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Activation of Nuclear Receptors: A Perspective from Structural Genomics

Review

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Crystal structures of more than two dozen different nuclear receptor ligand binding domains have defined a simple paradigm of receptor activation, in which agonist binding induces the activation function-2 (AF-2) helix to form a charge clamp for coactivator recruitment. Recent structural studies present a surprising contrast. Activation of the mouse LRH-1 receptor is independent of a bound agonist despite its large ligand binding pocket, whereas the activation of the *Drosophila* DHR38 receptor is dependent on ecdysteroids even though the receptor lacks a ligand binding pocket. These new findings shed light on the diverse structural mechanisms that nuclear receptors have evolved for activation, and have important implications in their respective signaling pathways.

The complete human genome contains 48 nuclear receptors that include receptors for classic endocrine ligands such as steroid hormones, retinoic acids, vitamin D, and thyroid hormone (Table 1). These classic receptors are DNA binding and ligand-dependent transcriptional factors that modulate gene expression involved in a broad spectrum of physiology. One distinguishing fact about these classic receptors is that they are among the most successful molecular targets in the history of drug discovery. Every receptor has one or more cognate synthetic ligands currently being used as medicines.

The human nuclear receptors also include a class of orphan receptors for which no ligand was known when the receptor was cloned, such as liver receptor homolog 1 (LRH-1, or NR5A2/FTF/PHR) and the nerve growth factor-induced clone B (NGFI-B) receptors. Given the prominent role of the classic receptors in physiology and their successes as drug targets, there has been enormous interest from both academia and the pharmaceutical industry in pursuing orphan receptors as drug targets. The result of intense research in the past few years has been the emergence of a class of so-called "adopted" orphan receptors for which either natural or synthetic ligands have been identified. In turn, these newly identified ligands have been exploited as chemical tools to elucidate the biology through the approach of "reverse endocrinology" (Kliewer et al., 1999), and have established the equally important role of these orphan receptors in physiology, especially in the metabolism of glucose, lipids, and xenobiotics (Chawla et al., 2001).

All nuclear receptors contain at least one of the two highly conserved domains: the centrally located DNA binding domain (DBD) and the C-terminal ligand binding domain (LBD). The LBD plays a pivotal role in ligand-mediated signaling. In addition to ligand recognition, the LBD contains dimerization motifs and a ligand-dependent activation function, which requires the proper position of the activation helix (the AF-2 helix, or helix 12) located at the C-terminal end of the receptor. When bound to an activating ligand, the AF-2 helix is stabilized in the active conformation, which allows the LBD to recruit coactivator proteins such as steroid receptor coactivators (SRC-1, 2, and 3) and the TRAP/DRIP mediator complex to activate target promoters. In contrast, the binding of an antagonist destabilizes the AF-2 helix from the active conformation and promotes the recruitment of corepressors such as nuclear corepressor (N-CoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) to repress transcription (Rosenfeld and Glass, 2001). Because ligand binding and ligand-mediated cofactor recruitment are crucial for functions mediated by nuclear receptors, the LBD has been the focus of intense structural study.

Structural Genomics of LBDs

The nuclear receptor superfamily has been grouped into six subfamilies based on sequence alignment and phylogenetic tree construction (NRNC, 1999). Since the first set of LBD structures of the apo-RXR and ligand-bound RAR and TR were published in 1995 by the Moras and Fletterick groups (Bourguet et al., 1995; Renaud et al., 1995; Wagner et al., 1995), more than two dozen LBD structures have been determined for the classic receptors and the adopted orphan receptors (Table 1). The structures of the *Drosophila* DHR38 (NR4A4, a homolog of the mammalian NGFI-B/Nurr1 receptors) and the mouse LRH-1, published in the current issues of *Cell* and *Molecular Cell*, together with the Nurr1 LBD structure recently published in *Nature*, not only expand the list of the LBD structures but also are the first representatives of their respective subfamilies (NR4 and NR5), which still remain as orphan receptors to date (Baker et al., 2003; Sablin et al., 2003; Wang et al., 2003). If we assume that the 48 human receptors can be divided into 28 subsets based on their ligand binding similarity (Willson and Moore, 2002), LBD structures have been determined for two thirds of these subsets (Table 1), which include representative structures from every subfamily of receptors except for the Dax/SHP subfamily. These structures are obtained with various LBDs in complex with agonists or antagonists, some with fragments of coactivators or corepressors, and in the form of monomers, dimers, or tetramers. The rich information provided by these structures has made it possible to develop a global view of the molecular basis of ligand binding and ligand-mediated regulation of nuclear receptors.

