

Chapter 2

Architecture of DNA Bound RAR Heterodimers

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Abstract Nuclear Retinoic Acid receptors (RARs) consist of three subtypes, α , β , and γ , encoded by separate genes. They function as ligand-dependent transcriptional regulators, forming heterodimers with Retinoid X receptors (RXRs). RARs mediate the effects of retinoic acid (RA), the active metabolite of Vitamin A, and regulate many biological functions such as embryonic development, organogenesis, homeostasis, vision, immune functions, and reproduction. During the two last decades, a number of in-depth structure–function relationship studies have been performed, in particular with drug design perspectives in the therapeutics for cancer, dermatology, metabolic disease, and other human diseases. Recent structural results concerning integral receptors in diverse functional states, obtained using a combination of different methods, allow a better understanding of the mechanisms involved in molecular regulation. The structural data highlight the importance of DNA sequences for binding selectivity and the role of promoter response elements in the spatial organization of the protein domains into functional complexes.

Abbreviations

AF-1	Activation function 1
AF-2	Activation function 2
ChIP	Chromatin immuno-precipitation

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DBD	DNA binding domain
DR	Direct repeat
Cryo-EM	Cryo-electron microscopy
FRET	Fluorescence resonance energy transfer
GR	Glucocorticoid receptor
HDX	Hydrogen deuterium exchange
IR	Inverted repeat
LBD	Ligand binding domain
LBP	Ligand binding pocket
NR	Nuclear receptor
NTD	N-terminal domain
PPAR	Peroxisome proliferator-activated receptor
RA	<i>All-trans</i> retinoic acid
RAR	Retinoic acid nuclear receptor
RARE	Retinoic acid nuclear receptor response element
RXR	Retinoid X nuclear receptor
SANS	Small angle neutron scattering
SAXS	Small angle X-ray scattering
VDR	Vitamin D nuclear receptor

Introduction

Following the pioneering work of Max Perutz and the British school of biocrystallography in the 1950s, protein crystallography has become one of the most powerful techniques for the analysis of the structure of macromolecules. Milestones have included resolution of the crystal structures of tRNA-Phe in 1974 [21, 47] and nucleic acid protein complexes (aminoacyl-tRNA synthetases/tRNAs, ribosome, RNA polymerase) that have enlightened the structure-function relationships of key steps of the translation of genetic information [9, 49, 50, 60]. The first structure of a membrane protein was resolved in 1984. This accomplishment paved the way for an incursion into an essential domain of biology [10]. Most of our knowledge of macromolecular interactions at the atomic level originates from these pioneering studies.

The field of structural investigation has expanded in the last decade to allow more ambitious questions, such as the study of transient complexes, to be undertaken. A three-dimensional view of molecular interactions, conformational changes, and dynamics of association can now be reconstructed at atomic or near atomic resolution using a variety of approaches and technologies that provide information at different time scales. Combination of these complementary data on the same molecular complex is called integrative structural biology [3].

The structural investigation of nuclear receptors (NR) started in the early nineties. There has been an impressive increase in knowledge about the structure and mechanism of action of NRs from the first reported atomic structure of a DNA binding domain [54] to our current appreciation of the structure of full length

