Structural insights into regulation of nuclear receptors by ligands

Eric H. Xu and Millard H. Lambert

Corresponding Author: Eric.Xu@vai.org

Laboratory of Structural Sciences, Van Andel Research Institute, Grand Rapids, Michigan 49503 (H.E.X) and Computational Chemistry, GlaxoSmithKline, Research Triangle Park, NC 27709 (M.H.L)

Nuclear receptors are DNA-binding transcription factors, the transcriptional function of many of which depends on the binding of ligands, a feature that distinguishes nuclear receptors from other transcription factors. This review will summarize recent advances in our knowledge of the interaction between selected nuclear receptors and their cognate ligands.

Received May 1st, 2003; Accepted June 2nd, 2003; Published June 15th, 2003 | **Abbreviations:** **AF-2:** activation function 2; **LBD:** ligand binding domain; **LXXLL:** nuclear receptor-interacting motif |

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Introduction

Nuclear receptors are DNA-binding transcription factors. The transcriptional function of most nuclear receptors depends on the binding of ligands, a feature that distinguishes nuclear receptors from other transcription factors. When bound to an activating ligand, nuclear receptors recruit coactivator proteins such as the Steroid Receptor Coactivators (SRC-1, 2, and 3) and the TRAP/DRIP mediator complex via their LXXLL sequence motifs [McKenna et al., 1999]. In contrast, the binding of an antagonist destabilizes nuclear receptor/coactivator complexes, instead promoting interactions with corepressors such as Nuclear CoRepressor (N-CoR) and Silencing Mediator for Retinoid and Thyroid hormone receptors (SMRT) [Hu and Lazar, 2000].

Coactivators generally promote gene transcription, whereas corepressors repress gene transcription. Coactivators and corepressors are thus the Yin-Yang factors that determine the transcriptional functions of nuclear receptors and subsequent ligand-mediated physiological responses. The molecular mechanisms of ligand-mediated recruitment of coactivators or corepressors are highly conserved across the nuclear receptor superfamily. In this review, we illustrate the basic principles of these mechanisms with X-ray structures of Peroxisome Proliferator-Activated Receptor– α (PPAR-α) in complex with either an agonist and coactivator or an antagonist and corepressor.

PPAR-α

PPAR-α is a key factor in fatty acid metabolism, and is responsible for mediating the lipid-lowering effects of fibrate drugs [Issemann and Green, 1990]. As a nuclear receptor, the PPAR-α LBD contains both the ligand binding and the ligand-dependent transcription activation function (AF-2), which is located at the extreme C-terminal end of the domain. The crystal structure of the PPAR-α LBD (Figure 1A) bound to a potent agonist, GW409544, reveals that the domain is mainly composed of α -helices that are folded into a three-layer helical sandwich [Xu et al., 2001]. The middle layer of helices is present in the upper half of the domain, but absent from the lower half of the domain, an arrangement that creates the ligand-binding pocket. Despite the conservation of the overall fold in nuclear receptors, the size and shape of the ligand binding pocket varies greatly from receptor to receptor, thus accounting for the specificity of ligand recognition and the corresponding signaling pathways.

