately requires that information move from outside of the target cell to its interior, so that cellular function can be altered to meet the needs of the organism. Specifically, the concentration of the substance must be detected by the target cell and converted into a change in cellular activity, a process known as *signal transduction*. The strategies used by hormones to affect cellular function are analogous and, in many cases, identical to those used by other extracellular agents such as neurotransmitters, drugs, and metabolites. However, classic endocrinology defines itself as the process by which extracellular signaling molecules use the bloodstream to travel from the organ of origin to the target tissue. By its nature, this process invariably results in dilution of the secreted molecule in the intravascular space, and thus, with rare exception, the target cell must be capable of detecting and responding to very low concentrations of hormone.

In spite of the vanishingly small concentrations of hormones present in the circulation, classic endocrine organs are usually uniquely equipped to secrete substantial amounts of hormone. Much of the history of endocrinology is defined by purification of hormones from these specialized secretory tissues. In the earliest days, the discovery of a hormone usually followed a stereotypical

course of events: (1) A syndrome, often resembling some human disease, was associated with removal of an endocrine gland; (2) the abnormal phenotype would be corrected by the reimplantation of the absent organ; (3) the same cure would be accomplished by administration of an extract from the organ of interest; and (4) the active agent would be purified from the organ. The discovery of insulin represents the prototype for this series of observations, but the same process led to the identification of other hormones such as thyroid hormone and cortisol.

Hormones can be divided into two groups on the basis of where they function in a target cell. The first group includes hormones that interact with receptors at the cell surface. All polypeptide hormones (e.g., growth hormone [GH], insulin), monoamines (e.g., serotonin), and prostaglandins (e.g., prostaglandin E_2) use cell surface receptors. The second group includes hormones that can enter cells. These hormones bind to intracellular receptors that function in the nucleus of the target cell to regulate gene expression. Classic hormones that use intracellular receptors include thyroid and steroid hormones.

It is worth noting that many molecules behave both as classical hormones that use the bloodstream to travel from their site of production to their site of action and as signaling molecules that do not meet that strict definition. For example, insulin-like growth factor 1 (IGF1) is produced and secreted by the liver under the positive influence of GH and circulates to target tissues like bone, but it is also produced locally by some tissues (e.g., chondrocytes at bone growth plates) to exert effects on neighboring cells. Similarly, norepinephrine is a neurotransmitter that is released at nerve endings and binds to cell surface receptors at postsynaptic membranes, but it is also secreted into the blood by the adrenal medulla, allowing it to act as a classic endocrine hormone. Testosterone is a nuclear receptor ligand that is produced by the Leydig cells of the testis; it can circulate as a hormone and act on muscle, bone, and other tissues, but it also acts as a paracrine agent on neighboring seminiferous tubules. Finally, many secreted molecules that are not regarded as classic hormones meet virtually all of the criteria used to define such agents. For example, cytokines are released by immune cells at the site of inflammation, but they also circulate in plasma and bind to cell surface receptors in the brain, evoking fever. In this sense, many circulating molecules, including those produced exogenously (i.e., obtained from the diet or synthesized by commensal bacteria), could be regarded as having hormonal properties. The key point is that a complete understanding of cell surface and nuclear receptor biology requires a more inclusive perspective than is typically achieved by adhering to a strict set of definitional criteria established decades ago. Having said that, in the interest of brevity, this chapter focuses primarily on receptors that bind classic hormonal ligands, with examples drawn from other systems as needed to provide a more comprehensive picture that reflects our current understanding of receptor biology.

Ligands That Act Through Cell Surface Receptors

The impermeability of the plasma membrane to peptides and small, water-soluble, charged molecules requires that receptors that recognize such substances be located on the outer surface of the cell. The limiting membrane of a typical eukaryotic cell is a 5-nm to 8-nm structure composed of proteins embedded in a bilayer of phospholipids and cholesterol, forming the fluid-mosaic membrane. The phospholipid polar head groups face outward from the membrane, interacting with the hydrophilic milieu that comprises the extracellular fluid and the cytoplasm. Buried between these two charged surfaces are the hydrophobic lipid tails of the phospholipids made up of acyl groups, which are long chains of hydrocarbons derived from fatty acids. The strongly nonpolar environment prevents the diffusion of water-soluble molecules, including many hormones, across the membrane. Thus surface proteins are needed to detect the presence of extracellular ligands that cannot diffuse and are not transported into the cell. Information from this hormone-binding process must then be transmitted across the plasma membrane so that intracellular signaling can commence.

TABLE 2.1 Hormones That Work on the Cell Surface

Peptides and Proteins

Adrenocorticotropic hormone (ACTH)
Antidiuretic hormone (ADH)
Atrial natriuretic peptide (ANP)
Calcitonin
Cholecystokinin
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Gastrin
Glucagon
Gonadotropin-releasing hormone (GnRH)
Growth hormone (GH)
Growth hormone–releasing hormone (GHRH)
Insulin
Insulin-like growth factor 1 (IGF1)
Luteinizing hormone (LH)
Oxytocin
Parathyroid hormone (PTH)
Prolactin (PRL)
Secretin
Somatostatin (SS)
Thyrotropin-releasing hormone (TRH)
Thyrotropin or thyroid-stimulating hormone (TSH)

Molecules Derived From Amino Acids

Dopamine (inhibits prolactin)
Epinephrine (also called adrenaline)
Norepinephrine (also called noradrenaline)
Serotonin

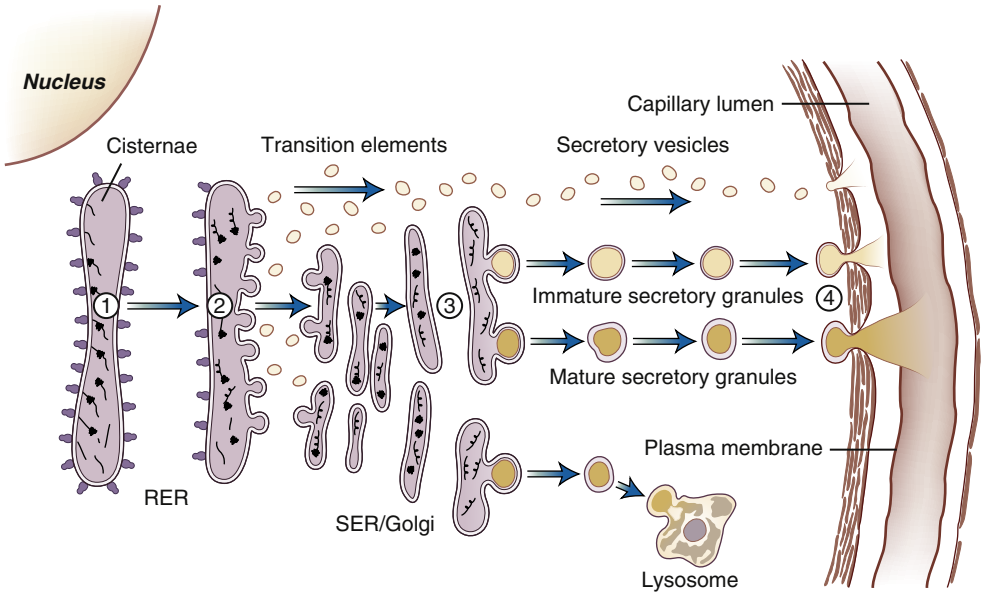
Eicosanoids

Prostaglandins: PGA_1, PGA_2, PGE_2

Classic Peptide Hormones

Most notable among the hormones that bind to cell surface receptors are the peptide hormones, which vary in size from a handful to hundreds of amino acids. Examples of peptide hormones include the glycoproteins and the GH family of proteins secreted by the pituitary, the pancreatic hormones glucagon and insulin, and numerous peptides secreted from nonglandular organs, such as leptin from adipocytes and atrial natriuretic peptide from the heart (Table 2.1).

A hormone's rate of secretion is closely tailored to its lifetime in the circulation and to its time course of action. In general, peptide hormones are released from endocrine glands quickly, as they are preformed and stored in secretory vesicles or granules. In the



• **Fig. 2.1** Subcellular organelles involved in transport and secretion of polypeptide hormones or other secreted proteins within a protein-secreting cell. (1) Synthesis of proteins on polyribosomes attached to rough endoplasmic reticulum (RER) and vectorial discharge of proteins through the membrane into the cisterna. (2) Formation of shuttling vesicles (transition elements) from endoplasmic reticulum followed by their transport to and incorporation by the Golgi complex. (3) Formation of secretory granules in the Golgi complex. (4) Transport of secretory granules to the plasma membrane, fusion with the plasma membrane, and exocytosis resulting in the release of granule contents into the extracellular space. Notice that secretion may occur by transport of secretory vesicles and immature granules or by transport of mature granules. Some granules are taken up and hydrolyzed by lysosomes (crinophagy). *Golgi,* Golgi complex; *SER,* smooth endoplasmic reticulum. (From Habener JF. Hormone biosynthesis and secretion. In: Felig P, Baxter JD, Broadus AE, et al, eds. *Endocrinology and Metabolism*. New York: McGraw-Hill; 1981:29–59.)

course of synthesis, peptide hormones are diverted to secretory vesicles via a regulated secretory pathway (Fig. 2.1). The cytoplasm of endocrine glands containing such secretory vesicles, such as the endocrine pancreas, the anterior pituitary, and the parathyroid glands, is filled with 200-nm electron-dense granules that represent the packaged hormone awaiting secretion. Just as secretion of hormones stored within vesicles can be evoked quickly, often within milliseconds, release can usually be terminated abruptly with great efficiency. Peptide hormones tend to have very short half-lives within the circulation, which allows blood levels to change rapidly in response to changes in secretion. Like the rapid changes in secretion and blood concentrations, initiation of signaling tends to be rapid, which is facilitated by high *on rates* for hormone binding to receptors. In contrast, the *off rate* is often slow, which results in a high equilibrium binding constant that enables the receptors to detect the relatively low levels of hormone in blood. However, a slow off rate is not compatible with the relatively rapid transient nature of peptide hormone–initiated signaling, suggesting mechanisms must exist for turning off the hormonal signal other than simple diffusion of the hormone off of the receptor.

A notable exception to the general rule that peptide hormones turn over quickly and have short durations of action is provided by IGF1. Unlike most peptide hormones, IGF1 circulates in the bloodstream bound to one or more binding proteins, which has two important consequences. First, the concentration of total IGF1 in blood is much greater than that of the unbound, biologically active hormone. Second, the lifetime of IGF1 is greatly extended, such that circulating levels of the hormone change slowly over the course of hours or days. As predicted by these properties, IGF1 primarily influences phenotypes that are modified over extended periods, such as growth and differentiation, and in marked contrast to its cousin insulin, most of the cellular targets of IGF1 are transcriptional.

Nonpeptide Hormones That Act at Cell Surface Receptors

In addition to peptide hormones, there are small, hydrophilic hormones related to monoamine neurotransmitters that bind cell surface receptors. These include adrenergic agents such as norepinephrine as well as other amino acid–derived water-soluble molecules such as melatonin, serotonin, and histamine. Like peptide hormones, these hormones can be stored in dense secretory vesicles, but they are more typically packaged into small, approximately 50-nm electron-lucent vesicles that are similar morphologically to vesicles in neural and neuroendocrine cells. The major difference is that in the presynaptic cleft, the vesicles are arranged in a tightly packed array at the membrane.

Interestingly, while most lipophilic molecules have intracellular receptors, there is at least one class of lipid that breaks this rule. The eicosanoids are a group of extracellular signaling molecules derived from 20-carbon fatty acids that includes the leukotrienes and prostaglandins. Many biologically active eicosanoids bind to cell surface receptors, which initiate their functions.[1]

The recent expansion of messenger types and the novel modes of interorgan communication have dramatically changed the traditional view of endocrinology such that all cell types can potentially both send and receive messages. One of the more interesting recent additions to the assortment of hormone-like molecules has

TABLE 2.2 Receptors for Metabolites and Ions

Metabolite	Receptor
Lactate	GPR81
Ketone bodies	GPR109A
3-Hydroxyoctanoate	GPR109B
Succinate	GPR91
α-Ketoglutarate	GPR80/99
Long-chain fatty acids	GPR40, GPR120
Medium-chain fatty acids	GPR84
Short-chain fatty acids	GPR41, GPR43
Calcium	CASR

been circulating metabolites such as lactate, ketone bodies, and succinate, as well as ions such as calcium[2] (Table 2.2). An even more distant modification of the original definition of *hormone* is the idea that metabolites produced by microbes in the gut, such as short-chain fatty acids, can signal by binding to cell surface receptors.[3]

Binding Properties of Cell Surface Receptors

When a hormone or hormone-like molecule arrives at a target cell, at least three critical components are required to induce the appropriate biologic response. First, there has to be recognition of the hormone as different from all other components of the extracellular milieu. This is an issue of specificity (the ability to distinguish the hormone from other structurally related molecules). Second, the receptor must be able to recognize the low concentration of hormone found in the blood, which is an issue of sufficient affinity of the receptor for the hormone. Third, the initial recognition step mediated by the receptor must be converted into a single action or a defined set of cellular events. Studies of the binding properties of hormones and neurotransmitters crystallized into a fundamental rule governing the action of extracellular agents: A biologic effect is directly proportional to the ligand occupancy of the receptor. A subtle but important modification to occupancy theory is the notion of *spare receptors,* which describes the situation in which a maximal biologic response is achieved by occupancy of only a portion of the available receptors. One consequence of the existence of spare receptors is that a decrease in the number of cellular receptors results in a change in the ED_{50} (effective dose for eliciting a 50% response) for a hormone but does not necessarily alter the maximal biologic response, as detailed later for insulin.[4,5]

As noted, a fundamental characteristic of a cell surface receptor is the ability to bind hormone with high specificity and high affinity. In addition, because there is a limited number of receptors, binding is saturable, such that adding additional ligand above a certain level results in no additional binding and no further increment in downstream biologic activity. Specificity, affinity, and saturability can be established experimentally by assessing the binding of ligands to receptors, using radioactive ligands in a variety of in vitro binding assays.[6] Authentic physiologic receptors for a given hormone will display a greater affinity for the cognate hormone than other potentially competing circulating molecules. In addition, the half maximal binding for a hormone to its real receptor will always be in the range of the circulating free concentration of that hormone.

Cell Surface Hormone Receptors

Cell surface receptors can be grouped conveniently into four classes: ligand-gated ion channel receptors, G protein–coupled receptors, receptors with intrinsic enzymatic activity, and receptors that associate with enzymes.

Ligand-Gated Ion Channels

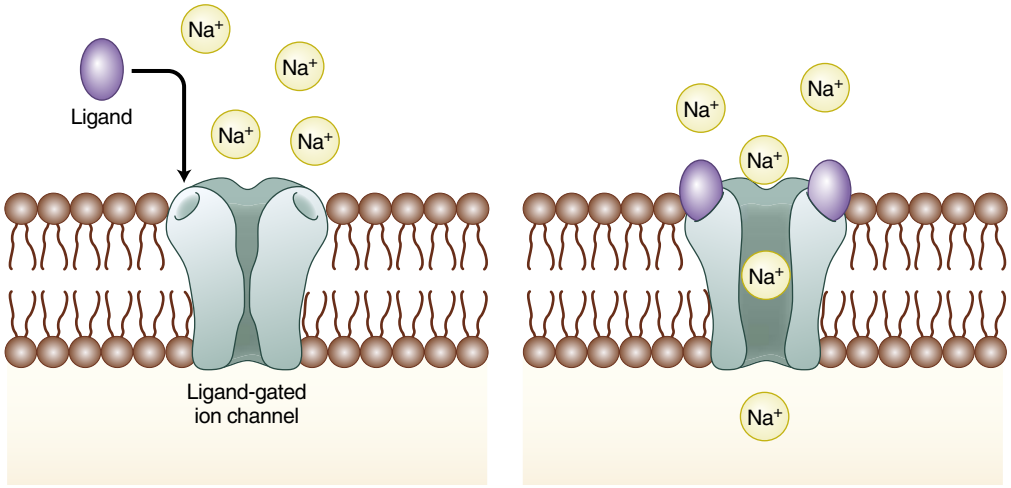
The simplest form of a cell surface signaling system is one in which both hormone-binding and signal-generating functions are provided by a single protein or complex of proteins. Ligand-gated ion channels fall into this category. They are made up of two key components: a ligand-binding domain accessible from the surface of the cell and a transmembrane domain containing a channel. Binding of ligand to the exofacial surface of the receptor generates a conformational change that results in the opening of a pore, allowing specific ions to travel through the channel across the plasma membrane (Fig. 2.2).

The prototype and founding member of the family of ligand-gated ion channels is the nicotinic acetylcholine receptor, which is present on some neurons and on the postsynaptic membrane of the neuromuscular junction.[7] When a nerve impulse arrives at the presynaptic terminal, depolarization leads to an increase in cytosolic calcium and secretion of acetylcholine. The secreted acetylcholine binds to its receptor on the muscle, which elicits a conformational change that opens the pore and allows sodium and potassium ions to pass in and out of the cell, respectively. This leads to depolarization and muscle contraction. The structure of the acetylcholine receptor, which is made up of four different peptides that constitute five subunits, defines a family of receptors that also includes the 5-hydroxytryptamine type 3 ($5HT_3R$), glycine, and inhibitory GABA type A receptors. Another shared characteristic of pentameric receptors is a conserved 15-amino acid dicysteine loop in the extracellular ligand-binding domain (LBD), giving this family its alternative name, the *cys-loop receptors*.[8]

Most ligand-gated ion channels, which when activated can elicit microsecond changes in signal transduction, serve as neurotransmitter receptors rather than receptors for classic hormones. A notable exception involves the receptors for some hypothalamic releasing factors, which are discharged from hypothalamic neurons into the portal circulation to regulate the secretion of hormones from the anterior pituitary. For example, it is thought that serotonin regulates release of prolactin by binding to the $5HT_3R$ receptor in lactotrophs of the anterior pituitary.[9] Glycine and GABA receptors are present in the pituitary gland, but their physiologic functions appear complex and remain imperfectly understood. Another class of ligand-gated ion channels, the purinergic cation receptors, are also expressed in the pituitary and most likely function in an autocrine/paracrine fashion in response to extracellular adenosine triphosphate (ATP).

