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Review

Structural studies on nuclear receptors

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Abstract. Nuclear receptors are DNA-binding factors which regulate the transcription of sets of specific genes in response to cognate ligands, usually small lipophilic molecules, thus controlling numerous physiological events in development, procreation, homeostasis, and cellular life. Their ligand-dependent activity makes nuclear receptors obvious targets for drug design in many therapeutic areas. Crystallographic studies have revealed the structure of isolated domains but not, yet, of a whole protein, probably due to an intrinsic flexibility at work in nuclear receptor action. The structure of DNA-binding domain dimers in complex with an oligonucleotide has brought insights into how nuclear receptors recognize and bind to their target sequences ('response elements'). The structure of several ligand-binding domains in different ligation states has provided evidence for a ligand-dependent transcriptional switch and a molecular basis for the mode of action of agonists and antagonists.

Key words. Nuclear receptor; ligand-dependent regulation of transcription; chromatin remodeling; DNA-binding domain; ligand-binding domain; ligand-induced conformational change; agonist; antagonist.

Introduction

In higher eukaryotes, the transcriptional machinery integrates a whole set of intra- and extracellular signals that converge to the nucleus following different pathways and which often interfere mutually, resulting in the adequate expression of each gene in each tissue. These intricate networks allow cells to take into account numerous informational messages coming from distant organs, neighboring cells, and their own interior (endocrine, paracrine, and intracrine regulation, respectively), and to achieve a coherent physiological response. The different signals exert positive or negative effects on transcription rates through a family of proteins called transcription regulators acting at the DNA level.

Nuclear receptors (NRs) form the largest known family of eukaryotic transcription regulators [1]. Present in vertebrates, arthropods, and nematodes, they control numerous processes involved in development, growth, procreation, cell differentiation, proliferation, and apoptosis, and the maintenance of homeostasis. In most cases, they activate transcription in response to the binding of a cognate ligand, in general a small, lipophilic molecule. Known ligands include steroid and thyroid hormones, vitamins, eicosanoids, oxysterols, and bile acids (fig. 1). Their ligand-dependent activity makes NRs obvious pharmacological targets; different agonists and antagonists are already used in contraception, contragestion, the control of inflammation, the prevention of osteoporosis, and in the treatment of various diseases, including diabetes, skin diseases, hormone resistance syndromes, and some cancers. NRs

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exhibit a modular structure composed of five to six domains designated A–F (fig. 2A). The most conserved domains are C, the DNA-binding domain [(DBD, ~70 amino acids (aa)], and E, the ligand-binding domain (LBD, ~250 aa). The N-terminal A/B region is highly variable in length (ranging among classical NRs from 23 aa in VDR to 602 in MR) and sequence and is involved in regulation; it harbors the ligand-independent transactivation function (AF-1). Most NR isoforms are N-terminal variants arising from the use of different promoters and/or alternative splicing, showing distinct promoter and cell specificities [see refs 2, 3]. D is

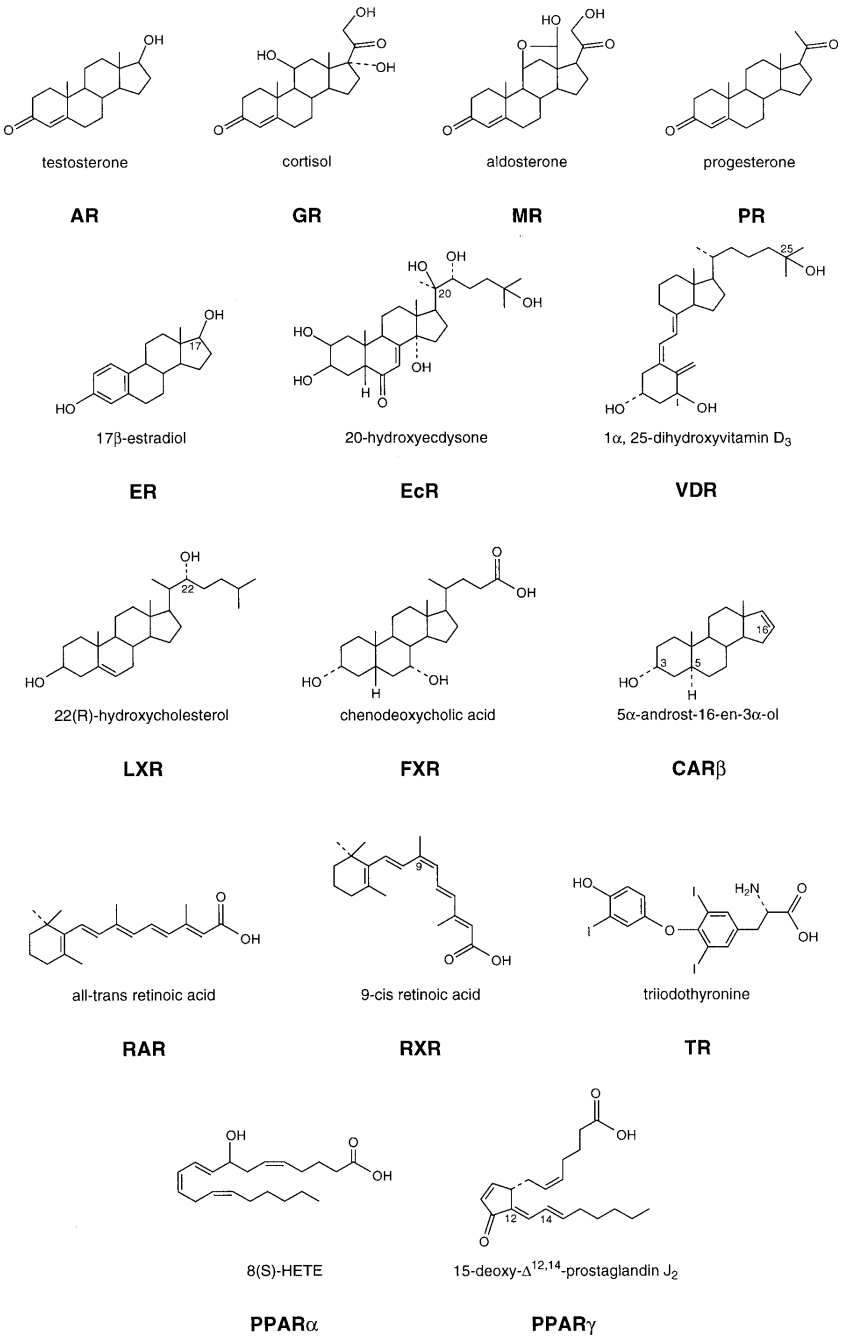

Figure 1. Known natural ligands for NRs.

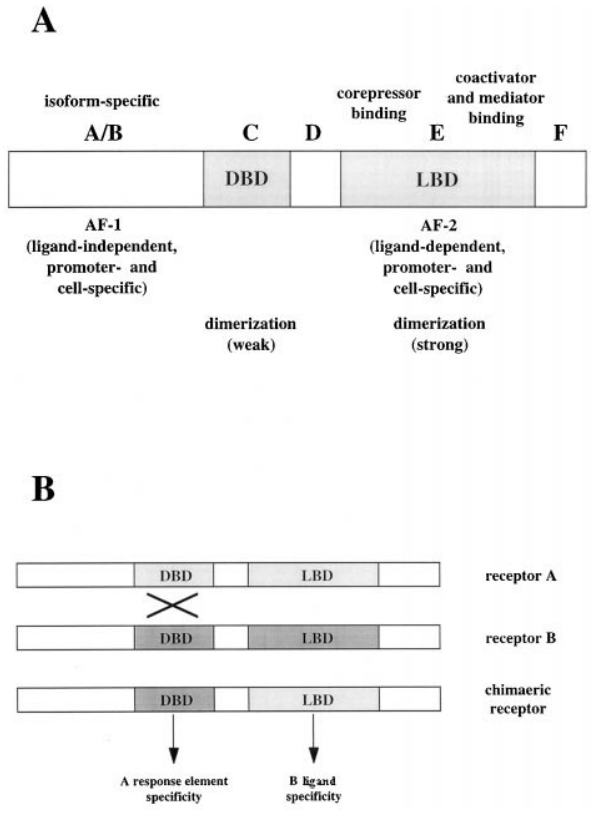

Figure 2. (*A*) Modular structure of NRs. (*B*) Principle of domain-swapping experiments.

a hinge region connecting the DBD and LBD; it is also highly variable in length and sequence, and contains nuclear localization signals. The F region is not always present and its function is poorly understood. The LBD is functionally the most important domain: it contains not only the ligand-binding site but also the ligand-dependent transactivation function (AF-2), and possesses interaction surfaces for multiple partners, including the homo-/heterodimeric partner, corepressors, coactivators, and other bridging factors that participate in signal transduction towards the basal transcription machinery. The modular character of NRs was demonstrated by domain-swapping experiments [4] (fig. 2B).

From the NR sequences deposited in databases, several phylogenetic trees have been constructed [5–8]. The most recent study [8] led to a classification into six subfamilies (fig. 3): 1, a large family containing RAR, TR, VDR, PPAR, EcR, and orphans such as ROR/RZR and Rev-erb; 2, grouping RXR, COUP, HNF4, EAR2, TR2, TR4, and TLL; 3, gathering steroid receptors, ER, and ERR; 4, the NGFIB group; 5, the SF1 group; 6, with GCNF1 alone. The problem of multiple naming for the same gene in the case of orphan receptors has prompted the launching of a unified nomenclature system based on this phylogenetic tree [9]. In this system, a separate group was created (subfamily 0) with all the unusual receptors lacking either a classical DBD (DAX, SHP) or a classical LBD (KNI, KNRL, EGON).

NRs are involved in chromatin remodeling and transcription initiation

In general, NRs activate transcription in the presence of their ligand. On the other hand, some receptors that heterodimerize with RXR such as RAR and TR act as 'silencers' in the absence of ligand: they repress basal transcription. The search for additional factors led to the characterization of two classes of NR-associated proteins called coactivators and corepressors [reviewed in ref. 10]. Some unliganded NRs (as well as some antagonist-bound steroid receptors) can recruit a corepressor such as NCoR or SMRT, which is part of a larger complex including NCoR/SMRT, Sin3, and RPD3, and possesses a histone deacetylase activity, thus contributing to maintain chromatin in a compact conformation to which transcription factors have no access. The crucial event is ligand binding, which triggers core-

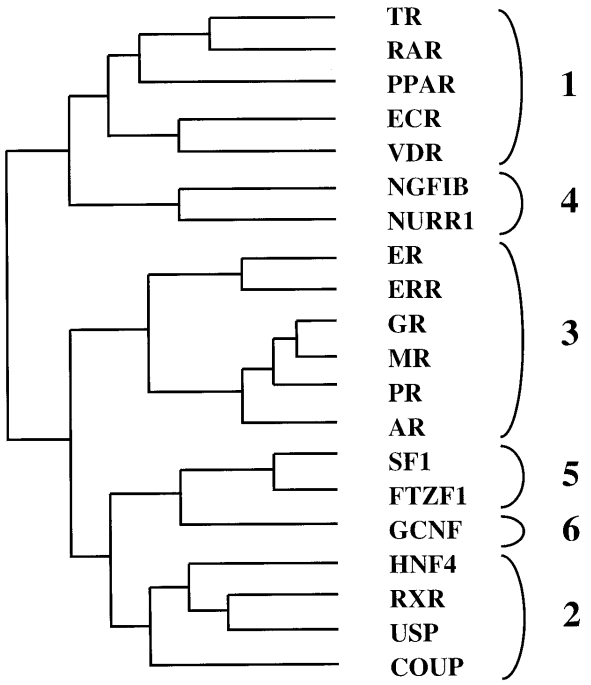

Figure 3. NR phylogenetic tree [adapted from ref. 8].

