
2 Receptors

Molecular Mediators of Hormone Action

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1. INTRODUCTION

The appropriate proliferation and differentiation of cells during development and the maintenance of cellular homeostasis in the adult require a continuous flow of information to the cell. This is provided either by diffusible signaling molecules or by direct cell–cell and cell–matrix interactions. All cells utilize a wide variety of signaling molecules and signal transduction systems to communicate with one another, but within the vertebrate endocrine system, it is the secreted hormones that are classically associated with cellular signaling. Hormones are chemical messengers produced from the endocrine glands that act either locally or at a distance to regulate the activity of a target cell. As discussed in detail elsewhere within this volume, prominent groups of hormonal agents include peptide hormones; steroid, retinoid, and thyroid hormones; growth factors; cytokines; pheromones; and neurotransmitters or neuromodulators.

Endocrine signaling molecules exert their effects by interacting with specific receptor proteins that are generally coupled to one or more intracellular effector sys-

tems. The presence of an appropriate receptor therefore defines the population of target cells for a given hormone and provides a molecular mechanism by which the hormone elicits its biologic actions. These hormone receptor proteins are the focus of this chapter. Section 2 considers general concepts of receptor action, including receptor structure, interaction with the hormone ligand, activation of cellular effector systems, and receptor regulation. Sections 3 and 4 then examine the major families of hormone receptors, grouped with respect to their structures and signaling properties, in greater detail, using specific examples that illustrate the general features of each family. Finally, Section 5 discusses some of the endocrinopathies that result from known alterations in hormone receptor structure or function.

2. GENERAL ASPECTS OF RECEPTOR ACTION

2.1. Receptors as Mediators of Endocrine Signals

The concept that hormone action is mediated by receptors is most often attributed to the work of Langley on the actions of nicotine and curare on the neuromus-

cular junction (Langley, 1906). Langley referred to the target for these compounds as the “receptive substance,” and postulated that “it receives the stimulus, and by transmitting it, causes contraction,” a clear description of the role of receptors in mediating the actions of signaling molecules. Of course, Langley could not know the nature of these receptive substances, suggesting only that they were “radicals of the protoplasmic molecule.” Indeed, despite a wealth of physiologic evidence in support of the receptor concept, firm biochemical evidence for the existence of specific receptors was not forthcoming until radiolabeled hormones became available. For hormones unable to traverse the cellular membrane, such as the polypeptide hormones, specific binding sites could be demonstrated, but their membrane association and low abundance precluded their characterization for many years. Only with the advent of molecular cloning techniques was it possible to establish firmly the structures of the hormone receptors, and to show that these proteins could, in effect, convert a nontarget cell into a target cell by conferring to the cell the ability to bind and, in some cases, appropriately respond to the corresponding hormone.

A second key event in the development of the receptor concept was the demonstration by Sutherland (1972) that cyclic adenosine monophosphate (cAMP) could mediate the intracellular effects of many different hormones, establishing the notion of “second messengers” and providing a cogent molecular explanation of how hormones and hormone receptors might elicit their widespread effects on cellular activity. The notion that many different signaling molecules, working through distinct receptors, could stimulate a common intracellular signaling pathway, together with the subsequent discovery of additional effector enzymes and intracellular second messengers, provided a rational explanation for the tremendous diversity in cellular responses to hormones.

At about the time that second-messenger systems were being discovered, different concepts were evolving on the mechanism of action of the small lipophilic steroid hormones. Radiolabeled steroids, such as estrogen, were synthesized by Jensen and others and were found to accumulate preferentially in known target organs for the hormone (Jensen and Jacobson, 1962). Specific steroid-binding proteins were subsequently identified, and the important finding that binding of agonists and antagonists to these receptors correlated with their biologic activity provided evidence that they were direct mediators of steroid action. These steroid receptors were shown to be soluble intracellular proteins, differentiating them from the membrane-associated receptors for the polypeptide hormones, and

facilitating their early purification, beginning with the identification of the estrogen receptor by Toft and Gorski (1966). The findings that the steroid receptors associated with chromatin in the cell nucleus and that steroid hormones affected RNA and protein synthesis provided the basis for models of direct genomic actions of the hormone-receptor complex that we now know to be correct.

2.2. Membrane-Associated vs Intracellular Receptors

Substances that act as signaling molecules have extremely diverse structures and chemical properties. They include complex multisubunit proteins such as follicle-stimulating hormone (FSH), small peptides such as somatostatin, steroids such as estrogen, amino acid derivatives such as thyroid hormone or the catecholamines, vitamin derivatives such as the retinoids or calcitrol, fatty acid derivatives such as prostaglandins, and simple compounds such as nitric oxide (NO), to provide but a few examples. Despite this structural complexity, most hormones act on target cells through one of two basic mechanisms. Small lipophilic molecules such as the steroids, retinoids, calcitrol, and thyroid hormone are able to enter cells directly by diffusion across the lipid bilayer, where they bind to intracellular receptors that mediate their subsequent action by directly regulating gene expression. By contrast, hydrophilic molecules such as the protein and peptide hormones must bind to their receptor at the cell surface, and the receptors are therefore typically integral membrane proteins that activate intracellular signal transduction cascades. Thus, it is common to differentiate between membrane-bound and intracellular receptors when considering mechanisms of hormone action.

The consequence of hormone-receptor interaction is the activation of pathways that lead to an appropriate biologic response in the target cell, an area referred to as a signal transduction, which is discussed in depth in Chapter 3. Briefly, most intracellular receptors, such as the steroid hormone receptors, are ligand-activated transcription factors that directly regulate the transcriptional activity of target genes. By contrast, cell-surface receptors transduce a signal to the cell interior, directly or indirectly altering the activity of proteins within the cell. This action often involves the enzymatic generation of a second messenger, and the activation of protein phosphorylation or dephosphorylation cascades. More important, the eventual consequence of the activation of enzymatic signaling pathways by cell-surface receptors is often a change in gene transcription; conversely, changes in enzymatic

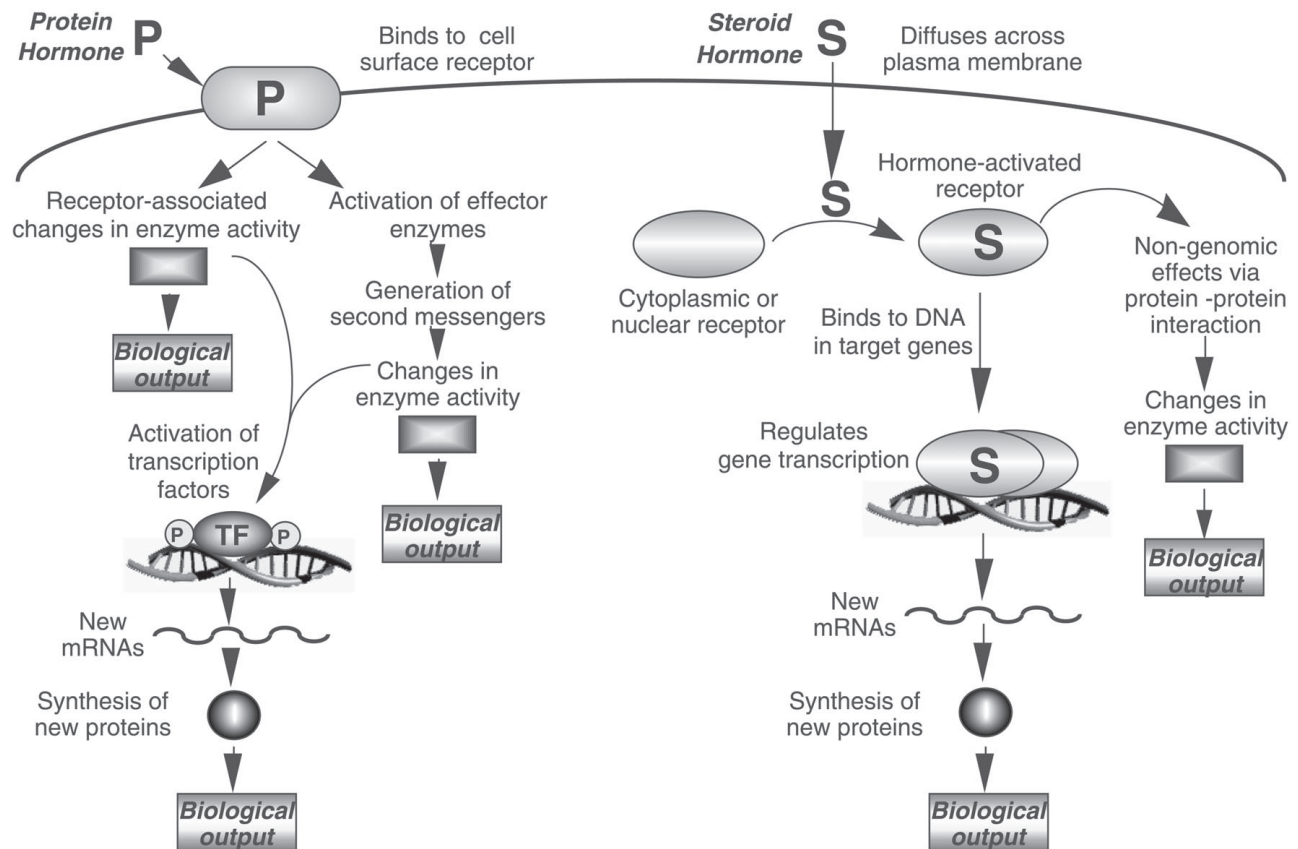


Fig 1. Hormonal signaling by cell-surface and intracellular receptors. Protein hormones (P) bind to cell-surface receptors that activate intracellular effector enzymes, often leading to the production of second messengers or the initiation of protein phosphorylation cascades. A longer-term effect is gene transcription and new protein synthesis. By contrast, steroid hormones (S), or other lipophilic ligands, bind to intracellular receptors that are ligand-activated transcription factors (TF) that directly mediate gene transcription, leading to new protein synthesis.

activities often follow as a consequence of altered gene activity when intracellular receptors are activated. Furthermore, there are many emerging examples of “cross talk” between cell-signaling pathways (Dumont et al., 2001). Finally, as our understanding of the complexity of receptor signaling increases, exceptions to these general modes of action of membrane-bound vs intracellular receptors are being identified. Thus, many of the historical mechanistic boundaries between cell-surface and intracellular receptors are breaking down. Nonetheless, these categories provide a useful way of organizing thinking about receptor action and are therefore used to classify the receptors in this chapter. Figure 1 illustrates some of the similarities and differences in hormone signaling through intracellular vs cell-surface receptors.

The membrane-associated receptors are an extremely diverse group of signaling molecules. Their common feature is the presence of one or more hydrophobic

domains that span the cell membrane and anchor the receptor at the cell surface. Extracellular regions of the protein often participate in hormone binding, and intracellular sequences generally either have direct enzymatic function or associate with intermediary proteins or effector enzymes. The functional cell-surface hormone receptor is often composed of multiple protein subunits that may play a role in hormone binding, signaling, or both activities. It is clear that the receptors for structurally and functionally diverse hormones can be closely related to one another, most commonly within their intracellular domains. Thus, a limited number of basic signaling strategies are used repetitively by a very large number of receptor proteins, defining distinct receptor families, which are considered in Section 3.

The intracellular receptors for the steroid hormones were the first to be biochemically purified. Following the molecular cloning of the receptors for all of the classic steroid hormones (Evans, 1988) it became clear that

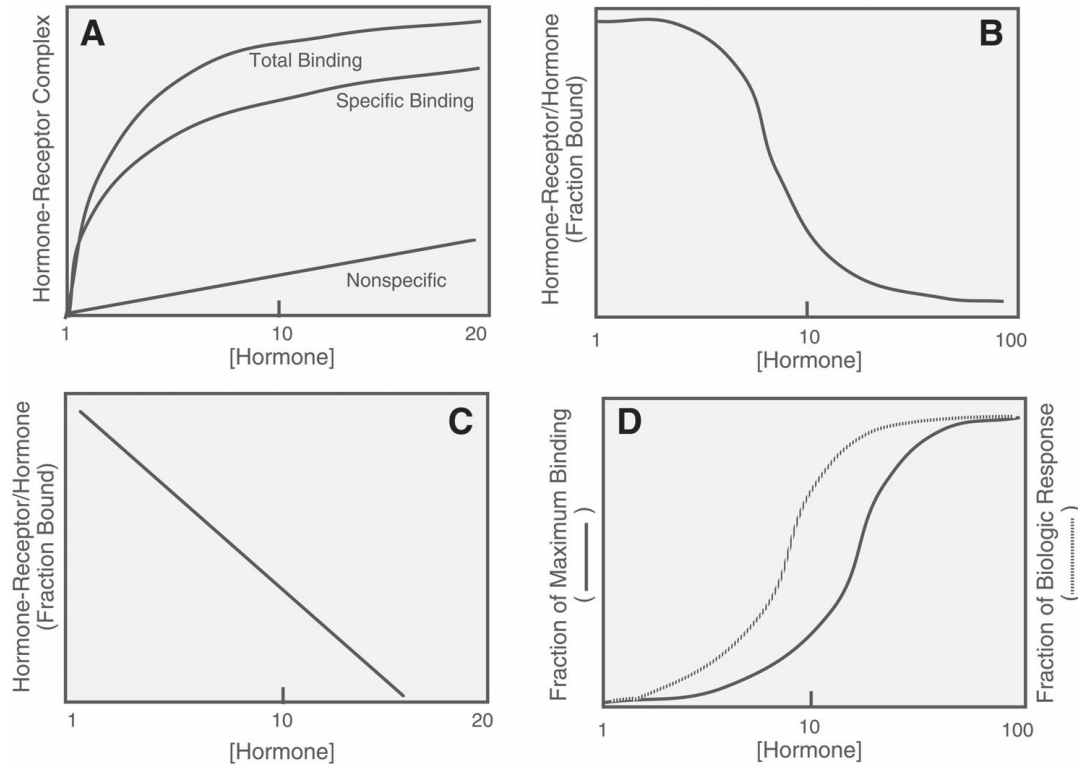


Fig. 2. Measurement of hormone-receptor interactions by binding analysis: (A) saturable binding to a target cell preparation as concentration of radiolabeled hormone is increased; (B) competition or displacement experiment in which binding of a radiolabeled tracer to the receptor is competed as excess unlabeled hormone is added; (C) Scatchard linear transformation of binding data, from which receptor number and affinity for hormone can be determined; (D) consequence of spare receptors. The biologic response is fully activated when only a fraction of the receptors is occupied by hormone. See text for details.

these receptors comprise a family of highly related proteins consisting of distinct structural domains. The highly conserved central DNA-binding domain (DBD) is generally flanked by a carboxyl-terminal region that is necessary for hormone binding by one or more additional domains that are important for activation of target gene transcription. The subsequent identification of the thyroid hormone, retinoic acid, and vitamin D receptors placed them in this nuclear receptor superfamily, along with an expanding number of “orphan receptors” that act in a ligand-independent fashion or for which appropriate ligands have not yet been identified (Willson and Moore, 2002). Not all intracellular receptors are structurally related to the steroid hormone receptors, some examples being the NO and arylhydrocarbon receptors.