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Table 1. Human Nuclear Receptors and the Solved LBD Structures

Classic Receptors	Structure[a]	Adopted Orphan Receptors	Structure	Orphan Receptors	Structure
AR	+	CAR	−	COUP-TF (I, II, III)	−
ER (α, β)	+	ERR (α, β, γ)	+	DAX	−
GR	+	FXR	+	GCNF	−
MR	−	HNF4 (α, γ)	+	LRH	+[b]
PR	+	LXR (α, β)	+	NGFI-B (α, β, γ)	+[c]
RAR (α, β, γ)	+	PPAR (α, γ, δ)	+	PNR	−
TR (α, β)	+	PXR	+	RevErbA	−
VDR	+	ROR (α, β, γ)	+	SF1	−
		RXR (α, β, γ)	+	SHP	−
				TLX	−
				TR2, TR4	−

[a] A "+" indicates the LBD structure for at least one of the subtypes is solved; a "−" indicates the structure is unsolved.
[b] The structure determined is mouse LRH-1.
[c] The structures determined are *Drosophila* DHR38 and human Nurr1.

All nuclear receptor LBD structures determined to date contain 11–13 α helices that are arranged into a three-layer antiparallel α-helical sandwich (Figure 1). The three long helices (helices 3, 7, and 10) form the two outer layers of the sandwich. The middle layer of helices (helices 4, 5, 8, and 9) is present only in the top half of the domain but is missing from the bottom half, thereby creating a cavity for ligand binding in most of the receptors. The C-terminal activation region also forms an α helix (AF-2), which can adopt multiple conformations depending on the nature of the bound ligand. Structural comparison reveals that the top half of the domain is highly similar among various LBDs, suggesting that the helix sandwich fold is evolutionarily selected for the binding of small molecules in most receptors.

Structural Basis of Ligand Recognition

The first step of nuclear receptor activation is initiated by ligand binding, and thus the ligand binding pocket is an important structural feature of nuclear receptors. The ligand binding pocket is generally located behind helix 3 and in the front of helices 7 and 10 (cyan surfaces in Figure 1). Despite the conserved fold of LBDs, the ligand binding pocket varies greatly in size, from 30 Å^3 in the DHR38 receptor to 1400 Å^3 in the subtypes of proxisome proliferator-activated receptors (PPARs). The trend seems that the adopted orphan receptors such as PPARs and pregnane X receptor (PXR) contain a large ligand binding pocket, whereas the classic receptors have a smaller pocket. This variation seems to be consistent with the biology mediated by these receptors. The large pockets in PXR and the PPARs allow these receptors to bind to diverse metabolites promiscuously and with a low affinity. In contrast, the small pocket in the classic receptors limits these receptors to recognize a specific ligand with a high affinity. Such high affinity and specificity of ligand recognition may be required for these classic receptors to mediate their physiological pathways.

The specificity of ligand binding is also determined by the shape of the ligand binding pocket, which also varies greatly from receptor subtype to subtype, to accommodate a variety of functions mediated by these receptors. The large pocket seen in PPARs has a distinct three-arm Y shape, allowing it to bind ligands with multiple branches (such as phospholipids and synthetic fibrates), or to bind singly branched ligands, such as fatty acids, in multiple conformations (Xu et al., 1999). In contrast, PXR has an elliptical shaped pocket with a