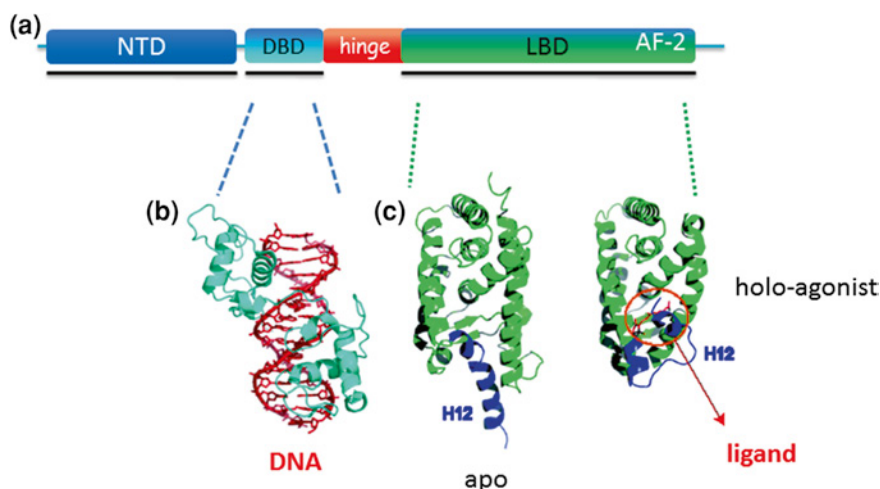


Fig. 2.1 Structural organization of RAR together with the atomic resolution structures of the isolated domain. **a** RAR, like other nuclear receptors, has a modular structure with an unstructured N-terminal domain (NTD) and two well-structured domains, the DNA binding domain (DBD) and the ligand binding domain (LBD). The LBD contains the ligand dependent activation function, AF-2. **b** Crystal structure of the RXR DBD homodimer bound to its DR1 DNA response element (PDB ID: 1BY4). Helices are represented as ribbon in cyan. **c** Structural changes in the ligand binding domain induced upon agonist ligand binding. Crystal structures of unliganded (apo) RXRα (PDB ID: 1LBD) and liganded (holo) RARγ bound to *all-trans* retinoic acid (PDB ID: 2LBD) are shown. Helices are represented as ribbon in green with the C-terminal helix H12 labeled and shown in blue

receptors bound to DNA and coactivator proteins (review in [17]). Crystallography has provided the bulk of the available information at atomic resolution with some interesting data contributed by researchers using Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (review in [19]).

History: Structural Analysis of Nuclear Receptor Isolated Domains

NRs control a large number of physiological events through their interactions with DNA sequence elements and downstream actions that are set in motion to regulate gene transcription. NR activation is controlled by ligands and cofactors that include repressors, activators, and bridging proteins [25, 39]. Additional fine-tuning is provided by post-translational modifications of NRs that result from cross-talk between different signaling pathways.

NRs share a common structural organization that is comprised of a variable N-terminal domain (NTD) harboring a ligand-independent activation function (AF-1), a conserved DNA binding domain (DBD) and a C-terminal ligand binding domain

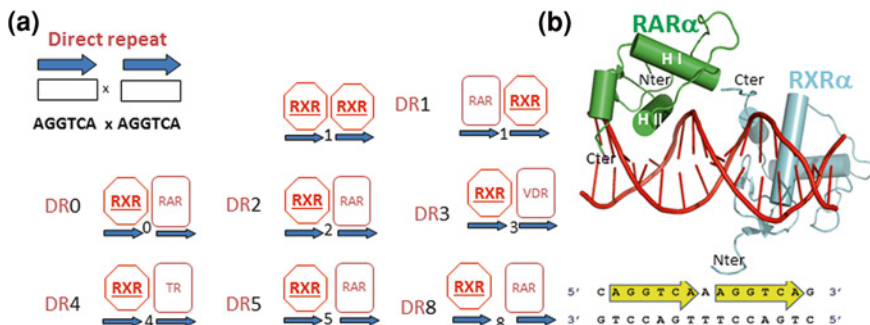


Fig. 2.2 DNA binding of RAR-RXR heterodimers to DNA. **a** DNA Retinoid response elements are composed of direct repeats (DR) of the hexanucleotide sequence (5'-(A/G)G(G/T)TCA-3') separated by separated by 0 (DR0), 1 (DR1), 2 (DR2), 5 (DR5) or 8 (DR8) nucleotides. RAR-RXR binds to these elements with a specific polarity. **b** Crystal structure of the heterodimer RARα (green)-RXRα (cyan) DBD in complex with the retinoic response element DR1. (PDB ID: 1DSZ). The DBD core is composed of approximately 66 amino acid residues which form a tertiary structure composed of an N-terminal β-hairpin and two α-helices (H I and H II) followed by a short C-terminal helix and an extension. The response elements are indicated with *yellow arrows*

(LBD). The LBD contains the ligand dependent activation function, AF-2 (Fig. 2.1). The LBD also harbors several interaction surfaces for homodimerization or heterodimerization and for the binding of coregulators. Structural studies of individual DBD and LBD have shed light on the molecular basis of transcription regulation by nuclear receptors. Unfortunately, structural information is still not available for the NTDs containing the ligand-independent function AF-1 or the hinge region connecting the DNA and ligand binding domains. These two domains are highly variable in size and sequence and show poorly defined secondary structures [30].