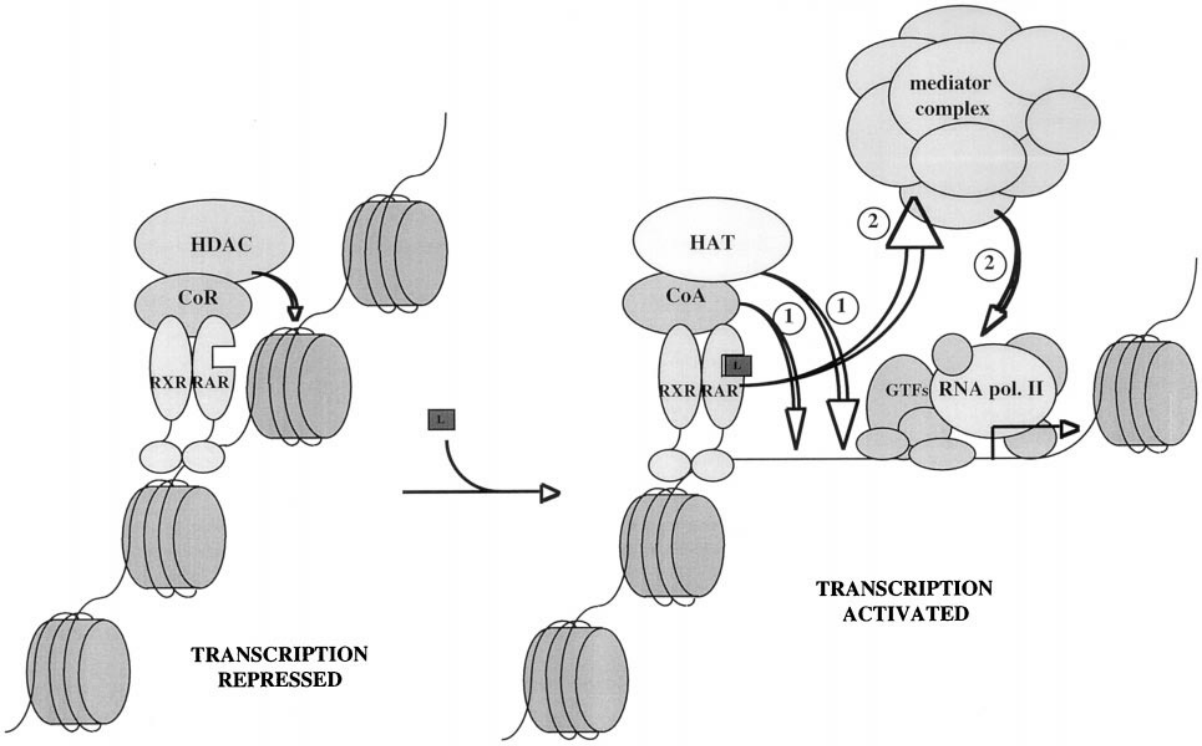

Figure 4. Schematic view of ligand-dependent activation of transcription by NRs. In the absence of ligand, the RAR-RXR heterodimer bound to its response element recruits a complex containing a corepressor (CoR) and a histone deacetylase (HDAC). Histone deacetylation contributes to maintaining chromatin in a compact state where transcription is repressed. Ligand binding triggers the dissociation of the corepressor complex and the recruitment of the coactivator complex, containing a p160 coactivator (CoA) and one or several histone acetyl-transferases (HATs). The p160 coactivators themselves exhibit weak HAT activity. Histone acetylation leads to a local unwrapping of chromatin (step 1). The coactivator complex then dissociates and the liganded NR heterodimer recruits a mediator complex that facilitates the assembly and/or stabilization of the preinitiation complex comprising the general transcription factors (GTFs) and RNA polymerase II (RNA pol. II), resulting in transcriptional activation (step 2).

pressor dissociation and coactivator binding. Among the ligand- and AF-2-dependent NR-interacting factors, the first bona fide coactivators to be identified were proteins of ~ 160 kDa (therefore called the p160 family), including SRC-1, TIF2/GRIP1, and pCIP/AIB1/ACTR/RAC3/TRAM1. The p160 coactivators possess an intrinsic histone acetyl-transferase (HAT) activity but, more importantly, they recruit signal integrators such as CBP/p300 which also exhibit HAT activity. CBP interacts directly with some NRs but this interaction is enhanced by the presence of p160 coactivators. Moreover, CBP/p300 associates with PCAF (p300/CBP-associated factor), another factor with HAT activity. All in all, DNA-bound, liganded NRs recruit a complex assembly of HATs, resulting in local chromatin unwrapping at the promoter level (in concert with ATP-dependent chromatin-remodeling factors such as the SWI/SNF complex) to allow the assembly of the preinitiation complex (PIC) comprising general transcription factors and RNA polymerase II. However, ligand-induced chromatin disruption is not sufficient for transcriptional activation [11]. Alternative biochemical approaches for identifying NR-associated protein complexes required for ligand-dependent transcriptional activation led to the discovery of very large, multisubunit complexes known as TRAP, DRIP, ARC, or SMCC ('mediator complexes') that are probably needed to stabilize the PIC, in particular by recruiting the C-terminal repeat domain (CTD) of the RNA polymerase II large subunit [12–14]. It thus appears that activation is a two-step process (fig. 4): first, ligand binding allows local chromatin disruption through histone acetylation by HATs; in the second one, mediator complexes help to assemble/stabilize the PIC, enhancing the initiation of transcription. A clue as to how the first complex becomes dissociated, allowing the binding of the second, has been provided by the demonstration of the hormone-induced acetylation of ACTR by p300/

CBP, leading to the disruption of the NR-p160 coactivator complex [15]. NRs thus transduce the absence versus presence of the ligand into repressed versus enhanced transcription.

Structural studies on NRs

The great flexibility of the hinge region between the DBD and the LBD certainly has a functional role (some heterodimers such as RXR-RAR or RXR-TR bind to direct, inverted, and everted repeats, implying that the DBD is rotationally flexible with respect to the LBD which carries the strong dimerization interface [16]), but this has hampered the crystallization of whole receptors. To date, only the structure of DBDs, alone or in complex with DNA, and of LBDs in different ligation states (without ligand or in the presence of agonists or antagonists) and recently in complex with a small peptide or a fragment of coactivator, could be obtained. Nevertheless, the modular structure of NRs allows a gross understanding of their mechanism of action from the present domain structures, even though the molecular basis for the mutual modulation between the DBD and the LBD, and between AF-1 in the N-terminal region and AF-2 in the LBD still awaits the structure determination of larger fragments.

The DBD and the DBD-DNA complexes

The NR DBD is a highly conserved 66-aa sequence comprising eight conserved cysteines which coordinate two Zn^{2+} ions, each one coordinated in a tetrahedral arrangement by four cysteines [17] (at positions 1, 4, 18, 21, and 37, 43, 53, 56, respectively; consensus DBD numbering, fig. 5A). The first structures of an NR DBD were solved by nuclear magnetic resonance (NMR): GR [18] and ER [19] (fig. 5B). Although the steroid receptors bind DNA as homodimers, the isolated DBD was found to be monomeric. The DBD contains two zinc-binding motifs (modules) that fold to form a single structural domain. In each zinc-binding motif, the third and fourth cysteine ligands are found at the N terminus of an amphipathic helix. The two amphipathic helices are almost perpendicular, crossing near their midpoint, and are held together mainly through the packing of hydrophobic side chains (at positions 24, 25, 58, and 61; fig. 5). This conserved, mostly aromatic core is surrounded by a shell of other conserved, hydrophobic residues (at positions 3, 13, 29, 35, 57, 62, and 66), the resulting extensive hydrophobic core stabilizing the DBD fold and fixing the relative orientation of the two substructures (fig. 5).

NRs bind to specific DNA sequences called response elements, which can be divided into three main classes according to the binding mode [16] (fig. 6): (i) some orphan receptors such as NGFI-B bind DNA as monomers; (ii) steroid receptors (as well as a few non-steroid receptors) bind DNA as symmetrical homodimers; (iii) non-steroid receptors including RARs (the retinoic acid receptors), TRs (the thyroid hormone receptors), VDR (the vitamin D_3 receptor), and PPARs (the peroxisome proliferator-activated receptors) bind DNA as asymmetric, oriented heterodimers with RXR. Accordingly, the response elements consist of a recognition motif derived from the archetypal AGGTCA sequence (the 'half-site') which is (i) an extended single motif in the case of monomers; (ii) duplicated as an inverted palindrome in the case of the steroid receptor homodimers; (iii) duplicated as an everted palindrome in some special cases; (iv) duplicated as a direct repeat in the case of the heterodimers with RXR (RXR itself and some orphan receptors also bind such direct repeats as oriented homodimers) (fig. 6). Mutations in the half-site sequence and variable spacer lengths give rise to a wide repertoire of response elements, allowing the specific binding of a given homo/heterodimer to multiple target genes with varying affinities, and leading to complex regulation networks involving mutual modulations between different signaling pathways ('cross-talk').

On the basis of the structure of the isolated DBD, models for the DBD dimer-response element complex consistent with genetic and biochemical data have been proposed [18, 19], where each DBD interacts with the DNA major groove at the level of a half-site through the first helix containing three exposed residues responsible for discrimination between different half-site sequences [20, 21], and that was therefore called the recognition helix. The first structures of a DBD dimer-DNA complex were later solved by crystallography for GR [22] and ER [23], confirming the main features of the models. Morever, the crystallographic structures revealed the details of the protein-DNA interactions and brought insights into the cooperative dimerization mechanism. Indeed, the main dimerization interface in NRs is found in the LBD and is DNA independent. Isolated DBD dimerization does not occur in the absence of DNA, but most NR DBDs dimerize cooperatively on their DNA targets. Briefly, the first zinc module is responsible for the half-site sequence recognition [proximal (P) box residues; 21] and the second is involved in dimerization and thus in the recognition of the half-site spacing and mutual orientation [distal (D) box residues; 21]. In the $(ER\ DBD)_2$-consensus ERE complex (fig. 7A), the ER DBD homodimer binds symmetrically to its fully specific palindromic binding site (two consensus half-sites separated by three intervening base pairs), the two DBDs binding to adjacent major grooves. The complex is stabilized by numerous interactions with the backbone phosphates, orienting the

recognition helix and allowing exposed residues to make sequence-specific contacts with DNA. Comparison of the NMR solution structure of the ER DBD monomer with the crystallographic structure of the ER DBD dimer bound to DNA shows that a 15-residue region disordered in solution is ordered in the complex, where it contacts both the DNA and the corresponding region of the other monomer, suggesting that it becomes ordered upon binding to the response element and is involved in the cooperative dimerization mechanism [24].

