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Journal of Steroid Biochemistry & Molecular Biology 74 (2000) 261–268

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

# A structural biologist's view of the oestrogen receptor

Ashley C.W. Pike, Andrzej M. Brzozowski, Roderick E. Hubbard *

*Structural Biology Laboratory, Chemistry Department, University of York, Heslington, York YO10 5DD, UK*

**Abstract**

Here we review the results that have emerged from our structural studies on the oestrogen receptor ligand-binding domain (ER-LBD). The effects of agonists and antagonists on the structure of ERα- and ERβ-LBDs are examined. In addition, the findings from structural studies of ER-LBD in complex with peptide fragments corresponding to the NR-box II and III modules of the p160 coactivator TIF2 are discussed in the context of the assembly of ER:coactivator complexes. Together these studies have broadened our understanding of ER function by providing a unique insight into ER's ligand specificity, it's ability to interact with coactivators and the structural changes that underlie receptor agonism and antagonism. 

*Keywords:* Agonism; Antagonism; Coactivator; Crystal structure; Oestrogen receptor

## 1. Introduction

Oestrogens play a critical role in the growth, development and maintenance of a diverse range of tissues. They exert their physiological effects via the oestrogen receptor (ER), which functions as a ligand-activated transcriptional regulator. ER is a member of a large family of eukaryotic nuclear receptor (NR) transcription factors [1]. Both the ER isoforms (ERα and ERβ) exhibit the characteristic domain organisation of NRs that includes a variable N-terminal transactivation (AF1) domain, a highly-conserved two zinc-finger DNA binding module and a C-terminal ligand-binding domain (LBD). The LBD is multifunctional and, in addition to harbouring a ligand recognition site, contains regions for receptor dimerisation and ligand-dependent (AF2) transactivation. In the absence of hormone, ER is sequestered in an inactive, repressed complex by molecular chaperones. Hormone binding to ER's LBD induces a conformational change in the receptor that releases it from the inactive complex, facilitates homodimerisation and subsequent binding to specific DNA sequences located in the regulatory regions of responsive genes. ER can also exert its effect by modulating the activity of the AP1 transcription complex [2]. The precise mechanism by which the ER affects gene transcription is poorly understood but, at least in the case of AF2 activation, it appears to be mediated by a host of nuclear factors that are recruited by the DNA-bound receptor (reviewed recently by O'Malley and colleagues [3]).

Structural studies on NR-LBDs have provided new insight into receptor function. At the time of writing (end of 1999), there are LBD structures for the unliganded retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor γ (PPAR) and various ligand-bound forms of the retinoic acid receptor γ (RAR), thyroid receptor (TR), oestrogen receptor (ER), δ and γ isoforms of PPAR and the progesterone receptor (PR) [4–12]. In each case, the receptor's cognate hormone binds within a hydrophobic cavity buried within the core of the molecule. Agonist binding induces a conformational rearrangement in the LBD resulting in the formation of a specific binding site for the helical NR interaction module (NR-box) of nuclear coactivators [5,10,13].

## 2. Structure of the LBD of ERα and ERβ

Table 1 summarises the structural information currently available for the ER-LBD. Three years ago we

* Corresponding author. Tel.: +44-1904-432569; fax: +44-1904-410519.

*E-mail address:* rod@ysbl.york.ac.uk (R.E. Hubbard).

0960-0760/00/$ - see front matter 
PII: S0960-0760(00)00102-3

Table 1
Summary of current structures of ER with various ligands listed in chronological order of publication

| ER isoform | Ligand(s) | PDB code | Ref. | Comments |
|---|---|---|---|---|
| ERα-LBD | $E_2$ | 1ERE | [8] | First steroid receptor structure with agonist bound. |
| ERα-LBD | RAL | 1ERR | [8] | First structure with SERM bound. Ligand promotes a novel orientation of H12 that provides a model for receptor antagonism. |
| ERα-LBD | $E_2$ | 1A52 | [9] | Hormone bound as in 1ERE but H12 alignment affected by non-native inter-molecular disulphide bridge |
| ERα-LBD | DES /peptide | 3ERD | [10] | First ER structure in complex with agonist and coactivator peptide. Illustrates the structural basis of agonist-dependent coactivator recruitment |
| ERα-LBD | OHT | 3ERT | [10] | Ligand induces a similar orientation in H12 to that seen with RAL. H12 blocks coactivator binding site. |
| ERβ-LBD | RAL | 1QKM | [14] | First structural description of beta isoform of ER. |
| ERβ-LBD | GEN | 1QKN | [14] | First structure exhibiting partial agonist conformation of H12. |
| ERα-LBD | RAL core/ peptide | – | – | Similar structure to 3ERD despite different ligand. |
| ERα-LBD | $E_2$/peptide | – | – | First description of the binding mode of a p160 NR-box III sequence. |