Studies of DNA Binding Domains

Retinoic acid receptors (RAR) are members of the NR family. The pleiotropic effects of retinoic acid (RA) are mediated through binding interactions with RARs. The RARs, as most of the NRs, function as dimers. RARs partner with retinoid receptors (RXR) to form heterodimers. A general model proposes that RARs bind to cognate RA-response elements (RAREs) both with and without RA bound to the receptor. Recent chromosome-protein precipitation analyses coupled to massive parallel sequencing and bioinformatics analyses carried out using different cell types have led to the identification of thousands of genomic RAR binding sites and RA-regulated gene networks [11, 18, 28, 29, 33]. Analyses of RAR bound loci have confirmed the presence of direct repeat consensus sequences composed of the hexanucleotide motif (5'-(A/G)G(G/T)TCA-3') separated by 1 (DR1), 2 (DR2) or 5 (DR5) nucleotides [2] (Fig. 2.2a). In vitro studies have also shown that a significant

number of RAR-RXR heterodimer-occupied sites in embryoid bodies or F9 embryonal carcinoma cells have divergent, non-canonical half-site spacings, including DR0, DR8 and inverted repeat 0 (IR0) elements [33]. RAR-RXR heterodimers bind to the asymmetric DRs with specific polarities [27, 41]. RAR DBDs bind to the half-site at the 3' end of DR5 or DR2 elements, while RXR binds to the 5' half-site of these RAREs. The polarity is reversed in the case of DR1, with RXR bound to the 3' half-site and RAR or other binding partners bound at the 5' end (Fig. 2.2a) [27, 41].

NMR provided the first 3D structure of estrogen NR DBDs [54], and crystallography unraveled the atomic details of DNA-DBD interactions in the glucocorticoid receptor (GR) [26]. It was determined that the DBD core is composed of approximately 66 amino acid residues which form a tertiary structure composed of an N-terminal β -hairpin and two α -helices followed by a short C-terminal helix and an extension (Fig. 2.1). The N-terminal α -helix (helix I) fits into the major groove of the DNA and makes direct and water mediated hydrogen bonds with the nucleotide sequence. In addition, there are a number of interactions between amino acid side chains and the phosphate backbone of the DNA. Helix II (Fig. 2.1) is perpendicular to the N-terminal helix I and stabilizes the core of the DBD.

Current understanding of DNA recognition by RAR-RXR at the atomic level is limited to the crystal structure of the RAR-RXR DBDs bound to a consensus DR1 with identical half-sites, as illustrated in Fig. 2.2b [45]. Unfortunately this structure does not clarify how non-canonical elements are recognized nor how flanking and spacer nucleotides influence the interactions. Consensus motifs could induce potential artifacts [26]. The use of natural DNA sequences from target genes for crystallization may be required to reveal the selectivity process. Such an approach has been used to study GR by the group of Yamamoto who demonstrated that the sequence of the GR binding sites differentially affects receptor conformation and transcriptional activity [32]. Although only minor structural changes could be determined by comparing the numerous GR crystal structures, the study showed that DNA can act as an allosteric effector to modulate GR activity [32]. A recent NMR study of GR DBD-DNA complexes confirms this allosteric mechanism [57]. The molecular structure of several other NR DBDs, such as the thyroid nuclear receptor (TR) or the Vitamin D nuclear receptor (VDR), either in their free states or bound to target DNA, have indicated that DNA sequences specify specific recognition and facilitate allosteric regulation [43, 51, 61].

Studies of Ligand Binding Domains

The first structures of LBDs to be determined were those of unliganded RXR α [5] and liganded RAR γ bound to all-trans RA [46]. These structures revealed a novel fold comprising 12 α -helices (H1 to H12) and a short β -turn, arranged in three layers to form an anti-parallel α -helical sandwich (Fig. 2.1). The overall fold has proven to be a prototype for the NR family [58].

The LBD is a key regulatory domain containing the ligand binding pocket (LBP) and multiple interaction surfaces for homo- or hetero- dimerization and for

interactions with corepressors, coactivators, and other cofactors that participate in sending signals to the basal transcriptional machinery [40]. Available structural data of NR dimers suggest a conserved interface in the LBD, with helices H7, H9 and H10 of each NR contributing a contact surface of between 1,000 and 1,500 square angstroms. In the absence of ligand, the RAR-RXR heterodimers are associated with corepressor complexes with histone-deacetylase activity that modify chromatin to establish and maintain a repressed transcriptional state [15, 34]. The binding of RA induces a structural transition in the LBD leading to release of the corepressors and the formation of a novel interaction surface for coactivators, including histone acetyltransferases and methyltransferases, as well as chromatin remodeling complexes or components of the basal transcription machinery [1].

