Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses

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Signal transduction via nuclear hormone receptors is unusual in that the hormone ligand forms an integral part of the protein complex involved in DNA binding and transcriptional activation. New structural and biochemical results have begun to unravel how these receptors produce different effects in different cells, and the structural changes involved in transcriptional activation.

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Introduction

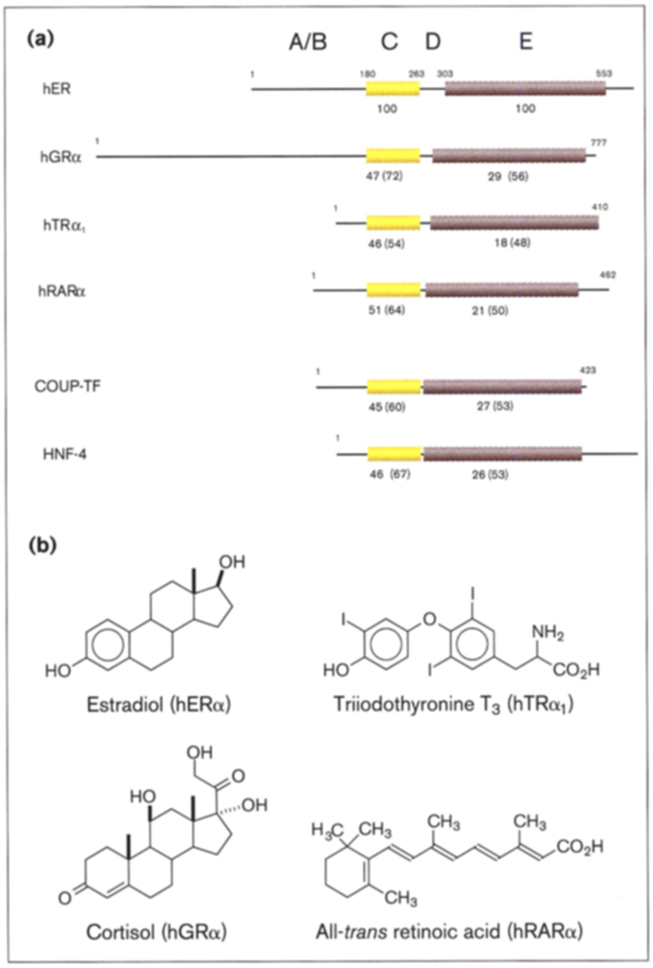
Radiolabeled steroid and thyroid hormones of high specific activity were first prepared in the late 1960's, and were used as probes to identify the sites of hormone action [1]. It has been known for nearly 30 years that these hormones act via intracellular receptor proteins whose principal target for action is in the nucleus. The receptor proteins were quickly surmised to be regulators of transcription [2–6], and are now known to be part of the nuclear receptor superfamily. This large group of transcription factors includes proteins that mediate the action of the steroid hormones (such as estrogens, androgens, glucocorticoids, mineralocorticoids and the insect steroid hormone ecdysone), as well as the non-steroid hormones (for example, thyroid hormone, vitamin D3 and the retinoids) and receptors that mediate the peroxisomal proliferation response to fatty acids and other factors (Fig. 1) [7–11].

Many other members of the superfamily have been identified by low stringency hybridization analysis; some of the genes thus identified encode proteins that are known to be expressed and have the conserved six-domain structure seen in the hormone receptors. Because the hormonal ligands for these proteins are unknown, they are termed 'orphan' receptors [12]. It is however possible that some of these so-called receptors may act as transcription factors alone, without ligands. To add to the complexity of the situation, most classes of receptors within this family contain more than one subtype (i.e., products of closely related genes); sometimes there are also different isoforms (i.e., products from alternate transcription start sites on the same gene) and products of mRNA splice variants. Both the concentration of these receptors and the relative ratio of subtypes and isoforms vary in different target tissues and at different stages of development.