Consensus ERE and GRE half sites (AG**GT**CA and AG**AA**CA, respectively) differ only at the two central base pairs. Comparison of the structures of the (ER DBD)$_2$-consensus ERE complex and of the specific side of the (GR DBD)$_2$-GRE$_{S4}$ complex has revealed how each receptor recognizes its cognate half-site sequence rather than the non-cognate sequence, and has emphasized that the mechanism of discrimination is more complex than suggested by previous mutational analyses. Among the three sequence-discriminating residues (Gly25, Ser26, and Val29 in GR; Glu25, Gly26, and

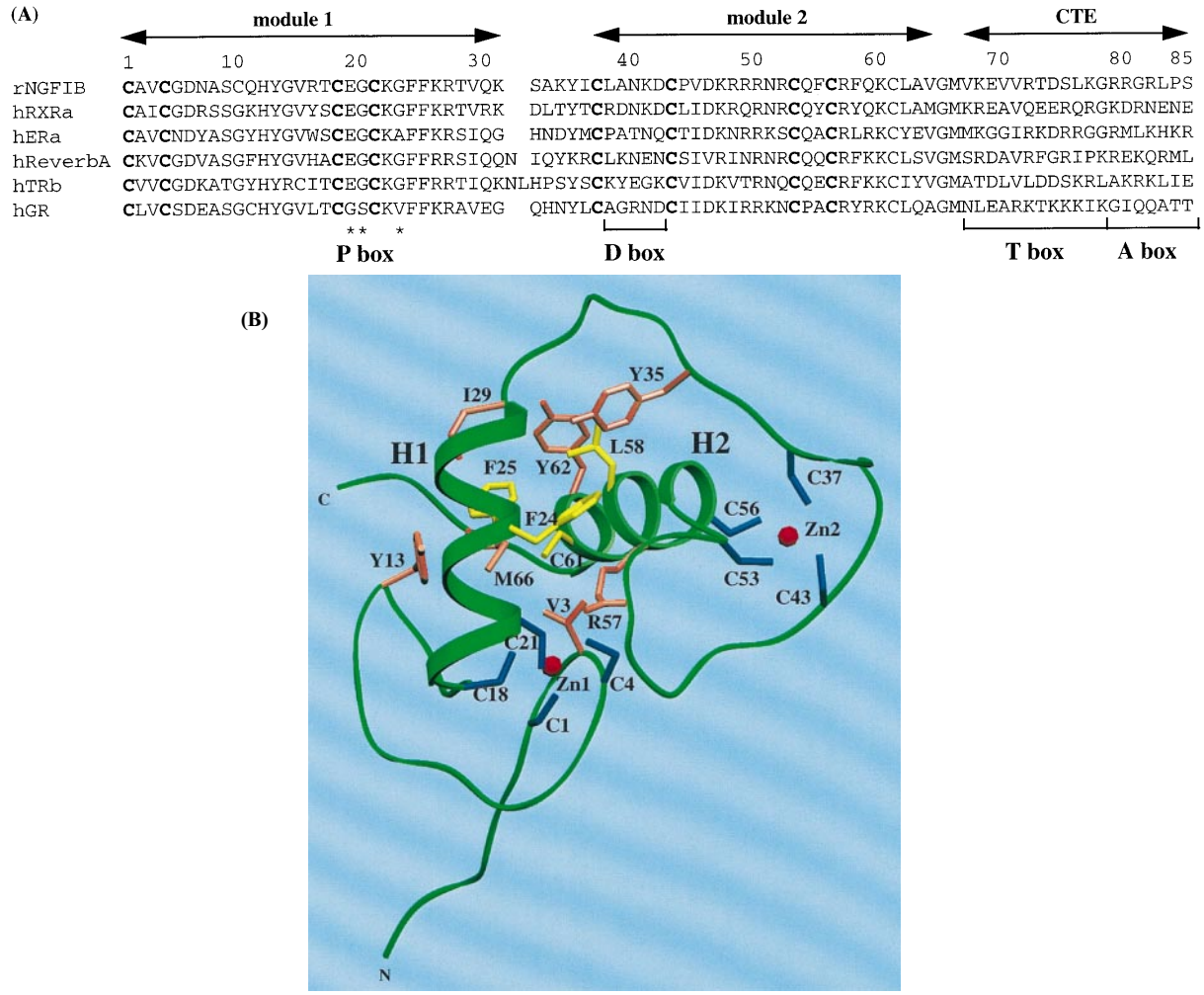

Figure 5. (*A*) DNA-binding domain (DBD) sequence alignment with consensus numbering. The eight conserved cysteines involved in Zn^{2+} coordination are shown in bold. The first zinc module is responsible for the half-site specificity [P (proximal) box residues, indicated by asterisks] and the second is involved in dimerization [D (distal) box]. The DBD C-terminal extension (CTE) contains the T (tandem) box (responsible for the cooperative dimerization on DNA) and the A (adenine) box (required for monomeric binding to extended half-sites). (*B*) ER DBD NMR solution structure [19; and PDB entry 1HCP]. The Zn^{2+} ions are depicted as red spheres, the conserved cysteines are shown in blue, the residues from the hydrophobic core in yellow, and the residues from the extended hydrophobic core in pink.

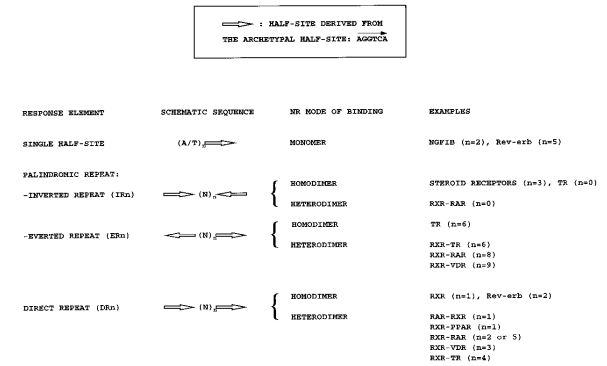

Figure 6. Different modes of DNA binding and corresponding response elements.

Ala29 in ER; fig. 8), only one makes base-specific contacts (Val29 in GR and Glu25 in ER), the others coming from conserved basic residues from the exposed face of the recognition helix (Lys28 and Arg33 in GR; Lys28, Lys32, and Arg33 in ER; fig. 8). Interestingly, Lys32 is also present in GR but not involved in GRE recognition. Thus specific recognition goes beyond the simple replacement of a triplet of amino acids by another one within a common framework, and conserved residues are sometimes used differently by different receptors. Moreover, physiological response elements rarely match the consensus sequence; thus NRs have evolved subtle mechanisms for recognizing several related DNA targets with varying affinities. Structural analysis of several complexes between a receptor and an imperfect response element have shed some light on these mechanisms [25, 26]. Thus Schwabe and colleagues [25] have solved the structure of an ER DBD homodimer in complex with an oligonucleotide derived from VitB1(2), a non-consensus response element found in the *Xenopus* vitellogenin gene B1, bearing a single mutation in one half-site (A**A**GTCA). Comparison with the structure of the $(ER\ DBD)_2$-consensus ERE complex [23] shows that the non-consensus sequence is recognized through the rearrangement of a lysine side chain leading to an alternative base contact. The crystal structure of an estrogen receptor-like DNA-binding domain bound to the wrong type of half-site (a glucocorticoid response element) reveals an interface that resembles the specific interfaces of the glucocorticoid receptor or estrogen receptor bound to their correct response elements [26]. The underlying stereochemical defect that weakens the non-cognate interface is a difference in the helical geometry of the incorrect DNA half-site which prevents a side chain contact and results in a gap which is filled by at least five additional fixed water sites, imposing a potential entropic burden on the stability of the interface. Thus an important distinction between specific and non-specific complexes is the number of ordered water molecules at the interface [27, 28].

The structure of some non-steroid NR DBDs has also been solved, including the solution structure of monomers (RXR [29], RAR [30], and ERR2 [31]) and the crystallographic structure of a monomer on a single half-site (NGFIB [32]), heterodimers on a direct repeat (RXR-TR on DR4 [33], RAR-RXR on DR1 [34]), and homodimers on a direct repeat (Rev-erb on DR2 [35], RXR on DR1 [36]). Even though their sequence is highly conserved, NR DBDs are able to arrange in tandem either head-to-head (on palindromes) or head-to-tail (on direct repeats), forming different interfaces in each case. Homodimers on palindromic response elements are symmetrical complexes, each DBD contributing the same region to the dimerization interface. In the $(ER\ DBD)_2$-ERE and the $(GR\ DBD)_2$-GRE crystal structures, the homodimerization interface is based on shape complementarity and a few receptor-specific interactions. Homo- and heterodimers on direct repeats are asymmetric complexes where different regions of the 5′ and the 3′ subunits participate in the dimerization interface. In the (RXR DBD-TR DBD)-TRE DR4 structure [33] (fig. 7B), the RXR DBD binds the upstream half-site and the TR DBD the downstream half-site, as is the case for most heterodimers with RXR [37]. The C-terminal extension (CTE) of the TR DBD (past the core DBD) contains a connecting loop corresponding to the T box of RXR (responsible for the cooperative binding of the RXR DBD homodimer to a DR1 tandem repeat [38]) and a long helix corresponding to the A box of NGFIB (required for the recognition of adenines 5′ to the single half-site to which NGFIB binds [38]), which projects here across the minor groove, making extensive contacts with DNA. The structure reveals the molecular basis of cooperative binding to DNA in the case of this heterodimer: the subunits interact through a DNA-supported interface involving the T box of the TR DBD and the second zinc module of the RXR DBD, leading to a mutual reinforcement of protein-protein and protein-DNA interactions. In the (RAR DBD-RXR DBD)-DR1 structure [34], the polarity is reversed, with the RAR DBD binding upstream and the RXR DBD downstream, the dimerization interface involving this time the T box of the RXR DBD and the second zinc module of the RAR DBD. In the RXR DBD solution structure, a third helix was found at the C terminus (T box) and was proposed to be responsible for the homodimeric binding of RXR to DNA [29, 39]. However, a subsequent NMR study on an RXR DBD monomer in complex with a half-site indicated that the additional helix unwinds upon binding [40]. Indeed, in the $(RXR\ DBD)_2$-DR1 crystallographic structure [36], the alpha helix in the T box is

disrupted, allowing efficient DNA binding and DBD dimerization. In the NGFIB DBD-extended single half-site structure [32] (fig. 7C), the A box, which is essential for monomeric binding, forms an extended structure which interacts with the minor groove of the DNA target, especially at the level of the 5′-flanking A-T base pairs. An analogous interaction between the CTE of the DBD and the 5′-flanking DNA sequence is seen in the $(\text{Rev-erb DBD})_2$-DR2 structure [35] (fig. 7D), where the A box of the downstream subunit also forms an extended loop lying in the DNA minor groove. In this case, several residues in the CTE simultaneously form dimerization and DNA contacts, thus promoting the cooperative assembly of the complex.