G Protein–Coupled Receptors

The largest family of cell surface receptors is defined by their use of heterotrimeric G proteins for signaling, leading to their designation *G protein–coupled receptors* (GPCRs). These receptors



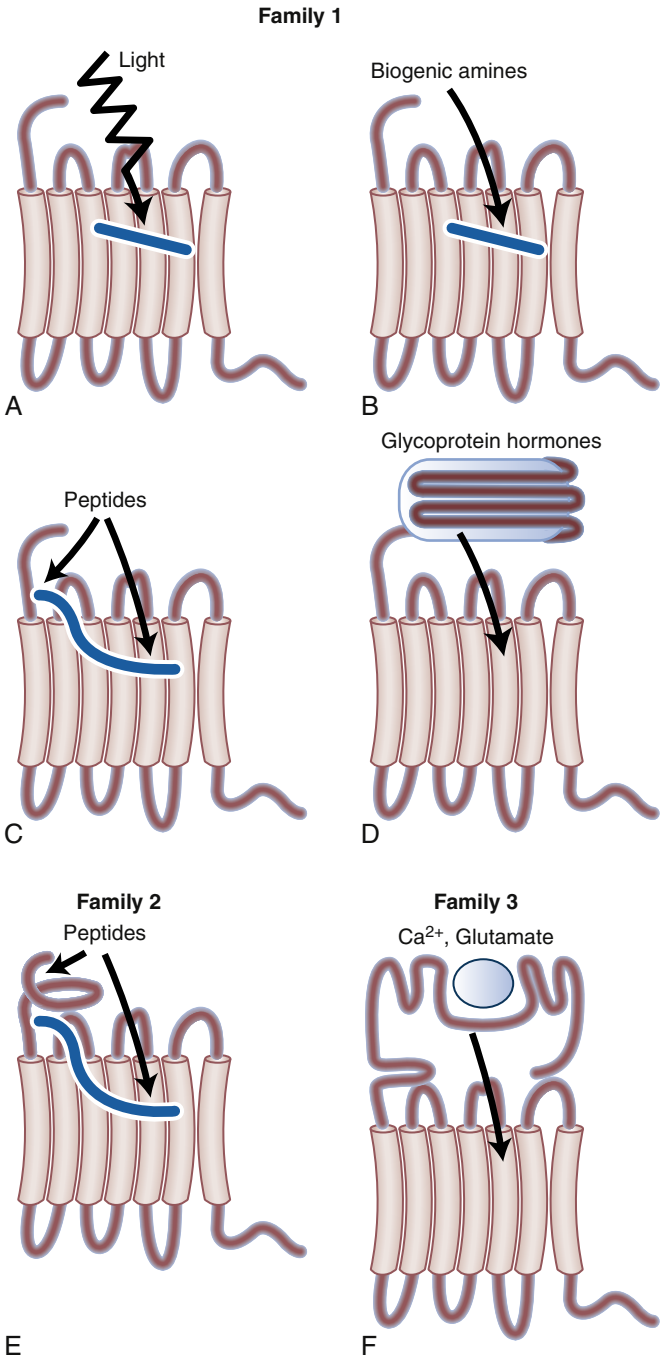
• **Fig. 2.2** Ligand-gated ion channels are transmembrane proteins that comprise at least two domains, a ligand-binding domain and a membrane-spanning domain capable of functioning as a pore. When a ligand binds, it induces a conformational change in the receptor such that the pore opens to the passage of ions, in this case sodium ions, down their electrochemical gradient.

have seven 25–amino acid α-helical segments that pass through the plasma membrane, with the amino (N)-terminus and carboxy (C)-terminus outside the cell and in the cytoplasm, respectively, leading to the name *seven transmembrane* (7TM) proteins.[10] There are more than 800 GPCR family members, with the vast majority being olfactory receptors. The diversity of ligands capable of binding to GPCRs is remarkable, ranging from a single photon to large proteins and including ions, odorants, amines, peptides, lipids, nucleotides, and metabolic intermediates. The smaller hormones, including catecholamines, bind to their GPCRs within the transmembrane-spanning region, oriented parallel to the cell surface; larger hormones bind to the extracellular N-terminus, which itself can range in size from 10 to 600 amino acids,[11] in addition to interacting with the transmembrane-spanning region (Fig. 2.3). The GPCR family has been divided into five subfamilies based on primary sequence and phylogeny, named the glutamate, rhodopsin, adhesion, frizzled/taste2, and secretin families.[12] Many hormones, including some hypothalamic releasing factors, the glycoprotein hormones secreted by the pituitary, and the amines, bind to members of the rhodopsin-like family. On the other hand, glucagon, parathyroid hormone (PTH), calcitonin, and some hypothalamic hormones, such as GH-releasing factor and corticotropin-releasing factor, bind to members of the secretin-like family. For many GPCRs, the endogenous ligand and its function are not known; these GPCRs are known as *orphan receptors*.

Signaling by Heterotrimeric G Proteins

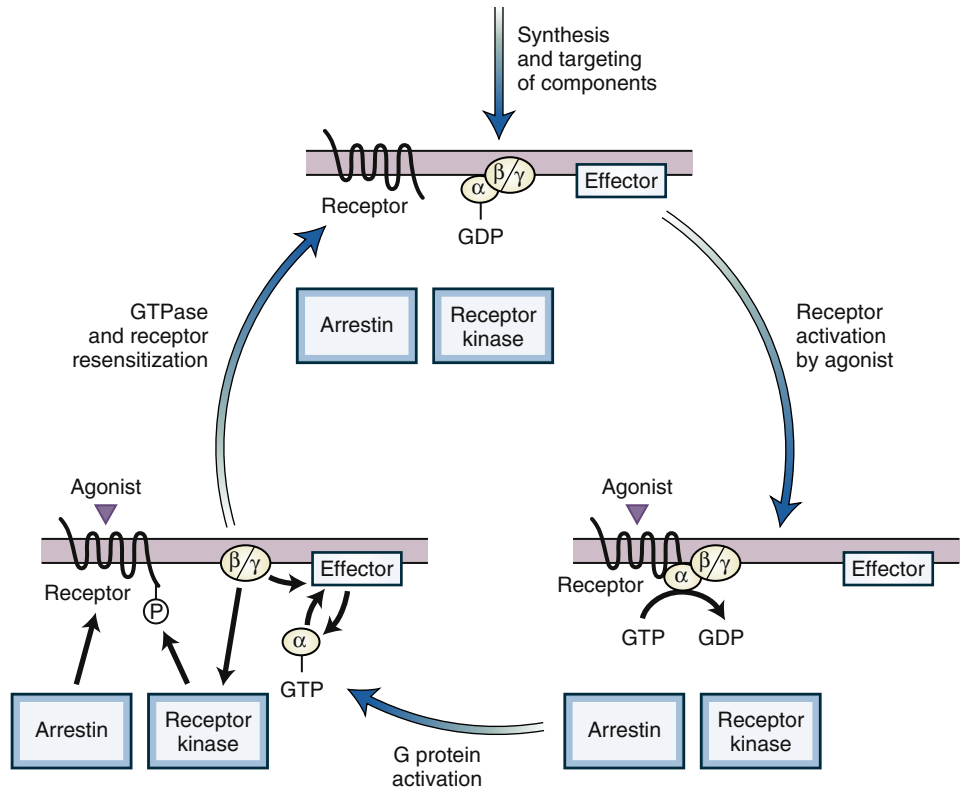
An important advance in the understanding of GPCRs occurred when Bourne and associates took advantage of the lethality of cyclic adenosine monophosphate (cAMP) toward lymphoma cells to select mutant lines resistant to the actions of the β-adrenergic agent isoproterenol.[13] Because the mutant cell lines lost responsiveness to a number of nonadrenergic agonists, it was clear that the genetic lesion did not reside in the β-adrenergic receptor but in a downstream component. When the signaling module that restored hormone responsiveness to the deficient membranes was purified, it turned out to be a heterotrimeric G protein complex, now known as G_s.[14] G_s binds a single guanosine triphosphate (GTP) to its α-subunit, which causes the α-subunit to dissociate from its other two (β, γ) subunits. The GTP-bound α-subunit of G_s is necessary and sufficient for activation of its downstream target, adenylyl cyclase. Like G_s, all G protein complexes are composed of one member each of the α-subunit, β-subunit, and γ-subunit families. Which exact subunit family member determines the downstream effector(s). Sixteen distinct genes encode about 20 different G protein α-subunits, which can be divided into four groups based on both structure and function: $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, and $G\alpha_{12}$.[10] The $G\alpha_s$ family has only two members, $G\alpha_s$ and the G protein for the olfactory receptor, $G\alpha_{olf}$; both couple to activation of adenylyl cyclase. The $G\alpha_i$ group of eight includes three $G\alpha_i$ proteins, all of which inhibit adenylyl cyclase; two $G\alpha_0$ proteins that are abundant brain proteins whose multiple targets are still not completely defined; two $G\alpha_t$ proteins that couple photoreceptors to cAMP phosphodiesterase (PDE); and $G\alpha_z$, which inhibits potassium channels. The $G\alpha_{q/11}$ subfamily consists of six members, all of which activate the enzyme phospholipase C beta (PLCβ), generating the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3). $G\alpha_{12}$ and $G\alpha_{13}$, which inhibit and activate the guanine nucleotide exchange factor, RhoGEF, respectively, form the final group. The combinational possibilities are complex, with 5 β-subunit isoforms and over 12 γ-subunit isoforms.

The key operational feature of G protein signaling is that the system behaves like a timed switch. Engagement of hormone with its cognate receptor promotes its association with a heterotrimeric G protein comprised of subunits Gα, Gβ, and Gγ (Fig. 2.4). This stimulates dissociation of guanosine diphosphate (GDP) from the α-subunit, allowing GTP to bind to the unoccupied site as a result of its greater intracellular concentration compared to GDP. The occupied receptor then detaches from the G protein. GTP loading of the G protein also induces the trimeric G protein complex to dissociate into the α-subunit and a dimeric β/γ-subunit, at least in vitro; it is not clear that dissociation actually occurs in an intact cell. In most cases, the α-subunit modulates an associated amplifier, which in the case of G_s is adenylyl cyclase, but other targets of α-subunits include those referred to previously. The β/γ-dimer can also interact with and regulate downstream signaling molecules. For example, the β/γ-dimer activates potassium channels following ligand binding to the muscarinic acetylcholine receptor.



• **Fig. 2.3** The G protein–coupled receptor (GPCR) superfamily: diversity in ligand binding and structure. Each panel depicts members of the GPCR superfamily. The seven-membrane-spanning α-helices are shown as cylinders, with the extracellular amino (N)-terminus and three extracellular loops above them and the intracellular carboxy (C)-terminus and three intracellular loops below. The superfamily can be divided into three subfamilies on the basis of amino acid sequence conservation within the transmembrane helices. Family 1 includes the opsins (A), in which light *(arrow)* causes isomerization of retinal covalently bound within the pocket created by the transmembrane helices *(bar)*; monoamine receptors (B), in which agonists *(arrow)* bind noncovalently within the pocket created by the transmembrane helices *(bar)*; receptors for peptides such as vasopressin (C), in which agonist binding *(arrow)* may involve parts of the extracellular N-terminus and loops and the transmembrane helices *(bar)*; and glycoprotein hormone receptors (D), in which agonists *(oval)* bind to the large extracellular N-terminus, activating the receptor through undefined interactions with the extracellular loops or transmembrane helices *(arrow)*. (E) Family 2 includes receptors for peptide hormones such as parathyroid hormone and secretin. Agonists *(arrow)* may bind to residues in the extracellular N-terminus and loops and to transmembrane helices *(bar)*. (F) Family 3 includes the extracellular Ca^{2+}-sensing receptor and metabotropic glutamate receptors. Agonists *(circle)* bind in a cleft of the Venus flytrap–like domain in the large extracellular N-terminus, activating the receptor through undefined interactions with the extracellular loops or transmembrane helices *(arrow)*.

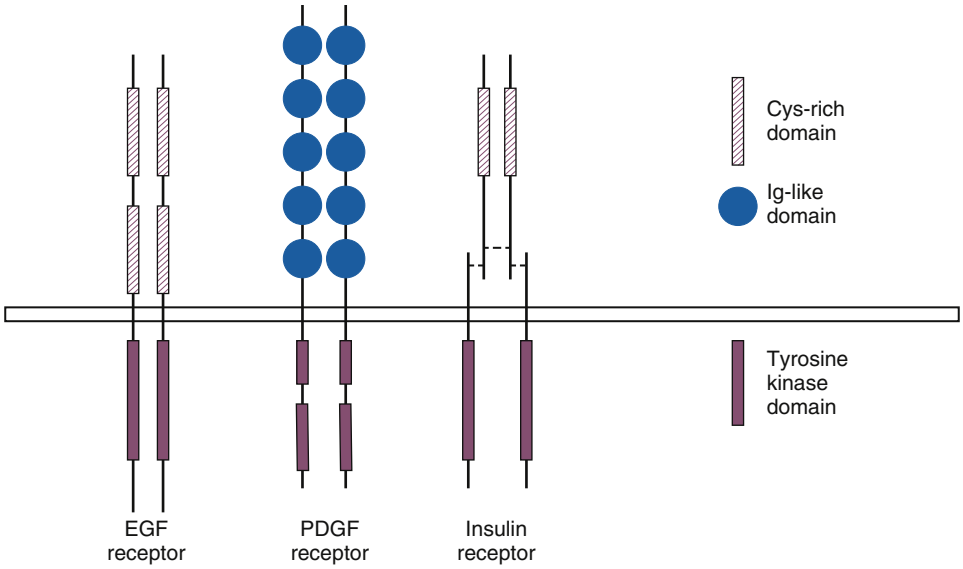
Critical to signal transduction by G proteins is that they remain active as long as GTP is bound. The rate of conversion of nucleotide GTP to GDP determines both the timing for inactivation of signaling and reassembly of subunits. Thus the G protein can exist in two distinct states: bound to GTP and active or bound to GDP and inactive; the time spent in each condition defines the strength of signaling. G protein α-subunits have low levels of intrinsic GTPase activity, but this can be enhanced by association with the regulators of G protein signaling (RGS) proteins.[15] Thus RGS proteins, which function as GTPase accelerating proteins



• **Fig. 2.4** The G protein guanosine triphosphatase (GTPase) and G protein–coupled receptor (GPCR) desensitization-resensitization cycle. In each panel, the *shaded area* denotes the plasma membrane, with the extracellular region above and the intracellular region below. In the basal state, the G protein is a heterotrimer with guanosine diphosphate (GDP) tightly bound to the α-subunit. The agonist-activated GPCR catalyzes release of GDP, which permits guanosine triphosphate (GTP) to bind. The GTP-bound α-subunit dissociates from the βγ-dimer. *Arrows* from the α-subunit to the effector and from the βγ-dimer to the effector indicate regulation of effector activity by the respective subunits. The *arrow* from effector to the α-subunit indicates regulation of its GTPase activity by effector interaction. Under physiologic conditions, effector regulation by G protein subunits is transient and is terminated by the GTPase activity of the α-subunit. The latter converts bound GTP to GDP, thereby returning the α-subunit to its inactivated state with high affinity for the βγ dimer, which reassociates to form the heterotrimer in the basal state. In the basal state, the receptor kinase and arrestin are shown as cytosolic proteins. Dissociation of the GTP-bound α-subunit from the βγ-dimer permits the dimer to facilitate binding of receptor kinase to the plasma membrane (*arrow* from βγ-dimer to receptor kinase). Plasma membrane binding permits the receptor kinase to phosphorylate the agonist-bound GPCR (*P,* depicted here as occurring on the carboxy-terminal tail of the GPCR, although sites on intracellular loops are also possible). GPCR phosphorylation facilitates arrestin binding to the GPCR, resulting in desensitization. Endocytic trafficking of arrestin-bound GPCR and recycling to the plasma membrane during resensitization are not shown.

(GAPs), serve to shorten the duration of signaling by G proteins, providing another important site of regulation. Many members of the large family of RGS proteins contain within their primary sequences canonical domains indicative of other functions and undergo complex post-translational modification. Modulation of the levels of RGS proteins affords a mechanism for signaling pathways to communicate with each other. For example, both thyroid-stimulating hormone (TSH, thyrotropin) and PTH signal through a G_s-cAMP pathway to increase expression of RGS2, which feeds back to inhibit G_s and to antagonize other pathways that depend on G_q.

Another GPCR regulatory system involves a family of proteins called arrestins (see Fig. 2.4). Two of the four arrestins (1 and 4) have been designated visual arrestins because they are expressed only in photoreceptor cells, while two arrestins (2 and 3) are expressed ubiquitously; the latter two are also called β-arrestins 1 and 2. Ligand binding to a GPCR not only signals the dissociation of the G protein complex as described earlier, but also promotes a conformational change in the GPCR that often leads to phosphorylation of the receptor by a G protein receptor kinase (GRK).[16] GRKs are represented by a family of seven related kinases. Phosphorylation of the GPCR at serine and threonine residues by GRKs allows the binding of an arrestin, which sterically uncouples the GPCR from the G protein, terminating the signal. Binding to the receptor also alters the conformation of the arrestin such that it interacts with components of the endocytosis system such as clathrin.[17] The GPCR is escorted to the sorting endosome where it either recycles back to the cell surface or is targeted to the lysosome for degradation. This system provides an efficient mechanism for homologous desensitization, in which there is receptor-specific downregulation of signaling pathways. This mechanism stands in contrast to negative regulation by second messenger–dependent protein kinases, which phosphorylate and inhibit all susceptible GPCRs regardless if occupied by ligand. In addition to its role in the modulation of G protein signaling, β-arrestin has a well-defined function as a signaling intermediate.



• **Fig. 2.5** Receptor tyrosine kinases. Three of the 16 families of receptor tyrosine kinases are represented. All receptor tyrosine kinases possess an extracellular domain containing the ligand-binding site, a single transmembrane domain, and an intracellular portion containing the tyrosine kinase domain. Several structural motifs (i.e., cysteine-rich domain, immunoglobulin-like domain, tyrosine kinase domain) in these receptor tyrosine kinases are indicated on the right side of the figure. *Dotted lines* indicate disulfide bonds. *Cys,* cysteine; *EGF,* epidermal growth factor; *Ig,* immunoglobulin; *PDGF,* platelet-derived growth factor.

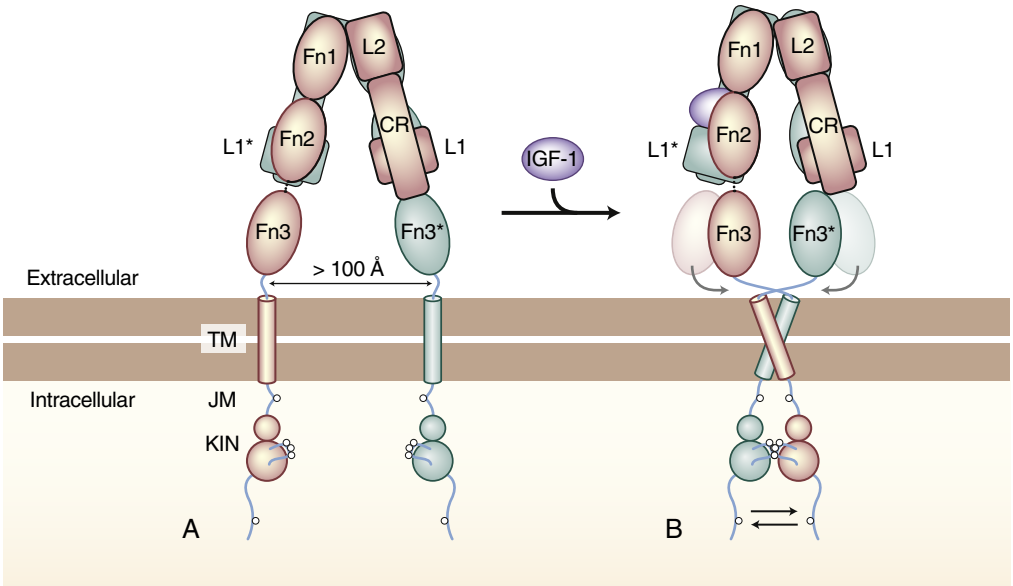
β-arrestin is now known to bind multiple members of the Src family of tyrosine kinases as well as other proteins, such as mitogen-activated protein kinases (MAPKs, also known as extracellular regulated kinases [ERKs]), phosphoinositide 3-kinase (PI3K), Akt, PDE4, and c-Jun N-terminal kinase-3.[18,19]

One of the most interesting aspects of GPCR signaling is the ability of GPCRs to undergo functional selectivity (also known as biased signaling), defined as the ability of ligands to stimulate distinct downstream signaling pathways, presumably due to stabilization of distinct conformational states of the receptor.[20] For GPCR receptors that can activate multiple G proteins, biased signaling refers to the ability to preferentially activate pathways downstream of a subset of the G proteins. For GPCR receptors that can activate arrestins, biased signaling usually refers to the ability of the receptor to favor the G protein response and minimize the arrestin response. Most of the research activity around biased signaling has taken place in the pharmaceutical industry, where the principle has been used in attempts to develop more specific therapeutics. For example, attempts have been made to develop opioid agonists that activate the analgesic effects mediated by G protein signaling but are devoid of arrestin-dependent desensitization and tolerance.[21,22] A similar strategy is being attempted to dissociate G protein–mediated opioid analgesia from arrestin-mediated constipation and respiratory depression.[21,22] Which downstream pathways are initiated by activation of a given GPCR is affected by the type and concentration of the ligand itself, and also the recruitment of specific GRKs, the subcellular location of the GPCR, and the time after ligand exposure.[23]

Receptor Tyrosine Protein Kinases as Cell Surface Receptors

The receptors that make up the receptor tyrosine protein kinase (RTK) family use a number of strategies to accomplish the same goal: to convert the binding of ligand to the exofacial portion of the receptor to a change in the activity of a tyrosine protein kinase domain residing in the interior of the cell. All of these receptors are type I transmembrane proteins with an N-terminal hormone-binding domain on the outside, a 25–amino acid hydrophobic segment that spans the membrane (the transmembrane domain), and a carboxy portion of the protein containing a kinase domain extending into the cytoplasm[24] (Fig. 2.5). The intracellular catalytic domain transfers phosphate from ATP to tyrosine residues in proteins, including the receptor itself. The 58 RTKs expressed in humans can be divided into about 20 subfamilies based on structural features. One of these groups is exemplified by the insulin receptor, which, unlike other RTKs, exists as a disulfide-linked tetramer in the basal state. Receptors in all other subgroups of RTKs, including receptors for fibroblast growth factor, platelet-derived growth factors (PDGFs), and epidermal growth factor (EGF), exist as monomers, though there is evidence that many associate noncovalently into larger structures in the basal state.