Structure and function of the nuclear receptors

The signature of the nuclear receptor family is a six-domain structure, the most highly conserved portion of which is the small (~70–80 amino acids) domain, C, that is responsible for DNA binding (Fig. 2). This domain has been known for some time to have a helix-loop-helix structure containing two zinc atoms, each chelated by four cysteine thiols at the start of each helix. Three residues at the start of the first helix in this domain 'read' a five to six base pair code in a DNA hormone-response element; the mechanism of this sequence-specific recognition is becoming increasingly clear through structural analysis of domain C–oligonucleotide complexes by X-ray crystallography [13]. The large (~250 amino acid) domain, E, which

Figure 1



Structures of nuclear receptors and their ligands. **(a)** Common domain structure of representative members of the nuclear receptor superfamily, human estrogen receptor α (hERα), human glucocorticoid receptor α (hGRα), human thyroid hormone receptor (hTRα$_1$), human retinoic acid receptor γ (hRARγ), and two orphan receptors COUP-TF and HNF-4. The DNA-binding domain C and ligand-binding domain E are shown with their percent sequence identity (or similarity, in parentheses) to hERα. **(b)** The natural ligands for the first four receptors in (a) are shown; there are no known ligands for the orphan receptors COUP-TF and HNF-4.

is moderately conserved across members of the family, is responsible for hormone binding and dimerization, and is critical in the regulation of transcription (see below). The other domains (the amino-terminal A/B domains, the hinge domain D, and sometimes a carboxy-terminal domain, F), which are poorly conserved in length and sequence across the family, are mostly involved in the modulation of receptor function.

Nuclear receptor ligands are directly involved in transcriptional regulation

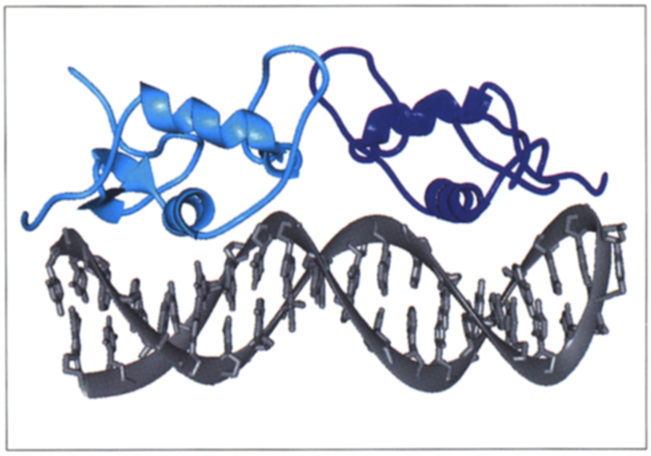
Recent advances have clarified the various ways in which these nuclear receptors can become activated, as well as some of the molecular details of the modulation of the transcriptional activity of specific genes. The essential and intricate role of the ligand in controlling the regulation of gene transcription by these receptors is also now becoming clearer (Fig. 3) [14,15]. Although hormones and growth factors that interact with receptors at the cell membrane may ultimately affect gene transcription, they require multiple-step signal transduction pathways to do so (Fig. 3a); the change in transcription factor activity takes place far away from the interaction between the receptor and the provoking hormonal agent. By contrast, a ligand that activates a nuclear receptor forms a part of the multicomponent complex that directly regulates gene transcription. Such direct interactions offer interesting opportunities for selective pharmacology [16].

There is evidence that high affinity binders for steroid hormones exist in cell membranes, especially in some brain, pituitary and cancer cells. These receptors appear to mediate some very rapid effects of steroid hormones, and it is not yet clear whether their modes of action are similar to or different from the nuclear receptors [17,18]. We will focus here exclusively on the nuclear receptors, since the information on this class is most complete.