2.3. Measurement of Receptor-Ligand Interaction

Hormone receptors are most commonly detected and measured using direct binding assays. This requires a labeled ligand, usually a radiolabeled hormone such as a tritiated steroid or an iodinated peptide; a source of the

receptor being analyzed, typically tissue extracts, cells, or cell membranes; and a physical means of separating the bound hormone from that which is unbound, commonly centrifugation or filtration techniques. As shown in Fig. 2A, when increasing amounts of a labeled polypeptide hormone are added to a constant amount of a cell preparation, the fraction of hormone bound to the cell surface increases and eventually approaches a maximum. In addition to specific binding to its cell surface receptor, the hormone can bind nonspecifically to other cellular constituents or to the reaction vessel, and this component is commonly measured by performing the binding reaction in the presence of a large excess of unlabeled hormone, to ensure complete displacement of the labeled hormone from specific sites. Specific binding is then established by subtracting the nonspecific binding from the total binding.

In a useful variation of this assay, a competition binding experiment, a constant, small amount of the labeled hormone is mixed with cells or cell membranes in the presence of increasing concentrations of unlabeled hormone. A typical competition or displacement curve re-

sulting from this type of experiment is shown in Fig. 2B. The binding that remains at high hormone concentrations represents the nonspecific binding, and the hormone concentration at which 50% of the radiolabeled tracer is displaced approximates the affinity constant of the receptor for its ligand, as described next.

The interaction of the hormone and its receptor can be described by the equation $H + R \rightarrow [HR]$, in which H is the free hormone, R is the free receptor, and HR is the hormone-receptor complex. Binding is assumed to be reversible, and the steady-state dissociation constant $K_d = [H][R]/[HR]$ provides a measure of the affinity of the receptor for its ligand. The total amount of receptor can be described as that which is free plus that which is liganded ($[R_T] = [R] + [HR]$), and the two equations together can be written in the form $[HR] = [R_T][H]/[H] + K_d$. When $[HR]$ is plotted as a function of $[H]$, the hyperbolic plot shown in Fig. 2A is obtained. The previous equation can be rearranged to the form $[HR]/[H] = [R_T]/[H] + K_d$, and when $[HR]/[H]$ is plotted as a function of $\log[H]$, the sigmoidal curve shown in Fig. 2B is obtained. Scatchard (1949) described a commonly used linear rearrangement of this relationship, $[HR]/[H] = -1/K_d [HR] + R_T/K_d$, and as shown in Fig. 2C, the plot of $[HR]/[H]$ as a function of $[HR]$ provides the total receptor number, R_T as the intercept with the abscissa, and the affinity constant, K_d , is derived from the slope, which is $-1/K_d$. The linear relationship shown in Fig. 2C becomes curvilinear when multiple binding sites with differing affinities are present or when cooperative binding interactions occur.

Most commonly, the K_d values for hormone receptors are near the physiologic concentration of the relevant hormone, meaning that fluctuations in the hormone level can elicit both positive and negative responses with respect to receptor activation. However, in many cases it has been shown experimentally that occupancy of only a small fraction of the cell-surface receptors is needed to elicit a maximal biologic response. The term spare receptors has evolved to describe this phenomenon, which is illustrated in terms of binding and activation curves in Fig. 2D. The ability of a small fraction of occupied receptors to stimulate fully a biologic response points to the tremendous amplification potential of the enzyme effectors that act downstream of the hormone receptors. Spare receptors may allow a response to be kinetically favorable even at very low hormone concentrations.

2.4. Cellular Signal Transduction Themes

Hormones, particularly those that act on cell-surface receptors, most commonly initiate a cascade of signaling events in their target cell, ultimately leading to an

appropriate biologic response. These cascades are referred to as signal transduction pathways, and although they are extremely diverse with respect to the specific molecules involved, they do have several common features. It is becoming clear that in many systems, homo- or heterodimerization of receptor proteins in response to binding of the hormone ligand is a key event in initiating cell signaling. This can lead to direct activation of a receptor's enzymatic function, or to the direct or indirect recruitment of additional effector proteins with enzymatic function. Through the actions of soluble second-messenger molecules, or through the direct enzymatic functions of the receptor or its associated proteins, protein kinases, protein phosphatases, or proteases are often activated and act to initiate cascades of protein modification that can be extraordinarily complex. Some of the modified target proteins play direct and rapid roles in the cellular response (e.g., an ion channel) whereas other modified target proteins play indirect or delayed roles in the cellular response (e.g., a transcription factor). Another common feature is the presence of regulatory mechanisms that attenuate the response to hormone and reset the signaling system. Chapter 3 provides a comprehensive treatment of signal transduction, and, therefore, only a few key points necessary for the subsequent discussion of hormone receptors and their actions are introduced in this section.

One can consider three broad classes of signal transduction themes downstream of cell-surface receptors: activation of second-messenger signaling pathways by G protein-coupled receptors (GPCRs), activation of tyrosine kinase or serine/threonine kinase receptors or receptor-associated proteins by growth factor and cytokine receptors, and activation of protein proteolysis or cell localization pathways by developmentally important receptors. Each of these is very briefly reviewed here and is considered in more detail in the following sections.

GPCRs are the largest single family of receptors in vertebrates, and derive their name from their common mechanism of action, which is to activate one or more G proteins following hormone stimulation. The G proteins are a large family of guanosine 5'-triphosphate (GTP)-binding proteins that are heterotrimeric, consisting of α -, β - and γ -subunits, and serve to stimulate or repress the activity of multiple cellular effector enzymes (Gilman, 1987). Some 16 distinct G protein α -subunits define major categories of cell responses; for example, stimulatory (G_s) and inhibitory (G_i) G proteins activate or repress, respectively, the enzyme adenylyl cyclase and thereby regulate production of the second-messenger cAMP (Schramm and Selinger, 1984), and G_q and G_{11} class G proteins regulate phospholipase C β activity

and control the production of inositol phosphate, diacylglycerol, and calcium second messengers (Berridge, 1993). Both the α and $\beta\gamma$ G protein subunits regulate enzyme effectors, and a cycle of GTP binding and subsequent hydrolysis to guanosine 5'-diphosphate (GDP) controls the subunit association and activity of these proteins. The second messengers that are produced as a result of G protein activation serve to regulate the activity of cellular protein kinases, such as the activation of cAMP-dependent protein kinase (protein kinase A [PKA]) and PKC by cAMP and calcium/phospholipids, respectively.

A second common signaling theme involves cell-surface receptors that have intrinsic protein kinase activity or that recruit and activate protein kinases. Three subclasses of these receptors can be considered. A large and historically important group is the growth factor receptors, which have cytoplasmic domains with intrinsic protein tyrosine kinase activity. On hormone binding, the receptor rapidly forms a dimer, and the cytoplasmic kinase domains rapidly cross-phosphorylate each other on tyrosine residues, which allows for the subsequent binding of a broad repertoire of cellular proteins that are involved in transducing the signal. A more recently characterized group is the transforming growth factor- β (TGF- β) superfamily receptors, which have cytoplasmic domains with intrinsic protein serine/threonine kinase activity. Dimerization of a hormone-binding type II receptor and a signaling type I receptor leads to the phosphorylation of target proteins of the Smad family, which transduce the signal. Finally, the cytokine receptors are not themselves protein kinases, but when activated they recruit soluble protein tyrosine kinases of the Janus kinase (Jak) family that phosphorylate both the receptor and receptor-associated effector proteins of the Stat family, which transduce the signal. Also in the same broad category are receptors that are protein tyrosine phosphatases, although much less is known about these receptors and their activation.

A third broad and emerging category includes pathways downstream of a variety of developmentally important signaling molecules such as the Wnt, Hedgehog, and DSL family ligands. The details are quite specific to each pathway, but common themes include retention of a key transcription factor or coregulator required for target gene expression in the cytoplasm until ligand activates the receptor, a switch from target gene repression to activation, and involvement of irreversible proteolysis as a key regulatory step.

2.5. Mechanisms of Receptor Regulation

It is clear that mechanisms must exist to regulate the levels of, or activity of, cell-surface receptors, allowing

for modulation or termination of the response to the hormone. In some cases, the biosynthesis of receptors is tightly regulated so as to generate additional receptors when they are required. For example, the low-density lipoprotein (LDL) receptor gene is activated by a protein that acts as both a sterol sensor and transcription factor, resulting in enhanced production of the receptor when sterol levels are low (Brown and Goldstein, 1999). In other cases, the degradation of receptors, and their corresponding ligands, is tightly regulated. Many hormones that act on cell-surface receptors are internalized through the process of receptor-mediated endocytosis, which is shown in Fig. 3A. The hormone-receptor complex is internalized into clathrin-coated vesicles that are acidified and lose their clathrin coat to form endosomes, where the low pH often results in hormone-receptor dissociation. The ligand is most commonly degraded in lysosomes, effectively removing the signal from the extracellular environment. The receptor typically has one of two fates: recycling to the cell surface, where it is again available to interact productively with hormone (e.g., the insulin receptor); or degradation in the lysosome (e.g., the epidermal growth factor receptor [EGFR]). In either case, internalization effectively reduces the number of cell-surface receptors, and the process is therefore a mechanism utilized to down-regulate cell-surface receptors.

A second common mode of regulation targets the ability of cell-surface receptors to bind the hormone or to subsequently transduce a signal. Probably the best-studied example of this is agonist-induced desensitization of the G protein-coupled β -adrenergic receptor. In the continual presence of the agonist epinephrine, the cAMP signal is attenuated, although the receptor continues to bind epinephrine. Desensitization is a reversible process, and it is mediated by phosphorylation of the receptor on cytoplasmic serine and threonine residues. Some of this phosphorylation is catalyzed by PKA, which is activated in response to the epinephrine-induced cAMP signal and acts in a feedback manner to downregulate the signaling pathway. Because activated PKA has the ability to phosphorylate many receptors, potentially attenuating their activity, this phenomenon is referred to as heterologous desensitization. The agonist-occupied β -adrenergic receptor is also phosphorylated by a very specific cellular kinase called the β -adrenergic receptor kinase (β ARK), a member of a broader family of GPCR kinases, or GRKs (Clairg et al. 2002). These kinases are broadly expressed and likely to play an important role in the desensitization of many hormonal responses mediated by GPCRs. Because β ARK targets only the β -adrenergic receptor, this phenomenon is referred to as homologous desensitization. The phosphorylated β -adr-

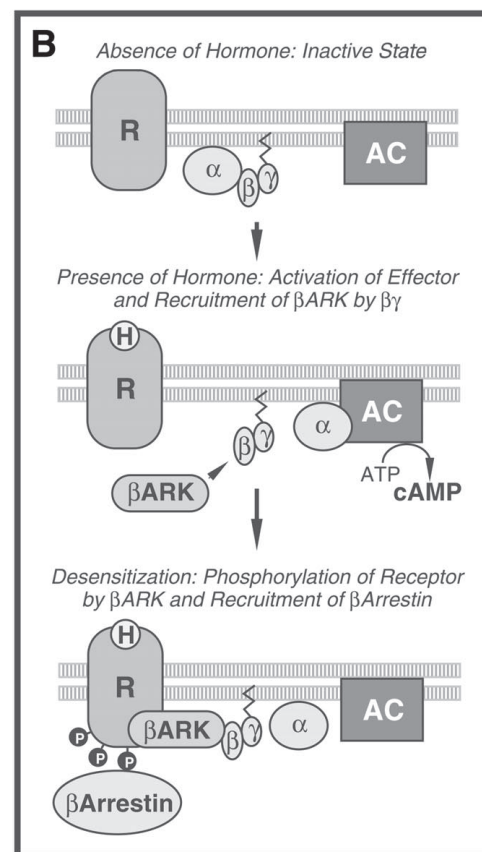
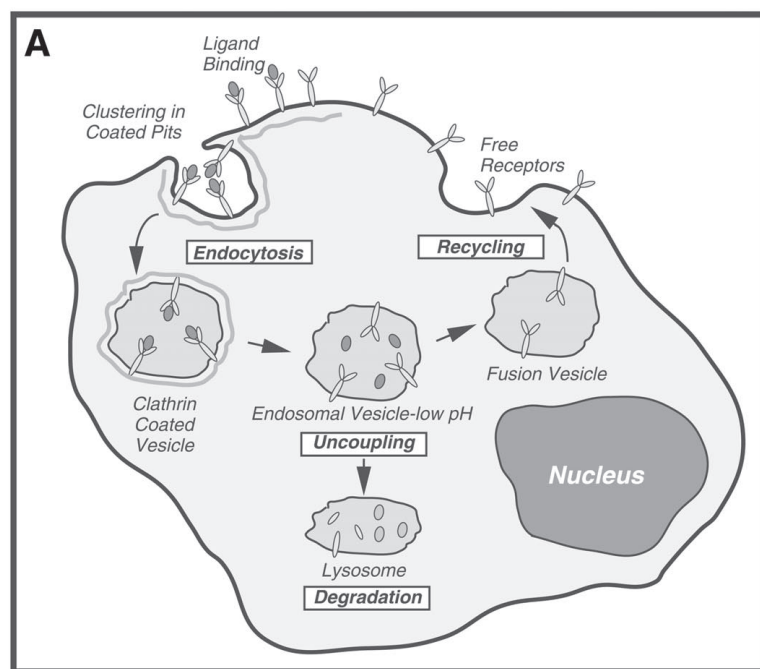