Biochemical experiments involving affinity cross-linking and biosynthetic labeling identified the structure of the insulin receptor and that of the highly related IGF1 receptor as a heterotetramer, composed of two 125-kDa α-subunits and two 90-kDa β-subunits linked by disulfide bonds[25,26] (Figs. 2.5 and 2.6). The receptor is synthesized as a single peptide with a cleavable signal sequence directing insertion cotranslationally into the membrane, and it is glycosylated and cleaved into the α and β chains in the Golgi complex.[27] Even though they exist as two separate peptides in the mature protein, each pair of α and β chains behaves much like a receptor monomer found in other growth factor receptors. Affinity labeling by insulin shows cross-linking to both the α-subunit and β-subunit, indicating that both are accessible to substances at the surface of the cell. Insulin binding has been long recognized to exhibit *negative cooperativity,* which means that the affinity for additional hormone decreases as the population of receptors binds more ligands.[28] In structural terms, this is explained by the presence of four binding sites on



• **Fig. 2.6** How insulin-like growth factor 1 (IGF1) activates its receptor. Each IGF1 receptor is made up of two half-receptors, which are linked by disulfide bonds (not shown). The six domains in the extracellular region of the first half-receptor (orange) are L1, CR, L2, Fn1, Fn2, and Fn3; the domains in the second half-receptor (green) are the same and labeled with an asterisk. The L1, CR, L2, and Fn1 are in the α-chain, and the Fn3 and the transmembrane and intracellular domains make up the β-chain of each half-receptor; the Fn2 domain is made up of contributions from both chains. The intracellular region comprises the juxtamembrane region (JM) and the tyrosine kinase domain (KIN). Sites of transphosphorylation are shown as circles. (A) When IGF-1 is not bound to the receptor, an interaction between L1* of the second half-receptor and Fn2 and Fn3 of the first half-receptor (and vice versa) is thought to maintain a large separation between the transmembrane (TM) helices *(double arrow)*. (B) When IGF1 binds to L1* (or to L1), it disrupts the L1*-Fn2 (or L1-Fn2*) interaction. This allows Fn2 and Fn3 of each half-receptor to pivot *(curved arrows)* toward each other (the previous positions of Fn2 and Fn3 are shown semitransparently). This in turn facilitates the dimerization of the TM helices in the membrane, which juxtaposes the kinase domains for efficient transphosphorylation *(black arrows)*. Binding of a single IGF1 molecule (shown as binding to the left side) is sufficient to activate the receptor, but exactly how this asymmetry affects the conformational changes in the receptor is unclear. It is believed that the same mechanism also applies to activation of the insulin receptor. (Modified from Hubbard SR, Miller WT. Closing in on a mechanism for activation. *eLife.* 2014;3:e04909.)

each holoreceptor—two of low affinity and two of high affinity. Insulin initially binds to a low-affinity site before binding to a high-affinity site on the contralateral α/β-dimer, thus effectively cross-linking the two halves of the receptor such that the stoichiometry of this high-affinity complex is one insulin molecule per insulin receptor. This stable structure prevents binding of hormone to the second high-affinity site. This structural organization for binding is largely conserved in the association of IGF1 with its receptor.[29] Other classes of RTKs use alternative strategies for ligand binding. For example, activation of the EGF receptor appears to require binding of one EGF molecule to the outer surface of one of two noncovalently associated EGF receptors,[30,31] while PDGF binds as a dimer to two noncovalently associated PDGF receptors.[32]

In general, activation of RTKs requires the formation of a receptor dimer. In some cases, such as the insulin, IGF1, FGF, and EGF receptors, the unbound receptors appear to consist of preformed dimers. In other cases, bivalent ligand binding to two receptor monomers is thought to promote dimer formation. Examples of receptors thought to act this way include the receptors for PDGF, vascular endothelial growth factor, and nerve growth factor.

Because some of the RTK receptors exist as a dimer in the basal state, it is clear that dimerization alone is not sufficient to activate RTKs; there must also be some fundamental change in the interaction between the two halves of the receptor. In the case of the insulin and IGF1 receptors, the extracellular portions of the unbound receptor exist in an inverted V conformation formed by the α-subunits and part of the β-subunits.[33] The base is continuous with and anchored by the transmembrane domains of the β-subunits. Insulin or IGF1 binding to its low-affinity site removes a brake on a molecular hinge, allowing the V to close and bring the transmembrane domains closer to each other[34,35] (see Fig. 2.6). This conformational change is transmitted to the cytoplasmic domains, where it has the effect of bringing the two kinase domains into closer proximity. In the unbound state, each kinase domain is inactive due to an intramolecular peptide, the *activation loop,* which is buried in the catalytic cleft and sterically hinders entry of substrates.[36] When the two cytoplasmic portions of the receptor domains are brought sufficiently close together, the kinase domain of one β-subunit phosphorylates the other on a cluster of tyrosine residues in the activation loop, forcing the loop out of the catalytic cleft, thus activating the kinase domain.[37] This is possible because of the kinetic nature of the receptor's inactive state, in which the catalytic site is always alternating between open and closed conformations, though in the basal state the activation loop is inaccessible most of the time. However, when the contralateral kinase domain is brought sufficiently close, it can phosphorylate the activation loop during the brief period it is in the extended

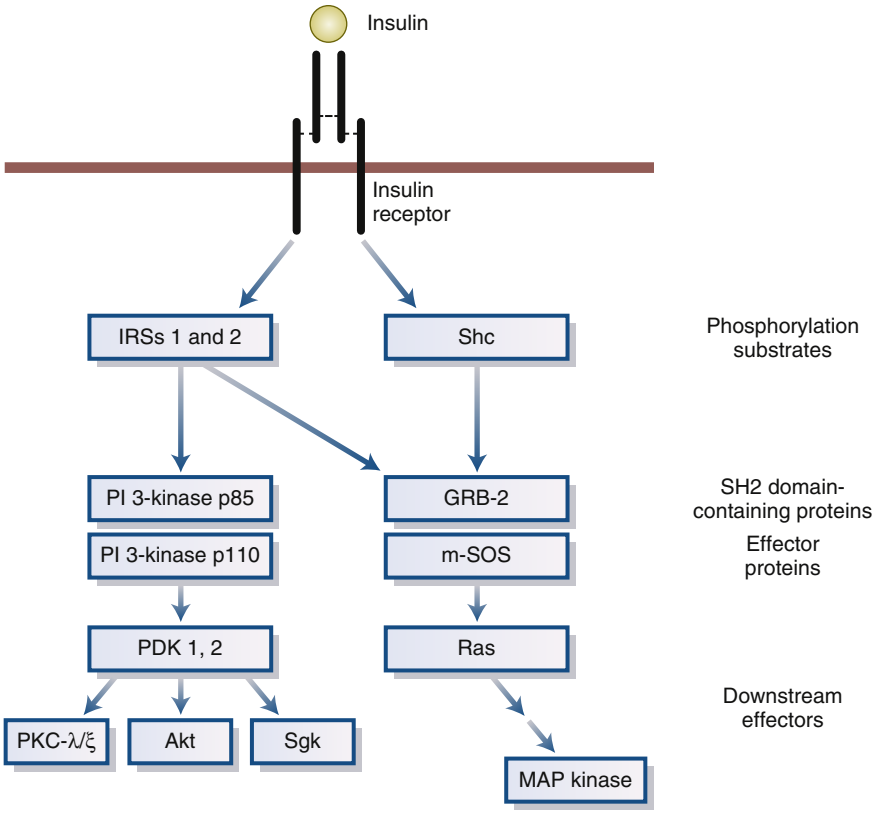
position, converting this to the more stable conformation. In this way, phosphorylation of one-half of the receptor increases its kinase activity, allowing the kinase in that half of the receptor to phosphorylate the activation loop in the other half and, ultimately, exogenous substrates.[38] Proximity-driven phosphorylation and activation of one monomer by the other are common features of RTK activation, but the precise strategies utilized to achieve this vary. Thus, although the active conformations of all tyrosine protein kinases are similar, the configurations of the inactive states differ enormously. An exception to the rule of activation by transphosphorylation is provided by the EGF receptor, in which activation depends on allosteric regulation of the kinase domain of one monomer by the other monomer, once again brought about by a conformational change bringing the two domains into adjacency. The critical interaction is between the C lobe of the activator kinase and the N lobe of the receiver kinase, which disrupts an autoinhibitory interaction present in the inactive monomer.[39]

Signaling by Receptor Tyrosine Protein Kinases

Because the insulin receptor is an enzyme with catalytic activity residing on the cytoplasmic surface of the plasma membrane, it stands to reason that it would transmit its signal by phosphorylating protein substrates within the cell. Nonetheless, though autophosphorylation sites within and outside the cytoplasmic kinase domain of the β-subunit have been long recognized, it proved difficult to identify robust, physiologically significant phosphorylation of tyrosine residues in other proteins. This seeming paradox is partially explained by the underlying mechanism of activation of signaling pathways by RTKs, which signal by recruiting a variety of signaling proteins to the different phosphorylated tyrosines in the receptor. These signaling proteins contain motifs such as the Src homology 2 (SH2) domain and the phosphotyrosine binding (PTB) domain that bind to phosphorylated tyrosines in specific contexts. In the case of the SH2 domain, a phosphorylated tyrosine residue in concert with some amino acids C-terminal to the phosphotyrosine serves as the binding interface for SH2 domains and therefore provides much of the specificity of the interaction.[40] For example, after PDGF binds to its receptor, autophosphorylation of tyrosines within the PDGF receptor in a context defined by the sequence tyrosine–methionine–any amino acid–methionine (YMXM) generates a binding site for the SH2 domains of the regulatory subunit of PI3K.[41] PI3K comprises a regulatory subunit that contains two SH2 domains in tandem and a catalytic subunit. Recruitment of PI3K to a phosphorylated receptor present in the plasma membrane both activates PI3K and brings the PI3K into proximity to its major physiologic substrate, the lipid phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$), which resides on the inner surface of the plasma membrane. PI3K phosphorylates $PI4,5P_2$ on the 3′-position of its inositol ring, generating phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a potent signaling molecule by virtue of its ability to recruit protein kinases and other signaling molecules to the membrane. This illustrates an important principle governing RTK signaling: The initiation of intracellular events is often driven primarily by the spatial relationship of proteins and lipids rather than changes in the specific activity of assembled components. Although in some cases the hormone-bound receptor will modulate the activity of target protein by phosphorylation, the more important event is often the establishment of adjacency between two or more critical signaling molecules, such as PI3K and its substrate, $PI4,5P_2$. An additional example of this signaling mechanism is provided by activation of another proto-oncogene, c-Ras. In this case, signaling is initiated by recruitment of the adapter protein SH2 domain-containing protein (SHC) or growth factor receptor-bound protein 2 (GRB2) via their SH2 and/or PTB domain. When SHC is recruited, it is in turn tyrosyl phosphorylated by the RTK, enabling it to recruit GRB2 via its SH2 domain. GRB2 contains two Src homology 3 (SH3) domains that remain constitutively bound to a polyproline sequence in the son of sevenless (SOS) protein, which is thus, in turn, carried to the plasma membrane.[42,43] Association of SOS with the plasma membrane is necessary and sufficient for activation of the small G protein Ras.[44] SOS is a guanine nucleotide exchange factor (GEF) protein that activates Ras by catalyzing the removal of GDP from inactive Ras to allow binding of GTP. As noted earlier, the critical event that determines the activity of Ras is the positioning of SOS in proximity to Ras.[45,46]

The insulin and IGF1 receptors signal using a variation of the strategy described previously for the PDGF receptor (Fig. 2.7). Rather than assembling a signaling complex on the cytoplasmic domain of the receptor, they assemble the complex on members of a family of scaffolds called insulin receptor substrate (IRS) proteins.[47] There are at least three members of this family in humans, but IRS1 and IRS2 are thought to be the most important to physiologic signaling by insulin and IGF1. Like other members of the group, IRS1 and IRS2 lack intrinsic enzymatic activity; they serve solely as docking proteins to bring signaling molecules together into a multimeric complex. IRS1 and IRS2 are heavily tyrosine phosphorylated by activated insulin receptor, generating binding sites for the SH2 domains of PI3K, GRB2, and the phosphotyrosine phosphatase SHP2. A pleckstrin homology (PH) domain and PTB domain located at the N-terminus of IRS1/2 are instrumental in bringing the protein to the receptor.[48] Upon ligand engagement of the insulin or IGF1 receptor, IRS1/2 is rapidly phosphorylated on tyrosine residues and more slowly on serine/threonine residues, the latter by a number of cytoplasmic kinases, including protein kinase C (PKC), c-Jun N-terminal kinase (JNK), and pp70 S6 protein kinase. Serine/threonine phosphorylation of IRS proteins provides a strong negative feedback signal as it is thought to block further tyrosine phosphorylation and in some cases induces degradation of the protein.

There is some evidence that the insulin receptor is capable of signaling through scaffolds other than the IRS proteins, though the physiologic significance of these pathways remains unclear. The insulin receptor recruits SHC to a phosphotyrosine motif via SHC's PTB domain and phosphorylates SHC to generate a docking site for the SH2 domain of GRB2; this leads to activation of Ras as described earlier.[49] GRB10, and most likely its close relative GRB14, is an SH2 domain–containing protein that binds to the insulin receptor with high affinity.[50] However, unlike the IRS proteins, GRB10 binds to the three phosphorylated tyrosine residues in the activation loop and blocks the activity of the insulin receptor, inhibiting the insulin-dependent production of PIP_3.[51] GRB10 is stabilized via phosphorylation by mammalian (mechanistic) target of rapamycin complex 1 (mTORC1), itself activated downstream of insulin, providing another form of negative feedback.[52,53] Disruption of GRB10 in mice yields embryonic overgrowth, consistent with its role as a negative regulator of IGF1 signaling.[54] Both SH2B1 and SH2B2 (formerly known as APS) bind directly to the phosphorylated insulin receptor and both enhance the actions of insulin in vivo. However, while insulin sensitivity is decreased in SH2B1-deficient mice, consistent

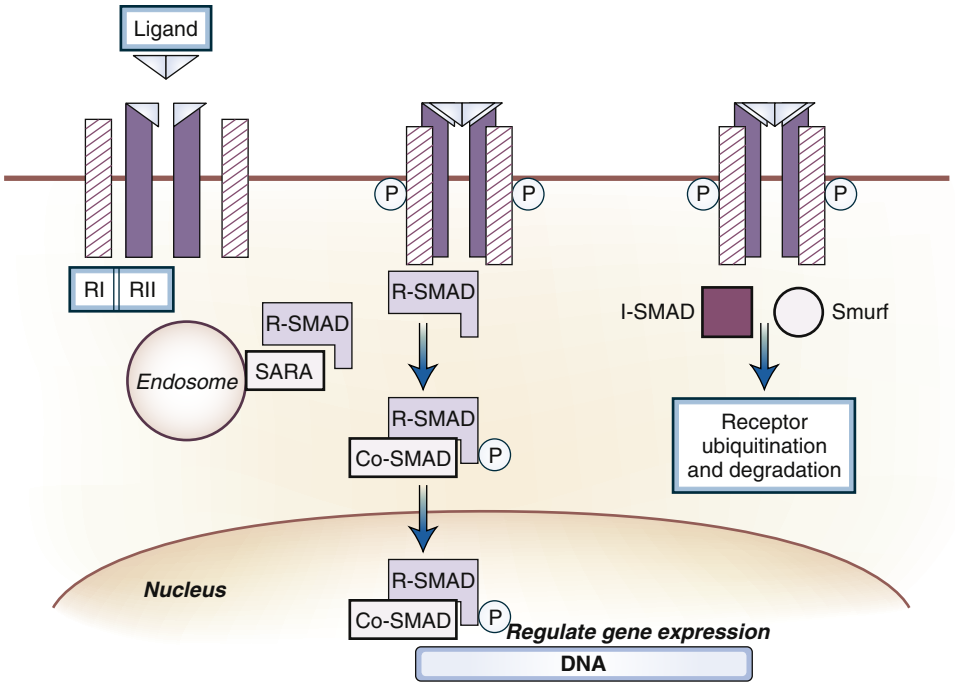


• **Fig. 2.7** Simplified model of signaling pathways downstream from the insulin receptor. Insulin binds to the insulin receptor, activating the receptor tyrosine kinase to phosphorylate tyrosine residues on insulin receptor substrates (IRSs), including IRS1 and IRS2. The phosphotyrosine residues in the IRS molecules bind to Src homology 2 (SH2) domains in molecules such as growth factor receptor–binding protein 2 (GRB2) and the p85 regulatory subunit of phosphoinositide (PI) 3-kinase (PI3K). These SH2 domain–containing proteins initiate two distinct branches of the signaling pathway. Activation of PI3K leads to activation of phosphoinositide-dependent kinases (PDKs) 1 and 2, which activate multiple protein kinases, including Akt/protein kinase B, atypical protein kinase C (PKC) isoforms, and serum-induced and glucocorticoid-induced protein kinases (Sgk). GRB2 interacts with m-SOS, a guanine nucleotide exchange factor that activates Ras. Activation of Ras triggers a cascade of protein kinases, leading to activation of mitogen-activated protein (MAP) kinase. *Shc,* Src, homology domain–containing protein.

with SH2B1 enhancing the actions of insulin, insulin sensitivity is modestly increased in SH2B2-deficient mice, suggesting that the SH2B2 gene product(s) may negatively regulate insulin sensitivity in animals.[51]

Receptor Serine/Threonine Protein Kinases

One of the more interesting variants on signaling by intracellular protein kinases is provided by a class of integral membrane receptors possessing intrinsic serine/threonine protein kinase activity. Ligands for these receptors are members of the transforming growth factor-β (TGFβ) family of first messengers. These 42 agonists encoded in the human genome can be classified into distinct groups typified by TGFβ itself, activin, inhibin, bone morphogenetic protein (BMP)/growth and differentiation factor (GDF), nodal, myostatin, and antimüllerian hormone. Each ligand is composed of a dimer of two peptides joined by hydrophobic interactions and often disulfide bonds. The hormone inhibin was isolated as an activity produced by gonadal tissue that blocks the secretion of follicle-stimulating hormone (FSH) from the pituitary.[55] Like other members of the TGFβ family, it is composed of two chains, an α-subunit and one of two related β-subunits. The hormone activin, which promotes the release of FSH, is formed by the assembly of homodimers of the β-subunit.[56] Like inhibin, activin was originally identified as a product of the gonads but is now known to be secreted by many tissues and to function in an autocrine or paracrine manner as well. The first indication that the TGFβ family of ligands exerts its actions via membrane protein kinases arose from the cloning of a complementary DNA encoding the activin receptor and recognition of a canonical kinase domain.[57] Like all receptors for ligands in the TGFβ superfamily, the activin receptor is composed of four transmembrane glycoproteins, two type 1 receptors and two type 2 receptors. Type 1 and 2 receptors have a similar primary structure, the major difference being an insertion of a conserved 30–amino acid sequence rich in glycine and serine (the GS domain) in the type 1 cytoplasmic domain preceding the kinase domain, which binds the immunophilin FKBP12. Activin interacts initially with type 2 receptors, which brings the type 1 and type 2 receptors into proximity so that the type 2 receptors can phosphorylate the GS domain of the partner type 1 receptors. This alleviates steric hindrance of the type 1 receptor kinase catalytic site and releases FKBP12, the two changes working in concert to activate the type 1 receptors, which allows the receptors to phosphorylate target substrates.[58] Inhibin exerts its inhibitory action by recruiting the transmembrane glycoprotein betaglycan (also called the type III receptor) to form a



• **Fig. 2.8** Mechanism of action for receptor serine kinases. Binding of dimeric ligand to the type II receptor (RII) subunit triggers assembly of the receptor into the heterotetrameric $[(RI)_2(RII)_2]$ state. RII transphosphorylates the type I receptor (RI), thereby activating phosphorylation of the receptor-regulated SMAD (R-SMAD) protein that is bound to the SMAD anchor for receptor activation (SARA) in endosomes. The phosphorylated R-SMAD associates with a comediator SMAD (Co-SMAD). Eventually, the R-SMAD is translocated into the nucleus, where it binds to DNA, enabling it to regulate gene transcription. The inhibitory SMAD (I-SMAD) can also bind to the activated receptor, promoting ubiquitination and degradation of the receptor. *P,* phosphorylation; *Smurf,* SMAD ubiquitination regulatory factor.