Variations on a theme

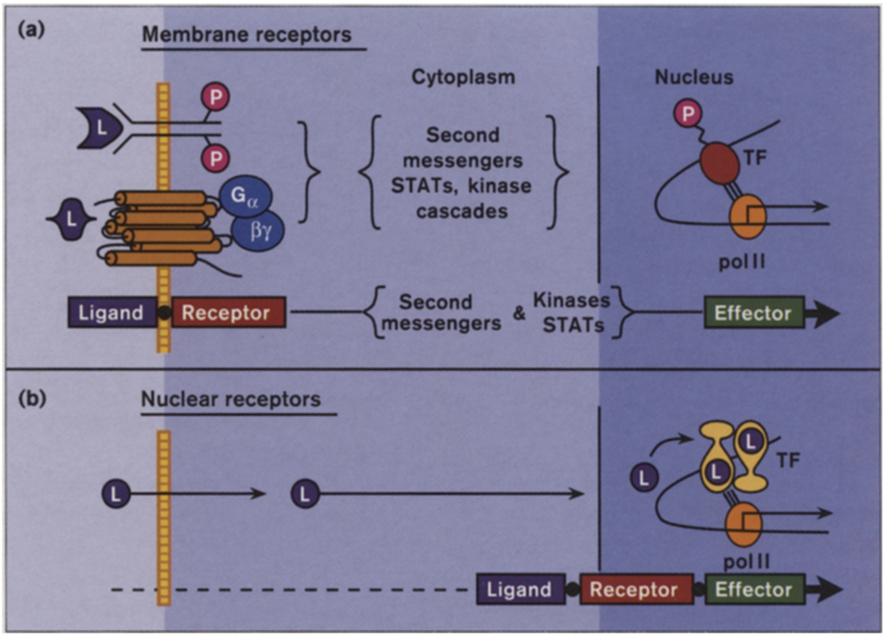
The classical picture of gene activation via nuclear receptors (Fig. 3b) is straightforward. The hormonal ligand binds to the receptor; the receptor–ligand complex thus formed binds (usually as a dimer) to a hormone-response element in the promoter region of a regulated gene, and the transcription of the gene connected to the promoter is thus activated.

Figure 2



A ribbon structure representation of the human glucocorticoid receptor DNA-binding domain dimer complexed with a glucocorticoid response element (GRE). The DNA contact helices, shown edge on, interact with the palandromic DNA sequences of the GRE in adjacent major grooves.

Figure 3

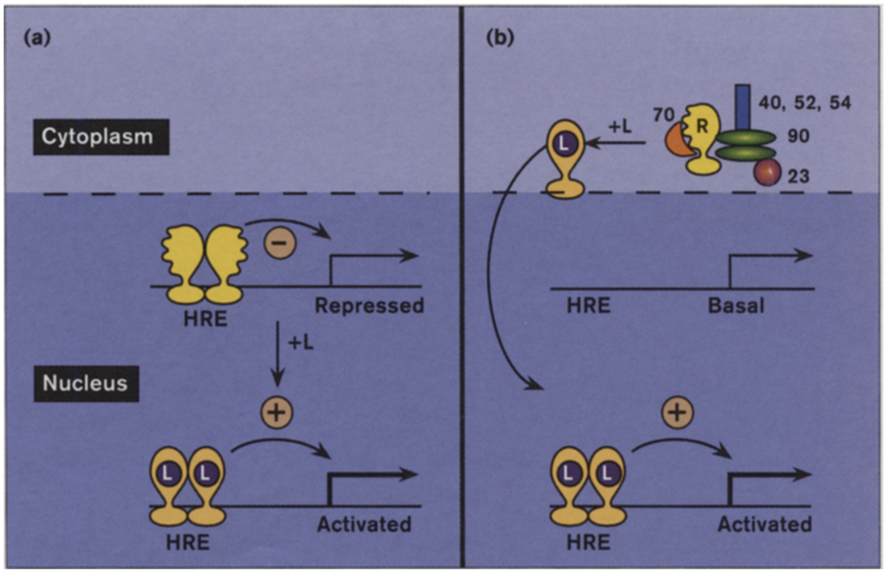


Both membrane receptors and nuclear receptors modulate gene transcription, but nuclear receptors do so more directly. **(a)** Membrane receptor signaling; **(b)** nuclear receptor signaling. In a membrane receptor signaling system, the signal resulting from the binding of the ligand (L) to the receptor must be transduced to the nucleus via complex signal-transduction cascades, which typically involve second messengers, kinase cascades and/or phosphorylation (P) of intermediary proteins (such as STATs) in the cytoplasm. The end result is a change in the activity of a transcription factor (TF) in the nucleus, affecting the rate of initiation of RNA polymerase II (pol II). The effects of a hormone that acts via a nuclear receptor are much more direct; the ligand and receptor form part of the multicomponent complex that modulates pol II activity.