Fig. 3. Mechanisms for regulation of cell-surface receptors. **(A)** Process of receptor-mediated endocytosis. Following ligand binding, receptors rapidly move into coated pits and are internalized by endocytosis. The resulting clathrin-coated vesicles are acidified in an endosomal compartment, leading to dissociation of the ligand from the receptor. Vesicular sorting leads to a segregation of the ligand into vesicles that will fuse with primary lysosomes, resulting in degradation or utilization of the ligand. The receptor either can be degraded, as often occurs for signaling receptors, or it can be recycled to the cell surface, as often occurs for transport receptors. **(B)** Steps involved in desensitization of β -adrenergic receptor. In the continual presence of hormone, the G protein $\beta\gamma$ -subunits recruit the β -adrenergic receptor kinase to the membrane, leading to specific phosphorylation of the receptor and an attenuation of signaling. The phosphorylated receptor is recognized by β -arrestin, further suppressing the signaling pathway. The pathway shown is homologous desensitization. As discussed in the text, heterologous desensitization involving receptor phosphorylation by PKA also occurs. ATP = adenosine triphosphate.

energetic receptor is unable to couple efficiently to its G protein, resulting in the observed attenuation of the signal. In addition, many phosphorylated GPCRs, including the β -adrenergic receptor, bind proteins of the arrestin family, which serve to suppress further the signal by promoting uncoupling of the receptor and its G protein (Claing et al., 2002). Some of the regulatory processes involved in receptor desensitization are outlined in Fig. 3B. Desensitization is a major factor controlling the efficacy of action of many therapeutic agents that target GPCRs such as the β -adrenergic receptor. Although negative regulatory mechanisms for other classes of receptors have not been studied in the same depth as those for the GPCRs, they certainly exist, and several are referred to in subsequent sections.

3. CELL-SURFACE RECEPTORS

3.1. Receptors Coupled to G Proteins

The GPCRs comprise the largest known family of cell-surface hormone receptors, and it is estimated that 50% of prescription drugs target GPCRs. Hundreds of these receptors have already been cloned and characterized, many of them orphan receptors of unknown function that represent significant targets for future drug development. Their common features are seven hydrophobic potential membrane-spanning domains, and the ability to stimulate the exchange of bound GDP for GTP on associated G protein α -subunits in response to agonist binding. They are also known as the heptahelical or serpentine receptors because of their mem-

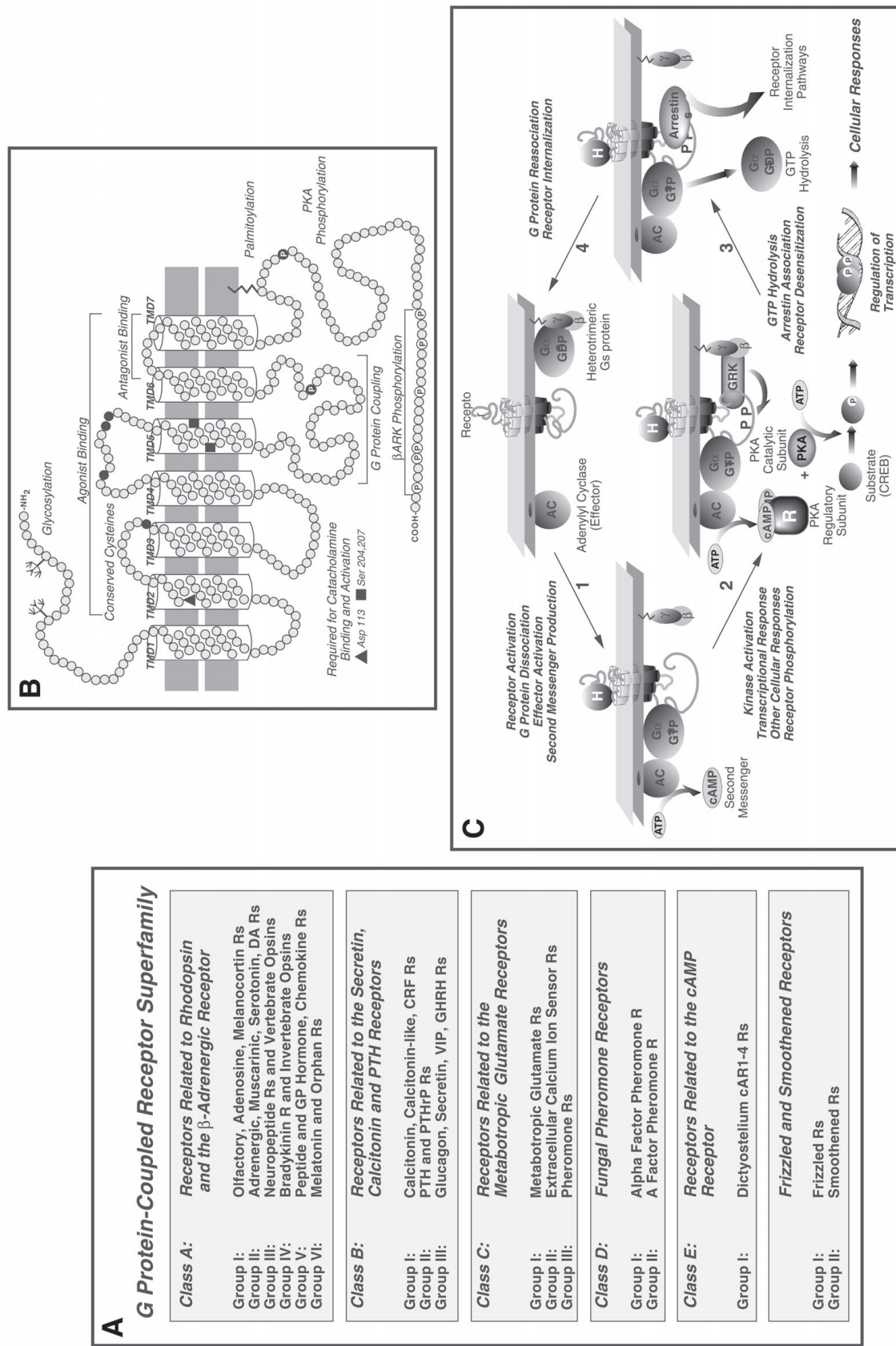


Fig. 4. Structure and signal transduction by GPCRs. (A) Major families and groups of GPCRs (GPCR Database; Horn et al. 2003). The mammalian receptors are largely confined to families A, B, and C. Family A is the largest and includes the diverse odorant receptors as well as prototypic GPCRs such as rhodopsin and the β -adrenergic receptor. (B) Schematic structure of one of the most extensively characterized GPCRs, β -adrenergic receptor. Major structural features are indicated and are expanded on in the text (After Duhlman et al., 1991). (C) Model for signaling through a GPCR. A receptor coupled to the cAMP pathway is illustrated, and CREB is used as an example of one substrate for PKA. This model is generic, and not all aspects will apply to all systems.

brane topology. As indicated in Fig. 4A, the GPCR superfamily can be divided into several major families that share significant sequence similarities. The three predominant mammalian families are class A (receptors related to rhodopsin and the β_2 -adrenergic receptor), class B (receptors related to the secretin, calcitonin, and parathyroid hormone [PTH] receptors), and class C (receptors related to the metabotropic glutamate and pheromone receptors). Further subdivisions into groups are indicated in Fig. 4, as are receptor classes D–E. Class A is by far the largest family of GPCRs, and its prototypes, rhodopsin and the β_2 -adrenergic receptor, are among the best-characterized receptors; therefore, much of what is known about the structure, function, and regulation of GPCRs in general comes from studies of these model proteins.

Tremendous diversity is a hallmark of the GPCR superfamily. Many of the neurotransmitter and peptide hormone receptors are encoded by multiple genes that produce related yet distinct receptors. For example, five distinct somatostatin receptors have been characterized. These are expressed with unique but overlapping tissue and cell specificity, and they are able to mediate signaling through several different G proteins. An extreme example of this is represented by the olfactory odorant receptors, a family that likely numbers in the hundreds. Other receptors are produced from a single gene, but alternative RNA processing results in the generation of multiple receptor isoforms, either produced in specific tissues or able to couple differentially to G protein-mediated signaling pathways. For example, the dopamine D2 receptor exists as two splice variants expressed in unique tissue-specific patterns, and the pituitary adenylate cyclase activating peptide receptor exists as five splice variants that differ in their ability to activate adenylate cyclase vs phospholipase C β (PLC β) effectors. Further diversity may be generated by recent findings that both homo- and heterooligomerization of GPCRs is common and can impact function, potentially generating receptors with novel properties through interaction of characterized receptors with known properties (George et al., 2002).

The major structural features of a model GPCR, the β_2 -adrenergic receptor, are shown in Fig. 4B. The extracellular domain is relatively short, although for some class A receptors, those for the glycoprotein hormones FSH, luteinizing hormone, and thyroid-stimulating hormone, this domain is more than 300 amino acids long. There are typically several sites for asparagine-linked glycosylation within the extracellular domain. The seven membrane-spanning domains of the receptor create three extracellular loops and three cytoplasmic loops that can be quite variable in length among different

receptors. The carboxyl-terminal cytoplasmic domain is typically short and is often associated with the plasma membrane through palmitoylation of a conserved cysteine residue. As discussed above, phosphorylation plays an important role in the regulation of receptor activity, and potential sites for phosphorylation by PKA as well as the specific GRK kinases are found within the third cytoplasmic loop and the carboxyl-terminal tail of the receptor.

Mutagenesis has been extensively applied to elucidate features of the β_2 -adrenergic receptor important for ligand interaction and for subsequent G protein coupling. A strategy that has been particularly useful for the analysis of the GPCRs has been the generation of chimeras between two homologous receptors with distinct ligand-binding or signaling properties. Such chimeras between the β_2 -adrenergic and α_1 -adrenergic receptors have implicated the transmembrane domains and associated extracellular loops in specific agonist binding and have shown that transmembrane domain seven is particularly important in specifying antagonist binding. Similar experiments to examine signaling through the stimulation (β_2 -adrenergic receptor) or inhibition (α_1 -adrenergic receptor) of adenylate cyclase by G $_s$ and G $_i$, respectively, led to the conclusion that the regions between transmembrane domains five and six, including the third cytoplasmic loop, were particularly important in determining G protein recognition. Additional mutagenesis has indicated that the carboxyl-terminal domain of the receptor, a major site of potential phosphorylation, is particularly important for desensitization, and that several conserved residues are critical for catecholamine binding and activation, including an aspartic acid residue in transmembrane domain two and two serine residues in transmembrane domain five of the β_2 -adrenergic receptor (Dohlman et al., 1991).

In general, much of what has been learned from model receptors such as the β_2 -adrenergic receptor has been applicable to additional GPCRs. The basic membrane topology of the β -adrenergic receptor, discerned through proteolysis and antibody epitope mapping studies, holds for other members of the superfamily that have been examined. There are differences in ligand-binding determinants, and, in general, receptors that interact with small molecules utilize the transmembrane domain segments more extensively for binding, and receptors that interact with larger peptides or proteins make greater use of the amino-terminal and extracellular loops. An extreme example of this is ligand recognition by the glycoprotein hormone receptors, where the large amino-terminal extracellular domain, expressed independently of the transmembrane domains, has the ability to bind the ligand with high affinity. As additional members of

this family are characterized, novel modes of hormone binding and receptor activation are also being observed. For example, thrombin proteolytically cleaves its GPCR in the extracellular domain, revealing a new N-terminus that acts like a ligand to mediate activation of the receptor.

The mechanisms by which ligand binding leads to an ability of the receptor to interact productively with a G protein remain largely unknown. The first (and to date only) structure for a GPCR is that of rhodopsin (reviewed in Filipek et al., 2003), and this has provided an important foundation for modeling studies of other GPCRs. Based on a wealth of biophysical and mutagenesis data, researchers believe that activation of GPCRs involves disruption of stabilizing contacts between membrane-spanning helices, causing changes in the relative orientation of several of these helices; in particular, there is a movement of transmembrane helix 6 with respect to transmembrane helix 3, opening up the receptor in a manner that is thought to expose cytoplasmic determinants for G protein interaction. As discussed in Section 5, mutations in GPCRs that cause constitutive activity of the receptor are a common cause of disease. In addition, many GPCRs are partially active in the absence of ligand and can be further activated when overexpressed. This has been explained in terms of a two-state model in which an equilibrium exists between a signaling active and inactive state of the receptor. Agonists bind to and stabilize the active conformation, whereas inverse agonists bind selectively to the inactive conformation and stabilize it; this latter class of compounds is a potential target for drug development in regulating the activity of constitutively active mutant receptors (Strange, 2002).