LBD crystal structures for most NRs in different oligomeric states have now been determined, and the changes brought about by the binding of a large number of natural and synthetic RAR and RXR selective ligands, including agonists, antagonists, and inverse agonists, have been characterized (review in [19]). The RAR and RXR ligands can be classified by their actions on coregulator recruitment and dissociation. Ligand binding induces allosteric conformational changes that promote or repress receptor-coregulator interactions. Structural data analyses have clearly linked (1) coactivator recruitment with receptor binding of agonists (2) coactivator dissociation with receptor binding of antagonists and (3) corepressor stabilization with receptor binding of inverse agonists.

Agonist ligands induce a unique closed conformation of RAR or RXR LBD with the LBP sealed by helix H12 allowing coactivator to interact. This conformation is referred to as the “holo” or “active” conformation. Comparison of the unliganded RXR α LBD and the “holo” RAR γ LBD suggest a ligand-triggered activation mechanism that is accompanied by a repositioning of the C-terminal helix (Fig. 2.1) [46]. Helix H12 (which contains the residues of the AF-2 domain) of Apo-RXR α extends outwards to the solvent, whereas this helix in RA-bound RAR LBDs folds back over the ligand binding pocket (LBP) such that the ligand is entirely buried in a predominantly hydrophobic pocket. Structures of liganded RXR confirm the proposed mouse trap mechanism [12].

Some synthetic ligands bind to RAR or RXR with high affinity, but in contrast to natural ligands that act as agonist, they fail to stabilize the receptors’ active conformation and prevent coactivator recruitment using two molecular mechanisms. (1) Inverse agonists induce a conformational change of the receptor protein that stabilizes its interactions with the corepressor. The compound BMS493 that strengthens corepressor interaction with RAR α is an example of this type of ligand [24]. (2) Antagonists prevent helix H12 from adopting the active conformation, which disrupts the interaction surface with coactivators. The structural basis of antagonism was provided by the structures of RAR α LBD in complex with the synthetic antagonist BMS614 [6] and those of RXR α LBD in complex with LG100754 [52].

The first crystal structure of a functional heterodimer, RAR-RXR showed that hydrophobic interactions play an important role in the relative positioning and stabilization of the dimers [6]. RAR-RXR is a non-permissive heterodimer meaning

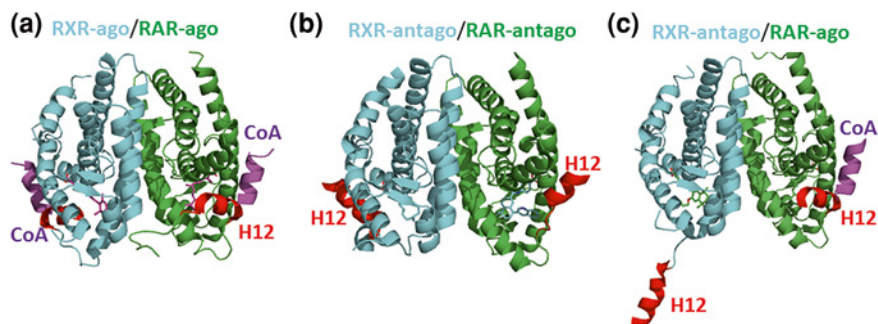


Fig. 2.3 Dimers of RAR-RXR LBDs. The crystal structures of RAR-RXR heterodimers with (a) both LBDs bound to agonists (ago) (RAR-9-*cis*-RA-RXR-9-*cis*-RA) (PDB ID: 1XDK), or with (b) both LBDs bound to antagonists (antago) (RAR-BMS614-RXR-oleic acid) (PDB ID: 1DKF), or with (c) one LBD bound to an agonist (RAR-*all-trans*-RA) and one LBD bound to an antagonist (RXR-LG100754) (PDB ID: 3A9E) have been reported. RXR and RAR are shown as ribbon in *cyan* and *green*, respectively with the C-terminal helices H12 shown in *red*. The coactivator peptide (CoA) that binds to the agonist-bound NR is shown in *pink*

that RAR agonists can activate transcription upon binding to the RAR LBD even if a ligand is not bound to the RXR LBD. In contrast, full responses to RXR ligands only occur if the RAR LBD is occupied by an agonist [13]. Although transactivation will not occur, RXR ligands are able to bind to the heterodimer even in the absence of RAR ligand [20].