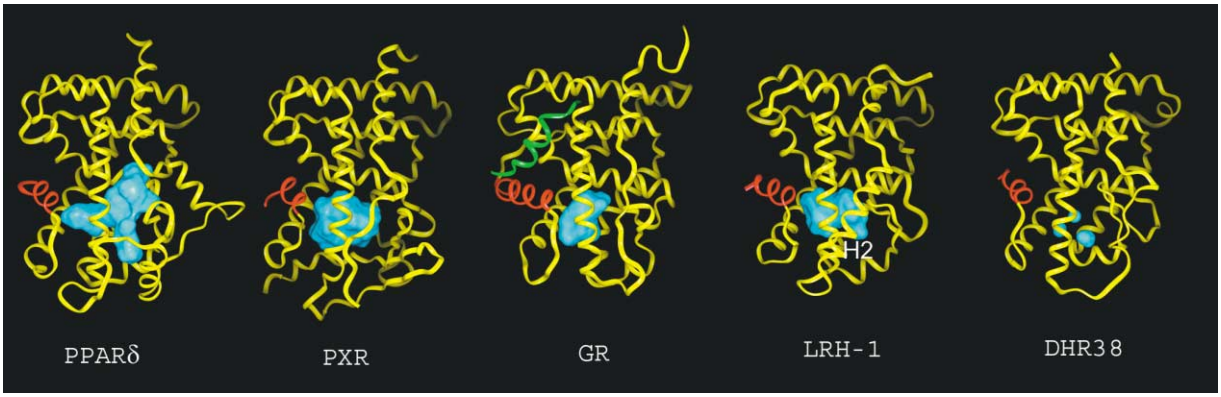

Figure 1. Overall Structures and Ligand Binding Pockets of Nuclear Receptor LBDs

The LBDs of PPARδ, PXR, GR, LRH-1, and DHR38 are illustrated by ribbons and their ligand binding pockets are presented in cyan surfaces. The C-terminal AF-2 helix is colored in red and the TIF2 LXXLL motif in the GR structure is colored in green.

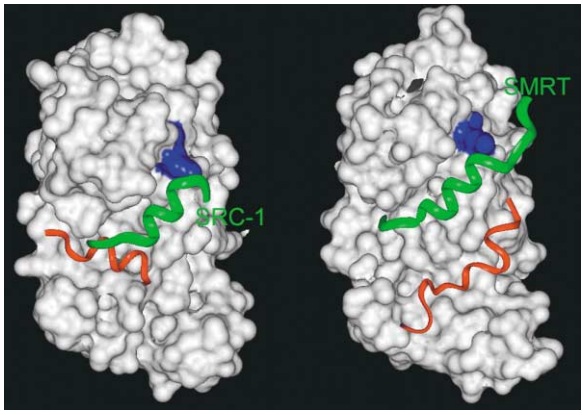

Figure 2. Structural Comparison of the PPARα LBD Bound to an SRC-1 Coactivator Motif or an SMRT Corepressor Motif

The surface of the PPARα LBD, excluding the AF2 helix, is shown in white. The AF-2 helix is in red and the charge clamp lysine from helix 3 is in blue.

volume of 1200 Å^3, allowing it to bind to the cholesterol-lowering drug SR12813 in three different conformations (Watkins et al., 2001). Although the PXR pocket is smaller than the PPAR pocket, the conformational flexibility of PXR apparently allows the pocket to expand to accommodate larger ligands, such as hyperforin and the antibiotic rifampicin, the largest ligand known for any nuclear receptor. The spherical feature and the plasticity of the PXR pocket have thus allowed this receptor to serve as the key regulator in response to binding of diverse xenobiotic chemicals, which do not come with the same shape or the same hydrogen-bonding groups.

Besides the size and shape, the hydrophobic/hydrophilic nature of the pocket surface also plays a determining role in ligand binding specificity. This is exemplified by the steroid hormone receptors GR, AR, PR, and ER (Bledsoe et al., 2002). These receptors share a high degree of sequence identity and similar three-dimensional structures, yet they are able to distinguish between several very similar endogenous steroid hormones to mediate their dramatically different physiological functions. The GR LBD structure reveals that the GR pocket has evolved to form specific hydrogen bonds with every polar group of dexamethasone, which has an identical set of polar groups as the endogenous glucocorticoid cortisol. Structural comparison reveals that such a complete set of hydrogen bonds between GR and dexamethasone cannot be constructed in steroid receptors other than GR because of a different distribution of polar atoms in the pocket surface. The situation is even more dramatic between AR and ER, where the only major difference in their respective hormones is a C3 ketone in testosterone versus a C3 hydroxyl in estradiol. In AR, the C3 ketone of testosterone accepts two hydrogen bonds from residues Q711 and R752, which are also conserved in GR and PR. In ER, the corresponding arginine is conserved but the corresponding glutamine residue is a glutamate, which prefers to accept a hydrogen bond from the C3 position of the ligand. The replacement of glutamine by glutamate therefore explains the selectivity of ER for a hydroxyl group at the C3 position, which also serves as hydrogen bond donor to the corresponding arginine residue. Taken together, nuclear receptors have evolved remarkably down to the single-residue level to recognize specific ligands by changing the size, shape, and polar/nonpolar nature of their ligand binding pockets.