The LBD in different ligation states

The second most conserved region in NRs, the LBD, roughly 250 amino acids long, is responsible for ligand specificity. It is also by far the most complex and functionally important domain of NRs as it contains the ligand-binding pocket (LBP), the main dimerization interface, and the ligand-dependent activation function AF-2, and it interacts not only with the ligand but also with various partners, including the dimeric LBD partner, corepressors, coactivators and, as shown more recently, mediators. Unliganded steroid receptor LBDs bind HSP90 in vitro but these complexes may not be stable in vivo and their physiological relevance remains controversial. Finally, LBD interactions with the DBD or the N-terminal A/B region have not yet been fully characterized although allosteric effects between these domains have been reported.

The main question that arises is: how does the mere binding of a small molecule promote the complete reorganization from a repressive complex to an activating complex, both based on the same DNA-bound NR dimer? For a long time the assumption was that upon

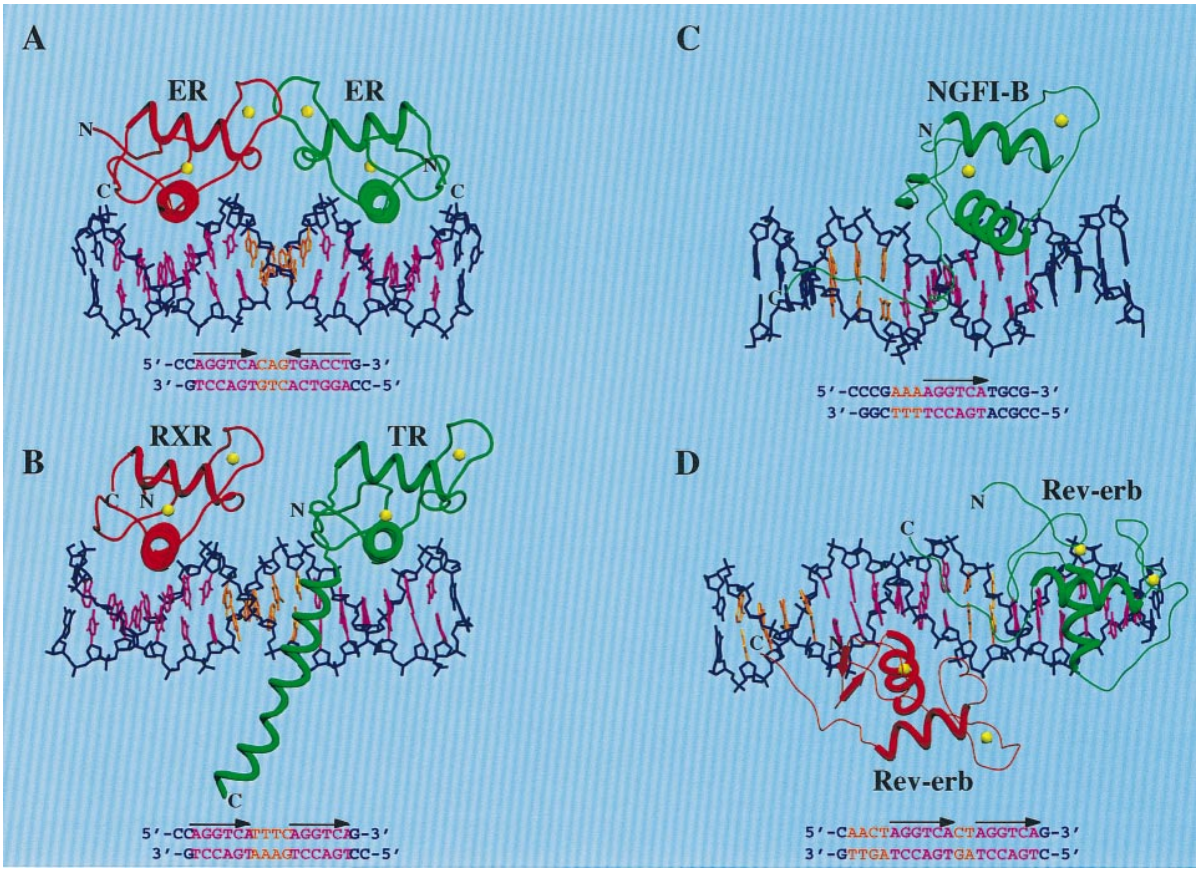

Figure 7. Crystallographic structures of DBD-DNA complexes. The DBD monomers are in red and green, the Zn^{2+} ions are shown as yellow spheres, the DNA is in blue except the half-site base pairs (in purble) and the intervening and 5′-flanking base pairs (in orange). (*A*) ER DBD homodimer-ERE [23; and PDB entry 1HCQ]. (*B*) RXR DBD-TR DBD-DR4 [33; and PDB entry 2NLL]. (*C*) NGFIB DBD-extended half-site [32; and PDB entry 1CIT]. (*D*) Rev-erb DBD homodimer-DR2 [35; and PDB entry 1A6Y].

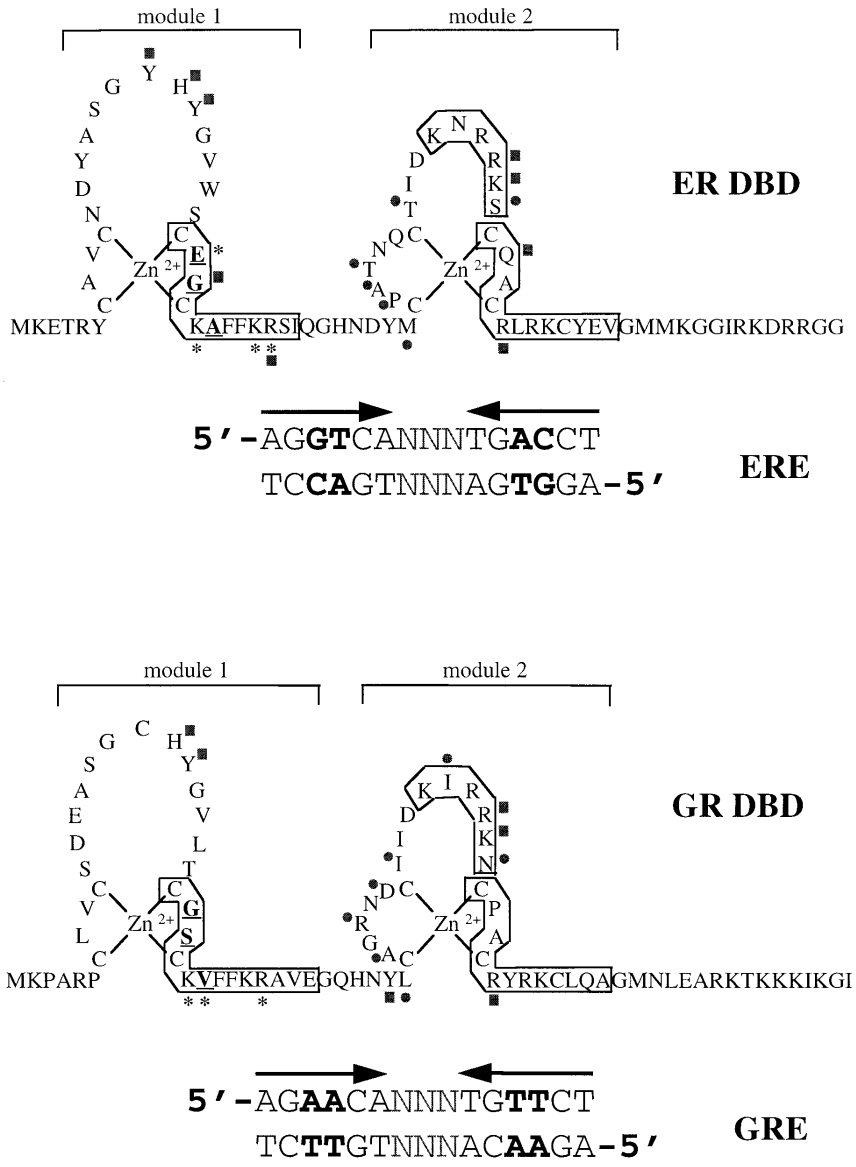

Figure 8. Secondary structure of the ER and GR DBDs. The helical segments are boxed. The P-box residues that direct the half-site recognition are shown in bold and underlined. Residues that contact bases are indicated by asterisks, residues that interact with phosphates by squares, and residues that participate in the dimer interface by circles.

ligand binding, the receptor undergoes a conformational change leading to its transcriptionally active form. Circular dichroism studies [41] showed that ligand binding induces a structural change in the receptor, but the conformational alteration elicited by the ligand remained unclear. Other studies revealed an increased resistance to proteolytic digestion [42–44] and an increased gel mobility of the LBD upon ligand binding [42], pointing to a more compact conformation of the liganded versus the unliganded LBD. Moreover, mutation and deletion studies had already emphasized the crucial role of a conserved segment at the C terminus of the LBD that was correctly predicted as an amphipathic helix, the integrity of which was shown to be absolutely required for the ligand-dependent activation of transcription [45–48]. It was therefore called the 'activation helix' or 'AF-2 activating domain.' Crystallographic studies have now given a structural basis to all these results, linking them together: ligand binding results in a repositioning of the activation helix, leading to a more compact structure.

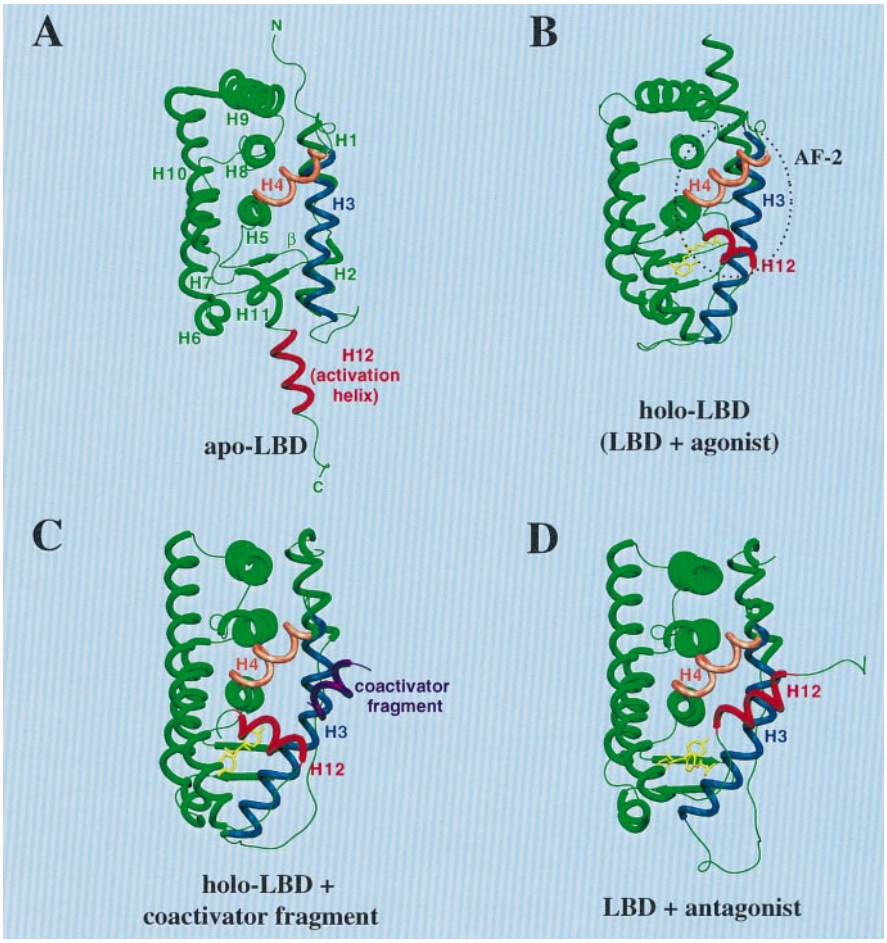

Figure 9. Crystallographic structures of NR LBDs in different ligation states. (*A*) RXRα apo-LBD [49; and PDB entry 1LBD]. (*B*) RARγ holo-LBD (in complex with the natural agonist all-*trans* retinoic acid; [55; and PDB entry 2LBD]). (*C*) ERα holo-LBD (in complex with the synthetic agonist diethylstilbestrol) bound to a coactivator peptide (the NR box 2 of GRIP1) [59; and PDB entry 3ERD]. (*D*) ERα LBD + antagonist (in complex with 4-hydroxytamoxifen; [59; and PDB entry 3ERT]).