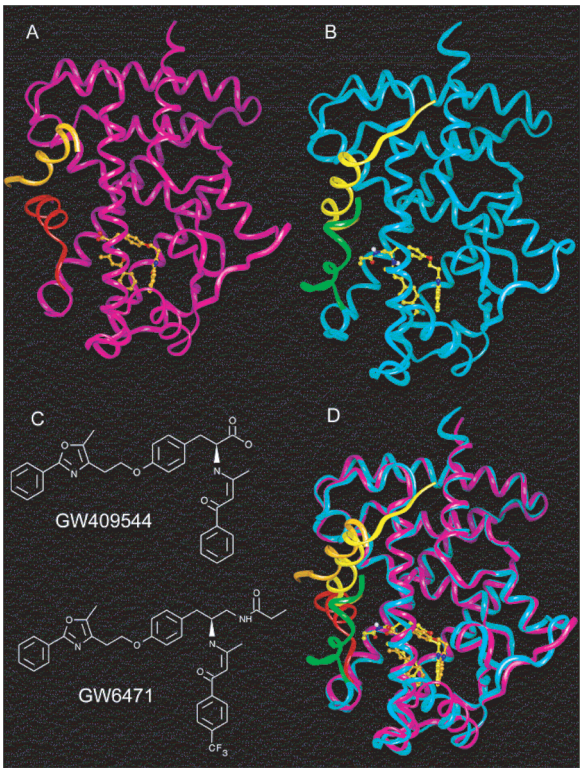

Figure 1. Structural views of ligand-mediated recruitment of coactivators and corepressors to nuclear receptors. A, structure of the PPAR α LBD bound to GW409544 and a SRC-1 coactivator motif (gold); B, structure of the PPAR α LBD bound to GW6471 and a SMRT corepressor motif (yellow); C, chemical structures of PPAR α agonist GW509544 and antagonist GW6471, D, comparison of corepressor and coactivator peptides bound to PPAR α.

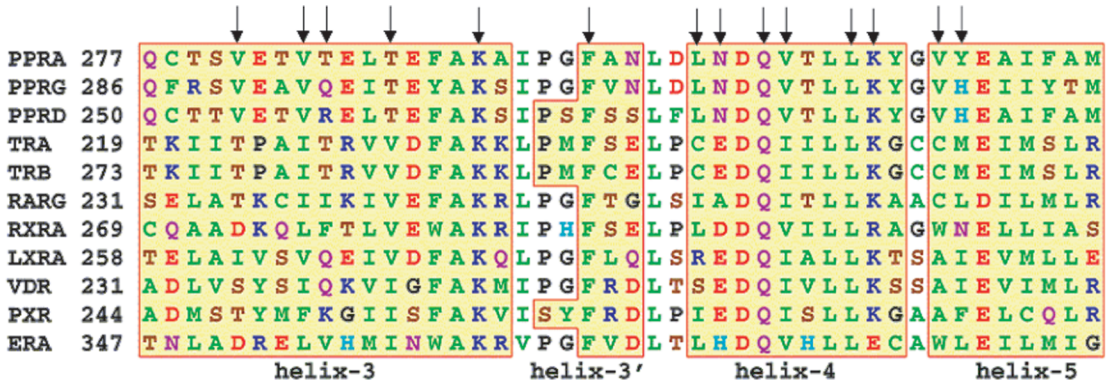

Figure 2. Conservation of coactivator and corepressor binding pockets in nuclear receptors. The residues that contact the SMRT corepressor motif are noted with arrows.

GW409544 agonist and PPAR-α

GW409544 is a synthetic acid that activates PPAR-α with an EC50 of 5 nM [Xu et al., 2001]. The PPAR-α LBD structure reveals that the acidic group of GW409544 makes a direct hydrogen bond with tyrosine-464 of the C-terminal AF-2 helix (red helix in Figure 1A), thereby locking the AF-2 helix in the active conformation. The same mode of receptor activation is also observed with PPAR γ and PPAR Δ [Gampe et al., 2000; Nolte et al., 1998; Xu et al., 1999]. In the case of PPAR γ, mutations that destabilize the active conformation of the AF-2 helix result in severe type II diabetes and dyslipidemia. Interestingly, this defect can be rescued by a potent PPAR γ agonist, which helps to re-stabilize the active AF-2 conformation [Kallenberger et al., 2003]. These results highlight the important role of ligands and the AF-2 helix in nuclear receptor regulation.