reported the crystal structures of human ERα (hERα) -LBD in complex with ER's endogenous hormone 17β-oestradiol ($E_2$) and with the selective oestrogen receptor modulator (SERM) raloxifene (RAL) [8]. Since then several other ligand bound forms of ER-LBD have been described including complexes with the synthetic agonist diethylstilbestrol (DES) and the SERM tamoxifen (OHT) [9,10]. More recently, we published the first structural description of the beta isoform of ER in the presence of the phyto-oestrogen genistein (GEN) and RAL [14]. In addition, we have determined the structures of ERα-LBD in complex with peptides derived from both the NR-box II and box III regions of a p160 coactivator (unpublished results).

We will discuss these structural results in the context of the following important questions on ER function:

- What is the nature of the dimerisation of ER?
- What is the structural basis of the distinctive ligand binding properties of ER?
- What are the differences between ERα and ERβ?
- How do SERMs such as RAL and OHT antagonise ER?
- What are the mechanisms for coactivator recruitment?

The three-dimensional structures of ERα- and ERβ-LBD are very similar and reflect their high sequence identity. In both cases, the polypeptide chain is folded into the canonical α-helical sandwich topology associated with NR-LBDs [15]. This compact 'wedge-shaped' domain is composed of 12 helices (H1–H12) that are arranged into three anti-parallel layers. The observed helical arrangement acts as a molecular scaffold that maintains a sizeable buried cavity of approximately 500 $Å^3$ at one end of the LBD. While the overall conformation of the ER-LBD is remarkably similar in the various ligand complexes, one aspect of the LBD, namely the orientation of the C-terminal transactivation helix (H12), is highly sensitive to the nature of the bound ligand (Fig. 1).

### 2.1. Dimerisation

All liganded ER-LBD complexes determined to date form homodimers within the crystal. The dimerisation surface is extensive and encompasses about 14–16% of each monomer's accessible surface area. The interface is dominated by the H11 helices from each respective monomer which interact via a stretch of conserved hydrophobic residues at their N-terminal ends [8]. Additional dimer interactions are provided by regions of H8, the loop between H9 and H10 and H10. This dimeric organisation appears to be the common quaternary state for NR-LBDs as similar arrangements are observed for unliganded RXR and both unliganded and liganded PPARγ [4,5]. ERα- and ERβ-LBDs have very similar dimer interfaces although there are indications that the arrangement of molecules within the ERβ-LBD dimer can be influenced by the bound ligand (unpublished observations). While both the ER isoforms readily form homodimers, their overlapping cell and tissue distribution raises the possibility of heterodimerisation. Formation of mixed dimers has been demonstrated both in vitro and in vivo [16]. Although no clear physiological role has been ascribed to ER heterodimers, the compatibility of the α and β isoform's dimer-forming interfaces suggests that their quaternary structure will be similar to ER homodimers.

### 2.2. Ligand binding

ER binds a wide repertoire of compounds with remarkable structural and chemical diversity [17]. Typically, ER ligands comprise two hydroxyl groups separated by a rigid hydrophobic linker region. In addition, effective ligands possess a phenolic hydroxyl group. Structures of ER-LBD in complex with $E_2$ [8], DES [10], RAL core, genistein [14], raloxifene [8,14] and tamoxifen [10] have been determined (see Table 1).