It cannot, however, be this simple. The target of the ligand–receptor complex can clearly vary with cell type, which would be impossible in the rudimentary scheme described above. For example, when estrogen binds to the estrogen receptor in breast cancer and uterine cells the result is the stimulation of transcription from some early response genes, such as *c-myc*, and genes for growth factors (such as TGF-α or pS2) or growth factor receptors (such as the EGF receptor) that are involved in the stimulation of cell proliferation evoked by the hormone [19]. The same ligand-binding event in pituitary and liver cells results in activation of other genes. In the pituitary, the expression of various secreted proteins such as prolactin is increased, whereas in the liver the level of vitellogenin, among others, is increased.

The variations on the classical picture occur at all levels. One source of variability in the effect of ligand binding is the cellular distribution of the receptor in the absence of ligand. The receptors for certain non-steroid ligands (e.g.,

Figure 4



The subcellular location of unliganded nuclear receptors affects the way that they modulate transcription. **(a)** The unliganded receptors for nonsteroid ligands such as thyroid hormone and retinoic acid are typically bound as dimers to their hormone response elements (HREs), even in the absence of ligand, and can act as transcriptional repressors without ligands or transcriptional activators with ligand. **(b)** The unliganded receptors for some steroid hormones, such as glucocorticoids, are largely held as monomers in the cytoplasm by heat-shock proteins (90, 23), chaperonins (70) and immunophilins (40, 52, 54); in this state they have no effect on transcription. Ligand binding releases the receptors from the cytoplasmic aggregate, and the activated receptors bind as dimers to the HREs and activate transcription.

thyroid hormone and the retinoids) appear to be already bound to their response elements (Fig. 4) [20]. Ligand binding may strengthen DNA binding, and may alter the structure of the receptor so as to enhance transcription (see below). In the absence of ligand, these DNA-bound receptors repress gene transcriptional activity [21,22]. In contrast, many of the steroid nuclear receptors (e.g., the glucocorticoid receptor) are largely cytoplasmic in the absence of ligand. They are held in the cytoplasm in complex with heat-shock proteins, chaperonins, and various other proteins such as immunophilins [23]. Ligand binding helps the receptor to shed these proteins, move into the nucleus, dimerize, and interact with appropriate hormone response elements (Fig. 4). In such a scheme, the unliganded receptor cannot be used as a transcriptional repressor, as it is held in the cytoplasm, away from the DNA. The degree of nuclear versus cytoplasmic localization of unliganded receptors varies with different receptors and in different cells, so the effect of the unliganded receptor on transcription will depend on the cell and response in question.

A second level of variation in our originally simple scheme is the way in which the receptor forms a dimer. The nonsteroid nuclear receptors for thyroid hormone, vitamin D and retinoic acid can either form homodimers or heterodimerize with the retinoid X receptor [12,20]. The receptor for the insect steroid hormone ecdysone, on the other hand, is active only as a heterodimer with the protein ultraspiracle, a homolog of the retinoid X receptor (RXR). The preference of the thyroid, vitamin D and retinoic acid receptors for pairing with themselves or with another partner depends on several factors, including the relative concentration of the monomer components (not forgetting the different subtypes and isoforms) and of their cognate ligands. Ligand binding can, in some situations, modulate the formation of specific complexes [24]. A further factor is the structure of the DNA response elements with which the homo- or heterodimers interact [20,24].

The dimerization of steroid receptors at first appeared to be less complicated, since heterodimerization between receptors that bind different ligands (like the thyroid receptor and the RXR) does not seem to occur. Nevertheless, heterodimerization is clearly possible between receptor subtypes (which may have some differences in ligand-binding specificity) and between receptor isoforms (which often have distinctly different transcriptional activities). Examples of subtypes and isoforms that heterodimerize are glucocorticoid receptor α and β, and progesterone receptor A and B forms, respectively. Receptor dimerization and receptor stability are important points for pharmaceutical regulation of transcription via nuclear receptors, and several hormone antagonists (some antiestrogens and antiprogestins, for example) appear to act at this level [25–28].