When the AF-2 helix is positioned in the active conformation, it contributes a conserved glutamate residue to form an essential part of a “charge-clamp” pocket with a lysine from helix 3. The coactivator binds to nuclear receptor through an LXXLL motif, which adopts a two-turn α -helix (gold helix in Figure 1A) with the hydrophobic leucine side-chains docking into the hydrophobic pocket between the two charge-clamp residues. The docking of the coactivator helix is further stabilized by helix-capping interactions with the charge-clamp residues at both ends of the helix. This same mode of coactivator binding was also observed in the previous structures of the PPAR γ homodimer [Nolte et al., 1998], the RXR/PPAR γ heterodimer [Gampe et al., 2000], and receptors for thyroid hormone and estrogens [Darimont et al., 1998; Shiau et al., 1998], indicating that the charge clamp pocket is the common mechanism for coactivator recruitment by nuclear receptors.

GW6471 antagonist and PPAR-α

Corepressors bind to nuclear receptors through an LXXXIXXXL/I sequence motif. This is similar to the LXXLL motif of coactivators, suggesting that corepressors might utilize a binding mode similar to that of coactivators [Perissi et al., 1999]. Expecting that corepressor recruitment to PPAR-α would be enhanced by an antagonist, we used knowledge from agonist-bound PPAR structures to design an antagonist, GW6471, to facilitate crystallization with SMRT [Xu et al., 2002]. The chemical structure of GW6471 is similar to that of GW409544 except that the carboxylate group of GW409544 has been modified into an amide group in GW6741 (Figure 1C). In its complex with PPAR-α, the GW409544 carboxylate makes a key interaction with Tyr464 in the AF-2 helix that is critical for receptor activation. The ethyl amide group in GW6471 was designed to block this key hydrogen bond, and to protrude into the volume normally occupied by Tyr464, thereby displacing the AF2 helix (green helix in Figure 1B and D). This prevents activation, and converts the compound from an agonist into an antagonist, greatly promoting the binding of corepressors to PPAR-α.

The crystal structure of the PPAR-α LBD bound to the antagonist GW6471 and a SMRT corepressor motif reveals several general features of antagonist-mediated assembly of the receptor/corepressor complex. First, the corepressor motif adopts a three-turn α -helix (yellow helix in Figure 1B), instead of the two-turn helix observed for coactivator motifs. Second, the corepressor-binding site overlaps the coactivator-binding site, with the additional helical turn of the corepressor motif extending into volume normally occupied by the AF-2 helix if it were in the active conformation. This provides a simple mechanism that makes coactivator and corepressor binding mutually exclusive and dependent on the position of the AF2 helix. Third, the binding of GW6471 pushes the AF-2 helix out of its active position, opening a larger binding grove to accommodate the extended corepressor helix. The repositioning of the AF-2 helix reinforces the importance of the highly flexible nature of this helix, allowing its conformation to be modulated by the binding of small molecules, either agonist or antagonist, to activate or to repress transcription.

The X-ray structure shows that SMRT makes key interactions with PPAR-α through the side-chains of its L+1, I+5 and L+9 residues. Alanine scanning mutagenesis through the SMRT corepressor motif confirms that these three core residues are the most critical for corepressor binding to PPAR-α and TR, especially the I+5 residue.

In addition, the PPAR-α residues that make up the corepressor binding pocket are also highly conserved across the nuclear receptor family (Figure 2), and the PPAR-α structure may provide a model for understanding ligand-mediated recruitment of coactivators and corepressors in other nuclear receptors.

Conclusion

In summary, ligand-dependent regulation of nuclear receptors is mediated through the highly mobile AF-2 helix, the position of which determines the ability of nuclear receptor to recruit coactivators or corepressors. Both coactivators and corepressor use a short helical motif to interact with nuclear receptors. In the presence of an agonist, the AF-2 helix is stabilized in the active conformation that forms a charge-clamp pocket to facilitate the binding of coactivator helix. In contrast, the binding of an antagonist keeps the AF-2 helix out of the active position, resulting in a larger pocket that destabilizes coactivator binding but fits better with the three-turn α -helix formed by the corepressor motif. The AF-2 helix thus serves as a ligand sensor to regulate nuclear receptor function. Furthermore, the ability to modulate the AF-2 helix by ligands has provided a great opportunity to design various ligands to control the biological processes mediated by the receptors and their related disease conditions.

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