As discussed earlier, the functional commonality among GPCRs is that following hormone binding they interact with the G protein so as to induce the release of GDP from the G protein α -subunit, allowing GTP to bind and facilitating dissociation of the α -subunit from the $\beta\gamma$ complex. Several G protein structures have been solved using crystallography, and these provide substantial molecular detail into how guanine nucleotide binding impacts subunit interactions. Both the free α -subunit and the $\beta\gamma$ complex participate in signaling, activating one or more effector enzymes. Eventually, the intrinsic guanosine 5'-triphosphatase (GTPase) activity of the α -subunit hydrolyzes GTP to GDP, promoting reassociation with the $\beta\gamma$ complex and return to the inactive state. For some G proteins with slow intrinsic GTPase activity, this process is facilitated by GTPase-activating proteins called regulators of G protein signaling. Aspects of signaling by GPCRs are summarized in Fig. 4C, which illustrates the best-studied

signaling pathway activated by this class of receptors, the G_s -stimulated cAMP pathway.

Given the diversity of the GPCRs, it is perhaps not surprising that many endocrinopathies can be attributed to mutations in specific GPCRs (Shenker, 1995). This connection first became apparent through the analysis of G proteins that these receptors interact with. It was demonstrated that mutations that constitutively activate $G_s\alpha$ by inactivating its intrinsic GTPase function were causative in acromegaly in association with pituitary adenoma (a late somatic mutation confined to the pituitary gland) as well as in McCune-Albright disease (an earlier somatic mutation affecting many endocrine systems). Subsequently, mutations in many GPCRs have been identified, both in human diseases and in animal models of human disease. These represent both loss-of-function and gain-of-function mutations in the targeted GPCRs. For example, inactivating mutations of the calcium-sensing receptor cause familial hypocalciuric hypercalcemia in the heterozygous state and neonatal severe hyperparathyroidism in the homozygous state, whereas an activating mutation in this same receptor causes a dominant form of hypocalcemia. In some cases, analysis of these mutations, which generally have a known phenotype, has revealed much about important structural features of these receptors. For example, more than 50 different mutations of the vasopressin V2 receptor in patients with nephrogenic diabetes insipidus have been found, providing a wealth of information on residues essential for hormone binding or activation of this receptor.

3.2. Receptors With Tyrosine Kinase Activity

The receptor protein tyrosine kinases (RTKs) are found in all multicellular organisms and mediate the actions of a broad spectrum of hormones and growth factors on cell growth, metabolism, and differentiation. They can be subdivided into several families, based largely on their structural characteristics. Examples of some of the major subfamilies, by one classification (van der Geer et al., 1994), include the platelet-derived growth factor receptor (PDGFR) subfamily, the fibroblast growth factor receptor (FGFR) subfamily, the insulin receptor subfamily, the EGFR subfamily, the nerve growth factor receptor (NGFR) subfamily, the hepatocyte growth factor receptor (HGFR) subfamily, and a series of additional receptors for which ligands have not yet been identified. Figure 5A shows examples of the major families of RTKs.

The RTKs are type I transmembrane proteins, having an external aminoterminal and a single membrane-spanning domain. The extracellular ligand-binding domains (LBDs), of these receptors are quite distinctive, but one

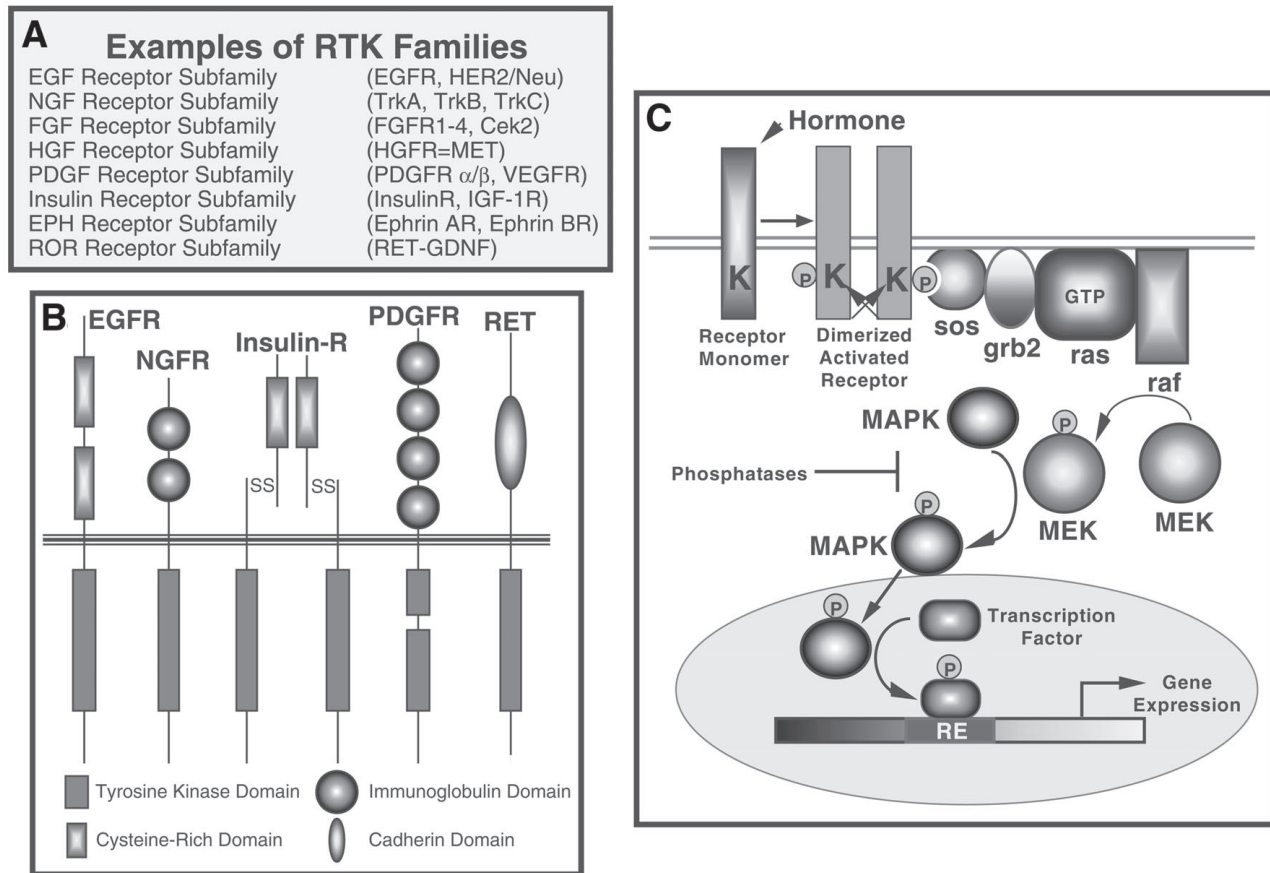


Fig. 5. Structure and signal transduction by RTK: **(A)** major families and specific examples of RTKs; **(B)** schematic of structures of several types of RTKs and key modular domains involved in ligand interaction; **(C)** example of generic signaling through a prototypic RTK, leading to activation of a MAPK signaling pathway as exemplified by ERK kinase.

or more of a variety of recognizable structural motifs appear in most of the LBDs. These domains include cysteine-rich regions, leucine-rich regions, immunoglobulin-related domains, fibronectin type II repeats, and EGF-like repeats, among others (Fig. 5B). For the most part, the precise roles of these extracellular domains in hormone recognition have not been well established, but structural studies are beginning to shed new light on the processes of hormone binding. The cytoplasmic catalytic domain of the RTKs is much more highly conserved, and a number of amino acid residues critical to its enzymatic function are absolutely conserved. Based on the crystallographic structures of several soluble protein kinases as well as the insulin receptor and EGFR tyrosine kinase domains, it appears that the receptor protein tyrosine kinase catalytic domains are likely to consist of two lobes, one that binds Mg^{2+} /adenosine triphosphate (ATP) utilizing a GXGXXG motif, and one that forms the actual catalytic loop. In some receptors in this family, such as the PDGFR, the tyrosine

kinase catalytic domain is interrupted by a spacer region (Fig. 5B).

The RTKs all exhibit a hormone-dependent dimerization that is crucial for subsequent signal transduction. Crystallographic studies of the extracellular domains of the EGFR and its relatives ErbB2 (Neu/HER2) and ErbB3 (HER3) demonstrate that growth factor binding to each receptor monomer promotes a structural rearrangement to expose a dimerization interface. For ErbB2, which does not have a ligand, the dimerization domain is constitutively exposed (Burgess et al., 2003). Some ligands for these receptors are themselves dimers (e.g., PDGF), whereas others are monomers (e.g., EGF), and this may affect the dimerization mechanism. The functional consequence of receptor dimerization is to bring the cytoplasmic tyrosine kinase domains into close proximity, which leads to cross-phosphorylation of the receptors and the subsequent recruitment of substrates having affinity for the tyrosine-phosphorylated receptor. These include a broad reper-

toire of cellular proteins that have an Src homology 2 (SH2) domain that specifically recognizes tyrosine phosphorylation sites on the receptor. Some of these SH2 domain proteins are direct substrates for phosphorylation by the receptor and can serve as effector molecules in transducing the signal. Examples of substrates include the phosphatidylinositol-3-kinase regulatory subunit, the Src family protein kinases, the protein tyrosine phosphatase Syp, and PLC γ . Other SH2 domain proteins act simply as molecular adapters; they bind to the phosphorylated receptor and also associate with additional cellular proteins, commonly through a protein interaction motif referred to as the SH3 domain. Examples include Grb2, Nck, and Crk, which bind to a variety of hormone receptors. Several proteins, including the adapter Shc and the insulin receptor substrate family of docking proteins, utilize a distinct phosphotyrosine-binding domain that recognize an Asn-Pro-x-pTyr motif in target receptors. Yet other adapter and effector proteins in this signaling pathway contain pleckstrin homology domains, which may play a role in tethering these proteins at the plasma membrane, or contain WW protein interaction motifs. Use of modular interaction domains is a common theme in cell signaling and is particularly apparent in the RTKs (Pawson and Scott, 1997).

Adapter proteins mediate a broad spectrum of cellular responses, but a common and well-characterized response leads to activation of the small GTP-binding protein Ras. Ras activation initiates a kinase cascade, the mitogen-activated protein kinase (MAPK) cascade, which eventually results in the phosphorylation and activation of nuclear transcription factors able to alter gene expression in the target cell. At many of the key regulatory steps, specific protein tyrosine phosphatases counter the actions of the kinases and, thus, exert negative control over activation, a likely component of inhibitory feedback mechanisms. A generic example of signaling of an RTK through the MAPK pathway leading to extracellular-regulated kinase (ERK) activation is shown in Fig. 5C.

The physiologic functions of many of the receptors in the tyrosine protein kinase family have been explored through gene disruption approaches in mice, or by the identification of naturally occurring mutations in these receptors in animal models or in people. It should be realized that many of the RTKs were initially discovered as transduced retroviral oncogenes that represent mutated, truncated, or inappropriately expressed versions of their cellular counterparts (Carbone and Levine, 1990). Although a complete discussion of the physiologic functions of receptor tyrosine protein kinases is beyond the scope of this chapter, a few examples related

to several important model receptors (the EGF, PDGF, and insulin receptors) are briefly considered. The EGFR played a key role in defining the relationship of cellular signaling pathways to retrovirus-induced cellular transformation and oncogenesis. A truncated and transduced form of the EGFR represents one of two oncogenes (v-erbB) of the avian erythroblastosis virus, and the highly related Neu tyrosine kinase receptor (also referred to as HER2 or erbB2) is mutated or amplified in a wide variety of human cancers. Thus, inappropriate activation of the signaling pathways mediated by these tyrosine kinase receptors can lead to cell transformation and tumorigenesis. Consistent with this, the PDGFR is involved in a number of reciprocal chromosomal translocations that lead to its constitutive activation and are associated with myeloproliferative disorders. The insulin receptor is subject to mutation in patients with insulin resistance, and this has led to the identification of many different types of mutations that affect the synthesis or function of this RTK. These mutations are associated with type A insulin resistance, leprachauism, and lipoatrophic diabetes.

3.3. Receptors With Serine and Threonine Kinase Activity

Receptors with serine and threonine kinase activity were identified relatively recently, beginning with the expression cloning of the activin type II receptor in 1991 (Mathews, 1994). Activin is a protein hormone in the TGF- β superfamily, and this superfamily includes hormones such as Müllerian-inhibiting substance (MIS), the bone morphogenetic proteins (BMPs), and nonmammalian homologs such as *Drosophila* decapentaplegic (*dpp*) and *Xenopus* Vg-1. Receptor nomenclature has been based on early crosslinking studies with TGF- β that revealed three distinct protein bands designated type I, II, and III receptors. The type III receptor is a large proteoglycan, also known as beta glycan, which is thought to play a role as a coreceptor for TGF- β , inhibin, and possibly other family members by facilitating delivery of the hormone to the signaling receptors. Both the type I and II receptors are involved in signaling. Distinct combinations of type I and II proteins form the functional receptor for each ligand of the TGF- β superfamily, and the relative roles of the two receptor subunits vary somewhat, depending on the ligand with which they interact. Figure 6A shows several representative ligands of the TGF- β superfamily along with the type I and II receptors that mediate their actions.