The crystal structures of RAR-RXR heterodimers with both LBDs bound to agonists (RAR-9c RA-RXR-9c RA) or of RAR-RXR with both LBDs bound to antagonists (RAR-BMS614-RXR-oleic acid), or with one LBD bound to an agonist (RAR-at RA) and one LBD bound to an antagonist (RXR-LG100754) have been reported (Fig. 2.3) [6, 42, 52]. The observation that the RAR α antagonist, BMS614, prevented adoption of the open, active, RAR α conformation in full antagonist-occupied heterodimers indicated that helix H12 in RAR α occludes the coactivator binding site when the receptor is not activated [6]. Previous studies showed that binding of LG100754 to RXR α led to transactivation mediated by RAR, an effect referred to as the “phantom effect” [23]. Examination of the RXR-LG100754 interactions indicated that this ligand acts as a true RXR antagonist; that is, it prevents helix H12 of the RXR α from folding into an agonist position and instead, causes helix H12 to flip out to the solvent. The antagonism of LG100754 on the RXR LBD does not affect dimerization of RXR with RAR nor does it have any effect on RAR adopting an active conformation upon binding RA.

Taken together, structural studies have indicated that RAR can bind RA and activate transcription, but only if it is interacting with RXR; RXR does not need to have a ligand. On the other hand, RXR can bind a ligand even when RA is not bound to RAR, but the complex will be transcriptionally inactive. Thus, the ‘phantom ligand effect’ of LG100754 is explained by the fact that direct binding of RA to RAR induces coactivator binding and transcriptional activation independent of RXR LBD antagonism [52].

Development of the Field: Towards Structural Characterization of Full-Length Proteins

The crystal structure of the PPAR γ -RXR α heterodimer bound to DNA provided the first atomic resolution model of a full-length NR complex [7]. The solution structures of RAR α -RXR α -DNA were also reported by Rochel and colleagues in 2011. These last structures were obtained using a combination of different structural methods, including small angle X-ray (SAXS), neutron scattering, and electron microscopy methods. The cryo-EM structure of VDR-RXR α -DNA provided another high resolution view of the complex that fitted the SAXS data [36]. The work has produced a clear picture of receptor architecture and receptor interaction dynamics with DNA and coregulators [48].

NRs have well-defined domains separated by unstructured linkers that make them inherently flexible. Their N-terminal domains (NTD) are highly variable in both length and sequence and characteristically, unfolded. The hinge domains connecting the DBD to the LBD are also flexible (a requirement for the NRs to recognize and adapt their conformations to DNA response elements of various topologies). Additionally, their conformations are adaptable. These features make it a challenge to determine the full-length structure of NR proteins using crystallographic methods and, even more so, to trap a meaningful functional conformer in the process [31]. As well, the quaternary structure of macromolecules can be affected by crystal packing forces that create artifacts in the crystal structure [55]. Alternate approaches have been developed to address these challenges. Small-angle scattering of X-rays (SAXS) is a method that is specifically tailored for the structural analysis of multi-domain proteins with flexible linkers [44, 56]. SAXS can determine the low-resolution, three-dimensional structure of a macromolecule in close-to-native conditions in a time-resolved manner that also provides information about the kinetics and dynamics of interactive elements and biological processes. Data from NMR [16], mutagenesis [14], fluorescence resonance energy transfer (FRET), or small angle neutron scattering (SANS) are often used to complement and validate SAXS models [44]. The SANS method has the unique capability of measuring diffraction data from samples where part of the multi-component complex can be masked. Another promising method that provides macromolecular solution structures at near atomic resolution is cryo-electron microscopy (Cryo-EM). This method can attain high resolution while avoiding the pitfalls of crystal packing artifacts.

Dynamics of functional complexes have been illustrated using proton exchange methods [22]. In the case of the VDR-RXR-DNA complex, Zhang and colleagues observed that binding of ligand to VDR or RXR causes changes within both the cognate receptor LBD and the receptor partner LBD. A number of these changes map to dimerization regions as well as more distant regions in the complex [61]. These studies suggest that crosstalk between the DBD and LBD promotes allosteric regulation of receptor binding with DNA and cofactors that ultimately tune gene expression.