Ligand-Mediated Activation versus Repression

The second step of nuclear receptor activation is ligand-induced recruitment of coactivator complexes, which contain chromatin-modifying enzymes required for transcription. Nuclear coactivators such as SRC-1 contain multiple LXXLL motifs that interact with LBDs. X-ray structures of various LBDs bound to agonists and peptides with LXXLL motifs reveal a conserved mode of coactivator binding. In these structures, the LXXLL binding pocket comprises two parts: the constant part (helices 3, 3′, 4, and 5) and the variable part (AF-2 helix). The constant part adopts essentially the same conformation in all the LBD structures, and does not change with the binding of different ligands. In contrast, the AF-2 helix adopts different conformations, depending on the nature of the bound ligand. As seen in the struc-

Figure 3. Conservation of the Charge Clamp Pocket

Sequence alignment of cofactor binding pockets in nuclear receptors reveals the conserved nature of the charge clamp pocket and the AF-2 helix. Positively charged residues are labeled in blue, negatively charged residues are in red, polar residues are in green, and the hydrophobic residues are in black. The arrows indicate the positions of the charged clamp residues.

ture of PPARα, when bound with an activating ligand, the AF-2 helix is packed against helices 3 and 10, becoming an integral body of the LBD (Xu et al., 2001). In this active conformation, a highly conserved glutamate residue from the AF-2 helix, together with a lysine residue from the end of helix 3, forms a charge clamp pocket to interact with the coactivator motifs (Figure 2).

The LXXLL coactivator motif adopts a two-turn α helix with its three-leucine side chains fitting into the hydrophobic pocket between the two charge clamp residues, which further stabilize the coactivator helix by capping both helical ends. The high degree of amino acid sequence conservation in the coactivator binding pocket suggests that this mode of coactivator binding represents a general mechanism for the activation of nuclear receptors (Figure 3). In addition, nuclear receptors can achieve specific recognition of coactivators by interacting with the variable residues within or flanking the LXXLL motifs. In the case of GR, the specific recognition of the TIF2 third LXXLL motif is mediated by two additional charge residues of GR that form a second charge clamp to interact with the charge residues specific to the TIF2 third motif (Bledsoe et al., 2002).

On the other hand, the position of the AF-2 helix also plays a key role in recruiting corepressors such as N-CoR and SMRT. These nuclear corepressors bind to LBDs via a conserved LXXXIXXXL/I motif, which is similar to the LXXLL coactivator motif but has an N-terminal extension. In comparison to coactivators, the longer corepressor motif adopts a three-turn α helix instead of two turns for the coactivator motif, and binds to the same overlapped site as for the LXXLL helix (Xu et al., 2002). The additional turn of the corepressor helix extends into space that would normally be occupied by the AF-2 helix when it is in the active conformation. Thus the binding of corepressors and the active AF-2 conformation is mutually exclusive. The AF-2 helix must shift to some alternative position to accommodate the larger corepressor helix. In the case of PPARα, rearrangement of the AF-2 helix is achieved by an antagonist, which pushes the AF-2 helix from its active position to provide additional space for binding of the corepressor helix. The rearrangement of the AF-2 helix from its active position also allows the corepressor helix to dock closer into the charge clamp pocket. The binding mode of corepressors, similar to that of coactivators, is also highly conserved among nuclear receptors (Figure 3). It seems clear that binding of coactivators and corepressors is tightly modulated by the position of the AF-2 helix. The conformational flexibility of this helix allows it to sense the presence of the bound ligand, either an agonist or an antagonist, and to recruit the coactivators or corepressors that ultimately determine the transcriptional output of nuclear receptors.