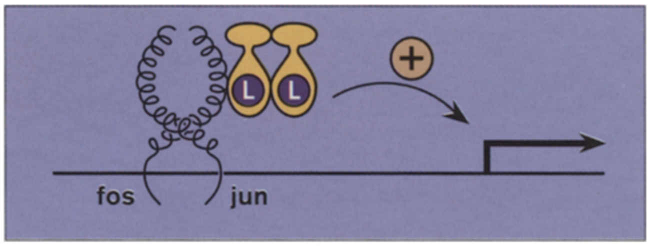
Variations also occur at a third level, the interaction of nuclear receptors with the DNA response elements. Although the response elements are often portrayed as consensus sequences — inverted or direct repeats of a defined five- to six-nucleotide sequence, with various spacers between the repeats — the response elements found in responsive genes are often nonconsensus in sequence; some are half-sites and others have multiple repeats. Often the response elements are found in complex, upstream-enhancer regions, clustered together or even overlapping with response elements for other known transcription factors, which may synergize or compete with the nuclear receptors. Sequences that flank the core response elements can also affect the DNA binding of these receptors (see, for example, [29]). And the structure of the DNA response element, since it affects the recognition between the receptor and the DNA, may also affect the interaction between the receptor and the ligand.

Given all the sources of variation described above, especially the fact that nuclear receptors may interact with or compete with a number of other sequence-specific transcription factors, it is not surprising that the response to a specific hormone depends on both the cell in which it is acting and the gene whose activity it modulates [16].

Nuclear receptor activation without direct DNA binding or without ligand binding

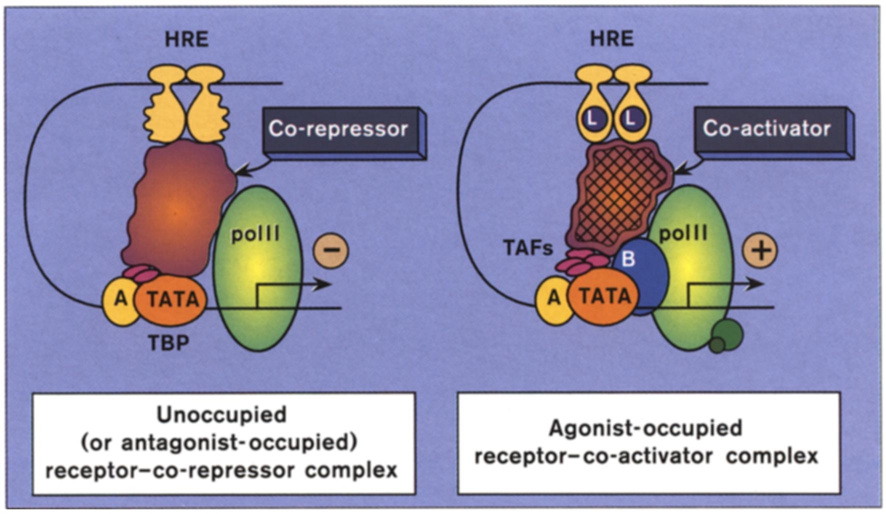
A curious but major deviation from the classical scheme for nuclear receptor action is gene activation in the absence of direct DNA-binding by the receptor. In this situation the promoter for a gene whose activity is clearly regulated by a nuclear receptor and its hormone appears to have no hormone-response element for the receptor, and does not, in fact, require direct DNA binding by the receptor. The hormone–receptor complex seems to function by binding to DNA indirectly via other DNA-tethered transcription factors (see, for example, [30–32]), thus acting as a ligand-modulated co-regulator, rather than a ligand-modulated transcription factor (Fig. 5).

Figure 5



Nuclear receptor gene activation can occur without direct DNA binding. The nuclear receptor is tethered to DNA by a protein–protein interaction with another sequence-specific transcription factor, such as fos/jun (AP1). In such a case, the nuclear receptor has the role of a ligand-modulated co-activator of transcription.