Both the type I and II receptors have conserved cytoplasmic domains with protein serine or threonine kinase activity. For those ligands most closely related to TGF- β , including activin and nodal, the type II receptor is the

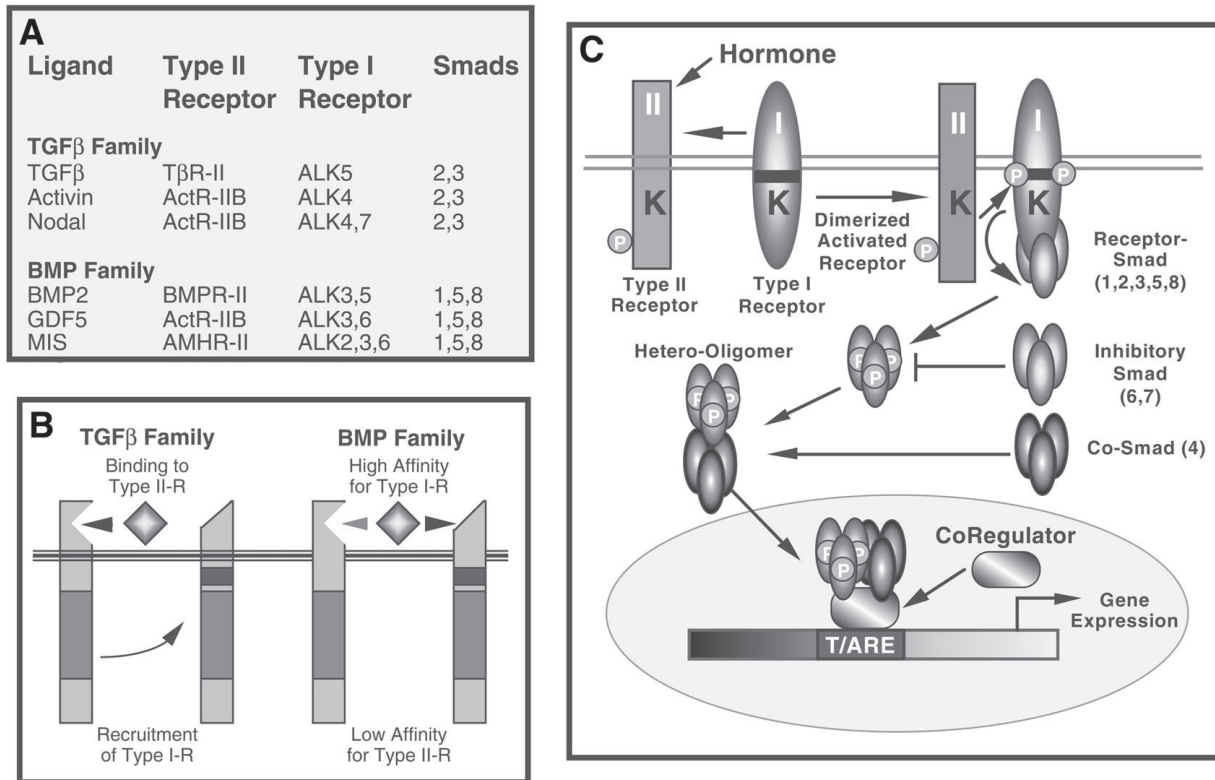


Fig. 6. Structure and signal transduction by receptor serine/threonine kinases: **(A)** representative ligands in TGF- β superfamily, their type II and I receptors, and Smad proteins they activate; **(B)** mechanisms of ligand binding to receptors for the two major subclasses of TGF- β ligands, TGF- β class and BMP class; **(C)** example of generic signaling through the Smad pathway, although this example of ligand binding illustrates initial binding to type II receptor, which is characteristic of TGF- β class.

high-affinity ligand-binding determinant. Binding of hormone allows the type II receptor to interact with the type I receptor, most likely in the form of a heterotetramer that interacts with the homodimeric ligand (Fig. 6B). The type II receptor, which has constitutive kinase activity, then phosphorylates the type II receptor in a serine-rich juxtamembrane region known as the GS box, leading to activation of the type I receptor kinase. By contrast, for ligands related to the BMPs, including the growth and differentiation factors (GDFs) and MIS, it is the type I receptor that has a higher affinity for ligand, although the type II receptor does contribute to ligand binding (Fig. 6B). Oligomerization leads to phosphorylation and activation of the type I receptor. Substantial insight into the interactions between ligand and receptor in this system has emerged from crystallographic and nuclear magnetic resonance structural studies (Shi and Massague, 2003). There appears to be a well-conserved mechanism for binding of BMP-related ligands to the type I receptor, but there is substantial diversity in the way in which TGF- β -related ligands interact with the type II receptor.

The major transducers of signaling downstream of the serine and threonine kinase type I receptors are a family of proteins known as the Smads in vertebrates. The name derives from the related proteins in *Caenorhabditis elegans* (Sma) and *Drosophila* (Mad). There are eight known Smad proteins that play distinct roles in signaling between the cell-surface receptor and the nucleus. Smads 1, 2, 3, 5, and 8 are known as receptor-regulated Smads (R-Smads), and they are direct targets for phosphorylation within a C-terminal SSXS motif by the type I receptor. The TGF- β -related ligands lead to phosphorylation of Smads 2 and 3, and the BMP-related ligands lead to phosphorylation of Smads 1, 5, and 8. The phosphorylated Smad proteins dissociate from the receptor and form heteromeric complexes with a common cytoplasmic Smad protein, Smad4. This heteromeric complex moves to the nucleus and acts to modulate transcription of target genes. Although there is evidence for direct DNA-binding by Smads 3 and 4, the predominant actions of Smads are thought to be mediated by their interaction with nuclear DNA binding proteins and stabilization of ternary DNA-bound complexes

among the R-Smads, Smad-4, and the target transcription factor (Attisano and Wrana, 2000). The *Xenopus* transcription factor FAST-1 was the first of these interacting proteins to be identified, but it is likely that the Smads target many transcriptional regulatory proteins. Smads 6 and 7 are known as inhibitory Smads, and they act to inhibit the activation of the R-Smads by binding to the type I receptor. Smads 6 and 7 are up-regulated by BMP and TGF- β pathway signaling, respectively, and are likely part of a negative feedback system. Finally, there are numerous examples of additional proteins that interact with or modify the activity of the serine and threonine kinase receptors, and signaling crosstalk is likely a very important component in generating specificity of response. Figure 6C summarizes aspects of signaling downstream of serine and threonine kinase receptors.

There are several reports of mutations in these receptors in association with human disease. Mutations in the BMP type II receptor and in ALK1 are found in pulmonary hypertension, and mutations in ALK1 are also found in hereditary hemorrhagic telangiectasia type II. Loss of expression of the TGF- β type II receptor through microsatellite instability or as a result of transcriptional repression is found in a variety of human cancers. Much more frequent are mutations in the downstream Smad proteins, indicating a role for these proteins as tumor suppressors. Smad2 mutations are found in colorectal and lung cancers, and Smad4 mutations are found in many cancers but are particularly prevalent in pancreatic carcinoma, in which Smad4 was first identified as the tumor suppressor deleted in pancreatic cancer (dpc)-4.

3.4. Receptors That Recruit Tyrosine Kinases

Many well-characterized cell-surface receptors lack apparent enzymatic functions within their cytoplasmic regions, and their activity therefore depends on the recruitment of signaling proteins to the receptor. This family includes the receptors for the interleukins (ILs), interferons (IFNs), erythropoietin (EPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), growth hormone (GH) and prolactin (PRL), among others, and is known as the cytokine receptor superfamily (Fig. 7A). The functional receptors include single-chain type I transmembrane proteins (GH, PRL, EPO), those that have a ligand-specific subunit that interacts with a common shared subunit (ILs, GM-CSF, LIF) and those that have multiple unique subunits (IFNs). Some examples of cytokine family receptors of each type are shown in Fig. 7B. Although these receptors all lack tyrosine kinase activity, conserved membrane-proximal regions of their

cytoplasmic domains mediate ligand-dependent interaction with one or more cytoplasmic protein tyrosine kinases, the Janus kinases, or Jaks (Ihle and Kerr, 1995). These kinases, including Jak1, Jak2, Jak3, and Tyk2, have amino-terminal homology domains of unknown function, and two carboxyl-terminal kinase domains, although the first of these lacks critical residues necessary for catalytic activity and is inactive. The Jak kinases are tyrosine phosphorylated and activated following ligand binding to the cytokine receptors, and this requires an association of the Jak kinases with two conserved membrane-proximal motifs in the receptor referred to as box 1 and box 2. It is thought that receptor dimerization brings the associated Jak kinases into close proximity and allows cross-phosphorylation and activation to occur, and, thus, two Jak kinases associate in the same manner as two cytoplasmic kinase domains of the RTKs associate. In addition to Jak kinase phosphorylation, the activation process is associated with phosphorylation of the receptor itself on specific tyrosine residues, which can lead to activation of some of the same pathways activated by the conventional RTKs, such as the MAPK pathway.

The pathway from Jak kinase activation to changes in target cell gene expression was completed through the analysis of proteins mediating the transcriptional responses to IFN, a family of proteins known as the Stat (signal transducer and activator of transcription) proteins (Darnell et al., 1994). Following cytokine receptor phosphorylation by a Jak family kinase, Stat proteins are recruited to the receptor and phosphorylated on a single tyrosine residue near the C-terminal transactivation domain of the protein. The phosphorylated and activated Stat proteins form homo- or heterodimers, translocate to the nucleus, and bind to specific DNA elements (originally called γ IFN activation sequences, or GAS elements) near target genes to regulate transcription. Stat proteins include a highly conserved SH2 domain as well as an SH3-related domain in the C-terminal regions, in addition to the conserved C-terminal tyrosine phosphorylation site, and are reported to interact with a broad spectrum of other transcription factors and coregulators. Seven Stat proteins that fall into three groups have been identified, and it appears that these serve as the major link between cytokine receptor activation and cellular transcriptional responses. There are likely several modes of negative regulation of this pathway, but a major mechanism involves Stat protein induction of the suppressor of cytokine signaling (SOCS) family of proteins, which then act through mechanisms that remain unclear to block Stat signaling, most likely at the level of the receptor or Jak kinase (Cooney, 2002).

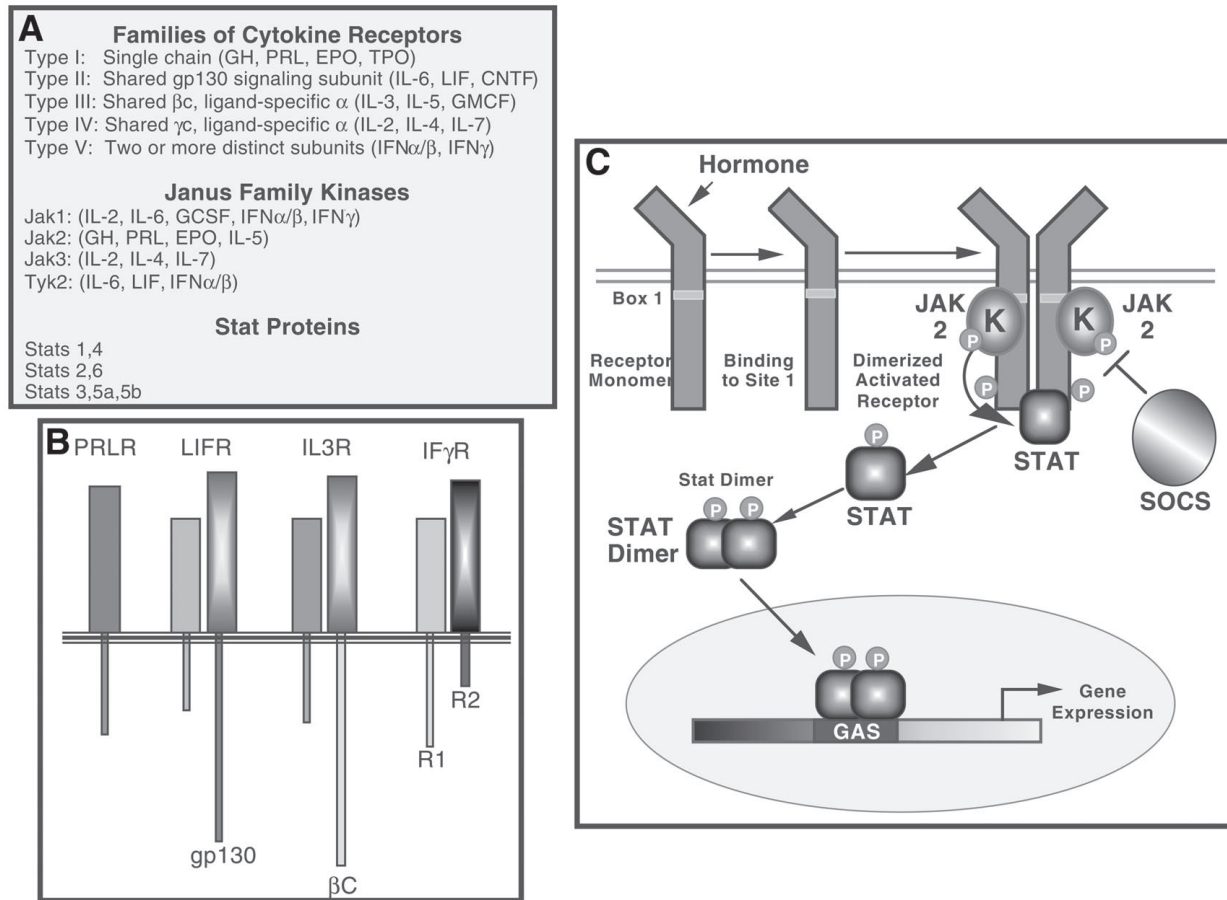


Fig. 7. Structure and signal transduction by cytokine superfamily receptors: (A) examples of some of the major families of receptors, along with Janus family kinases and Stat proteins they activate; (B) representative schematic structures demonstrating subunit composition of various types of cytokine superfamily receptors; (C) example of generic signaling through a type I cytokine receptor to the Jak-Stat pathway.