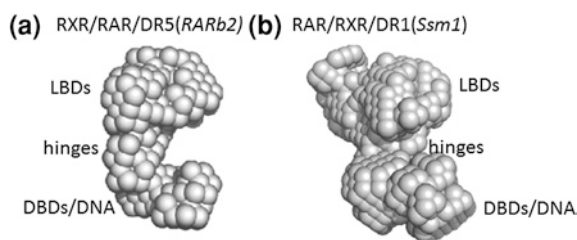


Fig. 2.4 Molecular envelopes calculated from Small Angle X-ray Scattering data of full-length complexes. **a** RAR-RXR-DR5. **b** RAR-RXR-DR1. These envelopes correspond to the low resolution architecture of the complexes showing the positioning of the two main regions of the dimer—the ligand-binding domains (LBDs) and the DNA-binding domains (DBDs) bound to the corresponding DNA

Current State of the Field: Architecture of Full-Length RAR/RXR/DNA Complexes

The atomic model of full-length PPAR γ -RXR α bound to a canonical DR1 response element [7] confirmed previous structural information obtained from studies of isolated DBDs bound to DR1 [45] and PPAR γ -RXR LBD heterodimer complexes [35, 59]. The PPAR monomer adopted a ‘closed’ conformation with extensive interactions between the PPAR LBD and DBD and the RXR LBD, hinge, and DBD [7]. In contrast, the RXR monomer had an ‘open’ conformation with the hinge region extended to create a surface for PPAR binding. The hinge regions adopt different conformations when different ligands are bound, whereas the PPAR LBD exhibits an agonist conformation even when antagonists serve as ligands. In all cases, the NTD was not visible in the electron density map and is probably unfolded. Variable conformations of the hinge regions were observed when comparing the three crystal structures of PPAR γ -RXR α in complex with different ligands. The functional correlation of the novel interdomain interactions is limited to a single point mutation having an observable effect on transactivation.

The ‘closed’ conformation of the PPAR-RXR complex was not observed in solution structure studies carried out using SAXS [48, 38]. Analysis of a number of RXR-NR heterodimers bound to different response elements using SAXS, SANS, and FRET clearly established the existence of a single, or largely dominant, conformer in solution. In contrast with the crystal structure, the solution structures of all heterodimers exhibited an extended asymmetric shape without additional interdomain contacts between the DBDs and LBDs beyond the connection through the hinge regions. The calculated molecular envelopes of RAR-RXR complexes illustrate this important result (Fig. 2.4). For both complexes with DR5 (Fig. 2.4a) and DR1 (Fig. 2.4b), the LBD dimers are positioned at the 5′ end of the target response element with an orientation orthogonal to the DNA axis. The hinge regions are in extended conformations permitting the ordering of the LBDs over the 5′ half-site of the DNA element. The pseudo-atomic models of RAR-RXR-DNA complexes have