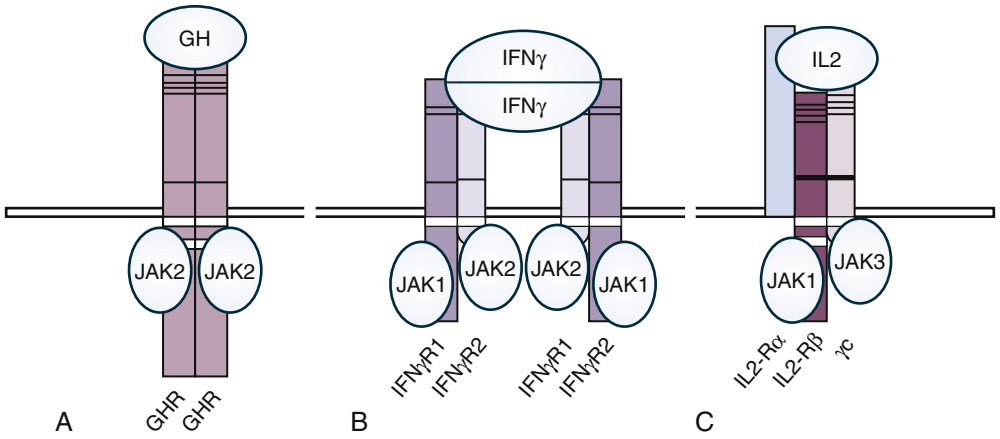
stable complex with type 2 receptors, thus sequestering them and preventing activation of the partner type 1 receptors.[59]

The major intracellular signaling mechanism utilized by all members of the TGFβ family involves SMAD proteins, which function as the major substrates for type I receptors (Fig. 2.8). There are eight human genes coding for SMAD proteins. Five of the human SMAD proteins, termed the receptor regulated SMADs or R-SMADs (SMADs 1, 2, 3, 5, and 8/9), contain a Ser-X-Ser phosphorylation site at their C-terminal tail and serve as substrates for the type I receptors. The activin receptor phosphorylates SMAD3 (and possibly SMAD2). Two R-SMADs then form a trimer with the common partner SMAD or co-SMAD (SMAD4) and are transported to the nucleus.[60] It is likely that other SMAD isoforms contribute to activin regulation of gene expression in vivo in a tissue-specific manner. Upon import into the nucleus, SMAD proteins are modified at their so-called linker domains by a complex set of phosphorylation events that serve both to enhance binding of SMAD proteins to transcriptional regulatory proteins and to target SMAD proteins for ubiquitin-dependent proteasomal degradation. SMAD proteins bind directly to DNA through a conserved N-terminal domain and interact with other transcription factors, which, in concert with the SMAD proteins, exert control over a transcriptional network defined by the cell type and activating ligand. A third class of SMADs, the inhibitory or I-SMADs (SMADs 6 and 7), can bind to the activated receptor and promote ubiquitination and degradation of the receptor.

One particularly interesting member of the TGFβ family is the hormone myostatin, formerly known as GDF8. Myostatin is secreted by skeletal muscle and negatively regulates muscle growth through binding to a type II (ActR-IIB) receptor and type 1 receptors (ALK4 and ALK5), which phosphorylate SMAD2 and SMAD3.[61] A deficiency of myostatin is responsible for the "double-muscled" phenotype of Belgian Blue and Piedmontese cattle, and deletion of its gene in mice and humans leads to massive muscle hypertrophy and hyperplasia.[62]

Signaling by Receptors That Associate With Enzymes

Another mode of signal transduction across the plasma membrane is provided by receptors that possess no intrinsic catalytic activity but that associate with a cytoplasmic, non–membrane-spanning tyrosine kinase. The best example of this is the family of class I and class II cytokine receptors, which are type 1 transmembrane proteins with the N-terminus on the outside of the cell and a cytoplasmic C-terminus (Fig. 2.9). As for RTKs, dimerization or higher order oligomerization appears important for activation of the receptor. In many cases, including the GH receptor, a single ligand molecule contains two distinct recognition sequences. The initial binding is to a high-affinity site, which is followed by a second lower affinity association with a site located on a second, associated monomer. The two monomers that compose the activated receptor make significant contact with each other, again in the exofacial domain close to where the receptor inserts in the membrane. For GH, prolactin, leptin, thrombopoietin, and erythropoietin (EPO), the receptor is a homodimer with two identical subunits. However, for some cytokines, the receptors consist of a ligand-specific monomer and one or more transmembrane chains shared with other cytokine receptors (see Fig. 2.9). For example, the interleukin 2 (IL2) receptor consists of an IL2 receptor–specific

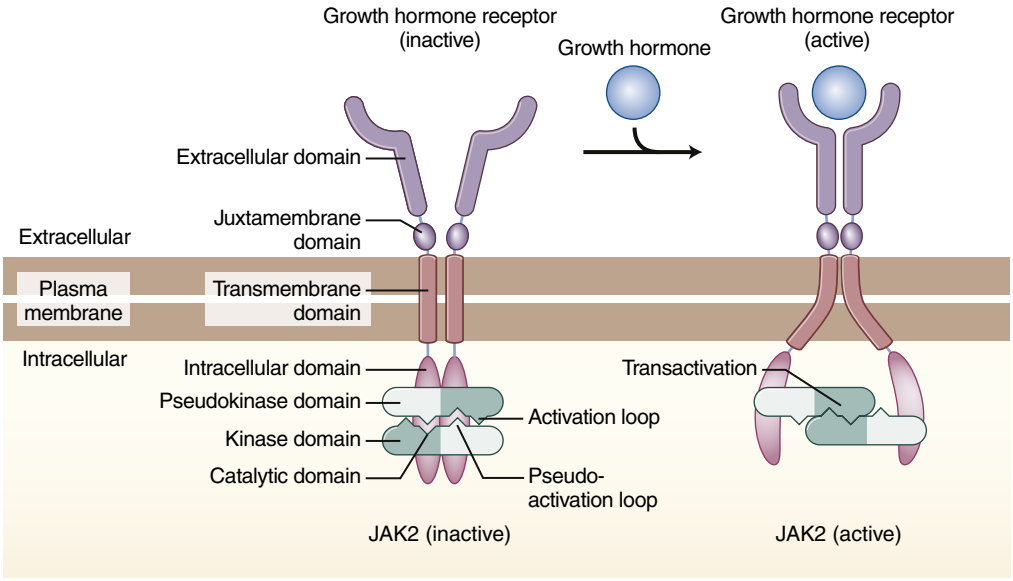


• **Fig. 2.9** Cytokine receptors are composed of multiple subunits and bind to one or more members of the Janus kinase (JAK) family of tyrosine kinases. (A) Growth hormone (GH), such as prolactin and leptin, binds to growth hormone receptor (GHR) homodimers and activates JAK2. (B) Interferon-γ (IFNγ) homodimers bind to their ligand-binding γR1 subunits. The γR2 subunits are then recruited, leading to activation of JAK1, which binds to the γR1 subunit, and JAK2, which binds to the γR2 subunit. Both subunits and both JAKs are necessary for responses to IFNγ. (C) Interleukin 2 (IL2) binds with high affinity to receptors composed of three subunits: a γc subunit shared with receptors for IL4, IL7, IL9, IL15, and IL21; an IL2Rβ subunit shared with the IL15 receptor; and a noncytokine receptor subunit, IL2Rα. IL2 activates JAK3, bound to the γc subunit, and JAK1, bound to IL2Rβ. Extracellular regions of homology are indicated by the *black lines* and *colored patterns*. Intracellular regions of homology are indicated by the *small white boxes*. Identical subunits are indicated by *identical colors*.

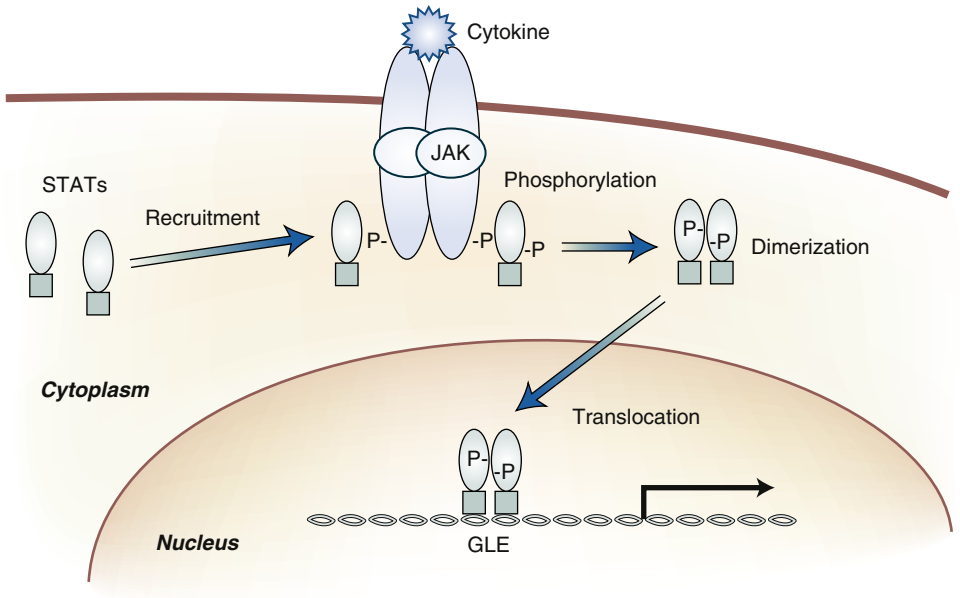
subunit (IR2Rα), a second subunit shared with the IL15 receptor (IL2Rβ), and a γc subunit that is shared with the receptors for IL4, IL7, IL9, and IL15. The IL6 receptor has a unique subunit but shares a glycoprotein 130 (GP130) subunit with at least five other receptors. As with RTKs, oligomerization appears important for activation of these receptors, as indicated by the observation that bivalent, but not monovalent, antibodies are capable of activating the receptors. However, also like RTKs, dimerization alone is insufficient to activate this class of receptors. This was recognized when the EPO receptor and subsequently the GH and prolactin receptors were examined in situ and found to exist as preformed dimers even in the unbound state.[63] The importance of dimerization of the GH receptor is illustrated by the effectiveness of the GH antagonist pegvisomant in treating acromegaly, a disease of excess GH secretion. Pegvisomant competes with native GH for its receptor and prevents functional dimerization.[64]

Proximal to the membrane on the inside of the cell, the class I and class II cytokine receptors have a conserved sequence that is critical to binding a protein tyrosine kinase of the JAK family. There are four members of this family: JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2), with JAK3 largely restricted to cells of the hematopoietic lineage.[65] For those receptors that function as homodimers, JAK2 is the predominant isoform involved in signaling. Cytokine receptors that function as heterodimers or higher order oligomers tend to bind more than one JAK family member. For example, the IFNγR1 subunit of the IFNγ receptor binds JAK1 and the IFNγR2 subunit binds JAK2, while the IL2 receptor recruits JAK1 to the IL2Rβ subunit and JAK3 to the γc subunit (see Fig. 2.9). The JAK proteins associate with a cytoplasmic, juxtamembrane portion of the cytokine receptors via a conserved, N-terminal domain structure called the FERM domain (named for its presence in Band 4.1 protein, ezrin, radixin, and moesin).[66] The carboxy half of JAK consists of two homologous regions in tandem, a pseudokinase domain followed by a kinase domain. The former has many of the conserved sequences that define a protein kinase, but it also has mutations of amino acids that are essential for catalytic activity. It is believed that in the non–ligand-bound receptor, the intracellular portions of two monomers are arranged in a way such that each pseudokinase domain binds to and suppresses the catalytic activity of the kinase on the other subunit, and vice versa. Binding of GH to its receptor results in a conformational change in the extracellular domain of the receptor, which induces a motion intracellularly like the opening of scissors, causing sliding of the two subunits of JAK in opposite directions. This relieves the allosteric inhibition of the kinases[67,68] (Fig. 2.10).

The major consequence of releasing the block to GH receptor-associated JAK2 activity is the JAK2-catalyzed transphosphorylation of the contralateral receptor subunit and its associated JAK2.[65] This allows binding of the SH2 and/or PTB domains of a number of signaling molecules, including IRS1/2 and PLCγ, thus recruiting them to the receptor and plasma membrane.[69] However, more important than these to the actions of GH on growth are members of the signal transducers and activators of the transcription (STAT) family (Fig. 2.11). The GH receptor binds a number of STAT family members, but STAT5b is most critical to its growth-promoting actions. There are seven STAT proteins with a shared domain structure. The N-termini are composed of four helical coils that function in binding to other proteins, followed by a DNA-binding domain (DBD).[70] The carboxy half of the proteins consists of a linker region, an SH2 domain, and a transcriptional transactivation domain. Several of the tyrosine residues in the GH receptor that undergo phosphorylation by JAK2 in response to ligand binding serve as docking sites for STAT5b. Once recruited to the receptor, STAT5b is itself phosphorylated, resulting in dimerization, with each STAT5b protein binding via its phosphorylated tyrosine to its partner's SH2 domain. At the same time, STAT5b dissociates from the receptor and translocates into the nucleus where it can regulate gene transcription. In addition to this basic pathway, there are numerous other layers of regulation. Serine/threonine protein kinases such as members of the MAPK and PKC families also phosphorylate STAT proteins; in some cases, this latter phosphorylation is required for maximal



• **Fig. 2.10** Scissor model for activation of the human growth hormone (hGH) receptor. In the basal state, the hGH receptor exists as an inactive dimer in which the two subunits are held together through weak interactions in the transmembrane membrane domain (TMD) and poised in the inactive state through electrostatic repulsion in the extracellular juxtamembrane domain (JMD) and pseudokinase inhibition in the associated JAK2 dimer *(left)*. Binding of hGH to the receptor *(right)* clamps the JMD such that it avoids the electrostatic repulsion and mechanically alters the TMD such that the intracellular domain is splayed outward. Splaying pulls on the JAK2 molecules to align their kinase domains. This triggers a wave of phosphorylation events, including the STAT proteins critical to receptor signaling. (Modified from Wells JA, Kossiakoff AA. New tricks for an old dimer. *Science*. 2014;344:703.)



• **Fig. 2.11** Cytokines activate signal transducers and activators of transcription (STATs). STAT proteins are latent cytoplasmic transcription factors. STATs bind through Src homology 2 (SH2) domains to one or more phosphorylated tyrosines (P) in activated receptor–Janus kinase (JAK) complexes. Once bound, STATs themselves are tyrosyl phosphorylated, presumably by the receptor-associated JAKs. STATs then dissociate from the receptor-JAK complex, homodimerize or heterodimerize with other STAT proteins, move to the nucleus, and bind to gamma-activated sequence-like elements (GLEs) in the promoters of cytokine-responsive genes. *P,* phosphorylation. (Modified from J. Herrington, used with permission.)

transcriptional activation. Using a different mechanism, SH2B1 binds to and enhances JAK2 activity.[69,71] STAT proteins can also heterodimerize with other STAT proteins or other transcription factors. For example, STAT5b has been shown to dimerize with the glucocorticoid receptor, with the latter acting as a coactivator for STAT5b to promote expression of GH-regulated genes (e.g., *IGF1*) implicated in body growth.[72]

Another important hormone that uses the JAK/STAT signaling pathway is leptin. Leptin is secreted by adipocytes; it acts on the arcuate nucleus of the hypothalamus as well as other regions in the brain to suppress appetite and, in rodents, increase metabolic rate. Humans deficient in leptin display massive obesity early in life.[73] Like GH, leptin binds to homodimers of a class I cytokine receptor and activates JAK2.[74] However, in contrast to the GH receptor, the leptin receptor recruits STAT3 as its primary signaling molecule, which binds to phosphotyrosines in a YXXQ motif. The phosphorylated leptin receptor also binds STAT5 and the SH2-containing protein tyrosine phosphatase 2 (SHP2; PTPN11). The latter is thought to act as a positive signaling module by mediating the first step in the activation of the ERK cascade.[75] On the other hand, the tyrosine phosphatase PTP1B dephosphorylates the leptin receptor and inhibits leptin action, and thus its deletion in mouse brain leads to obesity and insulin resistance.[76] JAK2 also phosphorylates IRS proteins, thereby engaging the PI3K pathway. The roles of the different signaling pathways activated downstream of leptin and JAK2 have been investigated using mice in which specific tyrosine residues in the receptor have been mutated. Replacement of tyrosine 1138 by serine completely blocks recruitment of STAT3, generating mice similar in their degree of obesity to those lacking leptin receptors, showing that STAT3 signaling is critical to the regulation of appetite and energy metabolism.[77]

Termination of class I cytokine signaling occurs in response to dephosphorylation of key phosphotyrosines; it is also promoted by the transcriptional induction of the suppressors of cytokine signaling, or SOCS proteins. The eight members of the SOCS family are direct targets of the STAT transcription factors and provide a potent negative feedback signal by binding to phosphorylated tyrosines in the receptors via the SOCS SH2 domain. Upon interacting with the receptors, SOCS proteins inhibit their action by reducing JAK activity, by competing for binding of other signaling molecules, and/or by inducing the degradation of receptor via the ubiquitin pathway due to the conserved SOCS box located at the C-terminus of the protein.[78] Mice deficient for SOCS2 appear normal when young but after weaning grow substantially larger than their wild-type littermates, consistent with enhanced GH signaling.[79] Cytokine signaling via STAT proteins can also be downregulated by members of the protein inhibitor of activated STAT (PIAS) family, which have been shown to regulate transcription through several mechanisms, including blocking the DNA-binding activity of transcription factors, recruiting transcriptional corepressors, and promoting protein sumoylation.