The first crystallographic structure of an NR LBD was that of the RXR apo-LBD [49; fig. 9A], which revealed a new fold containing 12 helices and a single β hairpin. The fold, called an antiparallel α-helical sandwich, is a three-layer structure with H4, H5, H8, H9, and H11 sandwiched between H1, H2, and H3 on one side and H6, H7, and H10 on the other. In this structure, the C-terminal activation helix, H12, points away from the LBD core (note that H11 and H12 were originally seen as a continuous, kinked helix called H11). The structure also revealed the dimeric interface as the RXR apo-LBD was found as a homodimer in the crystal (fig. 10A). Each monomer contributes 11% (945 Å^2) of its solvent-accessible surface to the dimeric interface comprising H10, H9, and loop 7-8. Nine hydrophobic heptad repeats spanning from s2, the second β strand, to H10 and conserved among NR LBDs have been proposed to constitute a dimerization motif [50]. In particular, the ninth heptad repeat was shown to be critical for dimerization in various receptors [51–53]. Indeed, the ninth heptad is found in H10 which is the major structural component of the dimeric interface. None of the other heptad repeats is involved in dimerization. In fact, the hydrophobic residues at positions 1, 5, and 8 in the other heptad repeats do not contribute directly to the dimeric interface, but rather play a crucial role in stabilizing the LBD architecture through numerous interhelical van der Waals contacts [49].

Then came the structures of the TR LBD in complex with DIMIT, a synthetic thyroid hormone analog [54] and of the RAR LBD in complex with its natural ligand all-*trans* retinoic acid (ATRA) [55]. First, both structures are very similar and obviously share a common fold with RXR (fig. 9B). Second, they revealed the

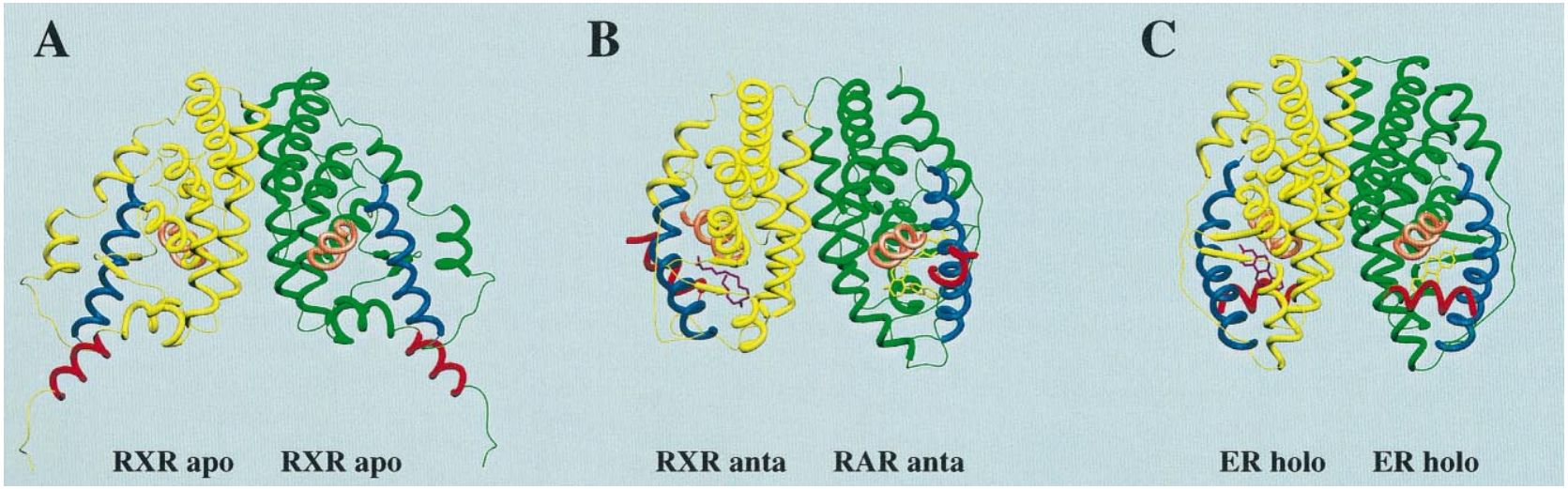

Figure 10. NR LBD homo- and heterodimers. H3 is shown in blue, H4 in pink, and H12 in red as in figure 9. (*A*) RXRα apo-LBD homodimer [49; and PDB entry 1LBD]. (*B*) Heterodimer between RXRα F318A LBD in complex with oleic acid and RARα LBD in complex with BMS614 [49; and PDB entry 1DKF]. In the RARα LBD, the α-specific antagonist BMS614 induces the 'antagonist' conformation of H12 seen in the structure of the ERα LBD in complex with raloxifene [57] or tamoxifen [59]. In the mutant RXR LBD, the partial agonist oleic acid cannot stabilize H12 in the 'agonist' conformation, and H12 also occupies the 'antagonist' conformation as in the structure of the ERβ-genistein complex [60]. The fortuitous presence of a ligand in the mutant RXR ligand-binding pocket probably accounts for its apparent constitutivity [124]. (*C*) ERα holo-LBD homodimer (in complex with the natural agonist 17β-estradiol) [57; and PDB entry 1ERE].

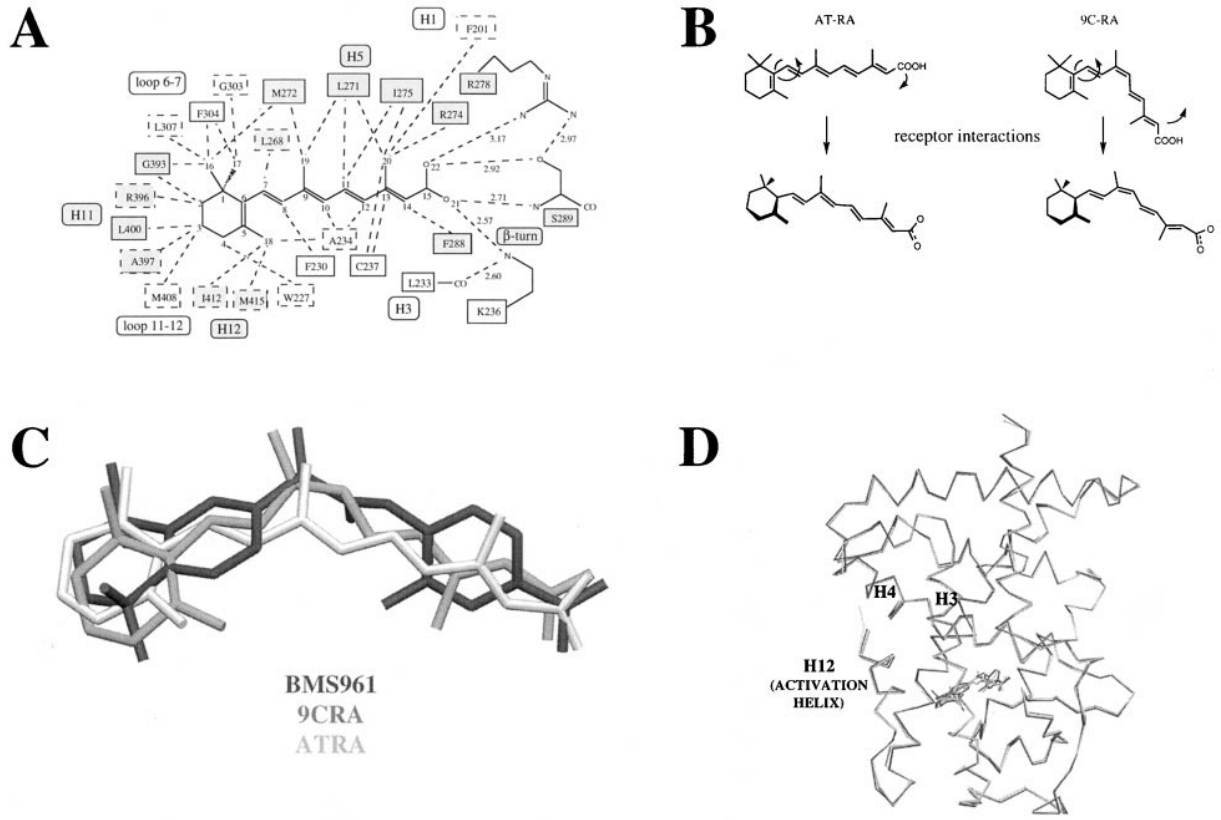

Figure 11. (*A*) van der Waals contacts and hydrogen bonds between the RARγ ligand-binding pocket and ATRA [reprinted from ref. 55 with permission]. A solid box indicates a residue within 4.0 Å and a dotted box a residue within 4.5 Å. (*B*) Conformational adaptation of the two natural agonists ATRA and 9-*cis* retinoic acid (9CRA) to the RARγ ligand-binding pocket [adapted from ref. 78]. (*C*) Superposition of ATRA, 9CRA, and the synthetic agonist BMS961 in the RARγ ligand-binding pocket [adapted from ref. 78]. (*D*) Superposition of the structures of the RARγ LBD in complex with ATRA, 9CRA [78; and PDB entry 3LBD], and BMS961 [78; and PDB entry 4LBD]. Only the Cα traces and the ligands are shown.

location of the LBP and the way the ligand is held there, namely through numerous van der Waals contacts with the mostly hydrophobic residues lining the cavity, except for the carboxylate group of the ligand which is engaged in polar interactions (fig. 11A). Moreover, the ligand was found completely buried in the LBD and the question was raised: how does the ligand enter the pocket? Third, a striking difference between the two liganded LBDs and the RXR apo-LBD was the position of the activation helix, which is folded against the LBD core in the TR and RAR structures. Thus a second question arose: are the differences between the apo and the holo structures, especially in the position of H12 and the Ω loop, due to differences between RXR and RAR/TR or are they a consequence of ligand binding?