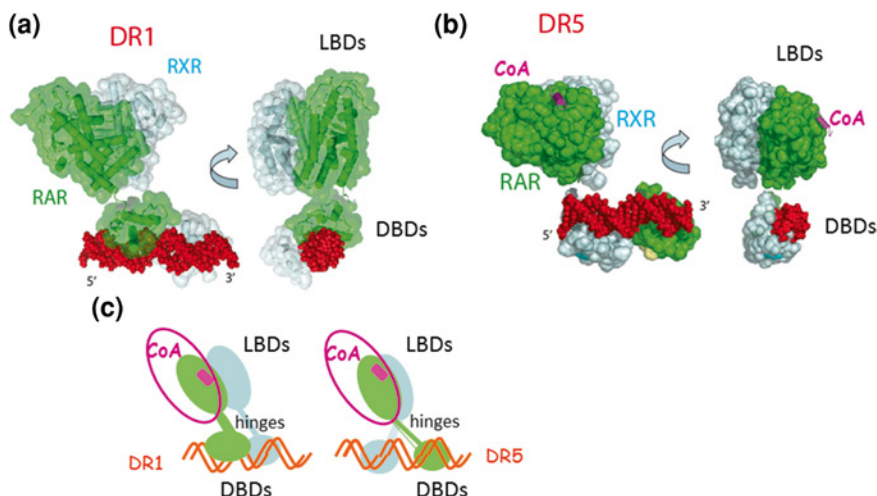


Fig. 2.5 Solution structures of full-length RAR-RXR-DNA complexes. **(a, b)** Two views of the solution structure of RAR-RXR (NTDs are truncated from the NRs) in complex with DR1 **(a)** and DR5 **(b)** DNA response elements. RAR is shown in *green*, RXR in *cyan*, DNA in *red*, and the coactivator peptide (CoA) interacting with RAR is shown in *pink*. The molecular models were obtained by docking the crystal structures of the LBD heterodimer and the DBDs bound to the DNA into the *ab initio* envelopes. The structures were refined as two rigid bodies using experimental diffraction data. Note that in the DR1 complex, the RAR and RXR DBDs are positioned on opposite side on the DNA, whereas in the DR5 complex, the RAR and RXR DBDs are positioned side by side on the DNA. **c** Schematic view of the two complexes that summarizes the main features of the structures: the absence of contacts between the DBDs and LBDs, the same orientation of the LBD heterodimer despite the different polarities of the DBD-DNA, and relative positions of the two DRs. In both cases, the relative position of the coactivator, represented by a *pink circle*, is similar

been determined, combining the available crystal structures of the domains, the SAXS analysis, and biophysical data (Fig. 2.5). Regardless of the different polarity of the bound RAR/RXR heterodimers on DR5 or DR1, the full-length RAR-RXR complexes exhibit a similar extended asymmetric conformation with the LBD dimer positioned on top of the 5' hexanucleotide (Fig. 2.5a for the RAR-RXR-DR1 and Fig. 2.5b for the RAR-RXR-DR5). In both cases, the two domains form an L-shaped structure with the dimer LBD's pseudo two-fold symmetry axis nearly orthogonal to the DNA-bound DBDs. The LBDs can rotate around the two-fold axis with the position controlled by the hinge domains that link to the DBDs. The DBDs are anchored to the DNA response elements and their location is dictated by the relative position of the binding motifs. Addition of one base pair to the spacer sequence induces a shift of approximately 3.5 Å and a rotation of 36° of the second hexameric target. Different orientations of heterodimer LBDs on RAREs is a consequence of the geometry dictated by the DNA sequence.

Cryo-EM provided a model of the VDR-RXR-DNA complex (frozen solution) that fits perfectly with the SAXS solution data and gives near-atomic details for the full-length heterodimer complex [36]. The results from these analyses confirmed the

flexibility of the full-length receptors, pointed to a lack of secondary structure in the connecting hinge domains of RXR, and underscored the importance of the hinge region in the positioning of the LBDs. Altogether the structural studies suggest that the discrepancy between the crystal and the solution structures are due to crystal packing artifacts. The latter could also explain the structural similarity between agonist and antagonist bound PPARs, a minor conformer present in the crystallization solution being trapped and stabilized by crystal packing forces. The recently published crystal structure of an HNF-4a homodimer bound to a consensus DR1 [8] revealed an asymmetric conformation that is very similar to the one observed in the solution structure of the RAR-RXR-DR1 complex, with a similar positioning of the LBDs and extended conformations of the hinges. The result confirms the concept of common architecture for DNA bound NRs and its extension to homodimers.

The ability of NRs to modulate the expression of target genes results from a combinatorial, coordinated, and sequentially orchestrated exchange between NRs and their coregulators [40]. Several structural models have been proposed for the binding of coactivators to a conserved anchoring cleft within the AF-2 in the LBD. Based on the finding that the primary sequence of the cofactor binding domain usually exhibits two or three LXXLL binding motifs, it was postulated that either two cofactors could bind to one heterodimer (RAR-RXR) or only one to both receptors using two motifs (the “hat model”). In numerous crystal structure models of LBD dimers in complex with short cofactor peptides, the stoichiometry is always 2:2, supporting both models. This observation may be an artifact arising from the addition of excess peptide during crystallization and the low binding affinity of the complex for the peptide compared to that of a larger coactivator domain. The first unambiguous structural evidence for a 2:1 stoichiometry for the receptor-coactivator complex was provided by solution studies of full-length receptors (RAR-RXR and VDR-RXR bound to DNA) bound to DNA and large coactivator protein fragments [37, 48]. Each heterodimer was shown to bind only one coactivator protein via the RXR partner. This preferential binding was controlled by affinity rather than by steric exclusion. Indeed, RAR antagonists prevent coactivator binding, whereas mutation of residues in the RXR coactivator binding cleft of RXR have no effect on the stoichiometry. The molecular model resulting from the experimental diffraction data indicate that the coactivator interacting domain is on one side of the DNA opposite to the RXR LBD and DBDs (Fig. 2.5c).