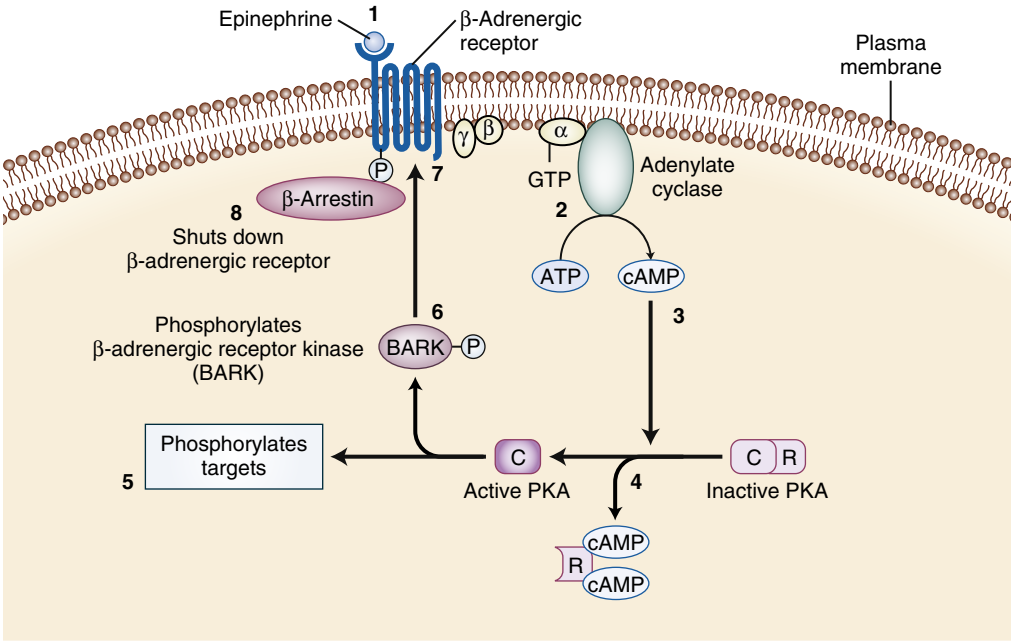
Coupling of Cell Surface Receptors to Intracellular Signaling

Downstream Signaling by Cyclic Adenosine Monophosphate

For the many hormones that bind exclusively to the outer surface of cells to carry out their actions, there must be some means of translating the extracellular signal into an intracellular response. The first example of a transduction system that was understood in some detail derived from investigating one of the key features of the fight-or-flight response, the mobilization of stored carbohydrate in the liver. The physiologic response to stress requires a supply of readily consumable energy, best provided in the form of blood glucose, which is stored as polysaccharide glycogen primarily in the liver. β-Adrenergic stimulation of hepatocytes by epinephrine leads rapidly to the hydrolysis of glycogen and the release of free sugar; glucagon also stimulates the breakdown of hepatic glycogen. The mechanism used to transmit this response is the prototypical example of a second messenger system, in which the agonist that interacts with the outside of the cell, in this case glucagon or epinephrine, is considered a *first messenger,* and a soluble, intracellular signaling molecule generated by hormone-receptor association is called a *second messenger*.[80] For hepatic glycogen breakdown in response to glucagon or β-adrenergic agents, the second messenger is cAMP, which is produced by a plasma membrane enzyme, adenylyl cyclase, from ATP (Fig. 2.12). Adenylyl cyclase is a direct target of $G\alpha_S$, which becomes GTP loaded and active in response to receptor occupancy.

The scope and diversity of hormones and other extracellular signals that activate adenylyl cyclase and increase the level of intracellular cAMP are remarkably extensive. Included in the long list of hormones that signal through this mechanism are β-adrenergic agents, glycoprotein hormones such as TSH, glucagon, adrenocorticotropic hormone (ACTH), hypothalamic hormones, and antidiuretic hormone. Moreover, the range of physiologic and biochemical events modulated by cAMP is equally vast. Thus, although the second messenger cAMP defines a commonly used mechanism for transducing signals from extracellular hormones, it also presents another problem in signaling: How do cells maintain selectivity in the way they respond to a given hormone? Much of this is accomplished by the subcellular compartmentalization of signaling complexes. A-kinase anchoring proteins (AKAPs), which are scaffolds localized to distinct intracellular sites, bind a number of proteins that modulate the actions of cAMP, including degrading enzymes and target kinases.[81] The regulated assembly of higher order structures confers a spatiotemporal resolution to cAMP signaling that can allow multiple biologic responses to exist within the same cell. For example, β-adrenergic agents and prostaglandin E_1 both act on the heart through elevations in cAMP, but each regulates a different cardiac function. This is accomplished through stimulation of distinct populations of cAMP target kinases, such that β-adrenergic agents are more potent than prostaglandins in their effects on the particulate fractions of the heart cell.[82] It is likely that AKAPs confer this specificity to the cardiomyocyte.

cAMP is degraded to AMP and phosphate by a specific PDE, and the balance between synthesis and degradation of cAMP determines the levels of the cyclic nucleotide. Although hormones generally use adenylyl cyclase as the means for modulating cAMP levels within the cell, the PDEs provide an additional site of regulation.[83] The cyclic nucleotide PDEs are a large and complex family of enzymes, whose diversity in both tissue and subcellular localization has made them favorite targets for the development of therapeutics. Caffeine and theophylline were two of the first drugs recognized to be inhibitors of PDE, but more recently, selective inhibitors of PDE5, an enzyme that degrades cyclic guanosine monophosphate (cGMP), have been widely used for the treatment of erectile dysfunction. In addition, PDE inhibitors are either currently being used or are in development for the treatment of a wide variety of diseases, including asthma, neurologic diseases, and pulmonary hypertension.



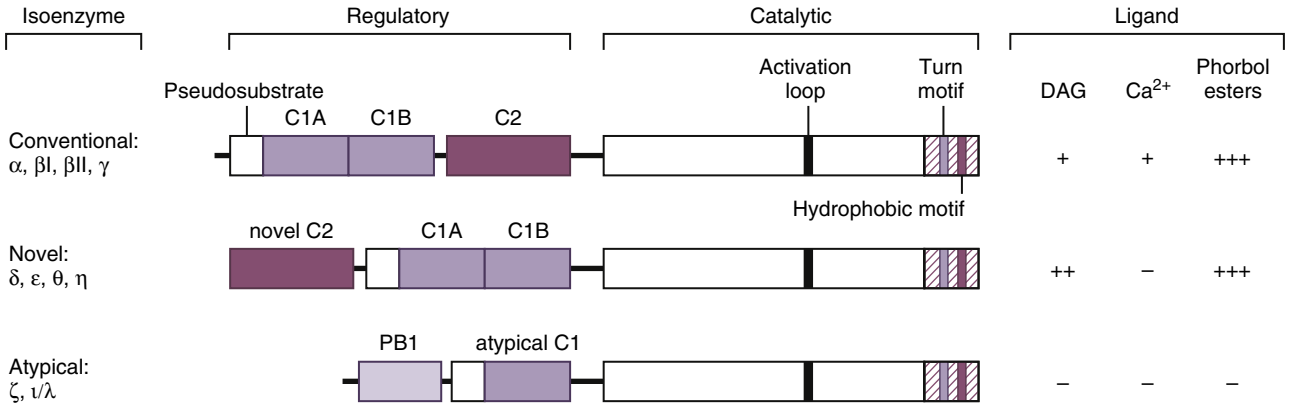
• **Fig. 2.12** Adenylate cyclase, protein kinase A (PKA), and β-adrenergic receptor kinase (BARK) activation by epinephrine. Step 1: Upon binding of epinephrine to the β-adrenergic receptor, G_s is activated. Step 2: $G_s\alpha$ binds to and stimulates adenylate cyclase. Step 3: Adenylate cyclase catalyzes the conversion of ATP to cAMP. Step 4: cAMP binds to the regulatory subunit (R) of PKA, releasing free catalytic subunit (C), which is active. Step 5: C phosphorylates a number of intracellular substrates in a manner determined by its location in the cell. Step 6: C phosphorylates serine and threonine residues on BARK. Step 7: BARK, itself a serine/threonine kinase, phosphorylates serine and threonine residues on the β-adrenergic receptor. Step 8: β-Arrestin binds to the phosphorylated receptor, which blocks further activation of G_s. β-Arrestin also initiates signaling cascades, which are not shown.

Glycogen metabolism in liver and muscle provided the initial example of a common mode of signaling initiated by second messengers—activation of a cascade of intracellular protein kinases. Signal transduction by protein phosphorylation stands as one of the most critical regulatory mechanisms in biology. The state of a phosphoprotein is regulated dynamically, determined by the relative rates of phosphorylation and dephosphorylation by protein kinases and phosphatases, respectively. In most cases, the turnover of the phosphate is rapid, allowing regulation by either the kinase or phosphatase, or in many instances both coordinately. Numerous endocrine signals exert control over intracellular metabolism, growth, and other functions via modulation of protein kinase activity. Originally, protein kinases were found to phosphorylate proteins on serine and threonine residues, but, as described earlier, tyrosine phosphorylation has emerged as another mode of signaling.

One advantage of such series of kinases is signal amplification. Amplification occurs because each individual kinase molecule can modify many downstream target proteins. When these downstream targets are also kinases that become activated upon phosphorylation, each one of them can in turn modify and activate many more proteins. In the case of cAMP-initiated signaling, one receptor:ligand pair creates multiple cAMP molecules. The multiple cAMP molecules activate the serine/threonine kinase protein kinase A (PKA), which in turn phosphorylates multiple downstream effector proteins. In the case of glycogen metabolism, PKA phosphorylates and activates glycogen phosphorylase kinase, which in turn phosphorylates and activates glycogen phosphorylase, which releases glucose-1-P from glycogen. In muscle, phosphorylase kinase is also stimulated by calcium, which is released from the sarcoplasmic reticulum during electrical stimulation and contraction. The mechanism by which cAMP activates PKA illustrates another theme in signal transduction: displacement or dissociation of intramolecular pseudosubstrates or substrates as a means to activate protein kinases, a mechanism also used by such protein kinases as PKC and myosin light chain kinase. cAMP binds to the two regulatory subunits of the heterotetrameric PKA, which causes them to dissociate from two catalytic subunits. A domain in the regulatory subunit resembles a PKA phosphorylation sequence but with the critical serine replaced by an alanine, which lacks the hydroxyl group required for transfer of the phosphate from ATP. When PKA is assembled into a heterotetramer of two regulatory subunits and two catalytic subunits, this pseudosubstrate interacts with the catalytic subunit, preventing it from phosphorylating target proteins.[84]

In addition to enhancing glycogen breakdown, PKA mediates the effects of a number of hormones in various tissues, including the positive inotropic and chronotropic effects of epinephrine on the heart, the trophic effects of the anterior pituitary hormones TSH and ACTH, and the effects of antidiuretic hormone on the permeability of the renal collecting duct to water. PKA also translocates into the nucleus to regulate gene transcription.[85] The best studied nuclear target of PKA is the cAMP-response element–binding protein (CREB), though it is still not clear how many of the physiologic actions of cAMP require this transcription factor to be phosphorylated. PKA also phosphorylates a number of coregulatory proteins, which also contribute to transcriptional outputs.

Importantly, cAMP also has actions that are independent of PKA. One of these is the direct regulation of ion channels;



• **Fig. 2.13** Domain structure and ligands of protein kinase C (PKC). The PKC family can be divided into three classes: the conventional, or classic, PKCs (cPKCs); the novel PKCs (nPKCs); and the atypical PKCs (aPKCs). The C1 domains bind diacylglycerol (DAG) or phorbol ester; the C2 domain binds calcium. A novel C2 domain in nPKCs does not bind calcium but mediates protein-protein interactions. Similarly, a PB1 domain in aPKCs is involved in protein-protein interactions. The aPKCs possess only one C1 domain and thus do not bind diacylglycerol. The conserved pseudosubstrate motif is represented by the *white boxes* in the regulatory domain. The activation loop and the turn and hydrophobic motifs are sites of regulatory phosphorylation.

another involves the exchange protein activated by cAMP (EPAC), which functions as a guanine nucleotide exchange factor (GEF) for the small GTP-binding protein Rap1.[86] Regulation of insulin secretion from pancreatic beta cells by glucagon-like peptide-1 (GLP1) and stabilization of the endothelial barrier by β-adrenergic agents are two processes thought to be mediated by EPAC.

Regulation by the Second Messengers Calcium and PKC

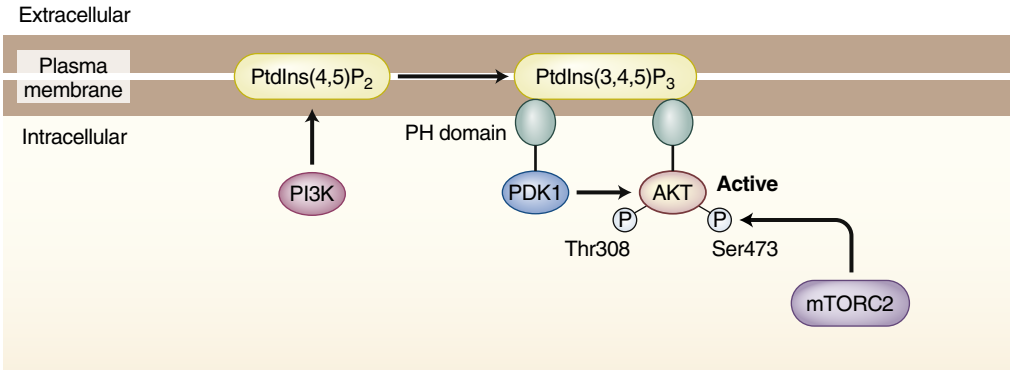
Many additional second messengers have been identified since the discovery of cAMP. These include calcium, cGMP, inositol polyphosphates, DAG, and nitric oxide. The calcium ion (Ca^{2+}) is one of the most common second messengers utilized by diverse cell types, and one that plays a particularly important role in the regulated secretion of hormones.[87] Ca^{2+} is maintained at low micromolar concentrations in the cytoplasm such that opening channels that lead to the outside of the cell or intracellular storage organelles results in a rapid increase in cytosolic Ca^{2+}. The heterotrimeric G proteins containing $G\alpha_q$ or $G\alpha_{11}$ cause increases in intracellular calcium by targeting the membrane-associated enzyme PLC. PLC catalyzes the hydrolysis of phosphatidylinositol 4',5'-bisphosphate into DAG and IP_3. Hormones that signal through G protein–dependent activation of PLC include angiotensin II, α-adrenergic catecholamines, growth hormone–releasing hormone (GHRH), and vasopressin. IP_3 binds to a receptor located on the cytoplasmic face of the endoplasmic reticulum, leading to the release of stored Ca^{2+} from that organelle. Ca^{2+} also interacts with the IP_3 receptor, further stimulating calcium discharge from the endoplasmic reticulum and providing a strong positive feedback loop. Another source of cytoplasmic Ca^{2+} is entry through receptor-operated channels in the plasma membrane, such as those activated by noradrenaline, endothelin, or histamine via heterotrimeric G proteins.

Ca^{2+} transmits its signal via a number of effectors, including protein kinases, in most cases through the intermediary binding protein, calmodulin, or its relative, troponin C. Calmodulin is a small, acidic protein that contains four copies of a canonical calcium-binding motif.[88] Calmodulin associates with and regulates in a Ca^{2+}-dependent manner glycogen phosphorylase kinase, myosin light chain kinase, and members of the family of calcium/calmodulin-dependent kinases. In addition to protein kinases, other calcium/calmodulin-dependent enzymes include the serine/threonine protein phosphatase, calcineurin, some adenylate cyclase and PDE isoforms, and nitric oxide synthase. Calcium interacts directly and independently of calmodulin with targets such as the protease calpain, synaptotagmin (a regulator of neurotransmitter and hormone exocytosis), and cytoskeletal proteins.

An important group of protein kinases directly activated by calcium is the PKC family. PKC, originally identified as the target of the tumor promoter phorbol ester, is a cyclic nucleotide-independent protein kinase regulated by the direct binding of DAG and calcium, two second messengers produced by the activation of PLC. The PKC family has been divided into three groups: classic (regulated by DAG, phosphatidylserine, and calcium), novel (regulated by DAG and phosphatidylserine), and atypical. All PKC proteins have a conserved kinase domain in their C-terminal portion and regulatory sequences in their N-terminal domain. For classic PKCs, the latter consist of a C1 domain, which binds DAG or phorbol ester, followed by a C2 domain, which associates with anionic lipids in a Ca^{2+}-dependent manner[89] (Fig. 2.13). Novel isoforms have a modified form of the C1 domain that confers a higher affinity for DAG than in the classic isoforms but lack the C2 domain, explaining the absence of Ca^{2+} regulation. Atypical PKCs have alterations in the C1 domain that eliminate DAG binding and also lack a site for Ca^{2+} binding. The regulation of PKC isoforms is complex, involving such covalent modifications as phosphorylation and proteolysis, as well as interaction with lipids and hydrophilic molecules other than those traditionally associated with activation of classic PKCs.[90]

Regulation of Protein Kinases by PI3K

Another important signaling pathway involves a family of related proteins that catalyzes phosphorylation of phosphoinositides on



• **Fig. 2.14** Mechanism of AKT activation. When phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) levels are low in the plasma membrane, AKT is in an inactive conformation in the cytoplasm and cannot be phosphorylated by the upstream activating 3-phosphoinositide-dependent protein kinase 1 (PDK1) (not shown). PtdIns(3,4,5)P_3 levels increase in the plasma membrane following the insulin-dependent recruitment to IRS1 and IRS2 of phosphoinositide 3-kinase (PI3K), which phosphorylates phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2). AKT binds PIP_3 through its pleckstrin homology (PH) domain and induces a conformational change within the AKT kinase domain, allowing PDK1 to phosphorylate the critical residue in the activation loop required for AKT kinase activity, threonine 308 (Thr308). Mammalian target of rapamycin complex 2 (mTORC2) also phosphorylates AKT at the carboxy-terminal serine 473 (Ser473) site to fully activate its kinase activity. PDK1 has a PH domain that can bind PtdIns(3,4,5)P_3, but this interaction is not essential for PDK1 catalytic activity. (Redrawn from Finlay D, Cantrell DA. Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol.* 2011;11:109.)

the 3′ position of the inositol ring.[91] All class I PI3Ks are comprised of a catalytic protein associated with a regulatory subunit and use PI4,5P_2 as a preferred substrate; these isoforms are most important to signaling by RTK, GPCRs, and tyrosine kinase oncogenes. Class II PI3Ks phosphorylate PI and PI4P in vivo and lack stable regulatory subunits but probably associate with other proteins as modulating factors. They have been implicated in a diverse set of physiologic responses, but the downstream targets are largely unknown. Class III PI3K, which has one catalytic member also known as vacuolar protein sorting 34 (Vps34), binds tightly to the regulatory protein Vps15, uses exclusively PI as a substrate, and is involved primarily in membrane protein trafficking related to endocytosis, phagocytosis, and autophagy.

Class IA PI3Ks are defined by regulatory subunits containing SH2 domains, which target them to activated RTKs. The heterotrimeric G protein subunit pair Gβγ, when free, activates those class I PI3Ks containing regulatory subunits not bearing SH2 domains.

Class IA PI3Ks are thought to be the most important PI3Ks for the actions of hormones, particularly insulin and IGF1.[92] Activation of the receptors for either hormone leads to phosphorylation of IRS1 or IRS2 at sites specialized for docking with SH2 domains in the p85 regulatory subunit associated with the p110α catalytic subunit of PI3K. The bound PI3K catalyzes the production of PIP_3 and possibly PI3,4P_2, which serve to recruit additional proteins (including protein kinases) to the membrane by binding their PH domains.[93] PH domains are best known for their ability to bind phosphoinositides with high affinity and specificity, although only a small portion have been proven to do so. The serine/threonine protein kinase Akt, also named *protein kinase B* because of its structural similarities to PKA and PKC, contains an N-terminal PH domain that preferentially binds to PIP_3 and PI3,4P_2.[94] When insulin acts upon a target cell, the PH domain of Akt associates with the PIP_3 generated on the cytoplasmic face of the plasma membrane. The binding of the PH domain to PIP_3 serves two purposes: to recruit Akt to the membrane and to relieve steric hindrance of Akt's phosphorylation sites and catalytic domain by the PH domain. Also at the plasma membrane via its own PH domain is the enzyme 3-phosphoinositide-dependent protein kinase (PDK1), which phosphorylates Akt on a threonine in its activation loop (Fig. 2.14). mTORC2 also phosphorylates Akt on a serine in its C-terminus. Together, the PDK1 and mTORC2 phosphorylation events confer full activity to Akt. mTORC2 appears to be regulated by insulin, but the mechanism is unknown.