Multiple Mechanisms of Nuclear Receptor Activation

A common mechanism for activation of nuclear receptors is mediated through the precise positioning of the AF-2 helix, which then forms a charge clamp pocket for the recruitment of coactivators. Within the framework of this general mechanism, however, nuclear receptors have explored a variety of structural mechanisms to stabilize the AF-2 helix in the active state. The most straightforward mechanism is direct interaction between the AF-2 helix and the bound ligand, as seen in the structures of PPARs and GR. In PPARs, diverse activating ligands contain an acidic group such as carboxylate or thiazolidinedione (TZD), which forms a direct hydrogen bond with the AF-2 helix and locks this helix into the active conformation (Gampe et al., 2000a). In GR, the active AF-2 helix is stabilized by hydrophobic interactions with the bound dexamethasone (Bledsoe et al., 2002). However, in many LBD/agonist complexes, the bound ligand does not make any direct contact with the AF-2 helix; instead, the ligand induces a conformational change in the receptor that allows a stable docking of the AF-2 helix. The most dramatic example of this mechanism is seen in the RXR structure. The apo-RXR forms a stable tetramer that is incompetent for binding of coactivators (Gampe et al., 2000b). Binding of 9-cis-retinoic acid induces a dramatic conformational change that results in dissociation of the tetramer and formation of active dimers that are capable of binding coactivators (Gampe et al., 2000a). Ligand-induced conformational changes are also seen in ER, where antagonist binding partially unwinds helices 3 and 10. By comparison, agonists appear to stabilize helices 3 and 10, which allows the active AF-2 helix to pack tightly against these two helices (Brzozowski et al., 1997; Shiau et al., 1998). The third mechanism is that the AF-2 helix is predisposed in the active conformation, and the regulation of these receptors is mediated primarily through negative mechanisms. ERRγ contains a small ligand binding pocket, and its AF-2 helix is constantly held in the active conformation by inserting a hydrophobic phenylalanine from this helix into its small pocket (Greschik et al., 2002). The binding of ERRγ antagonists (diethylstilbestrol or 4-hydroxytamoxifen) inactivates the receptor by rearranging the AF-2 helix from the active conformation. In HNF4, the bound palmitic acid serves as a nonexchangeable cofactor that stabilizes the AF-2 helix in active position (Dhe-Paganon et al., 2002; Wisely et al., 2002). Regulation of HNF4 activity could be mediated through posttranslational modifications such as phosphorylation and acetylation, which would control nuclear retention of the protein (Soutoglou et al., 2000).

The recent DHR38 and LRH-1 structures have added further complexity to these mechanisms and raised questions about the role of ligand binding. The activation of both DHR38 and LRH-1 apparently still requires the precise positioning of the AF-2 helix in the active conformation despite the dramatic contrast in their ligand requirements. LRH-1 is a true orphan receptor that plays key roles in homeostasis of bile acids and cholesterol (Goodwin et al., 2000; Lu et al., 2000). Whereas most nuclear receptors bind to DNA as dimers, LRH-1 binds to DNA and activates transcription as a monomer. Sablin et al. (2003) present a crystal structure of the mouse LRH-1 LBD that has set up a framework for understanding monomeric action of LRH-1.

However, the most surprising aspect of the structure is the presence of a large (~800 Å^3), completely enclosed, ligand binding pocket in the LRH-1 LBD. Although there is no ligand in the pocket, the AF-2 helix

is in the active conformation, in good agreement with the constitutive activation phenotype of LRH-1. Radical mutations that change the size or the shape of the pocket do not reduce the activation potential of the receptor, suggesting that the endogenous ligands (if they exist) can change shape and size to fit into the mutated receptors, or that there are no such ligands at all.