Figure 6



Co-regulators mediate the interaction between the nuclear receptor and components of the transcription complex. Unoccupied or antagonist-occupied receptors can recruit co-repressors (left); when an agonist ligand binds, the ligand–receptor complex can recruit co-activators (right).

Another major deviation from the classical scheme for activation of genes by nuclear receptors is ligand-independent gene activation. In certain systems there appears to be significant crosstalk between signal-transduction pathways that activate transcription. The result is that growth factors or hormones that operate through receptor tyrosine kinases or via cAMP or other second messengers can activate nuclear receptor regulated genes in a manner that requires receptor but not ligand [19]. In some cases, these alternative pathways may synergize with the normal ligand-mediated pathway [33]. The molecular mechanism for such action is not well understood, but it is possible that phosphorylation of specific sites on the nuclear receptors may enhance the transcriptional activity of the unliganded receptor [19,34].

Modulation of gene transcription

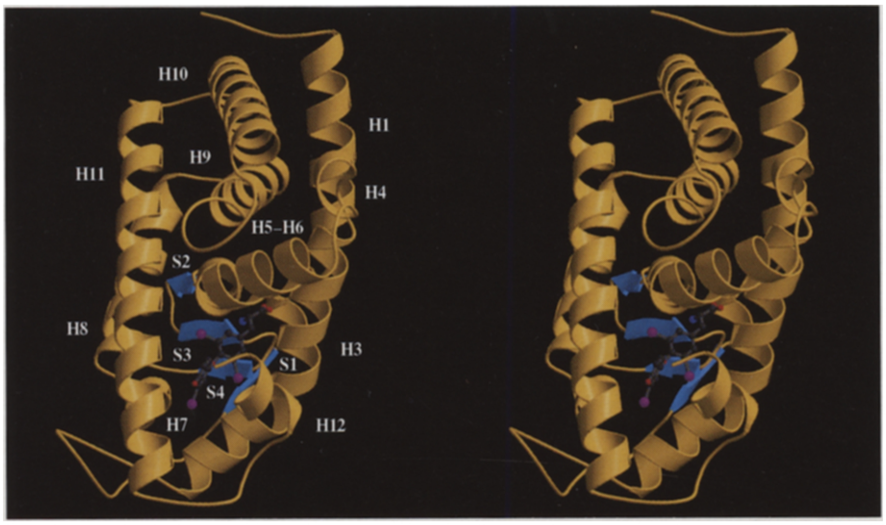
Once a nuclear receptor is bound to DNA, what happens next? The final step of the classical pathway, the process by which these receptors modulate the rate of gene transcription (Fig. 6), has its own sources of regulatory complexity. First, it is important to recognize that the rate at which a gene is transcribed depends both on the local chromatin architecture, and on the rate at which an active RNA polymerase preinitiation complex can be assembled. The nuclear receptors appear to affect both of these processes, both directly and indirectly via 'transcription intermediary factors' (TIFs) [9,35,36], although their effect on chromatin architecture is poorly understood. There is evidence that DNA-bound nuclear receptors interact directly with some of the proteins comprising the basal transcription machinery, such as TFIIB or TATA-binding protein associated factors (TAFs) [37–39]. If they suppress or stimulate a rate-limiting step in the assembly of an active RNA polymerase II preinitiation complex, this would result in repression or activation of transcription. In many cases the relevant interactions between nuclear receptors and basal transcription factors appear not to be direct, however, but are mediated by various co-regulators.

The co-regulators involved in nuclear-receptor modulation of gene transcription are diverse, and are being discovered at an increasing rate. They are often large multidomain proteins, with some homology to factors that are known to modulate chromatin structure; some have known protein-interaction domains, or have the ability to interact with various components in the general transcription apparatus [16]. Some also appear to fit nicely into the unliganded-repression/liganded-activation paradigm, in that one set of co-regulators binds to the unliganded thyroid and retinoid receptors to repress transcription [40,41], whereas another set binds to liganded receptor to enhance transcription [42–46]. In the case of the steroid receptors, the co-regulators appear to bind to either the amino-terminal or carboxy-terminal activation domain of the receptors. Some co-regulators interact with and influence the transcriptional activity of many steroid hormone receptors and other related receptors, such as RXR, whereas other co-regulators show a more restricted range of receptor interaction.