Akt is essential to many of the metabolic actions of insulin and growth effects of IGF1.[95,96] There are three Akt isoforms, each encoded by separate genes. Akt1 is the most widely expressed isoform and seems to be critical to the regulation of growth; Akt2 is enriched in insulin target tissues and is more important to the control of metabolism; and Akt3 is expressed primarily in the brain, where it controls growth of that tissue.[97] Indirect activation of mTORC1 by Akt and suppression of forkhead box (FOX)O–driven transcription are two of the critical targets for promoting organ growth, the Akt/mTORC1 pathway being particularly engaged in the regulation of cell size.[98] Members of the Rab GTPase-activating protein family, TBCD4 (also known as AS160) in fat cells and TBC1D1 in both muscle and fat, are phosphorylated and inhibited by Akt, contributing to the activation of glucose transport.[99]

Regulation of Protein Kinases by Ras

Routes to activation of Ras by GRB-SOS include both RTKs and GPCRs acting through β-arrestin.[100] GTP-bound Ras recruits several receptors to the plasma membrane, including the serine/threonine kinase Raf, which is activated by dimerization and a series of phosphorylation/dephosphorylation events.[101] Raf then phosphorylates MAPK/ERK kinase (MEK1), a tyrosine and serine/threonine–dual specificity protein kinase, initiating a protein kinase cascade centered on extracellular signal-regulated kinases 1 and 2 (ERK1/2). This represents one of four MAPK cascades,

the others involving c-Jun N-terminal kinase (JNK), the 38-kDa stress-activated kinases (p38), and ERK5. Specificity for MAPKs is conferred by scaffold proteins that bind most or all members of a given pathway, ensuring that each member phosphorylates only its appropriate target kinase.[102] Gonadotropin-releasing hormone, PTH, GH, angiotensin, and gastrin are just a few of the many hormones believed to signal at least in part through regulation of MAPKs.

Disease Caused by Defective Cell Surface Receptors

Numerous diseases develop as a result of dysfunctional binding to or signaling by hormone receptors. These *hormone resistance syndromes* invariably mimic the phenotype of the hormone-deficient state but present with high levels of biologically active hormones in the circulation.

Insulin Resistance Syndromes

The best studied inherited disease of hormone resistance is that caused by mutations in the insulin receptor. In addition to hyperinsulinism and the expected abnormalities in metabolism, patients with severe insulin resistance also display acanthosis nigricans (hyperpigmentation primarily in the skin folds) and often hyperandrogenism.[103] Beyond that, there is a range of syndromes that correlate with the degree of insulin signaling impairment. The strongest loss-of-function mutations result in leprechaunism, in which there are severe developmental defects presenting at birth. Some mutations of the insulin receptor gene cause a decrease in the number of receptors in the plasma membrane, in some cases accompanied by a decrease in the mRNA. Other mutations adversely affect hormone binding or the function of the kinase domain.[103] In contrast to insulin resistance caused by mutations in the receptor gene, sometimes referred to as type A insulin resistance, type B resistance presents at middle age, often with signs of autoimmunity such as vitiligo, alopecia, and arthritis. This syndrome is defined by the presence of antibodies directed against the insulin receptor; the levels of antibody correlate with the severity of the disease.

In many ways, the use of insulin resistance to describe the common syndrome associated with obesity or polycystic ovary syndrome (PCOS) is a misnomer. The term *resistance* was originally coined to describe the situation of hyperglycemia in the face of elevated concentrations of insulin in the blood.[105,106] However, the recognition that insulin has numerous physiologic actions in addition to those on carbohydrate metabolism has led to ambiguity in nomenclature. On the one hand, the term *insulin resistance* is often applied to abnormalities in insulin signaling to all outputs from the receptor; this typically occurs with mutations of the insulin receptor. However, in the insulin resistance of obesity or PCOS, some actions of insulin are preserved. This is demonstrated by a comparison of the phenotype of individuals with type 2 diabetes mellitus to those with genetically encoded partial defects in insulin receptor function.[107] Both groups share hyperglycemia, but only those with type A insulin resistance display defects in the regulation of hepatic lipid metabolism by insulin. Thus the metabolic phenotype associated with type A inherited insulin resistance is not faithfully phenocopied by the insulin resistance of obesity. Consistent with this, numerous pathologic mechanisms have been proposed to account for the insulin resistance associated with obesity, almost all of which involve a "post-receptor defect."

Defects in Cell Surface Receptors That Control Growth

One of the most clinically recognizable syndromes is resistance to the actions of GH, which results in shortness of stature. An inability to respond to GH results in Laron syndrome, characterized by high levels of circulating GH, very low levels of IGF1, and short stature.[108] Diverse molecular causes have been reported, including large deletions as well as missense, frameshift, and splicing mutations in the GH receptor. Similar syndromes of decreased growth can also result from mutations in STAT5b and deficiency in IGF-1 or defects in IGF-1 signaling. Recently, a syndrome has been described in which mutations in the *PIK3R1* gene, which encodes the p85α regulatory subunit of class I PI3K, lead to SHORT syndrome, which includes dysmorphic facial features and defects in growth (short stature, hyperextensibility, ocular depression, Rieger anomaly, and teething delay).[109] As might be expected by the similarities in IGF-1 and insulin signaling, individuals with SHORT syndrome also display lipodystrophy and insulin resistance.[110]

Diseases Caused by Mutations in GPCRs and G Proteins

A number of endocrine diseases can be attributed to mutations in the GPCR–G protein signaling system.[111,112] For GPCRs, many mutations are associated with some degree of loss of function and are inherited in a recessive manner (Table 2.3). Some examples include hypothyroidism from mutations in the thyrotropin-releasing hormone or TSH receptor, glucocorticoid deficiency from mutations in the melanocortin 2 receptor, extreme obesity from dysfunction of melanocortin 4 receptor, and infertility due to mutations in the receptor for luteinizing hormone or FSH. Gain-of-function mutations include those in the TSH receptor causing hyperthyroidism, in the α_2-adrenergic receptor, leading to diabetes mellitus, and in the calcium-sensing receptor resulting in hypoparathyroidism. Somatic activating mutations have been reported in the luteinizing hormone and TSH receptors.[111] A limited number of heterotrimeric G proteins are known to have mutations that cause human disease, and in all cases they affect the α-subunit. Mutation of the gene encoding the $G\alpha_t$ subunit of transducin is associated with night blindness. Dominant, activating mutations of $G\alpha_s$ cause pituitary adenomas, most often secreting GH, and more rarely, tumors of the thyroid, parathyroid, and adrenal glands.[112] Patients who inherit a loss of a functional allele in $G\alpha_s$ develop Albright hereditary osteodystrophy (AHO); those who inherit the mutant allele from their mothers also have pseudohypoparathyroidism type 1a in addition to AHO. This is due to imprinting of the $G\alpha_s$ gene, such that it is expressed preferentially from the maternal allele in a number of hormone target tissues, but biallelically in most other cell types.

Ligands That Act Through Nuclear Receptors

Many signaling molecules share with thyroid and steroid hormones the ability to function in the nucleus to convey intercellular and environmental signals. Lipophilic signaling molecules that use nuclear receptors include derivatives of vitamins A and D, endogenous metabolites such as oxysterols and bile acids, and

TABLE 2.3 Diseases Caused by G Protein–Coupled Receptor Loss-of-Function Mutations

Receptor	Disease	Inheritance
V_2 vasopressin	Nephrogenic diabetes insipidus	X-linked
ACTH	Familial ACTH resistance	AR
GHRH	Familial GH deficiency	AR
GnRH	Hypogonadotropic hypogonadism	AR
GPR54	Hypogonadotropic hypogonadism	AR
Prokineticin receptor 2	Hypogonadotropic hypogonadism	AD[a]
FSH	Hypergonadotropic ovarian dysgenesis	AR
LH	46 XY, intersex	AR
TSH	Familial hypothyroidism	AR
Ca^{2+} sensing receptor	Familial hypocalciuric hypercalcemia	AD
	Neonatal severe primary hyperparathyroidism	AR
Melanocortin 4	Obesity	AR
PTH/PTHrP	Blomstrand chondrodysplasia	AR

[a]With incomplete penetrance.

ACTH, Adrenocorticotropic hormone; *AD,* autosomal dominant; *AR,* autosomal recessive; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *GPR54,* orphan G protein–coupled receptor 54; *LH,* luteinizing hormone; *PTH,* parathyroid hormone; *PTHrP,* parathyroid hormone–related protein; *TSH,* thyroid-stimulating hormone.

TABLE 2.4 Nuclear Receptor Ligands and Their Receptors

Ligand	Receptor
Classic Hormones	
Thyroid hormone	Thyroid hormone receptor (TR), subtypes α, β
Estrogen	Estrogen receptor (ER), subtypes α, β
Testosterone	Androgen receptor (AR)
Progesterone	Progesterone receptor (PR)
Aldosterone	Mineralocorticoid receptor (MR)
Cortisol	Glucocorticoid receptor (GR)
Vitamins	
$1,25\text{-}(OH)_2$-vitamin D_3	Vitamin D receptor (VDR)
All-*trans*-retinoic acid	Retinoic acid receptor, subtypes α, β, γ
9-*cis*-retinoic acid	Retinoid X receptor (RXR), subtypes α, β, γ
Metabolic Intermediates and Products	
Fatty acids	Peroxisome proliferator-activated receptor (PPAR), subtypes α, δ, γ
Oxysterols	Liver X receptor (LXR), subtypes α, β
Bile acids	Bile acid receptor (BAR, also called FXR)
Heme	Rev-Erb subtypes α, β
Phospholipids	Liver receptor homologue-1 (LRH1) Steroidogenic factor-1 (SF1)
Xenobiotics	Pregnane X receptor (PXR) Constitutive androstane receptor (CAR)

chemicals not naturally encountered in the environment (i.e., xenobiotics). These molecules are referred to as *nuclear receptor ligands*. The nuclear receptors for all of these signaling molecules are structurally related and are collectively referred to as the *nuclear receptor superfamily.* They are all transcription factors, serving to activate or repress specific gene sets that mediate their physiologic effects. The study of these receptors is a rapidly evolving field, and more detailed information can be obtained by visiting the Nuclear Receptor Signaling Atlas website.[113,114]

General Features of Nuclear Receptor Ligands

Unlike polypeptide hormones that function through cell surface receptors, no ligands for nuclear receptors are directly encoded in the genome. All nuclear receptor ligands are small (molecular mass <1000 Da) and lipophilic, enabling them to enter cells by passive diffusion, although in some cases a membrane transport protein is involved. For example, several active and specific thyroid hormone transporters have been identified, including monocarboxylate transporter 8 (MCT8), MCT10, and organic anion transporting polypeptide 1C1 (OATP1C1).[115]

All naturally occurring nuclear receptor ligands are derived from dietary, environmental, or metabolic precursors. In this sense, the function of these ligands and their receptors is to translate cues from the external and internal environments into changes in gene expression. Their critical role in maintaining homeostasis in multicellular organisms is highlighted by the fact that nuclear receptors are found in all vertebrates and insects but not in single-cell organisms such as yeast.[116]

Because nuclear receptor ligands are lipophilic, most are readily absorbed from the gastrointestinal tract. This makes nuclear receptors excellent targets for pharmaceutical interventions. In addition to natural ligands, many drugs in clinical use target nuclear receptors, ranging from drugs used to treat specific hormone deficiencies to those used to treat common multigenic conditions such as inflammation, cancer, and type 2 diabetes.

Subclasses of Nuclear Receptor Ligands

One classification of nuclear receptor ligands is outlined in Table 2.4 and is described in the following paragraphs.

Classic Hormones

The classic hormones that use nuclear receptors for signaling are thyroid hormone and steroids. Endogenous steroid hormones include cortisol, aldosterone, estradiol, progesterone, and testosterone. In some cases (e.g., thyroid hormone receptor α and β genes [*THRA* and *THRB*], estrogen receptor α and β genes [*ESR1* and *ESR2*]), more than one gene exists for a given type of hormone receptor. Each receptor gene may in turn encode additional receptors for the same hormone by alternative promoter usage or by alternative splicing (e.g., *THRB1* and *THRB2*).

Some receptors mediate the signals of multiple hormones. For example, the mineralocorticoid receptor, also known as the

aldosterone receptor, has equal affinity for aldosterone and cortisol and probably functions as a glucocorticoid receptor in some tissues, such as the brain.[117] Likewise, the androgen receptor binds and responds to both testosterone and dihydrotestosterone (DHT).[118]

Vitamins

Vitamins are essential constituents of a healthful diet. Two fat-soluble vitamins, A and D, are precursors of important signaling molecules that function as ligands for nuclear receptors.

Precursors of vitamin D are synthesized and stored in skin and activated by ultraviolet light; vitamin D can also be derived from dietary sources. Vitamin D is then converted in the liver to 25(OH)D (25-hydroxyvitamin D, calcidiol), which is subsequently converted by the kidney to $1,25(OH)_2D_3$ (1,25-dihydroxyvitamin D_3, calcitriol), the most potent natural ligand of the vitamin D receptor (VDR).[119] The 1-hydroxylation of calcidiol is tightly regulated, and calcitriol acts as a circulating hormone, arising in the kidney and circulating through the bloodstream to act on target tissues such as intestine and bone.

Vitamin A is stored in the liver and is activated by metabolism to all-*trans*-retinoic acid, which is a high-affinity ligand for retinoic acid receptors (RARs).[120] Retinoic acid functions as a signaling molecule in both a paracrine and an endocrine manner. Retinoic acid is also converted to its 9-*cis*-isomer, which is a ligand for another nuclear receptor, the retinoid X receptor (RXR).[121] These retinoids and their receptors are essential for normal development of multiple organs and tissues, and they have pharmaceutical utility for conditions ranging from skin diseases to leukemia.[122]

Metabolic Intermediates and Products

Certain nuclear receptors respond to naturally occurring endogenous metabolic products. The peroxisome proliferator-activated receptors (PPARs) constitute the best-defined subfamily of metabolite-sensing nuclear receptors.[123] All three PPAR subtypes are activated by polyunsaturated fatty acids, and although specific lipid species may act as selective PPAR ligands, the PPARs may also function as integrators of the concentration of a number of fatty acids.[124]

The natural ligand for PPARα has not been clearly identified, but may be a fatty acid derived from lipolysis.[125,126] The fibrate class of lipid-lowering pharmaceuticals are potent ligands for PPARα, and the very name of this receptor is derived from its ability to induce the proliferation of peroxisomes in the liver, organelles that break down very long-chain fatty acids through β-oxidation.[127] Indeed, stimulation of fatty acid oxidation is an important physiologic role of PPARα.

The other PPARs (δ and γ) are structurally related to PPARα but do not induce proliferation of peroxisomes when activated by their respective ligands. PPARδ, also known as PPARβ, is ubiquitous, and its ligands—other than fatty acids—are not well characterized. Activation of PPARδ increases oxidative metabolism in fat and skeletal muscle.[128] PPARγ is expressed primarily in adipocytes and is necessary for differentiation along the adipocyte lineage.[129] PPARγ is also expressed in other cell types, including colonic epithelial cells, macrophages, and vascular endothelial cells, where it may play physiologic and pathologic roles. The natural ligand for PPARγ is not known, but PPARγ is a major tissue target of thiazolidinedione (TZD) antidiabetic drugs that improve insulin sensitivity.[130,131] These pharmaceutical agents bind to PPARγ with nanomolar affinities. Non-TZD PPARγ ligands are also insulin sensitizers, further implicating PPARγ in this physiologic role.

Another metabolite-responsive nuclear receptor, the liver X receptor (LXR), is activated by oxysterol intermediates in cholesterol biosynthesis. Mice lacking LXRα have a dramatically impaired ability to metabolize cholesterol.[132] A related receptor known as farnesyl X receptor (FXR) binds and is activated by bile acids, and it plays a role in the regulation of bile synthesis and circulation in normal conditions and in disease states.[132]

Endobiotics and Xenobiotics

Other nuclear receptors appear to function as integrators of exogenous environmental signals, including natural endobiotics (medicinal agents and toxins found in plants) and xenobiotics (compounds that are not naturally occurring). In these cases, the role of the activated nuclear receptor is to induce cytochrome P450 enzymes that facilitate detoxification of potentially dangerous compounds in the liver. Receptors in this class include sterol and xenobiotic receptor (SXR), also known as *pregnane X receptor* (PXR), and constitutive androstane receptor (CAR).[133] Unlike other nuclear receptors that have high affinity for specific ligands, xenobiotic receptors have low affinity for a large number of ligands, reflecting their function in defense against a varied and challenging environment. Although these xenobiotic compounds are not hormones in the classic sense, the function of these nuclear receptors is consistent with the general theme of helping the organism to cope with environmental challenges.

Orphan Receptors

The nuclear receptor superfamily is one of the largest families of transcription factors, with 48 members in humans. The hormones and vitamins just described account for the functions of only a fraction of the nuclear receptors. The remaining receptors have been designated as *orphan receptors* because their putative ligands are not known.[134]

From analyses of mice and humans with mutations in various orphan receptors, it is clear that many of them are required for life or for the development of specific organs, ranging from brain nuclei to endocrine glands. Some orphan receptors appear to be active in the absence of any ligand (i.e., constitutively active) and may not respond to a natural ligand. Nevertheless, all of the receptors known to respond to metabolites and environmental compounds were originally discovered as orphans. Therefore future research will likely find that additional orphan receptors function as receptors for physiologic, pharmacologic, or environmental ligands. For example, the nuclear receptor NR1D1 (also known as Rev-Erbα), which is a regulator of circadian rhythms,[17] has been shown to be a receptor for heme,[135,136] although the physiologic significance of this remains to be determined.

Variant Receptors

The C-terminal domain of the nuclear receptors is responsible for hormone binding. In a few nuclear receptors, including THRA and the glucocorticoid receptor, alternative splicing produces variant receptors with unique C-termini that do not bind ligands.[137,138] These variant receptors are normally expressed, but their biologic relevance is uncertain. They may modulate the action of the classic receptor to which they are related by inhibiting its function.

Other normally occurring variant nuclear receptors lack a classic DBD (discussed later). These types include NR0B1 (also known as DAX1), which is mutated in human disease,[139] and PTPN6 (also known as SHP1).[140] Their ligands have not been identified, and it is likely that NR0B1 and PTPN6 bind to and repress the actions of other receptors.