The LBD canonical structure

Superposition of the RXR and RAR LBD structures [55] allowed the correct alignment of all NR LBD sequences and to the proposal of a conserved fold throughout the superfamily [56]; indeed RXR and RAR are evolutionarily quite distant, even though they share a common ligand, 9-*cis* retinoic acid (9CRA) (figs 1, 3). This proposal has been confirmed by all subsequent LBD structures: ER [57–60], PR [61], PPAR [62–65], and VDR [66]. Analysis of the global sequence alignment revealed two common features in the LBD of NRs: a conserved hydrophobic core in the upper part and a hypervariable region corresponding to the ligand-binding site in the lower part [56]. The conserved core is composed of hydrophobic residues mostly clustered in a 34-aa peptide ranging from the middle of H3 to the middle of H5. This segment contains the LBD signature motif: [(F,W)AKxxxxFxxLxxxDQxxLL] in its simplified form, encompassing the C terminus of H3, loop 3-4, and the N terminus of H4 [56]. The conserved core serves a structural purpose, ensuring LBD stability. On the other hand, the high sequence variability at the level of the LBP constitutes the basis of ligand specificity. For

example, 24 residues contribute to the LBP in RARγ [55] and 22 in TRα [54]; 16 residues share a common position in the alignment and only 4 are identical (fig. 12). Thus, the LBD retains the memory of the ligand in the sequence. Indeed, the ligand remodels the LBD as it enters the pocket [67] and becomes an integral part of the hydrophobic core of the liganded LBD.

LBD fold conservation throughout the NR superfamily constitutes a sound basis for reasonable homology modeling, provided experimental data are available for validation. Homology modeling has been used for RXR [56], GR [56, 68], DAX-1 [69], VDR [70, 71], ER [72, 73], MR [74], GCNF [75], and Rev-erb/RVR [76]. In these studies, the model was correlated with experimental data including quantitative structure-activity relationships, mutagenesis studies, and labeling studies.

Ligand-pocket interactions/ligand recognition

An important aim in solving the structure of NR LBDs is to reveal the determinants of the specific recognition by a given receptor of its ligand(s). The LBP is topologically conserved in all the LBD structures known to date and comprises residues mainly from H3, H5, H7, H11, H12, and the β sheet, but the precise contacts are unique for each receptor-cognate ligand pair. Details of the ligand-pocket interactions in the known liganded LBD structures have been reviewed elsewhere [77]. In the case of RAR, ATRA fits snugly into the pocket, explaining its tight binding, and the good complementarity suggests that there is not much room for additional atoms, for example bulky substituents, or for alternative geometries of the ligand. So an intriguing question arose: how does 9CRA, an isomer of ATRA

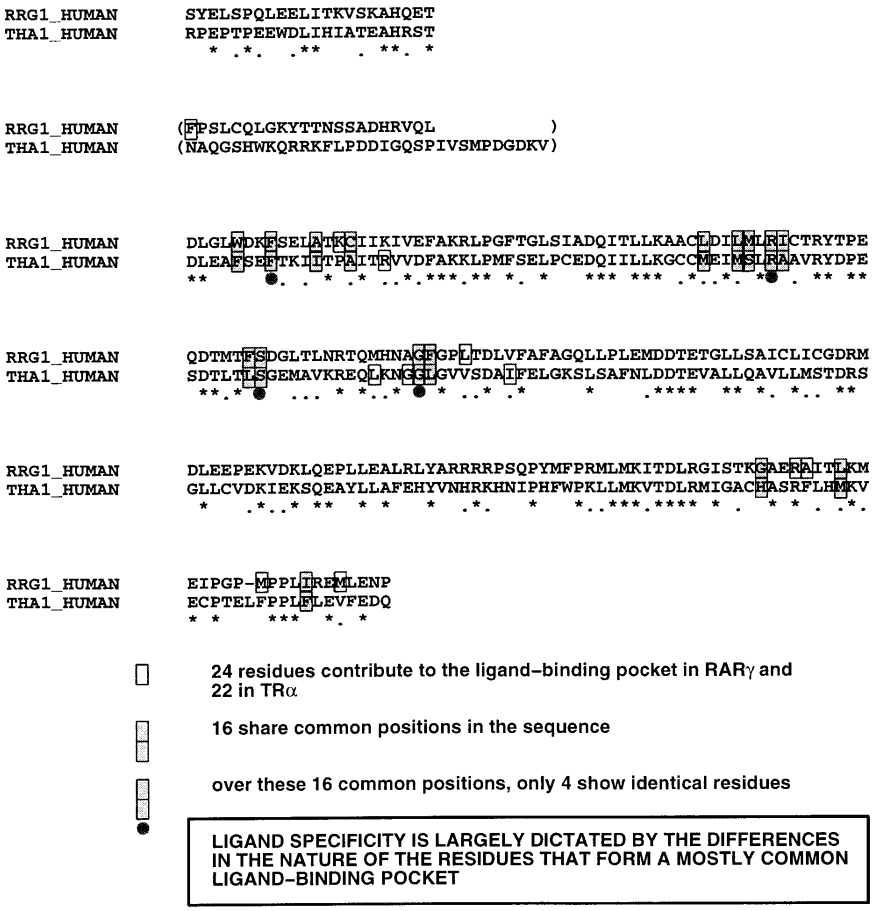

Figure 12. Structural alignment of the TRα LBD [54] and the RARγ LBD. Identical and similar residues are denoted by asterisks and dots below the alignment, respectively. Residues that contribute to the ligand-binding pocket are open boxed. The box of those sharing a common position in the sequence are shaded. Identical residues at common positions are indicated by starred dots.

with a very different geometry (fig. 1), bind to the same RAR LBD with a comparable affinity? To answer this question, the structures of the human RARγ LBD in complex with 9CRA and with a synthetic γ-specific agonist were solved [78]. Agonists were found to adapt to the LBP (fig. 11B). Thus ATRA is slightly bent by the protein constraints [55] and 9CRA is much less bent compared to the unbound state. Finally, the synthetic agonist adopts a geometry similar to that of the two natural agonists in their bound state (fig. 11C). On the other hand, the protein structure is essentially identical in the three cases, not only at the level of the LBP but also for the whole LBD, especially for the conformation of H12, suggesting again that the role of agonists is to reposition H12 to yield the transcriptionally active form of the LBD, the one that binds coactivators (fig. 11D). By contrast, PPARs show a very large pocket ($\sim$1300 Å^3 compared to $\sim$420 Å^3 for the RAR LBD), accounting for the diversity of their ligands [62–65]. Fatty acids probably bind this pocket in multiple conformations, as illustrated by the PPARβ LBD-eicosapentaenoic acid structure, where the fatty acid is found bound in two conformations [64]. Due to their capacity to bind numerous low-affinity fatty acids and fatty acid derivatives, PPARs have been suggested to behave as physiological lipid sensors, regulating transcription accordingly [79–81].

The known LBD structures have already been used for the rational design of new selective ligands [82, 83]. There is a particular need for isotype-selective ligands which could eventually be used as drugs and which are ideally devoid of unwanted side-effects. For example, ATRA controls different signaling pathways through RARα, β, and γ [84], which differ by only three residues in the LBP [55], and these residues have been demonstrated to be sufficient to control isotype selectivity, as this selectivity can be swapped upon mutation of these residues [85]. In one case (RARγ), the origin of the selectivity could be ascribed to the existence of a specific hydrogen bond between the ligand and the sulfur of Met272 (which is an isoleucine in RARβ and γ) [78, 86].

Mechanism of the ligand-dependent conformational switch: the mousetrap model

From the structural comparison between the RXR apo-LBD and the RAR holo-LBD, and from electrostatic considerations, a mousetrap mechanism was proposed, whereby ligand binding triggers a conformational change in NR LBDs which repositions the activation helix as it enters the pocket, thus creating a new surface where coactivators can bind [55]. In the RAR holo-LBD structure, H12 makes a lid to the cavity, directly contacting ATRA, and its displacement provides an obvious entry channel for the ligand. To gain further insight into the dynamic aspect of retinoic acid binding to RAR, molecular dynamic simulations were carried out [67]. In particular, enhanced sampling molecular dynamics allowed an investigation of the possible escape paths for the ligand using only the structure of the holo RAR LBD as a starting point and making no assumption. Figure 13 presents the result of a simulation with 20 ligand copies. After $\sim$100 ps, all the copies have escaped in the same direction, between H3 and H11, after H12 has moved away to open the lid, in good agreement with the mousetrap mechanism. Interestingly, with 50 copies, no other escape direction is taken by the ligand, making alternate entry paths very unlikely for RAR. The apo/holo switch has now been substantiated by the resolution of the RXR holo-LBD structure [87], which can be directly compared to the previously solved apo-LBD structure [49], confirming that the RXR LBD adopts the canonical agonist-bound conformation [56] upon 9CRA binding. Interestingly, in ER holo-LBD, H12 is also in the canonical agonist-bound conformation, forming the lid of the LBP, though it makes no direct contact with estradiol [57]. In the case of PPAR apo-LBDs, the ligand pocket is exposed to the solvent between H3 and the β sheet; moreover, the high flexibility of the loop between H2′ and H3 indicates that the channel may be even larger and thus suggests an alternative entry mode for the ligands [62, 64]. NR apo-LBDs in general probably have a more dynamic character and the conformation actually seen in the RXR apo-LBD crystal structure is probably imposed by the packing. Indeed, in the structure of the PPARγ apo-LBD dimer, H12 in one subunit has the same conformation as in the RXR apo-LBD, and H12 in the other subunit has the same conformation as in the RAR holo-LBD [62]. This suggests that there is an equilibrium between at least these two conformations of H12 in the apo state, and that the equilibrium is shifted and the holo conformation strongly stabilized upon ligand binding. In the PPARγ [63] and PPARβ [64] apo-LBD monomer structures, H12 is also packed against the LBD core. But in all three cases where H12 is in the 'holo' conformation in a PPAR apo-LBD, its conformation is slightly different from the agonist-bound conformation, emphasizing the crucial role of the direct or indirect contacts between the ligand and H12 for transactivation: the bound ligand tightens the position of H12 to provide a precise anchoring point for the coactivator together with the H3-H4 region.