It is clear that different receptors in the cytokine receptor superfamily preferentially associate with one or more of the four Jak kinases and subsequently preferentially activate one or more of the seven Stat proteins, but the mechanisms of specificity are largely unknown. The various Jaks and Stats have unique tissue distributions, and this likely explains in part why particular receptors signal through particular Jak-Stat pathways. Because the cytokine receptor superfamily is large, and the receptors utilize diverse signaling mechanisms, it is informative to consider one example focused on the endocrine system in greater detail. One of the best-studied receptors in this class is the GHR. It is representative of the single-chain class of cytokine receptor and has the two pairs of conserved cysteines in the extracellular domain that are a hallmark of this family. Interestingly, two receptor chains bind to a single GH molecule at distinct sites on the hormone, and thus a single ligand molecule catalyzes receptor dimerization and subsequent signal

transduction. This has been demonstrated by determination of the crystallographic structure of the extracellular domain of the GHR complexed with GH. Site 1 on GH must be occupied prior to occupancy of site 2. At high concentrations of hormone, saturation of all receptors by binding to site 1 on GH is predicted to inhibit receptor dimerization and the subsequent biologic response. This has been experimentally demonstrated in several systems and may represent an additional mode of receptor regulation. The GHR is unique in that the extracellular domain of the receptor also functions as a soluble serum-binding protein for GH. The extracellular domain is released from the mature receptor by proteolysis in some species, and it is encoded by a distinct mRNA generated by alternative RNA processing in other species. The activated GHR associates with the Jak2 kinase, leading to the phosphorylation and activation of Stats 1, 3, and 5. In addition, the MAPK pathway is also activated by GH, presum-

ably through the interaction of adapter proteins with the phosphorylated GHR, or with Jak2 itself. Signaling through a single-chain cytokine superfamily receptor such as the GHR is illustrated in Fig. 7C.

The GHR is also a good example of the potential involvement of receptors within this superfamily in endocrine disease. Mutations in the GHR/binding protein are responsible for the GH resistance observed in Laron-type dwarfism, in which serum GH is elevated in association with classic symptoms of GH deficiency, including reduced levels of insulin-like growth factor-1 (IGF-1) and short stature. Dozens of distinct mutations in the receptor have been found, and nearly all are in the extracellular domain and result in gross alteration in receptor structure, generally through premature termination of translation. Other cytokine family receptors involved in disease include the leptin receptor, which is mutated in a syndrome of obesity and pituitary dysfunction, and the GCSF receptor, which is truncated in severe congenital neutropenia. A particularly important example is mutation of the common γ c chain of the IL receptor in severe combined immunodeficiency. Not surprisingly, the downstream Jak and Stat proteins are also likely to be targets for mutation in human disease, and Jak2 and Stat5B are both reported to act as fusion partners in chromosomal translocations in particular forms of leukemia.

3.5. Receptors That Activate Developmental Signaling Pathways

Several highly conserved signaling pathways, in addition to those already described, play important roles in the development of metazoan organisms and have common features that warrant their consideration as a single group. These include DSL ligand signaling through the Notch receptor, Wnt signaling through the Frizzled receptor, and Hedgehog signaling through the Patched and Smoothened receptors. In each of these cases, receptor activation is associated with movement of a key regulatory molecule from the cell surface or cytoplasm to the cell nucleus, regulated proteolysis is a key event in transducing the signal and genes that are otherwise repressed become activated through a corepressor/coactivator switch.

The Notch family of receptors (Mumm and Kopan, 2000) are large type I transmembrane proteins with an extracellular LBD. However, the ligands for Notch are themselves cell-surface type I transmembrane proteins of the Delta, Serrate, and Lag2 (DSL) family, so that Notch mediates direct cell-cell signaling. Another surprising feature of the Notch receptor is that the cytoplasmic domain, which has signaling function, appears to act in the nucleus to modulate transcription. In response

to ligand binding, proteases of the presenelin family cleave the Notch receptor, releasing the Notch intracellular domain. This domain has a nuclear localization signal, and it enters the nucleus to form a complex with proteins of the CBF-1, Su(H), Lag-1 family (CBL) and converts these proteins from transcriptional repressors to transcriptional activators, stimulating target gene expression. Notch signaling is illustrated in Fig. 8A.

The Frizzled family of receptors (Wodarz and Nusse, 1998) bind to a large array of secreted Wnt ligands to mediate diverse effects on cell proliferation and differentiation. The Frizzled receptors are seven-membrane-spanning domain receptors and thus can be classed with the GPCRs, but there is still no strong evidence to indicate that these receptors are in fact G protein coupled. The best, although still incompletely, characterized pathway of Wnt signaling involves the stabilization of cytoplasmic β -catenin. In the absence of Wnts, β -catenin is phosphorylated by the kinase GSK3, which triggers its ubiquitination and degradation. This occurs within a multiprotein complex, and the activity of this complex is modulated by the Dishevelled protein, which is genetically downstream of Frizzled receptor signaling. In the presence of a Wnt ligand, the activity of the complex promoting β -catenin degradation is repressed. The net effect is an increase in cytoplasmic β -catenin levels, allowing β -catenin to enter the nucleus and interact with the transcription factor TCF/LEF. This interaction promotes the recruitment of transcriptional coregulators and stimulates the expression of Wnt target genes. This pathway of Wnt signaling is illustrated in Fig. 8B. In addition, there are reports of Wnt signaling through pathways as diverse as Jun N-terminal kinase activation and calcium mobilization.

The Hedgehog family of ligands, which in vertebrates includes Sonic, Indian, and Desert Hedgehog, signals through a mechanism that is similar in many respects to the Wnt pathway. In this example, the Hedgehog binding receptor, Patched, is a 12-transmembrane domain protein that modulates the activity of a coreceptor, the 7-transmembrane domain Smoothened receptor (Ingham and McMahon, 2001). There is no strong evidence for G protein coupling of the Smoothened protein, and it does not interact directly with the ligand Hedgehog. Instead, Hedgehog binding to Patched appears to relieve Patched-mediated repression of Smoothened, allowing activation of Smoothened and transduction of the signal. Events immediately downstream of Smoothened are unknown but act on a multiprotein complex to modify the proteolytic processing of a key signal transducer, a zinc-finger protein known as Ci (in *Drosophila*) or Gli (in vertebrates). In the absence of a Hedgehog signal, Ci/Gli is processed

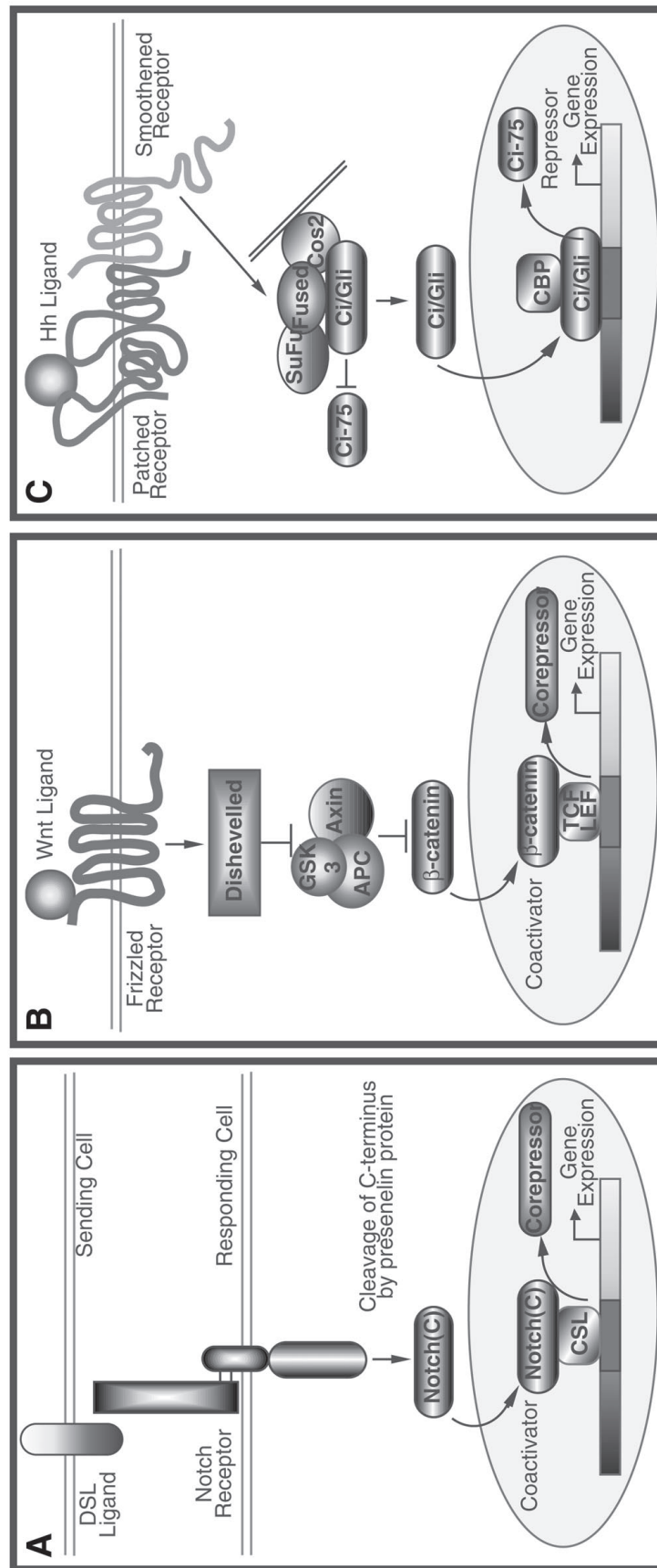


Fig. 8. Key developmental signaling pathways and their receptors. **(A)** signaling through Notch receptor in response to cell-bound DSL ligand; **(B)** Wnt signaling through Frizzled receptor; **(C)** Hedgehog signaling through Patched receptor and Smoothened coreceptor. These are generic pathways and in each case represent the best characterized of several signaling pathways that are activated by these ligands and receptors. The three pathways shown have many common features, as discussed in the text.

to a truncated form that enters the nucleus and acts to suppress Hedgehog target genes. When Smoothed is activated, the full-length form of Ci/Gli is stabilized, and it enters the nucleus and promotes transcription of Hedgehog target genes. Signaling through the Hedgehog pathway is summarized in Fig. 8C.

Although mutational studies of these related pathways have been largely the domain of model organisms, it is becoming appreciated that these receptors and signaling pathways are of key importance to development and organogenesis in vertebrates. Mutations in the Notch ligand JAG1 are causative in arteriohepatic dysplasia. A Frizzled-4 receptor mutation has been reported in familial exudative vitreoretinopathy. Mutations in the Patched receptor and, as documented in a few reports, the Smoothed coreceptor are a major cause of basal cell carcinoma.

3.6. Receptors With Protein Tyrosine Phosphatase Activity

A large number of protein tyrosine phosphatases have been identified by molecular cloning, and their structures suggest that at least seven of these are transmembrane proteins with the potential to act as ligand-regulated phosphatases (Fischer et al., 1991). They are distinct from the serine and threonine protein phosphatases and might be expected to participate in cellular signal transduction by countering the activities of the RTKs. Despite the striking similarity of these transmembrane protein tyrosine phosphatases to receptors, distinct ligands that might activate these proteins have not been identified. Some of these proteins have extracellular domains that include regions resembling segments of fibronectin or neural cell adhesion molecule, suggesting that the transmembrane protein tyrosine phosphatases might be involved in signaling in response to direct cell-cell communication or cell-matrix interaction.

The best characterized of the potential receptor protein tyrosine phosphatases is the leukocyte common antigen CD45. It consists of an external segment that is cysteine rich and resembles a ligand-binding motif, a single transmembrane domain, and two tandem cytoplasmic protein tyrosine phosphatase domains. Multiple forms of CD45 arise through alternative RNA-processing mechanisms, and these are differentially glycosylated and expressed on distinct subsets of lymphocytes. CD45 is known to be essential for antigen-stimulated proliferation of T-lymphocytes and for thymic development, and mutations in CD45 are implicated in autoimmunity. Recent studies indicate that members of the Src family of cytoplasmic protein tyrosine kinases are likely to be the major physiologic substrates for the CD45 tyrosine phosphatase.

3.7. Receptors With Guanylyl Cyclase Activity

As their name implies, the receptors in this small family have cytoplasmic domains that possess guanylyl cyclase (GC) activity, promoting the production of a cyclic guanosine 5'-monophosphate (cGMP) second messenger. They are distinct from, but related to, the soluble GCs. They are single-chain transmembrane proteins with an extracellular LBD, a membrane proximal cytoplasmic domain that resembles a protein kinase domain but is catalytically inactive, and the carboxyl-terminal GC domain (Garbers, 1999). There are seven identified transmembrane GCs. GC-A, GC-B, and GC-C all represent receptors for natriuretic peptides or heat-stable enterotoxins. The GC-A receptor binds atrial natriuretic peptide as well as the related BNP, GC-B binds the natriuretic peptide CNP, and GC-C binds heat-stable enterotoxin and probably binds the novel peptide guanylin. GC-D is expressed in olfactory sensory neurons; GC-E and GC-F are expressed within the eye; and GC-G is expressed in lung, intestine, and skeletal muscle. These latter four remain orphan receptors with no known ligand and suggest a subfamily of GC receptors (D, E, and F) restricted to sensory tissues. Interestingly, the GC receptors appear to exist in a dimeric or tetrameric state prior to activation. Ligand binding is thought to induce a conformational change that brings the catalytic guanylyl cyclase domains into proximity to form an active dimer. Phosphorylation strongly modifies receptor activity, although the kinase responsible has not been identified.