Structural and conformational changes on ligand binding

As the interaction between the co-regulators and the nuclear receptor is regulated by ligand binding, it is plausible that ligand binding elicits a conformational change in the receptor that may permit co-activator but not co-repressor binding in the presence of ligand (or co-repressor but not co-activator binding in the absence of ligand). Mutational mapping studies have begun to identify the different regions of the receptor that seem to be responsible for interaction with co-repressors and co-activators [35,43,47]. Most exciting are some of the structural features revealed

Figure 7



Stereoview of a ribbon structure of the ligand-binding domain of the rat thyroid hormone receptor complexed with thyroid hormone (T3), shown as a skeletal structure in the lower half of the protein. The regions of α-helical (H) and β-strand (S) secondary structure are designated.

in the recent X-ray crystal structures of three different receptor ligand-binding domains (domain E) (Fig. 7); these structures provide insight into the conformational reorganization that occurs upon ligand binding [48–51].

The ligand-binding domain of the nuclear receptors is large, larger than most single protein domains, with a unique antiparallel α-helix triple sandwich topology (Fig. 7). Approximately half of the domain consists of a rigid, tightly packed assembly of helices that appear to act as a fundament or fulcrum for the action of the remainder of the domain, which is more flexible and is involved in ligand binding. Although the three structures that have been described so far do not permit a direct comparison between the conformations of a single receptor in the liganded and unliganded state, certain general features have emerged that are likely to hold true for the ligand-induced conformational changes of all of the members of the superfamily.

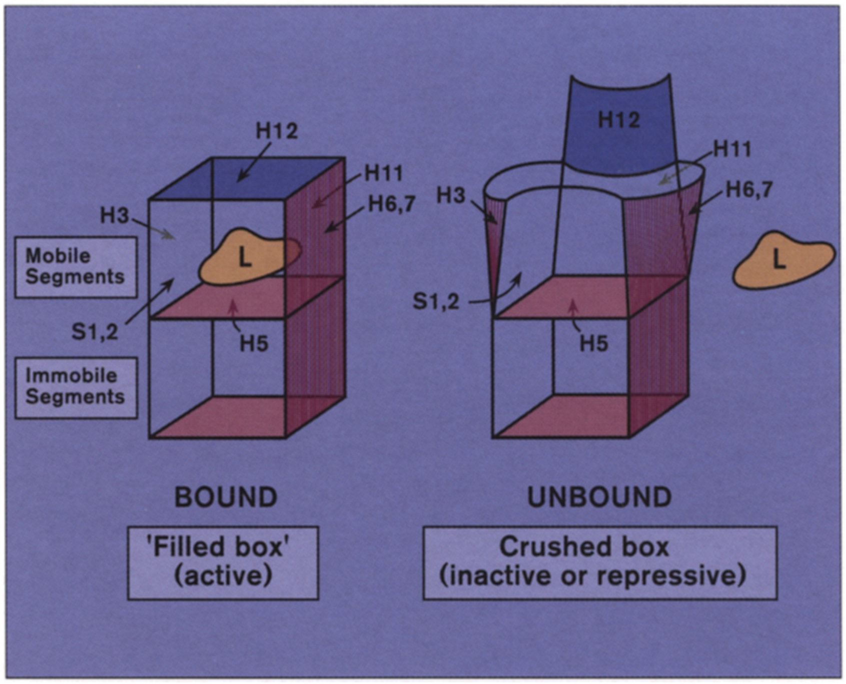
In the bound state, the ligand is completely engulfed by the flexible portion of the domain, and actually forms the hydrophobic core for this region [49,50]. Six segments of secondary structure, arranged roughly as the six sides of a box, surround the ligand, with more than 20 residues making direct contact with the ligand (Fig. 8). In the liganded state, the carboxy-terminal portion of this domain, an amphipathic helix, termed the activation function 2 activation domain (AF2-AD), interacts with the ligand and is positioned adjacent to two other helical portions of the receptor whose specific orientation is also dependent upon contacts with the ligand (see Fig. 7, helix 12). This composite surface, whose integrity appears to be critically dependent on ligand binding, is one likely site for co-activator binding.