How does the receptor stabilize the AF-2 helix in the presence of a large empty cavity inside the domain? The LRH-1 structure reveals a four-layer helix sandwich instead of the three-layer sandwich observed for other nuclear receptors. The additional layer of helix is composed of a long helix 2 that packs tightly alongside helix 3. The interactions with helix 2 would tend to stabilize helix 3, in a manner similar to that observed with ER, thus creating a stable docking surface for the AF-2 helix. The constitutive activation of LRH-1 is analogous to ERRγ and HNF4. However, in this case, evolution has designed a delicate negative system through the binding of SHP, which represses LRH-1 activation in response to activation of another nuclear receptor, FXR (Goodwin et al., 2000; Lu et al., 2000). In addition, the presence of the large empty pocket in LRH-1 not only raises the question of why nature designs such a pocket with no corresponding ligand, but also tempts structural chemists to design small molecules to dock into this pocket to achieve "super" activation. The feasibility of these artificial ligands as pharmaceutical agents, either destabilizing or further stabilizing the AF-2 helix, remains to be seen.

In the case of DHR38, Baker et al. (2003) have discovered a series of ecdysteroid derivatives that activate transcription through the receptor, some with potency two orders of magnitude better than that on the well-characterized ecdysone receptor. Although the physiological relevance of DHR38 activation in insect morphogenesis remains to be established, the authors have revealed several intriguing and puzzling aspects of DHR38 activation. DHR38 is the first receptor known whose activation requires the preactivated heterodimer partner, either USP in insect or RXR in mammals. This is completely different from the activation of other RXR heterodimer complexes. For example, activation of PPARγ/RXR heterodimer only requires the PPARγ AF-2 helix and its ligands (Schulman et al., 1998). The RXR ligand and its AF-2 helix are dispensable for the activation of the PPARγ/RXR heterodimer although the addition of an RXR agonist can synergistically activate the receptor. Why does activation of DHR38 by ecdysteroids require the preactivated RXR? The second puzzle is that the largest cavity in DHR38 is only 30 Å^3, too small to accommodate even a small benzene ring ($\sim$100 Å^3) despite its activation being dependent on ecdysteroids. Exhaustive biochemical efforts have failed to establish direct binding of ecdysteroids to the DHR38 LBD. What is the role of ligands in DHR38 activation? It is conceivable that ecdysteroid binding to DHR38 may involve the DBD or the N-terminal domain. However, this is ruled out by the fact that the GAL4 chimeras that contain only the DHR38 LBD can also be activated by ecdysteroids. The molecular basis of DHR38 activation by ecdysteroids remains to be further clarified.

The third puzzle is that despite the fact that the DHR38 AF-2 helix is required for its activation, it does not form a conventional charge clamp pocket for coactivator recruitment. The conserved glutamate in the AF-2 helix from other nuclear receptors is replaced by an asparagine in DHR38 and a lysine in the NGFI-B/Nurr1 receptors, and the conserved lysine at the end of helix 3 is replaced by a glutamate. It seems that NGFI-B contains a "reverse" charge clamp, but the topology of the reverse charge clamp pocket is altered too dramatically to accommodate an inverted LXXLL motif. Reversal of the reverse charge clamp in Nurr1 also fails to rescue the binding of LXXLL motif (Wang et al., 2003). In fact, no known coactivators have been shown to bind to DHR38 or NGFI-B/Nurr1. However, there must be a DHR38 coactivator given the requirement of its AF-2 helix for its activation. What is it? If such a putative coactivator exists, it must be highly conserved because the insect DHR38 is highly homologous to the mammalian NGFI-B receptors. Given the importance of NGFI-B receptors in the survival of dopaminergic neurons and the development of Parkinson's disease (Zetterstrom et al., 1997), the pursuit of the putative DHR38/NGFI-B-specific coactivator is particularly pressing for elucidation of the atypical DHR38/NGFI-B signaling pathway, for which many questions remain to be addressed.

Perspectives

Crystal structures for more than half of the human nuclear receptors have been determined. These structures reveal that nuclear receptors have evolved from a conserved sandwich fold to achieve specific recognition of diverse hormones and ligands. The structures also highlight the conformational flexibility of the AF-2 helix and how nuclear receptors explore this helix, in a variety of mechanisms, to sense diverse ligands and to cascade the signal from ligand binding to transcriptional regulation. Currently, there is only one classic receptor (MR) and one adopted orphan receptor (CAR) for which the LBD structure remains unsolved. In contrast, the structures of most orphan receptors remain unknown (Table 1). As much as the surprise we learned from DHR38, Nurr1, and LRH-1, the excitement for the structures of the remaining orphan receptors is yet to come.

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