TABLE 2.5 Mechanisms Regulating Ligand Levels

Precursor availability
Synthesis
Secretion
Activation (prohormone → active hormone)
Transport
Deactivation (active hormone → inactive hormone)
Elimination (hepatic, renal clearance)

Rare, naturally occurring mutations of hormone receptors can cause hormone resistance in affected patients. Inheritance of the hormone resistance phenotype is dominant if the mutant receptor inhibits the action of the normal receptor, as occurs with resistance to thyroid hormone or PPARγ ligands.[141] Inheritance is recessive if the mutation results in a complete loss of receptor function, as with the syndrome of hereditary calcitriol-resistant rickets, which is caused by mutations in the VDR.[142] Inheritance can also be X-linked, as with the mutated androgen receptor in androgen insensitivity syndromes.[143]

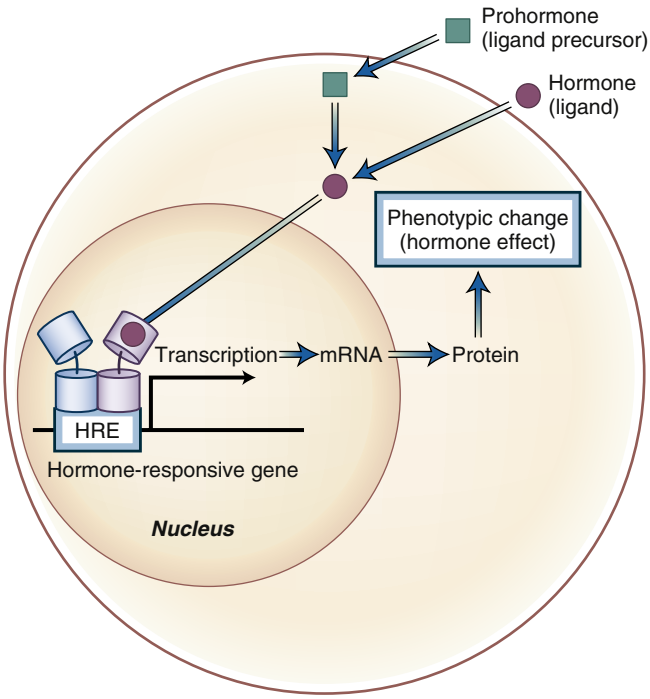
Regulation of Ligand Levels

Ligand levels can be regulated in several ways (Table 2.5). A dietary precursor may not be available in required amounts (e.g., hypothyroidism due to iodine deficiency). Pituitary hormones (e.g., TSH) regulate the synthesis and secretion of classic thyroid and steroid hormones. If the glands that synthesize these hormones fail, hormone deficiency can occur.

Many nuclear receptor ligands are enzymatically converted from inactive prohormones to biologically active hormones; examples include the 5′ deiodination of thyroxine (T_4) to triiodothyronine (T_3) (see Chapter 11). This can occur in the target cell itself or within other tissues that subsequently release T_3 to the circulation for action elsewhere in the body. In other cases, one hormone is a precursor for another, as illustrated by the aromatization of testosterone to estradiol. Biotransformation may occur in a specific tissue that is not the main target of the hormone, as with renal 1-hydroxylation of vitamin D (see Chapter 29), or it may occur primarily in target tissues (e.g., 5α-reduction of testosterone to DHT; see Chapter 19). Deficiency or pharmacologic inhibition of the enzymes responsible for these reactions can reduce hormone levels.

Transport into the target cell can also be a regulated process. T_3 and T_4, for example, do not penetrate the hydrophobic membrane by themselves; they require a transporter such as MCT8 or OATP1. Mutations in MCT8, for example, lead to neurologic issues, including severe intellectual disability and movement disorders with elevated serum T_3.[144] In this condition, it seems likely that the pathology is secondary to the inability of T_3 to enter neurons. Steroid hormones, by contrast, are believed to traverse the membrane by passive diffusion, although it remains possible that undiscovered binding proteins play a role.

Nuclear receptor ligands can be inactivated by hepatic or renal clearance or by more specific enzymatic processes. Mutations in genes encoding inactivating enzymes, or pharmacologic agents that inhibit these enzymes, can result in symptoms of hormone excess such as renal deactivation of cortisol by 11β-hydroxysteroid dehydrogenase (11βHSD). Because cortisol can activate the mineralocorticoid receptor, insufficient 11βHSD activity due to licorice ingestion, gene mutation, or extremely high cortisol levels causes syndromes of apparent mineralocorticoid excess.[145]



• **Fig. 2.15** Mechanism of signal transduction by hormones and other ligands that act through nuclear receptors. *HRE,* hormone response element; *mRNA,* messenger ribonucleic acid.

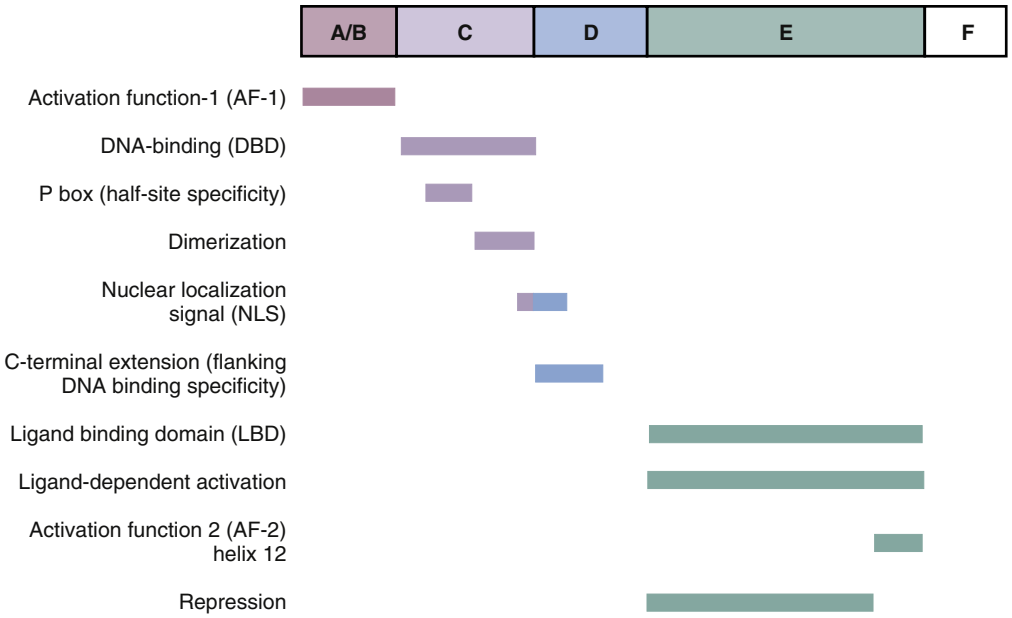
Nuclear Receptor Signaling Mechanisms

Nuclear receptors are multifunctional proteins that transduce the signals of their cognate ligands. General features of nuclear receptor signaling are illustrated in Fig. 2.15.

For hormone action, the ligand and the nuclear receptor must both get into the nucleus. The nuclear receptor also must bind its ligand with high affinity. Because a major function of the receptor is to selectively regulate target gene transcription, it must recognize and bind to promoter and enhancer elements in appropriate target genes. One discriminatory mechanism is dimerization of a receptor with a second copy of itself or with another nuclear receptor. The DNA-bound receptor must work in the context of chromatin to signal the basal transcription machinery to increase or decrease transcription of the target gene. In the regulation of signaling by nuclear receptors, some basic mechanisms are used by many or all members of the nuclear receptor superfamily, whereas other mechanisms impart the specificity that is crucial to the vastly different biologic effects of the many hormones and ligands that use these related receptors.

Domain Structure of Nuclear Receptors

Nuclear receptors are proteins with molecular masses between 50,000 and 100,000 Da. They share a common series of domains, referred to as domains A through F (Fig. 2.16). This linear depiction is useful for describing and comparing receptors, but it does not capture the roles of protein folding and tertiary structure in mediating various receptor functions. The structures of individual domains have now been solved for many receptors, as has the full-length structure of a more limited number of nuclear receptors.



• **Fig. 2.16** Domain structures of nuclear receptors.

Nuclear Localization

The nuclear receptors, like all cellular proteins, are synthesized on ribosomes that reside outside the nucleus. Import of the nuclear receptors into the nucleus requires the nuclear localization signal (NLS), which is located near the border of the C and D domains (see Fig. 2.16). As a result of their NLSs, most of the nuclear receptors reside in the nucleus, with or without their ligands. A major exception is the glucocorticoid receptor; in the absence of hormone, it is tethered in the cytoplasm to a complex of chaperone molecules, including heat shock proteins (HSPs). Hormone binding to the glucocorticoid receptor induces a conformational change that results in dissociation of the chaperone complex, allowing the hormone-activated glucocorticoid receptor to translocate to the nucleus by means of its NLS.

Hormone Binding

High-affinity binding of a lipophilic ligand is mediated by the C-terminal ligand-binding domain (LBD), domains D and E in Fig. 2.16. This region of the receptor has many other functions, including induction of dimerization and transcriptional regulation (see later discussions).

The structure of the LBD has been solved for a number of receptors. All share a similar overall structure consisting of 12 α-helical segments in a highly folded tertiary structure (Fig. 2.17A). The ligand binds within a hydrophobic pocket composed of amino acids in helices 3, 4, and 5 (H3, H4, and H5, respectively). The major structural change induced by ligand binding is an internal folding of the most C-terminal helix (H12), which forms a cap on the ligand-binding pocket (see Fig. 2.17B). Although the overall mechanism of ligand binding is similar for all receptors, the molecular details are essential for determining ligand specificity.[146,147] Ligand binding is the most critical determinant of receptor specificity.

Target Gene Recognition by Receptors

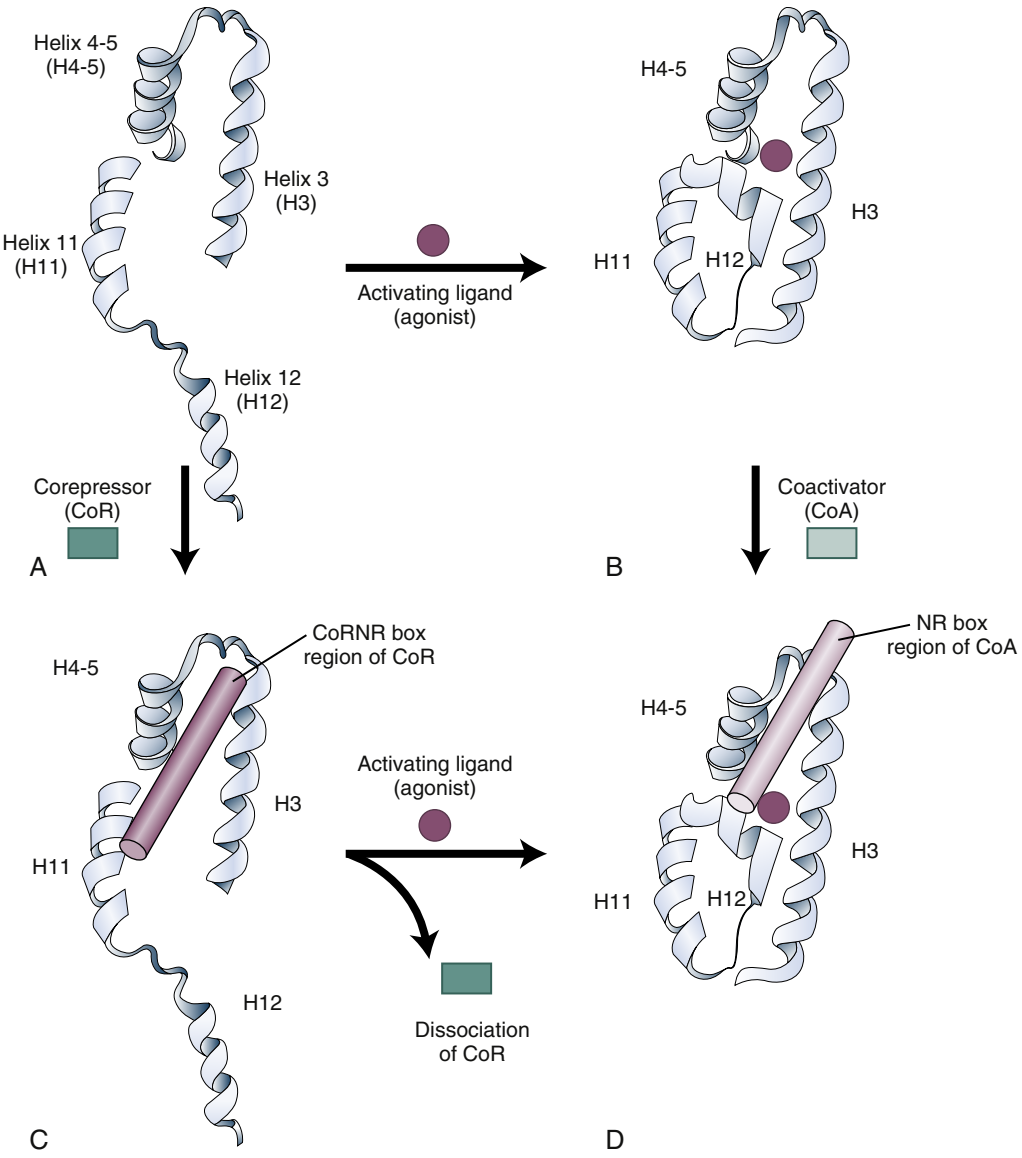
Another crucial specificity factor for nuclear receptors is their ability to recognize and bind to the subset of genes that is to be regulated by their cognate ligand. Target genes contain specific DNA sequences that are called *hormone response elements* (HREs). Binding to the HRE is mediated by the central C domain of the nuclear receptors (see Fig. 2.16). This region is typically composed of 66 to 68 amino acids, including two subdomains that are called *zinc fingers* because the structure of each subdomain is maintained by four cysteine residues coordinated with a zinc atom.

The first of these zinc-ordered modules contains basic amino acids that contact DNA; as with the LBD, the overall structure of the DBD is similar for all members of the nuclear receptor superfamily. The specificity of DNA binding is determined by multiple factors (Table 2.6). All steroid hormone receptors, except for the estrogen receptor, bind to the double-stranded DNA sequence AGAACA (Fig. 2.18).

By convention, the double-stranded sequence is described by the sequence of one of its complementary strands, with the bases ordered from the 5′ to the 3′ end. Other nuclear receptors recognize the sequence AGGTCA. The primary determinant of this specificity is a group of amino acid residues in the *P box* of the DBD (see Fig. 2.18). These hexamer DNA sequences are referred to as *half-sites*. The only difference between these hexameric half-sites is the central two base pairs (underlined in Fig. 2.18). For some nuclear receptors, the C-terminal extension of the DBD contributes specificity for extended half-sites containing additional, highly specific DNA sequences 5′ to the hexamer. Another source of specificity for target genes is the spacing and orientation of these half-sites, which in most cases are bound by receptor dimers.

Receptor Dimerization

The nuclear receptor DBD has affinity for the hexameric half-site or for extended half-sites; however, many HREs are composed of repeats of the half-site sequence, and most nuclear receptors bind these HREs as dimers.[148] Steroid receptors, including estrogen receptors, function primarily as homodimers, which preferentially bind to two half-sites oriented toward each other (i.e., inverted repeats [IRs]) with three base pairs in between (IR3) (see Fig. 2.18A). The major dimerization domain in steroid receptors is within the C domain, although the LBD contributes. Ligand



• **Fig. 2.17** Structural basis of nuclear receptor ligand binding and cofactor recruitment. (A and C) Apo-receptor (no ligand bound). (B and D) Ligand-bound receptor. (C and D) Structures showing the positional binding of a corepressor (CoR) (in C) or coactivator (CoA) (in D). *NR,* nuclear receptor.

binding facilitates dimerization and DNA binding of steroid hormone receptors. Most other receptors, including THR, RAR, PPAR, LXR, and VDR, bind to DNA as heterodimers with RXR (see Fig. 2.18B).

Heterodimerization with RXR is mediated by two distinct interactions, one involving LBDs and the other involving DBDs. The receptor LBD mediates the strongest interaction, which occurs even in the absence of DNA. These receptor heterodimers bind to two half-sites arranged as direct repeats (DRs) with a variable number of base pairs in between.

The spacing of the half-sites is a major determinant of target gene specificity; it results from the second receptor-receptor interaction, which involves the DBDs and is highly sensitive to the spacing of the half-sites. For example, VDR/RXR heterodimers bind preferentially to DRs separated by three bases (DR3 sites), TR/RXR binds DR4, and RAR/RXR binds DR5 with highest affinity.[149]

Studies of isolated DBD binding to DNA have shown that these spacing requirements are related to the fact that the RXR binds to the upstream half-site (i.e., farthest from the start of transcription). As a result of the periodicity of the DNA helix, each base pair separating the half-sites leads to a rotation of about 36 degrees of one half-site relative to the other. Subtle differences in the structure of the receptor DBDs make the interactions more or less favorable at different degrees of rotation.[150] Solution of the crystal structures of full-length nuclear receptor heterodimers bound to DNA has demonstrated remarkable diversity in the precise relationship between heterodimeric partners. For example, the PPARγ-RXR heterodimer forms a nonsymmetric complex, allowing the LBD of PPARγ to cooperate with both DBDs to enhance response element binding,[151] whereas the LXR-RXR heterodimer binds symmetrically to its target sequence.[152] Additional structures will be required to better understand the spectrum of RXR heterodimer binding to DNA.

The discovery of nuclear receptor binding sites has been largely empiric, based on the finding of binding sites in small numbers of known target genes. Unbiased analysis of thousands of

TABLE 2.6 Determinants of Target Gene Specificity of Nuclear Receptors

Specificity	Region of Receptor
1. Binding to DNA	DBD (C domain)
2. Binding to specific hexamer	P box in C domain (AGGTCA vs. AGAACA)
3. Binding to sequences 5′ to hexamer	Carboxy-terminal extension of DBD
4. Binding to hexamer repeats	Dimerization domain (C domain for steroid receptors; D, E, and F for others)
5. Recognition of hexamer spacing	RXR heterodimerization domain (nonsteroid receptors, D/E domains)
6. Cell-specific factors	Receptor-independent (cell-specific factors that open chromatin to permit receptor binding based on receptor-intrinsic properties above)

DBD, DNA-binding domain; *RXR*, retinoid X receptor.

nuclear receptor binding locations in living cells using chromatin immunoprecipitation followed by next generation sequencing has confirmed the canonical binding sequences for many nuclear receptors, including those of the estrogen receptor,[153] the androgen receptor,[154,155] the glucocorticoid receptor,[156] and PPARγ-RXR heterodimers.[157, 158] The complete set of cellular binding sites is referred to as the *cistrome*.[159] Although the sequence of the genome is the same in nearly all cells of the body, cistromes are context dependent, owing to cooperation with factors that open chromatin in a cell type or developmentally specific way, allowing the receptors to bind.

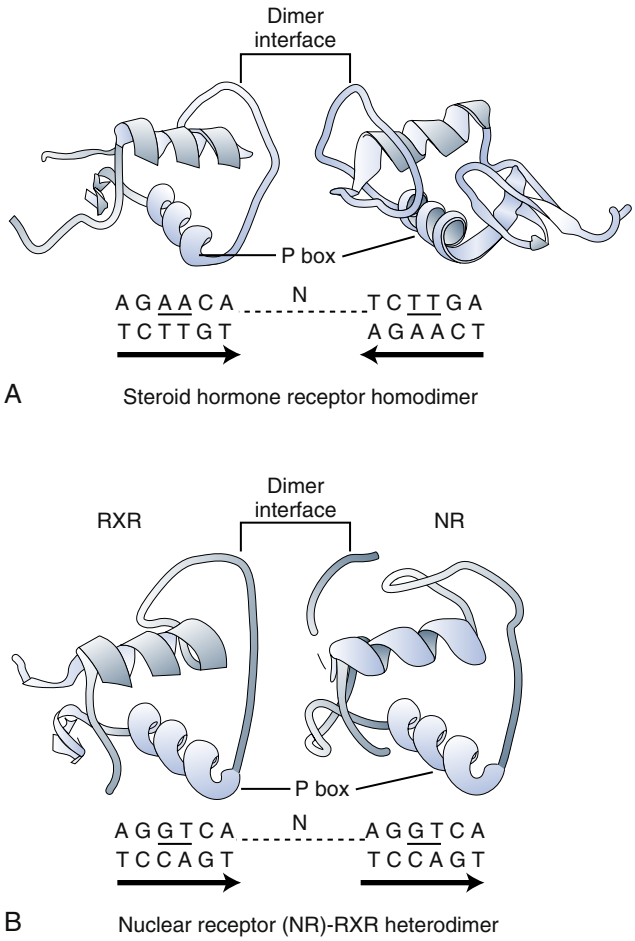
Receptor Regulation of Gene Transcription

Nuclear receptors mediate a variety of effects on gene transcription. The most common modes of regulation are ligand-dependent gene activation, ligand-independent repression of transcription, and ligand-dependent negative regulation of transcription (Table 2.7). Much of this regulation is mediated by interactions of nuclear receptors with proteins called *coregulators,* which include coactivators and corepressors.[160]

Ligand-Dependent Activation

Ligand-dependent activation is the best understood function of nuclear receptors and their ligands. The ligand-bound receptor increases transcription of a target gene to which it is bound. The DBD brings the receptor domains that mediate transcriptional activation to a specific gene. Transcriptional activation itself is mediated primarily by the LBD, which can function as an independent unit even when it is transferred to a DNA-binding protein that is not related to nuclear receptors. The activation function (AF) of the LBD is referred to as *AF2* (see Fig. 2.12).