By contrast, in the unbound state, the flexible portion of the ligand-binding domain lacks its hydrophobic core, namely, the ligand. In the one published structure for an unliganded receptor [48], the box-like structure of the flexible portion of domain E appears to have collapsed, with two sides tipping inward and two sides tipping outward; the activation helix is dislodged from its position between the other two helices, since their relative position is no longer supported by contacts with the ligand (see Fig. 8). The composite surface for co-activator binding is thus absent or at least substantially modified in the unliganded state. But in the collapsed state, various new topographical features have developed, providing potential sites for co-repressor binding.

X-ray crystallography provides static pictures of protein structure. It is thus possible that the flexible ligand-binding region of domain E in the unliganded state may be rather fluid, perhaps in a molten globule-like state. The binding of heat shock proteins (which normally bind only to unfolded or partially folded proteins) and immunophilins to the unliganded steroid receptors and the sensitivity of the unliganded receptor to proteolysis supports this view [52]. Further studies, especially ones in which a direct comparison can be made between structures of the liganded and unliganded state of the same receptor, will be needed to verify the generality of these conformational transitions.

Ligand binding affects receptor shape — thus, receptor shape reflects ligand shape. As co-repressor/co-activator binding responds to alterations in receptor shape, the ligand is the crucial factor in recruiting or disbanding these important co-regulators. The view that ligand shape determines receptor shape and thus receptor activity can also account for the spectrum of biological activity — from pure agonists to partial agonists/antagonists to pure antagonists — that is known for ligands for some of these nuclear

Figure 8



A 'box model' for the ligand-binding domain of a nuclear receptor. When an agonist ligand is bound, the upper box, made up of mobile segments with the ligand at its core, is 'filled'; in this conformation, it has a structure in which the activation domain (helix 12) is in the active state, where it can interact with co-activators, activating transcription. Without ligand, the upper box is empty and is 'crushed', so that two sides cave inward and two sides bulge outward; the activation helix is displaced from the active state, and the empty receptor is thus either inactive or recruits co-repressors to become repressive. Antagonists and partial agonists fill the top box in a different manner, such that the activation helix is fully or partially misoriented from the activating position. The conformation of the lower box is not affected by ligand binding. (Note that this schematic representation of the ligand binding domain of a nuclear receptor is shown in the orientation opposite to that of the thyroid hormone receptor–T3 complex shown in Figure 7. In Figure 7, the ligand-binding 'box' is at the bottom.)

receptors, such as estrogens and progestins. Given all this, the potential for pharmaceutical modulation of the transcriptional activity of nuclear receptors is obvious [15,16].

Pharmacological issues, however, extend beyond the ligand–receptor interaction. The biological effect that a particular ligand will have, acting via a given receptor, will also depend on the intracellular context (i.e., the levels of the relevant co-regulators and transcription factors with which the receptor cooperates) and the promoter for the specific gene being regulated (i.e., the structure of the hormone-response element and whether any other transcription factors bind to nearby sites). This 'tripartite receptor pharmacology', comprising ligands, receptors, and cell and promoter specific transcriptional effectors, offers rich possibilities for developing tissue- and response-specific pharmaceuticals [16].

The future

There is much more to learn. The details of the ligand-induced conformational changes within one receptor protein are yet to be revealed, and we do not yet know how all the different domains of a nuclear receptor interact with each other. A full appreciation of the molecular interactions involved in the gene-regulating action of the nuclear receptors will require reconstitution of multiprotein complexes involving the intact receptor (as a homo- or heterodimer) interacting with a complete gene regulatory region, together with other associated transcription factors, co-regulator proteins, and elements of the general transcription apparatus. Equally important will be biological studies detailing regulation of the levels and activity of receptors and their co-regulators as a function of physiological and developmental state in different hormonal target cells and tissues. Clearly, the major and perhaps the most exciting challenges still lie ahead.

Acknowledgements

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