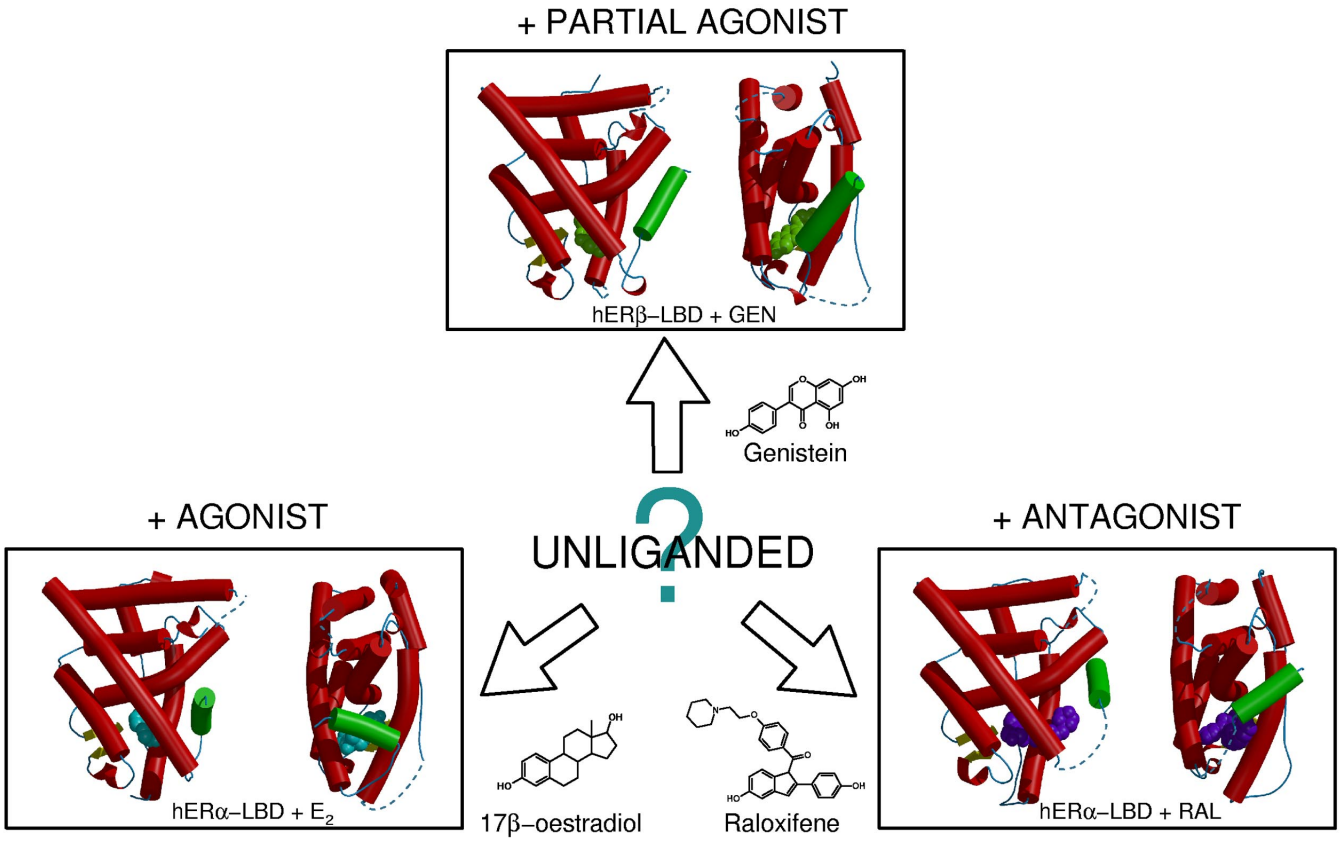


Fig. 1. Schematic representations of the three unique conformational states of ER. The conformations of ERα and ERβ-LBD in the presence of an agonist ($E_2$), a partial agonist (GEN) and a mixed agonist/antagonist (RAL) are depicted. In each ligand panel, the ER-LBD is viewed both from the side (left) and end-on (left, 90° clockwise rotation relative to side view). Helices are shown as red rods, strands as yellow arrows and coiled regions as thin blue lines. The bound ligands are shown in space-filling form and the C-terminal transactivation helix (H12) is coloured green. In the presence of agonists, H12 is orientated over the ligand-binding cavity. This helix alignment is prevented by the bulky sidechain substituent of the antagonist RAL which protrudes from cavity. Instead, H12 is redirected by the end of RAL's sidechain, rotating 120° from its agonist position, and binds in the shallow groove between H3 and H5. A third conformational state for H12 has been recently defined in the complex between hERβ-LBD and the partial agonist GEN [14]. In this complex, H12 adopts an unusual 'quasi' antagonist-like position between H3 and H5 so that it masks the coactivator recruitment site. Dotted lines highlight regions of the various crystal structures that are poorly ordered. The chemical structures of the relevant ligands are also shown.

Rather than describing the interactions made by each ligand individually, the following discussion examines the overall character of different regions of the cavity. For clarity, we will use the hERα sequence numbering scheme hereafter.