3.8. Tumor Necrosis Factor Receptors

A large and diverse family of receptors mediate the actions of the tumor necrosis factors (TNFs). There are 18 identified TNF family ligands that promote cell proliferation, cell survival, or cell death, and, remarkably, they bind to at least 30 distinct receptor proteins (Gaur and Aggarwal, 2003). Ligands appear to be able to bind to multiple receptors, and the receptors are expressed in a tissue-specific fashion. The receptors have homologous extracellular domains and can be divided into two broad groups based on the structure of their cytoplasmic domain. At least six receptors in the TNF family have an intracellular death domain. The death domain is responsible for recruiting a broad spectrum of intracellular signaling proteins that eventually result in the activation of caspases and initiation of the apoptotic response. TNF receptors that lack the death domain can also induce apoptosis, although the signaling mechanisms involved are not clearly understood.

A second major signaling pathway activated by all TNF receptors is the nuclear factor- κ B (NF- κ B) path-

way. This is mediated by the recruitment of TNF receptor-associated factors (TRAFs), of which at least six are activated in a ligand- and receptor-specific fashion. TRAFs in some manner lead to activation of the I κ B kinase, phosphorylation and degradation of the inhibitor I κ B, and release of NF- κ B to enter the nucleus, where it regulates genes that suppress apoptosis and are involved in inflammatory responses. TNFs induce the NF- κ B pathway in all cells, whereas they stimulate cell death in a smaller subset of cells. At least two TNF family receptors are implicated in human disease. Mutations in TNF-R1 cause a familial periodic fever syndrome, and mutations in the receptor Fas are found in malignant lymphoma and in autoimmune lymphoproliferative syndrome type I.

3.9. Transport Receptors

A significant number of cell-surface receptors may not have classic signaling functions but, rather, serve to transport substances into the cell from the plasma. These include the receptors for transferrin, LDLs, asialoglycoproteins, mannose-6-phosphate (M-6-P), and other plasma proteins. These receptors bind their ligand, cluster into coated pits, and are rapidly endocytosed into endosomal or lysosomal vesicles, where the ligand can be dissociated and utilized, and the receptor either degraded or recycled back to the cell surface, as described in Fig. 3A. Several of these receptors have proven to be important models for investigating the cell biology of cell-surface receptor trafficking. Two receptors that exemplify this family of transport receptors, the LDL receptor and the M-6-P/IGF-2 receptor, are discussed subsequently.

The LDL receptor extracellular domain consists of a cysteine-rich and negatively charged ligand-binding region, an EGF precursor homology domain, and a juxtamembrane domain rich in O-linked glycosylation sites (Brown and Goldstein, 1986). The cytoplasmic domain is short and has no obvious signaling features but is critical for clustering the receptor into coated pits for subsequent endocytosis, and eventual lysosomal hydrolysis of the cholesterol esters of LDL, to generate cellular stores of cholesterol. The importance of this receptor in the transport of LDLs is underscored by the finding of many different defects in the LDL receptor in patients with the disease familial hypercholesterolemia, in which plasma levels of cholesterol are highly elevated. These mutations affect nearly all aspects of receptor function, including synthesis, transport, binding, and internalization of the receptor.

The M-6-P/IGF-2 receptor is referred to as a multifunctional protein because it is known to bind at least two ligands utilizing distinct sites on the extracellular

domain of the receptor (Kornfield, 1992). The receptor is a large protein of 275 kDa, more than 90% of which is extracellular. Fifteen conserved repeat sequences of approx 150 amino acids, each with eight conserved cysteines and 16–38% overall identity, constitute the large extracellular domain of the receptor, and the cytoplasmic domain is small and includes multiple potential phosphorylation sites. The receptor binds proteins containing M-6-P and is important for both the sorting of newly synthesized lysosomal enzymes and the endocytosis of extracellular lysosomal enzymes. A second M-6-P receptor, the cation-dependent receptor, is a 46 kDa protein that has an extracellular domain that is a single copy of the conserved repeat sequence of the larger M-6-P/IGF-2 receptor, and it is important for the sorting of newly synthesized lysosomal proteins. Although IGF-2 binds to the M-6-P/IGF-2 receptor with high affinity at a site distinct from the M-6-P-binding site, the role of this receptor in IGF-2 signaling remains uncertain, and it has been suggested that IGF-2 may instead act through the IGF-1 receptor, a member of the RTK family. An additional feature of interest regarding the M-6-P/IGF-2 receptor is that the gene encoding this receptor is subject to genomic imprinting effects, and only the maternal allele is expressed.

4. INTRACELLULAR RECEPTORS

4.1 Nuclear Receptor Superfamily

The receptors in the nuclear receptor superfamily are structurally and functionally distinct from the cell-surface receptors, in that they are located in either the cytoplasm or nucleus, where they bind hydrophobic ligands that are able to diffuse passively through the plasma membrane of the cell. In response to hormone binding, these receptors undergo a conformational change and become competent to bind to specific DNA elements that act as hormone-dependent enhancers of gene expression. The steroid hormones and their receptors are considered in greater detail in Chapter 4; the intent of this section is to contrast briefly the structure and function of the intracellular receptors with the cell-surface receptors, rather than to review this class of receptors comprehensively.

The nuclear receptor superfamily includes the receptors for the classic steroid hormones such as estrogens, progestins, androgens, and glucocorticoids; the receptors for thyroid hormones, retinoids, and vitamin D; and a large number of orphan receptors that share basic features of the superfamily but that have no known activating ligand. Many receptors initially identified as orphans are now known to have specific ligands or physiologic functions (Willson and Moore, 2002). Some of the ~50 vertebrate nuclear receptors are listed in Fig. 9A. Most

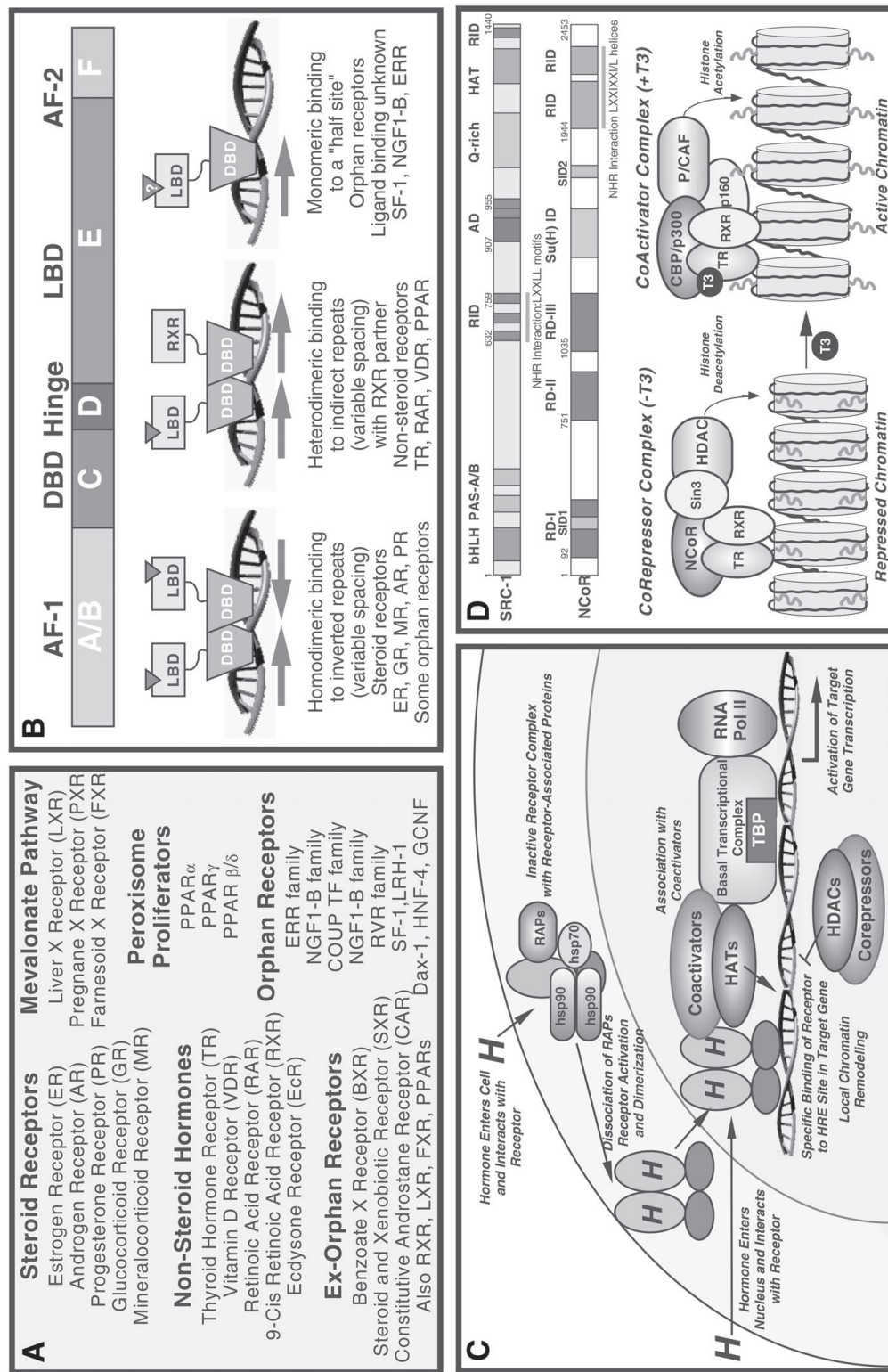


Fig. 9. Structural features and mechanism of action of nuclear receptors. **(A)** Examples of major classes of nuclear hormone receptors, including orphan receptors. **(B)** General features of nuclear receptors. The predominant domains are the DBD, the LBD and the transactivation domains AF-1 and AF-2, as discussed in the text. Also indicated in **(B)** are three potential modes of DNA binding. Some orphan receptors bind DNA in a monomeric form; steroid receptors bind DNA as homodimers; and thyroid, retinoid, and vitamin D receptors bind DNA as heterodimers with the partner RXR. Arrows indicate the number and orientation of core motifs in the DNA-binding site. **(C)** Generic model for signal transduction by steroid hormones and related compounds. The unliganded receptor can be either cytoplasmic or nuclear and is often found in a complex with chaperone proteins. Hormone binding leads to the dissociation of these chaperones and the formation of receptor dimers competent to bind to DNA. Binding of the receptor at HREs alters target gene transcription through interactions with the basal transcriptional complex that are likely to be mediated by coactivator proteins, which also serve to remodel chromatin. **(D)** Schematic structures of a representative coactivator (SRC-1) and corepressor (NcoR) protein, illustrating the distinct nuclear hormone receptor (NHR) interaction motifs. The bottom half of the panel is a schematic of the switch between corepressors and coactivators induced by binding of some hormones to their nuclear receptors, in this example, the TR.

of the nuclear receptors have a distinct and common structure composed of six conserved domains (A–F), shown schematically in Fig. 9B. The defining feature of these proteins is the presence of a highly conserved DBD that includes two zinc fingers of the Cys-4 type. Sequences within this domain, particularly those near the end of the first zinc finger and the beginning of the second zinc finger, are important for determining the DNA-binding specificity of each receptor. These receptors bind to specific DNA elements near target genes termed *hormone response elements* (HREs), which are usually either direct or inverted repeats of a 6-bp core consensus sequence, separated by a variable-length spacer, the combination of which is unique for each receptor class. Sequences within the DBD and the adjacent C-terminal domain are also required for nuclear localization of the receptor, and for dimerization. The receptors bind to HREs as either monomers (some orphan receptors such as NGFI-B), homodimers (steroid receptors such as the glucocorticoid receptor [GR] and estrogen receptor [ER]), or heterodimers (the thyroid hormone receptor [TR], retinoic acid receptor [RAR], and vitamin D receptor [VDR]). In the latter class, the retinoid X receptor (RXR) serves as a common heterodimerization partner. These modes of DNA binding are illustrated in Fig. 9B. There is a region of highly variable length and sequence N-terminal to the DBD, and in many receptors this region includes a domain important for transcriptional activation termed activation function-1 (AF-1). The C-terminal region of the receptor includes the specific LBD, as well as a second transactivation domain, AF-2, and regions necessary for dimerization.

In the absence of hormone, the steroid, thyroid, and retinoid receptors appear to be localized predominantly in the nucleus. Some, particularly those that partner with RXR, may be bound to the DNA in the absence of ligand. An exception is the GR, which appears to be predominantly cytoplasmic in the absence like the GR of hormone. Unliganded steroid hormone receptors are associated with an array of chaperone proteins such as the heat-shock proteins hsp90 and hsp70, and the immunophilin FKBP52. These chaperones likely play an important role in the folding of the receptor protein during synthesis, in the maintenance of the inactive receptor in a high-affinity steroid-binding conformation, and in the transformation to the active state of the receptor following hormone binding. A model depicting the dissociation of receptor-associated chaperone proteins on hormone binding, the dimerization of the receptor, its binding to HREs in DNA, and the regulation of target gene transcription by the hormone-activated receptor is presented in Fig. 9C.