On the other hand, that the ligand-induced conformational change of H12 is also directly causing corepressor release is suggested by the observation that an H12-truncated receptor constitutively binds the core-

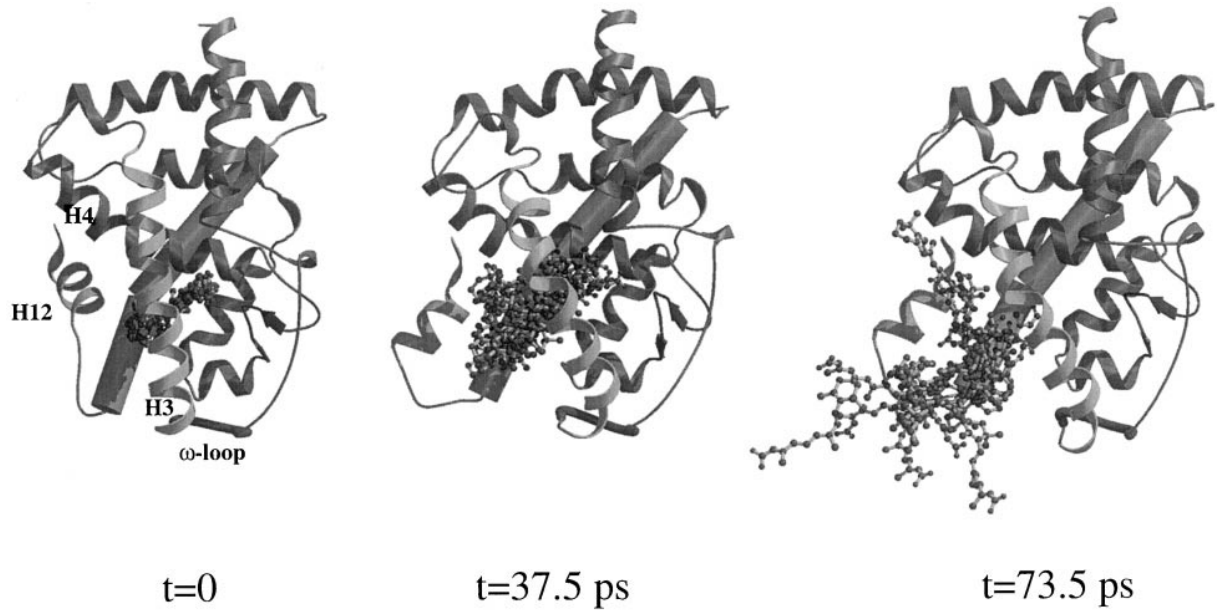

Figure 13. Enhanced sampling molecular dynamics simulation of ATRA escape from the RARγ LBD with 20 copies of the ligand [adapted from ref. 67]. ATRA is shown in ball-and-stick, H3 as a light-gray ribbon, H10 and H11 as cylinders.

pressor, which can no longer be released in the presence of the ligand [88], as was demonstrated in the case of TR by studies in solution [89].

NR-coactivator interactions and mechanism of action of agonists and antagonists

Demonstration of transcriptional interference ('squelching') between different steroid receptors was the first indication that cofactors in addition to the liganded receptor and the basal transcription machinery were needed to achieve gene activation [90]. A number of putative coactivators have been identified by several biochemical approaches [91]. Several studies had shown that a conserved segment at the C terminus of the LBD, called the AF-2 activating domain, was essential for ligand-dependent transcriptional activation: mutations were found in this segment that do not affect ligand binding or dimerization but severely impair transactivation, suggesting that it was involved in coactivator binding [45–48]. Accordingly, these mutations also impair interaction with coactivators [92, 93]. This conserved segment corresponds to H12 in the LBD structures. A highly conserved lysine from H3 was also shown to be important for transcriptional activation and was proposed to participate to the coactivator binding surface together with H12 [94]. Indeed, scanning surface mutagenesis in the case of agonist-bound TR demonstrated that a hydrophobic cleft formed between H3, H4, and H12 constitutes the coactivator binding surface [95]. Agonists are thus ligands that stabilize the activation helix in a proper, conserved position for the recruitment of coactivators. This has been confirmed by all the agonist-bound LBD structures solved to date [96]. On the cofactor side, Le Douarin et al. [97] reported that the NR-interacting proteins TIF1 and RIP140 contained a ten-amino acid-long sequence that was necessary and sufficient to bind NRs in a ligand- and activation helix-dependent manner. This conserved segment, also found to be present in TRIP3, another NR-interacting protein, was characterized by an LXXLLL motif and was termed the 'NR box' [97]. Other groups extended the study to p160 coactivators and CBP and showed that the shorter LXXLL motif was necessary and sufficient for the binding of liganded NRs [98, 99]. Indeed, LXXLL-containing peptides specifically block coactivator interaction with a liganded LBD in vitro [99] and transcriptional activation in vivo [98].

Recently, several structures of a complex between an agonist-bound LBD and a peptide containing a conserved LXXLL motif through which coactivators bind to NRs have been solved: the TR holo-LBD with a 13-amino acid peptide derived from the NR box 2 of GRIP1 [100], the ER holo-LBD and a peptide derived

from the NR box 2 of GRIP1 [59] (fig. 9C), and the PPARγ holo-LBD homodimer with an 88-residue fragment of SRC-1 [62]. In the TR and ER structures, the LXXLL motif is indeed included in an amphipathic α helix interacting with a hydrophobic cleft on the LBD surface formed between the C terminus of H3, H4, and the activation helix H12. In the PPAR structure, the SRC-1 fragment contains the NR boxes 1 and 2; each LXXLL motif binds one hPPARγ LBD monomer, the connecting region being disordered. In all three cases, the three leucines from the conserved motif point into the cleft on the LBD, making van der Waals contacts with hydrophobic residues, and the peptide is further stabilized by helix-capping interactions: first, the conserved glutamate in H12 makes hydrogen bonds with two amide groups at one end of the peptide main chain, and second, a conserved lysine at the end of H3 makes hydrogen bonds with two carbonyl groups at the other end of the peptide main chain. The glutamate in H12 and the lysine in H3, both highly conserved in NR LBDs, thus form a 'charge clamp' [62] that orients and stabilizes the NR box in its binding site on the LBD surface. This seems to be a common feature of NR-coactivator complexes, and explains how coactivators can bind different NRs. Conversely, this also explains how an NR can bind different coactivators, or different motifs within a coactivator, as illustrated by the observation that SRC-1 NR boxes 1 and 2 make identical contacts with both subunits of the hPPARγ homodimer [62]. Furthermore, NR boxes appear to bind differentially to NRs, thus allowing some selectivity of interaction [101–104]. Residues flanking both sides of the LXXLL core are presumably responsible for selectivity [100, 105], especially N-terminal basic residues [106]. Finally, the availability of the different coactivators constitutes an additional way to regulate transcription.

Conservation of the LBD conformation between the agonist-bound form alone and in complex with the NR box peptide shows that the coactivator interface is already formed upon ligand binding. This interface constitutes the whole AF-2, the ligand-dependent activation function. Thus, the AF-2 contains a static [59]/constitutive [95] part (H3, loop 3-4, and H4) and a dynamic part (H12), the role of agonist ligands being to trigger the relocation of H12 and/or to stabilize its final conformation in order to assemble and maintain the coactivator interaction surface. The recent structures of LBD-coactivator peptide complexes have brought deeper insight into the significance of the LBD signature. These conserved residues were first recognized to form a hydrophobic cluster that was important for LBD stability [56]. But in fact, the LBD signature is also conserved to ensure the stability of the H3-H4 corner, which constitutes the static part of the AF-2. And the conserved lysine in H3 is in fact important for transcriptional activation, as was shown by mutagenesis [94, 95, 106], because it contributes to the binding of the NR box peptide through the 'charge clamp,' together with the conserved glutamate in H12. The TRAP/DRIP complexes seem to be recruited by NRs in a similar fashion: one subunit, TRAP220/DRIP205, also contains LXXLL motifs and directly interacts with numerous NRs in a ligand-dependent manner [107]. Finally, acetylation by CBP of ACTR bound to estradiol-liganded ER induces coactivator release; systematic mutation revealed that neutralization of two lysine residues close to the LXXLL motif results in ER LBD-coactivator complex disruption [15]. This may explain in part the use of a coactivator complex with several acetyltransferases: acetylation would serve first to unwrap chromatin locally and then to dissociate the coactivator complex itself, allowing the second step of transcriptional activation to occur, namely the recruitment of the mediator (TRAP/DRIP) complex.

Antagonists still bind to NRs but they inhibit transcriptional activation. They are in general bulkier than the corresponding agonists, suggesting that they prevent the activation helix from adopting the conserved 'agonist conformation' through steric hindrance and induce a different positioning of H12 at the LBD surface. This was confirmed by the crystal structure of the ER LBD in complex with the antagonists raloxifene [57] and tamoxifen [59] (fig. 9D). The first study revealed that raloxifene, a selective estrogen receptor modulator (a ligand which functions as an antagonist in specific tissue and promoter contexts [108]), indeed binds at the same site as the natural agonist 17β-estradiol but induces a distinct conformation of H12: instead of making a lid to the LBP, as in the case of agonists, where its precise positioning generates a competent AF-2 surface capable of interacting with coactivators, H12 is displaced by the long side chain of raloxifene and lies in a hydrophobic groove formed by H3, loop 3-4, and H4; in this conformation, H12 partially buries the highly conserved lysine at the end of H3 (Lys362) required for transactivation [94], thus suggesting a structural basis for antagonism: raloxifene prevents the formation of the transcriptionally active AF-2 surface. Indeed, H12 is forced by the ligand to occupy the coactivator binding site, as was demonstrated in the second study, where the authors compared the structure of the ER LBD in complex with the synthetic agonist diethylstilbestrol and a coactivator peptide, and the ER LBD in complex with tamoxifen, another selective estrogen receptor modulator. In the ER LBD-diethylstilbestrol-NR box complex, H12 is in the agonist conformation, as seen in the ER LBD-estradiol complex structure [57]. In the ER LBD-tamoxifene complex, H12 adopts a conformation similar to that in

the raloxifen complex, mimicking the NR box peptide (fig. 14A). Indeed, H12 of NRs contains an LXXLL-like motif (fig. 14B), allowing its hydrophobic face to interact through van der Waals contacts with the hydrophobic groove of the coactivator binding site (fig. 14C). Note that in the crystal structure of the RXR apo-LBD and PPAR apo-LBD dimers, H12 in the extended conformation (in both subunits in RXR and in one subunit in PPAR) lies in the hydrophobic groove of the coactivator binding site of a neighboring molecule.

There are exceptions to the conserved position of H12 in agonist-bound LBDs. In the structure of the hERβ LBD-genistein complex [60], the small ligand is completely buried in the cavity and is bound in a manner similar to estradiol in the hERα LBD [57], but H12 occupies the NR box cleft rather than the pocket entrance, as in the case of the hERα LBD in complex with the antagonists raloxifene and tamoxifen. This preference may be correlated to the observation that genistein is a partial agonist for ERβ [109]. In the structure of the hPPARγ LBD-GW0072 complex, where GW0072 is a weak partial PPARγ agonist, the activation helix is found in a position closer to the apo than to the agonist position, and does not interact directly with the ligand, in contrast to the rosiglitazone complex [65]. Partial agonists would then not be able to stabilize the activation helix in the proper position, due to the lack of a few key interactions, and even a slight mispositioning of H12 may result in an attenuated transcriptional response.