Gene transcription is mediated by a large complex of factors that ultimately regulate the activity of ribonucleic acid (RNA) polymerase, the enzyme that uses the chromosomal DNA template to direct the synthesis of messenger RNA. Most mammalian genes are transcribed by RNA polymerase II using a large set of cofactor proteins that include basal transcription factors and



• **Fig. 2.18** Structural basis for nuclear receptor (NR) DNA-binding specificity is shown in the ribbon diagrams of receptor DNA-binding domains (DBDs). (A) Steroid hormone receptor binding as a homodimer to the inverted repeat *(arrows)* of the AGAACA half-site. The central base pairs are underlined. (B) RXR-NR heterodimer binding to the direct repeat of AGGTCA. The position of the P box, the region of the DBD that makes direct contact with DNA, is shown. *N,* number of base pairs between the two half-sites; *RXR,* retinoid X receptor.

TABLE 2.7 Regulation of Gene Transcription by Nuclear Receptors

Mode of Regulation	Examples
1. Ligand-dependent gene activation	DNA binding and recruitment of coactivators
2. Ligand-independent gene repression	DNA binding and recruitment of corepressors
3. Ligand-dependent negative regulation of gene expression	DNA binding and recruitment of corepressors, or coactivator redistribution

associated factors collectively referred to as *general transcription factors* (GTFs). Details about GTFs are of fundamental importance and are available elsewhere.

The ligand-bound nuclear receptor communicates stimulatory signals to GTFs on the gene to which it is bound. Ligands specifically recruit a subset of the coregulators to the nuclear receptor LBD. Positively acting coregulators, called *coactivators*,

specifically recognize the ligand-bound conformation of the LBD and bind to the nuclear receptor on DNA only when an activating (agonist) hormone or ligand is bound. A number of coactivator proteins that bind to liganded nuclear receptors have been described (Table 2.8).[161]

The most important determinant of coactivator binding is the position of H12, which changes dramatically when activating ligands bind receptors (see Fig. 2.17B). Along with H3, H4, and H5, H12 forms a hydrophobic cleft that is bound by short polypeptide regions of the coactivator molecules.[162–164] These polypeptides, called *NR boxes* (see Fig. 2.17D), have characteristic sequences of LxxLL, in which L is leucine and xx can be any two amino acids.[165]

Coactivators increase the rate of gene transcription. This is accomplished by enzymatic functions, including histone acetyltransferase (HAT) activity; some coactivators possess intrinsic HAT activity, while others act as scaffolds to recruit HAT proteins.[166] HAT activity is critically important for activation because chromosomal DNA is tightly wrapped in nucleosomal units composed of core histone proteins. Acetylation, as well as some other histone modifications, opens up this chromatin structure.

The best understood class of coactivator proteins is the p160 family, whose name is based on their protein size (approximately 160 kDa). The family contains at least three molecules, each with many names (see Table 2.8).[167] These factors possess HAT activity and recruit other coactivators, such as CREB-binding protein (CBP) and p300, which are also HATs. A third HAT, p300/CBP-associated factor (PCAF), is also recruited by liganded receptors. These HATs, along with a complex of SMARC molecules (SWI/SNF-related, matrix-associated, actin-dependent regulators of chromatin) that direct ATP-dependent DNA unwinding, create a chromatin structure that favors transcription (Fig. 2.19).[168]

Recruitment of multiple HATs may reflect different specificities for core histones and, potentially, for some nonhistone proteins. Some HATs also interact directly with GTFs and further enhance their activities. The mediator complex, which has also been called the thyroid hormone receptor–associated protein (TRAP) complex, and the vitamin D receptor–interacting protein (DRIP) complex link nuclear receptors to GTFs.[169] The HATs and TRAP factors are recruited to the liganded, target gene–bound receptor in an ordered manner that also involves cycling on and off the target receptor with a time scale of minutes.[170] Nuclear receptor interactions with the genome are even more complex, with on-off rates that have been measured to be on the order of milliseconds.[171]

TABLE 2.8 Nuclear Receptor Coregulators

Coactivators

Chromatin remodeling
SWI/SNF complex
Histone acetyltransferase p160 family (Srcs)
p300/CBP
PCAF (p300/CBP-associated factor)
Mediator

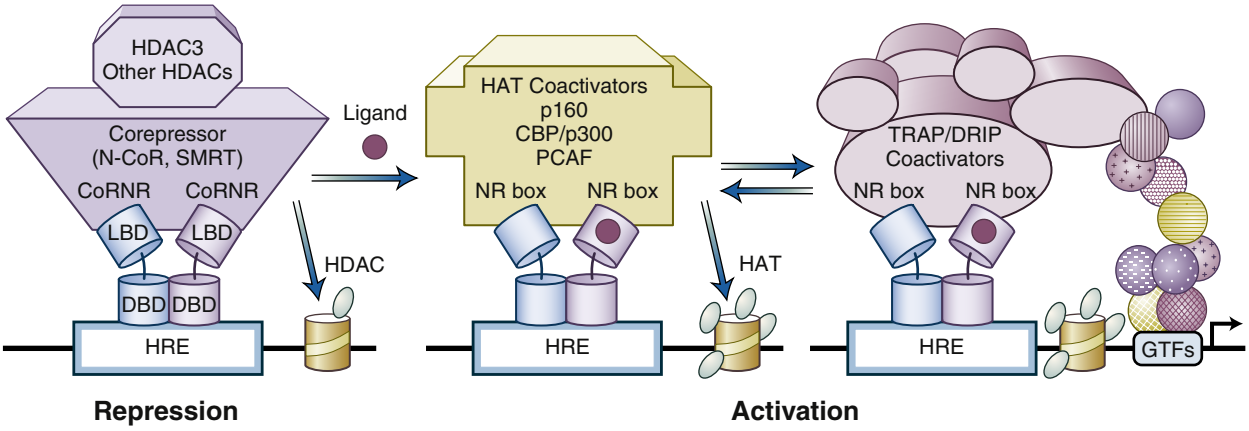
Corepressors

NCoR (nuclear receptor corepressor)
SMRT (silencing mediator for retinoid and thyroid hormone receptors)

CBP, CREB-binding protein (CREB = cAMP-response element–binding protein).

Repression of Gene Expression by Unliganded Receptor

Although DNA binding is ligand dependent for steroid hormone receptors, other nuclear receptors are bound to DNA even in the absence of their cognate ligand. In many cases, the unliganded, DNA-bound receptor actively represses transcription of the target gene. By reducing the expression of the target gene, this repressive function of the receptor amplifies the magnitude of the subsequent activation by hormone or ligand. For instance, if the level of gene transcription in the repressed state is 10% of the basal level in the absence of a receptor, hormone activation to 10-fold above that basal level represents a 100-fold difference of transcription rate between hormone-deficient (repressed) genes and hormone-activated genes (Fig. 2.20).[172] This phenomenon helps to explain why loss of hormone production can result in a much more profound phenotype than loss of the receptor. For example, hypothyroidism due to thyroid gland dysfunction or ablation or iodine



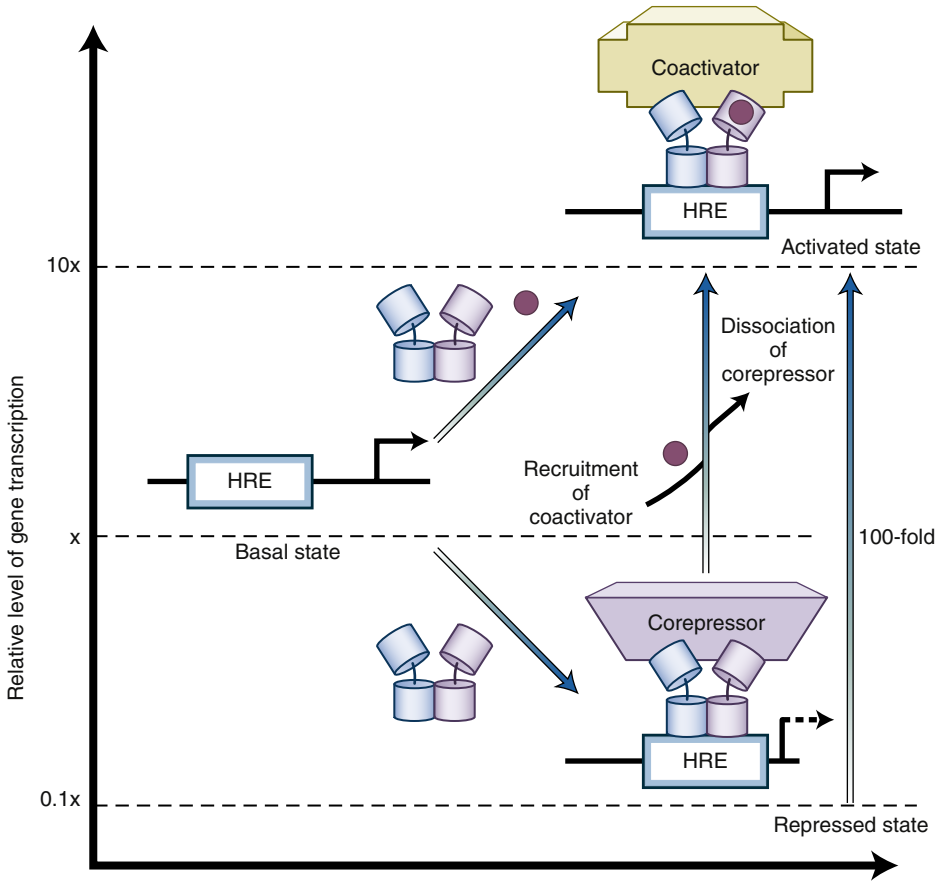
• **Fig. 2.19** Coactivators and corepressors in transcriptional regulation by nuclear receptors. *CBP,* CREB-binding protein; *CoRNR,* coreceptor nuclear receptor box; *DBD,* DNA-binding domain; *DRIP,* vitamin D receptor–interacting protein; *GTFs,* general transcription factors; *HAT,* histone acetyltransferase; *HDAC,* histone deacetylase; *HRE,* hormone response element; *LBD,* ligand-binding domain; *N-CoR,* nuclear receptor corepressor; *NR,* nuclear receptor; *PCAF,* CBP/p300-associated factor; *SMRT,* silencing mediator of retinoid and thyroid receptors; *TRAP,* thyroid hormone receptor–associated protein.

deficiency leads to severe consequences, up to and including cretinism, coma, and death. This is true in mice as well as people. On the other hand, mice lacking all thyroid hormone receptors are relatively mildly affected, with only moderate growth and fertility issues.[173,174] This discrepancy can be attributed to the fact that an unliganded receptor in the hormone deficient state exerts unchecked repressive activity, which has more severe consequences than loss of both repressive and activating functions of THR.

In many ways, the molecular mechanism of repression is the mirror image of ligand-dependent activation. The unliganded nuclear receptor recruits negatively acting coregulators, called *corepressors*, to the target gene. The two major corepressors are large (approximately 270 kDa) proteins: nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT, also known as NCoR2).[175] NCoR and SMRT specifically recognize the unliganded conformation of nuclear receptors and use an amphipathic helical sequence similar to the NR box of coactivators to bind to a hydrophobic pocket in the receptor.

For corepressors, the peptide responsible for receptor binding is called the *CoRNR box* (see Fig. 2.17C), and it contains the sequence (I or L)xx(I or V)I, in which I is isoleucine, L is leucine, V is valine, and xx represents any two amino acids.[176] The receptor uses helices 3 to 5 to form the hydrophobic pocket, as in coactivator binding, but H12 does not promote and even hinders corepressor binding. This highlights the role of the ligand-dependent change in the position of H12 as the switch that determines repression versus activation by nuclear receptors (see Fig. 2.19).[177]

The transcriptional functions of NCoR and SMRT are the opposite of those of the coactivators. The corepressors themselves do not possess enzyme activity, but they recruit histone deacetylases (HDACs) to the target gene, thereby reversing the effects of histone acetylation described earlier and leading to a compact, repressed state of chromatin. Although the mammalian genome contains multiple HDACs, several of which may play a role in nuclear receptor function, the main one involved in repression is HDAC3, whose enzyme activity depends on interaction with NCoR or SMRT.[178] The ability of NCoR to bind and activate HDAC3 is required for normal metabolic and circadian physiology.[179] The corepressors interact directly with GTFs to inhibit their transcriptional activities, and they also exist in large, multiprotein complexes whose range of functions is not fully understood. Biology is complex, however, and recent studies suggest that in brown adipose tissue, HDAC3 foregoes its corepressor role and acts as a coactivator in concert with the nuclear receptor ERRα. In this case, the effect of HDAC3 is mediated at least in part by deacetylation of the key coactivator protein PGC-1, rather than a histone.[180]



• **Fig. 2.20** Repression and activation functions augment the dynamic range of transcriptional regulation by nuclear receptors. The magnitudes of activation and repression were arbitrarily set at 10-fold for this theoretical example. In cells, these magnitudes vary as a function of coactivator and corepressor concentration and in a target gene–specific manner. *HRE,* hormone response element.

Ligand-Dependent Negative Regulation of Gene Expression: Transrepression

The ligand-dependent switch between repressed and activated receptor conformations explains how nuclear receptor ligands activate gene expression. However, many important gene targets of such hormones are turned off in the presence of the ligand. This is referred to as *ligand-dependent negative regulation of transcription,* or *transrepression,* to distinguish it from the repression of basal transcription by unliganded receptors.

The mechanism of negative regulation is less well understood than ligand-dependent activation, and there may in fact be several mechanisms. One mechanism involves nuclear receptor binding to DNA-binding sites that reverse the paradigm of ligand-dependent activation (i.e., negative response elements). For example, when the unliganded thyroid receptor binds to the negative response element of the genes for the β-subunit of TSH or TSH-releasing hormone, transcription is activated,[181] although more recent studies suggest that the role and recruitment of coregulators in this process are complex.[182] In other cases, negative regulation may result from nuclear receptors that bind to, and inhibit, other transcription factors without binding DNA. This interaction leads to redistribution of coactivators from the other transcription factors that positively regulate the gene. Recent evidence supports this model, whereby inhibition of the activity of the positively acting factors results in the observed negative regulation.[183,184] Nuclear receptors can also lead to inhibition of gene expression indirectly by activating a gene that encodes a transcriptional repressor.

Roles of Other Nuclear Receptor Domains

The N-terminal A/B domain of the nuclear receptors is the most variable region among all members of the superfamily in terms of length and amino acid sequence. Subtypes of the same receptor often have completely different A/B domains, and the function of this domain is the least defined. It is not required for unliganded repression or for ligand-dependent activation. In many receptors, the A/B domain contains positive transcriptional activity, often referred to as activation function 1 (AF1) (see Fig. 2.16). Its activity is ligand independent, but it probably interacts with coactivators and may influence the magnitude of activation by agonists or partial agonists. This AF is tissue specific and tends to be more important for steroid hormone receptors, whose A/B domains are notably longer than those of other members of the superfamily.[185] The F domain of the nuclear receptors is hypervariable in length and sequence, and its function is not known.

Cross-Talk With Other Signaling Pathways

Hormones and cytokines that signal through cell surface receptors also regulate gene transcription, often by activating protein kinases that phosphorylate transcription factors such as CREB. Such signals can also lead to phosphorylation of nuclear receptors. Multiple signal-dependent kinases can phosphorylate nuclear receptors, leading to conformational changes that regulate function.[186] Phosphorylation can lead to changes in DNA binding, ligand binding, or coactivator binding, depending upon the specific kinase, receptor, and domain of the receptor that is phosphorylated. The properties of coactivators and corepressors are also regulated by phosphorylation.[187]

TABLE 2.9 Factors Modulating Receptor Activity in Different Tissues

- Concentration of receptor
- Cell specificity
- Variation within a given cell type
- Post-translational modification of receptor (e.g., phosphorylation)
- Regulation of intracellular ligand levels (see Table 2.5)
- Tissue-specific factors that open chromatin
- Function of ligand
  - Agonist
  - Partial agonist
  - Antagonist
- Concentration and types of coregulators
  - Coactivators
  - Corepressors

Receptor Antagonists

Certain ligands function as receptor antagonists by competing with agonists for the ligand-binding site. In the case of steroid hormone receptors, the position of H12 in the antagonist-bound receptor is not identical to that in the unliganded receptor or in the agonist-bound receptor. In antagonist-bound steroid receptors, H12, which has a sequence that resembles the NR box, binds to the coactivator-binding pocket of the receptor and thereby prevents coactivator binding.[188] This antagonist-bound conformation of the receptor also favors corepressor binding to steroid hormone receptors.

Tissue Selectivity of Ligands Interacting With Nuclear Receptors

Many endogenous hormones that act through nuclear receptors do so in a tissue-specific manner. The most obvious mechanism is differential expression of the receptors, both in space (e.g., cell type specificity)[189] and time (e.g., circadian variation).[190] Ligand levels may be regulated intracellularly (see earlier discussion and Table 2.5), and post-translational modification (e.g., phosphorylation) influences cell-specific receptor function. Although nuclear receptors bind at thousands of sites on genomic DNA, the specific binding sites are regulated in a cell type–specific manner. For example, the estrogen receptor binds to overlapping but clearly different sets of genomic sites in the uterus and in the breast, probably because of the differential actions of so-called pioneer transcription factors, which open tightly compacted chromatin in a tissue-specific manner and enable nuclear receptor and other transcription factors to bind.[191]

Some ligands function as antagonists in certain tissues but as full or partial agonists in others. These selective receptor modulators include compounds such as tamoxifen, a selective estrogen receptor modulator (SERM). SERMs are estrogen receptor antagonists with respect to the functions of AF2, including coregulator binding, and they require the AF1 for their agonist activity.[192] This agonism, like AF1 activity, tends to be tissue specific and therefore has great therapeutic utility.[193] Table 2.9 summarizes factors contributing to the tissue specificity of receptor activity.

Nongenomic Actions of Nuclear Receptor Ligands

Some actions of steroids and other nuclear receptor ligands occur within seconds or minutes, far quicker than should be possible

using the transcriptional mechanisms described in the chapter. This suggests that some traditional nuclear receptor ligands may have a discrete set of nongenomic actions. There is now reasonable evidence that thyroid hormone, estrogen, androgen, and possibly other ligands can bind and activate receptors outside of the nucleus. In most cases, these extranuclear receptors are splice variants of the same genes that encode the traditional nuclear receptor, often with loss of the DNA binding domain and NLS. Binding of ligands to these receptors causes activation of many classic signaling pathways, such as Src, ERK, and Akt pathways.[194] In some cases there may be binding of ligands to receptors that are completely unrelated to the nuclear receptors, though this is less clear. The nongenomic and genomic actions of ligands may act cooperatively (e.g., by causing phosphorylation of the nuclear form of the receptor).[195]

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The complete list of references is available online at ExpertConsult.com.

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