Functional Relevance

The solution structures of NRs bound to RARE reveal two key features (1) the position of LBDs at the 5' end of the target DNAs is conserved regardless of the polarity of the response elements (2) the binding of only one coactivator molecule per heterodimer through the RXR partner [48]. The combination of these two features explains the key role of DNA in NR dependent transcription regulation. The response elements direct the relative position of the LBDs and the DNA helix, which in turn fixes the binding site of the cofactors (Fig. 2.6).

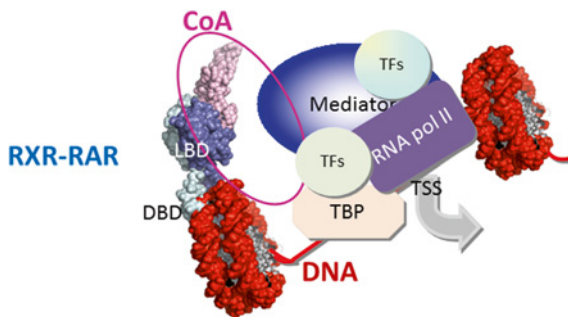


Fig. 2.6 Functional implication of the conserved relative positions of RAR and the bound coactivator. The model of RAR-RXR bound to the RAR β 2 promoter illustrates the importance of the DNA sequence in orienting the protein complex and its association with coactivator to the transcriptional initiation complex. To begin transcription, eukaryotic RNA polymerase II (RNA Pol II) requires the general transcription factors (TFs) to be associated at the promoter. The promoter contains a DNA sequence called the TATA box, located 25 nucleotides away from the site where transcription is initiated (TSS), that is recognized by the TATA box binding protein (TBP). The rest of the general transcription factors (TFs) as well as the Mediator assemble at the promoter. Docking of the complex RAR-RXR-DNA on a nucleosome of the RAR β 2 promoter using experimental DNA protection data shows that the position of the RAR LBD orients the bound coactivator (CoA) allowing its association with the transcription machinery

In summary the molecular structures show that DNA binding controls the architecture of the complexes. The polarity of the binding motifs and the number of dinucleotide spacers modulates rotation of the LBDs and the relative positions of the receptors. As a result, the environment of the accessible surface in the active complex is different for each receptor. In addition to tethering the NR near the transcription start site of target genes (Fig. 2.6), the architecture of the DNA response element can also serve as an allosteric regulator of receptor function and receptor association with coregulators [4, 32, 43, 51, 61].

The solution structures make it possible to address another functionally related question: is there a sequential order for complex formation? The structural data provide several snapshots of different functional states that suggest that the heterodimer forms first and then binds to DNA. Such a process, which combines two DBDs for a simultaneous recognition of the response element, is an efficient way to overcome the specificity problem with low affinity constants for each single DBD. The structural data also show that the extended conformation is recognized and maintained during the subsequent step, namely coactivator binding.

Future Directions

A combination of structural methods has elucidated the architecture of full-length RAR-RXR complexes bound to DR5 or DR1 RARE. However, the precise structural organization of RAR-RXR-coregulator complexes on consensus

and non-consensus DR and IR elements has not been determined. The polarity of the DNA molecule raises several questions in relation to the specific sequence of the target elements and their relative position on DR or IR. A complete understanding of the role and more specifically, the allosteric effects of DNA binding elements will require further data regarding the structure and organization of individual domains and full length NRs bound to different elements in different functional states. The capacity of NRs to specifically interact with numerous partners, such as DNA and protein cofactors, has functional consequences which are driven by mechanisms that are yet to be revealed. NRs and their coregulators are subject to post-translational modifications, including phosphorylation, acetylation, methylation and sumoylation that allosterically influence their functions. The physico-chemical details underlying the assembly and coordination of these large, transient, dynamic macromolecular complexes and the impact of post-translational modifications are yet unknown. Future studies will utilize multiple structural approaches to assemble information on complexes in multiple functional states, a first step towards “cellular structural biology”.

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Structure, Activation, and Function at the Molecular
Level

Asson-Batres, M.A.; Rochette-Egly, C. (Eds.)

2014, VIII, 230 p. 46 illus., 44 illus. in color., Hardcover

ISBN: 978-94-017-9049-9