Transcriptional control of gene expression by the nuclear receptors requires their interaction with coactivators or corepressors that stimulate or repress, respectively, expression of the target gene. The first coactivator identified was steroid receptor coactivator-1 (SRC-1), and many such proteins have now been identified (McKenna and O'Malley, 2002). Coactivators do not bind directly to DNA but likely facilitate interactions between the DNA-bound nuclear receptor and the basal transcription complex. Some coactivators have intrinsic histone acetyltransferase (HAT) activity, whereas others recruit HATs to the gene promoter. Acetylation of amino-terminal lysine residues of the core histone proteins of nucleosomes loosens their association with DNA and is associated with enhanced transcriptional activity. Conversely, deacetylation of histones is associated with a compacted chromatin structure and transcriptional repression, and several corepressor proteins exert their actions by recruitment of histone deacetylases (HDACs) to the gene promoter. Major corepressor proteins involved in steroid hormone action are the nuclear receptor corepressor (NCoR) and the silencing mediator of retinoic and thyroid receptors (SMRT). These coregulatory proteins play key roles in mediating NHR signaling. Figure 9D shows schematic structures of a key coactivator (SRC-1) and corepressor (NCoR) for nuclear receptor action. In many examples, the unliganded receptor is maintained in the repressed state through the actions of corepressors and the liganded receptor becomes active through both loss of corepressor interactions and establishment of coactivator interactions. This is best described for the non-steroid nuclear receptors such as TR and RAR. This concept as applied to thyroid hormone action is illustrated in Fig. 9D.

Many steroid hormone antagonists exert their actions by promoting a receptor conformation that favors interaction with corepressor proteins, whereas agonists promote interaction with a coactivator. The cellular composition and concentration of coregulatory proteins therefore have important influences on steroid hormone action, and this finding has been exploited in the development of selective steroid receptor modulators, drugs that act as agonists in some tissues and antagonists in others. Crystallographic structures of nuclear receptor LBDs, in the unliganded state and bound to agonists or antagonists, provide a likely explanation for these findings, in that the ligand induces structural changes, particularly in helix 12 within the AF-2 domain, that promote interaction with either a coactivator or corepressor dependent on the bound ligand.

There are mechanisms by which some of the steroid receptors, and other nuclear receptors, can be activated

in a ligand-independent fashion (Weigel and Zhang, 1998). Stimulators of cellular kinase activity (such as cAMP) or inhibitors of cellular phosphatase activity (such as okadaic acid) can activate some of the nuclear receptors in the absence of any ligand. For example, the PR, ER, and VDR can all be activated by the neurotransmitter dopamine in a manner that does not involve binding to the receptor, and the ER can be activated by a variety of polypeptide growth factors. In other cases, ligand-independent activation is not observed. The pathways mediating this activation appear to involve both receptor and coactivator phosphorylation. The steroid receptors are phosphoproteins, and some receptors such as the PR are inducibly phosphorylated on ligand binding by a variety of cellular kinases. Ligand-independent activation of these receptors may provide an important mechanism of cross talk between the steroid receptor pathway and other cellular signal transduction pathways. In addition, not all of the effects of steroid hormones are mediated by the nuclear receptors, and there is ample evidence for “nongenomic” actions of these hormones.

The nuclear receptors play critical roles in endocrine communication, and it is therefore not surprising that receptor mutations are associated with a range of endocrine disorders. Resistance to thyroid hormone can involve mutation or deletion of the TR β gene, and familial glucocorticoid resistance can be caused by deletions or point mutations in the GR gene. Hypocalcemic vitamin D-resistant rickets results from mutations in the VDR that can affect hormone binding, DNA binding, or nuclear translocation of the receptor. More than 200 distinct mutations in the AR have been found in patients with androgen insensitivity syndromes. A single example of mutation of the ER gene in a man with estrogen resistance has been reported, but no mutations of the PR in humans are known. Although these latter findings might imply lethality of ER or PR mutations, disruption of these genes in mice results in animals that live to adulthood, although they have impaired reproductive function. RAR α is a translocation fusion partner in acute promyelocytic leukemia. Mutations in the orphan receptor Dax-1 cause congenital adrenal hyperplasia, and mutation of the orphan receptor SF-1 results in XY sex reversal and adrenal failure.

4.2. Additional Mechanisms of Intracellular Hormone Action

Although the nuclear receptors are the largest and best studied of the intracellular receptors, several other examples that involve intracellular receptors that function as transcription factors are briefly mentioned in this section. The first of these is the arylhydrocarbon (Ah)

receptor. Ahs include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related dioxin-like environmental pollutants such as the polychlorinated biphenyls, as well as compounds such as indolo[3,2-*b*]carbazole, that are generated naturally through the digestion of plant metabolites. These toxic, carcinogenic, and highly persistent compounds act through specific binding to a soluble intracellular receptor, the Ah receptor, also known as the dioxin receptor, to modulate gene expression. It is not clear if the Ah receptor's primary function is in defense against these foreign chemicals, but there are no known endogenous ligands for this receptor, and target genes for Ah receptor regulation include enzymes that metabolize these foreign chemicals to forms that can be excreted. The Ah receptor is a ligand-activated transcription factor (Schmidt and Bradfield, 1996). Although it has a similar mode of action to the nuclear receptors described in the previous section, its structure is not consistent with it being a member of this family. The Ah receptor is instead a basic helix-loop-helix (bHLH) transcription factor that is a member of the PAS domain family (for *Per*, AhR, Ah receptor nuclear translocator [ARNT], and *Sim*). The related ARNT protein does not bind dioxins but, rather, acts as a heterodimerization partner for the Ah receptor. The unliganded Ah receptor is found in the cytoplasm and associates with hsp90, much like the steroid receptors. Following ligand binding, hsp90 dissociates, and the receptor translocates to the nucleus and binds to ARNT to form the heterodimer. The Ah receptor–ARNT complex binds to selective DNA elements known as xenobiotic response elements to regulate target gene transcription.

A somewhat similar example, although not involving a hormonal ligand, is the system for oxygen sensing in cells, with the key player being another PAS family bHLH transcription factor, hypoxia-inducible factor- α (HIF- α). Although HIF- α is not the direct receptor that senses oxygen tension, changes in oxygen tension lead to its posttranslation modification through asparagine and proline hydroxylation, stabilizing and activating the protein such that it can regulate target genes involved in the hypoxic response. Surprisingly, the ARNT protein acts as a heterodimerization partner for HIF- α much as it does for the Ah receptor (Semenza, 2002).

Sterol sensing in cells involves a membrane-bound sterol sensor called the sterol-response element binding protein (SREBP). The N-terminal domain of SREBP is a bHLH transcription factor that is proteolytically cleaved and liberated from the endoplasmic reticulum membrane, allowing it to enter the nucleus to regulate target genes necessary for sterol biosynthesis. High levels of sterols, in turn, inhibit the proteolysis and lead to

the degradation of any nuclear SREBP (Brown and Goldstein, 1999).

A final example of an intracellular receptor is the soluble form of GC (Koesling, 1999). These enzymes are α - β heterodimers and serve as the target for the signaling molecule NO, leading to the formation of a cGMP second messenger. A bound heme confers sensitivity to NO in these proteins. Thus, members of the GC family have functions as both membrane-associated and intracellular receptors for diverse signaling molecules.

5. RECEPTORS, HEALTH, AND DISEASE

Given the central roles of receptors in intercellular communication, it is not surprising that many of them are targets for mutation in diseases of the endocrine system. Illustrative examples have been pointed out in each of the previous specific sections, but to summarize some of this information, Table 1 lists some of the many mutations in cell-surface or intracellular receptors that disrupt their function and lead to human disease. This partial list is stunning in the diversity of conditions, both within and outside the endocrine system, that result from mutations in receptor proteins. A few points are worth considering. First, for many of these receptors, both gain-of-function and loss-of-function mutations have been reported. Gain-of-function mutations are most common in the GPCRs and generally result in constitutive activity of the receptor. Second, the combination of identifying disease-causing mutations in these receptors with the tremendous advances that are being made in protein structure determination has resulted in a powerful ability to understand structure-function relationships that impact receptor activity. Third, Table 1 lists only human disease-causing mutations, and many more of the receptor proteins discussed in this chapter have been mutated in a targeted way in the mouse or other model organisms, providing both animal models for human disease and a wealth of new information on the physiologic functions of the receptors.

In addition to genetic disease-causing mutations, many receptors are likely to be inappropriately expressed or regulated in human disease and, thus, are significant targets for pharmaceutical intervention. Agonists and antagonists of many of these receptors have critical therapeutic value. The GPCRs alone are thought to be the target of about half of all prescription drugs, and the nuclear receptors may represent the fastest-growing targets for prescription drug design today. Of importance, both of these families include many poorly characterized orphan receptors that represent tantalizing targets for future drug discovery. The RTKs have also recently been the target of substantial drug development efforts, particularly ligand antagonists or kinase inhibitors,

Table 1
Representative Mutations in Receptor Proteins
Associated With Human Disease ^a

<i>Condition</i>	<i>Receptor</i>	<i>Type</i>
Hirschsprung disease	Endorphelin B	GPCR
Pituitary dwarfism	HH	GPCR
Retinitis pigmentosa	Rhodopsin	GPCR
Diabetes insipidus	Vasopressin	GPCR
Glucocorticoid deficiency	ACTH	GPCR
Male precocious puberty	LH	GPCR
Hypercalcemia	Ca-sensing	GPCR
Hyperthyroidism	TSH	GPCR
Color blindness	Opsins	GPCR
Chondrodysplasia	PTH	GPCR
Gastric Carcinoma	CCK2	GPCR
Congenital bleeding	Thromboxane A ₂	GPCR
Ovarian failure	FSH	GPCR
Hypogonadism	GnRH	GPCR
Resistance to TH	TR β	NR
Glucocorticoid resistance	GR	NR
Hypocalcemic rickets	VDR	NR
Androgen insensitivity	AR	NR
Promyelocytic leukemia	RAR α	NR
Adrenal hyperplasia	Dax-1	NR
XY sex reversal	SF-1	NR
Pseudohypoadosteronism	MR	NR
Insulin resistance	PP	NR
Parkinson Disease	NR4A2	NR
S-cone syndrome	NR2E3	NR
MODY	HNF4A	NR
Insulin resistance	Insulin	RTK
Craniosynostosis	FGFR2	RTK
EN Type II	RET	RTK
Brachydactyly type B	ROR	RTK
Renal cell carcinoma	MET	RTK
Venous malformation	TIE2	RTK
Piebaldism	KIT	RTK
Hereditary	VEGFr3	RTK
CIPA	TrkA	RTK
Dwarfism	FGFR3	RTK
Pfeiffer syndrome	FGDR1	RTK
Pulmonary hypertension	BMPT2	STK
Telangiectasia type II	ALK1	STK
Colon carcinoma	TGF β R2	STK
Brachydactyly type A2	BMPT1	STK
Laron dwarfism	GH	CRS
Obesity/pit dysfunction	Leptin	CRS
Congenital neutropenia	GCSF	CRS
SCID	ILR- γ c	CRS
Benign erythrocytosis	Epo	CRS
Atopy/IgE syndrome	IL-4	CRS
Periodic fever syndrome	TNFR1	TNF
ALPS	Fas	TNF
Hypercholesterolemia	LDL	TR
Hepatocellular carcinoma	IGF-2	TR
Hemochromatosis type III	Transferrin-2	TR

^a The disease or condition is listed along with the receptor that is mutated and the classification of the receptor. GPCR = G protein-coupled receptor; NR = nuclear receptor; RTK = receptor tyrosine kinase; STK = serine and threonine kinase receptor; CRS = cytokine receptor superfamily; TNF = TNF receptor superfamily; TR = transport receptor.

given the critical roles that these receptors play in cell proliferation and in cancer.

6. SUMMARY

In assessing the structure and function of hormone receptors, several interesting issues emerge. It is clear that a fairly limited number of general strategies have been utilized repeatedly to generate the tremendous diversity of cellular responses to hormonal stimuli that are observed. Thus, superfamilies and families of receptors that have hundreds of members often represent subtle variations on a general structural or functional theme, such as tyrosine kinase activation, G protein coupling, or DNA binding. This knowledge is extremely useful for identifying novel receptors, based on conserved structural features, and for attempting to understand the mechanism of action of novel receptors once they are isolated. At a second level, aspects of receptor function such as heterodimerization with multiple partners or generation of multiple isoforms through alternative RNA processing further contribute to diversity. It is also apparent that substantial functional diversity can be generated at the level of the signal transduction cascades that receptors utilize. For example, coupling of a receptor to different G proteins, leading to the activation of distinct effectors, or interaction of a receptor with different adapter proteins, leading to the activation of distinct kinases, may occur in a tissue- or cell-specific fashion or in a developmentally regulated manner, generating dissimilar signaling responses to a single receptor.

In most of the examples considered in this chapter, our knowledge of the signaling pathways from hormone-receptor interaction to biologic response is still at best rudimentary. Despite tremendous progress in isolating receptors, in deciphering signal transduction cascades, and in unraveling the intricacies of gene transcription, there are relatively few examples for which a complete response pathway has been elucidated, and perhaps no example for which the complexities of the interaction of multiple signaling pathways in generating an appropriate cellular response is fully appreciated. It seems certain that substantial progress is needed to better characterize the signaling processes that are already known, and to uncover the novel receptor activation and signal transduction pathways that surely await discovery.

An important outcome of the quest to understand the actions of hormone receptors has been a recognition of the critical role that receptors play in cellular and organismal homeostasis and of the consequences of aberrant receptor function in endocrine diseases. The number of specific diseases or disorders associated with

gain-of-function, loss-of-function, or alteration-of-function mutations in hormone receptors has increased dramatically since the first edition of this volume. This knowledge has already had immediate application in the diagnosis of endocrine disease and is likely to have eventual application in the rational design of therapeutic strategies for the prevention or treatment of these diseases. Finally, receptor biology provides a focal point for the scientific investigation of many of the critical outstanding areas of question in biology, including the control of cell proliferation, cell differentiation, and cell death; the coordinated interaction of cells during the development of a multicellular organism; and the generation of appropriate cellular responses to environmental and sensory stimuli. Further understanding of receptor-mediated signal transduction promises to contribute greatly to these fundamentally important issues.

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