NR-corepressor interactions

Several NRs such as RAR and TR repress transcription in the absence of ligand. Corepressors such as NCoR [110] and SMRT [88] mediate this transcriptional silencing by unliganded RAR and TR, as well as by the orphan receptors COUP-TF and Rev-erb [10]. NCoR/SMRT are part of larger complexes with histone deacetylase activity [111, 112] which contribute to maintaining chromatin in a condensed state. Antagonist-bound steroid receptors such as ER and PR also interact with NCoR, suggesting an active repression mechanism in these cases [10]. An NR-interacting domain (NID) was first localized at the C terminus of NCoR (aa 2240–2300) and SMRT [88, 110]. NCoR and SMRT were then shown to contain a second NID in the same region (aa 2040–2239 in NCoR) [113, 114] but until recently, no information was available on the interface between NRs and corepressors. It was then reported that the two NIDs contain an (L/I)XX(I/V)I sequence, similar to the coactivator NR boxes and thus called the CoRNR ('corner') box, and that this CoRNR box is required for unliganded NR binding, NR specificity being determined by flanking sequences; accordingly, CoRNR box peptides specifically block corepressor interaction with unliganded LBDs in vitro and transcriptional repression in vivo [115]. Nagy et al. [116] have reported similar findings, with a corepressor signature motif containing a hydrophobic $\Phi XX\Phi\Phi$ core

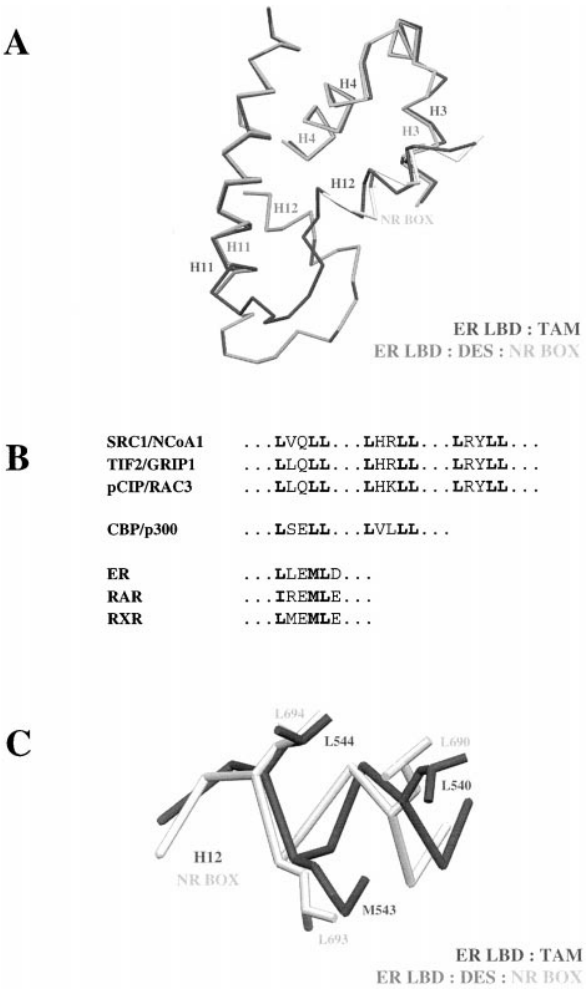

Figure 14. (*A*) Superposition of the Cα traces of the ERα LBD in complex with the selective antagonist 4-hydroxytamoxifen (in dark gray) and of the ERα LBD in complex with the agonist diethylstilbestrol (in medium gray) and the NR box 2 of GRIP1 (in light gray), showing that the antagonist-induced conformation mimics the coactivator peptide. The ligands have been omitted for clarity. (*B*) p160 coactivators and CBP/p300 (as well as TRAP220/DRIP205) bind liganded NR LBDs through conserved LXXLL motifs. H12 of NRs contains an LXXLL-like motif. (*C*) Detail of the superposition of the ERα LBD-4-hydroxytamoxifen and ERα LBD-diethylstilbestrol-NR box complexes, showing the hydrophobic side chains of the LXXLL motif of the coactivator peptide (in light gray) and of the LXXLL-like motif of H12 in the complex with the antagonist (in dark gray).

and sufficient for NR LBD binding and ligand-induced release; furthermore, they showed that mutation of NR LBD residues involved in coactivator binding also abolishes corepressor binding, suggesting at least partially overlapping binding sites. Perissi et al. [117] further showed that the two NIDs are involved in the cooperative binding of an RAR-RXR heterodimer, each NID binding one LBD of the heterodimer. They also proposed a corepressor signature motif with a longer consensus sequence, LXX(I/H)IXXX(I/L), binding to the same hydrophobic groove as the coactivators but as an N-terminally extended helix, which suggested a mechanism for the coactivator-corepressor exchange: the ligand-induced charge clamp being specific for the length of the coactivator helix, repositioning of H12 upon agonist binding would disrupt the NR-corepressor complex by preventing binding of the corepressor extended helix [117, 118]. Finally, the corepressor binding site has been characterized in the case of Rev-erb/RVR [76]. Rev-erb/RVR are orphan receptors that function as constitutive repressors, transcriptional repression being mediated by NCoR. Interestingly, Rev-erb/RVR lack the C-terminal activation helix H12. Homology modeling of Rev-erb/RVR LBD showed that the putative LBP is fully occupied by side chains, suggesting that Rev-erb/RVR lack a ligand, in good agreement with their constitutive repressing activity and the lack of H12. Modeling also revealed a large hydrophobic surface due to the absence of H12, comprising residues from H3, loop 3-4, H4, and H11. Mutation of residues from this surface severely impaired the in vitro and in vivo interaction of Rev-erbA/RVR LBD with NCoR, as well as transcriptional repression by Rev-erb/RVR. Hence, all these studies [76, 115–117] point to overlapping surfaces in NR LBDs for corepressor and coactivator association, the ligand-induced repositioning of H12 resulting in a switch from a corepressor- to a coactivator-binding surface (fig. 15).

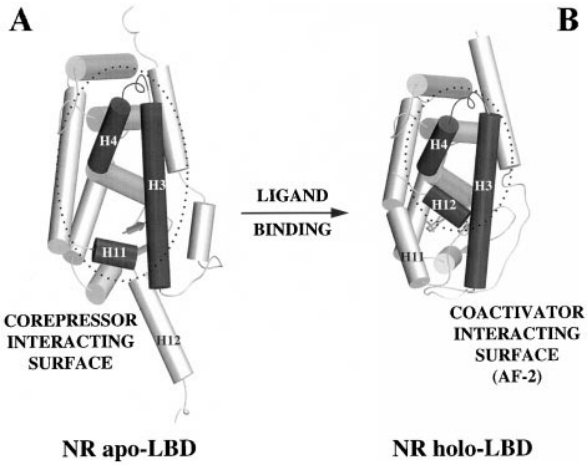

Figure 15. Differential binding of corepressors and coactivators to NR LBDs [adapted from ref. 76]. (*A*) In the absence of ligand, the corepressor is bound to the LBD (H3, H4, and H11), the 'activation helix' H12 not being involved [76]. This is exemplified by the RXR apo-LBD crystal structure, even though it may represent only one of the possible conformations in the apo state. (*B*) Upon ligand binding, H12 is locked in a conformation that is no longer compatible with corepressor binding, and the corepressor is therefore released. The newly formed AF-2 surface (H3, H4, and H12) can now recruit coactivators [59, 62, 100]. This is exemplified by the RAR holo-LBD crystal structure, which appears to be prototypical of the NR LBD canonical structure [56, 96]. Thus the ligand-induced repositioning of H12 results in a switch from a corepressor- to a coactivator-binding surface.

The homo-/heterodimeric interface

Most NRs are physiologically functional as a dimeric form, as homodimers for steroid receptors and some non-steroid receptors such as RXR and Rev-erb, and as heterodimers with RXR for most non-steroid receptors. The homodimeric interface was first seen in the RXR apo-LBD structure [49] (see above). The ER LBD also crystallized as a homodimer [57], as did the PPAR LBD in one crystalline form [62], but this latter homodimer is not functional. A distinctive interface including the H11-H12 region was seen in the crystal structure of the PR LBD homodimer [61], but it is much smaller than in the other cases since it excludes only ~ 700 Å^2 of solvent-accessible surface per monomer (compared with ~ 1700 Å^2 for ER) and its physiological relevance remains to be established. The first structure of an NR LBD heterodimer, namely RARα-RXRα, was published recently [119]. It reveals that the heterodimerization interface is topologically the same as the homodimerization interface in RXR, ER, and PPAR (fig. 10), involving mainly residues from H10, H9, loop 8-9, and H7. The buried surface is ~ 970 Å^2 for each monomer, close to the value for the hRXRα apo-LBD homodimer (~ 950 Å^2 per monomer) but smaller than that for the ER LBD homodimer (~ 1700 Å^2 per monomer). The weaker association within RXR homo- or heterodimers may allow RXR to switch between various partners [119]. A correlation was also found between residue conservation at the dimeric interface and dimerization characteristics of NRs [119]. A second structure of an NR LBD heterodimer, that of PPARγ-RXRα, was reported very recently [120]. The global structure is similar to the RARα-RXRα heterodimer. An interesting feature is the salt bridge between the C-terminal carboxylate of PPARγ (Y477, four residues downstream of H12) and K431 in H10 of RXRα. In the case of heterodimers, transcription may be regulated by both respective ligands. But some heterodimers are per-

missive for activation by RXR ligands, such as PPAR-RXR, whereas others are not, such as RAR-RXR and TR-RXR [121]. The interaction between the PPAR LBD C-terminus and H10 of RXR suggests a structural basis for permissiveness, as it may stabilize H12 of PPAR in the holo conformation allowing the recruitment of coactivators, even in the absence of a bound agonist [120].

Future goals for the structural biology of NRs

The first structural studies on NRs have brought insights as to how NRs recognize and bind to their target DNA sequences on one hand and to their ligand on the other hand, and have shed some light on the ligand-dependent transcriptional switch and on the mode of action of agonists and antagonists. The determinants of selectivity have been pinpointed in each individual case, paving the way for structure-based drug design, in particular for NRs with several isotypes.

NRs were first characterized on the basis of their ability to activate transcription in response to a given ligand. New members of the family were then cloned by different techniques, especially using their sequence homology with known receptors. As their ligand was unknown at the start, they have been named orphan receptors [122]. They represent in fact roughly 75% of the superfamily. In some cases, endogenous activators could be characterized, though it is not always clear whether they are really physiological ligands. Sometimes, only synthetic agonists have been found. Furthermore, some members of the family apparently possess no ligand and thus function as constitutive activators or repressors. A phylogenetic study suggests that the ancestral nuclear 'receptor' had no ligand and that ligand-binding ability was acquired during evolution [123]. Determining the structure of orphan receptors and deciphering their function is a promising challenge, where structural biology will be associated with many other disciplines. The discovery of ligands for some orphan receptors may unravel unidentified signaling pathways and help to define new pharmaceutical targets.

Finally, one major goal for structural biology in this domain is to determine the structure of multicomponent functional assemblies including whole receptors, DNA fragments, ligands, corepressors or coactivators, and possibly other partners, in order to reconstitute eventually the whole chain of signal transduction triggered by ligand binding to NRs.

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