The underlying determinants of ER's 'promiscuous' ligand binding preferences were revealed by the initial hERα-LBD complexes [8]. The ligand-binding cavity is formed by residues from H3, H6, the loop region between H7 and H8, H8, H11 and H12. All the ligands bind across the cavity between H3 and H11. Ligand recognition is achieved through a combination of specific hydrogen bonds and the complementarity of the hydrophobic residues that line the cavity to the non-polar nature of ER ligands. A triumvirate of hydrogen-bonding residues (Glu353, Arg394 and a structurally conserved buried water molecule) form a polar pocket between H3 and H6. Access to this polar pocket is somewhat restricted by a 'pincer-like' arrangement of the sidechains of Leu387 (H6) and Phe404 (S1) and consequently, the planar moieties of ER ligands, such as the A-ring of $E_2$, bind at this end of the cavity. In contrast to the extensive network of hydrogen-bonding residues at the 'A-ring' region of the binding cavity, interactions at its distal end, where the D-ring of $E_2$ lies, typically involve a single hydrogen bond to a histidine residue (His524) located in H11.

While the rigid protein architecture around the A-ring pocket imposes an absolute requirement on effective ER ligands to contain a planar ring group, the remainder of the binding cavity is quite accommodating. In particular, the distal end of the cavity is quite flexible and permits a variety of ligand-binding modes [8,10,14]. Even though the length and breadth of bound ligands are well-matched by the receptor, there are relatively large unoccupied hydrophobic cavities above and below the steroid skeleton. In fact, the binding cavity is nearly twice the volume of its cognate ligand.

This unusual feature is well-known [17] and high-affinity synthetic ER ligands, such as DES, possess additional moieties that occupy these regions of the binding cavity [10]. The discrepancy between the volume of the ER binding cavity and the size of its cognate ligand is intriguing. Such a situation is to be expected for NRs such as PPARγ which must recognise a variety of different-sized ligands, but is curious for a 'single-hormone' receptor such as ER. The binding cavities of other 'single-hormone' receptors, such as RAR and TR, are well-matched to their cognate ligands [6,7]. In essence, ER's hormone binding cavity appears to have evolved sufficiently so that it can discriminate between $E_2$ and the varied assortment of endogenous steroids whilst retaining some remnants of its ancestral character. This observation is consistent with the theory that the ligand-binding ability of NRs was acquired relatively late in their evolution [18]. A more controversial interpretation of the observed sub-optimal architecture in certain regions of the hormone-binding cavity is that perhaps novel endogenous ER modulators remain undiscovered.

### 2.3. Antagonist binding

SERMs, such as RAL, bind across the cavity in a similar manner to agonists. However, their large sidechain substituent (see Fig. 1 for the chemical structure of RAL), that is characteristic of ER antagonists, cannot be accommodated within the confines of the binding cavity. Instead, the substituent protrudes from the binding cavity resulting in the displacement of H12 (Fig. 1). In addition, the limited scope for positioning this bulky substituent, combined with the antagonist's rigid core framework, forces RAL to adopt a different binding mode at the D-ring end of the cavity. Based on these observations, we were able to propose a structural model for RAL antagonism [8]. The implications of such a displacement are clear; by repositioning H12, the region that contains the core of the AF2 transactivation function [19], RAL is in some way able to disrupt the recruitment of coactivators. However, at the time, the precise consequences of this structural disruption on the ER function were unclear. Subsequent studies of NR-LBDs in complex with fragments of coactivators revealed the importance of the precise positioning of H12 (see Section 3).

### 2.4. Ligand selectivity

It is not surprising that the two ER isoforms have similar ligand binding preferences given the invariant architecture of the binding cavity. However, certain ligands exhibit selective binding profiles [20]. Such compounds are of considerable pharmaceutical interest due to the different physiological roles of ER isoforms in mammalian development. We have recently determined the structure of hERβ-LBD in complex with the phyto-oestrogen genistein (GEN) [14]. This isoflavonoid displays a 7–30-fold higher affinity for ERβ over ERα but acts as a partial agonist through this isoform [21]. The structure of this complex displays several interesting features. Most notable, perhaps, is the orientation of H12 which lies in a 'quasi'-antagonist position along the H3/H5 coactivator binding cleft (Fig. 1). This alignment of the AF2 transactivation helix correlates with GEN's partial agonist character in ERβ and suggests that coactivators must displace H12 prior to binding. The ligand itself is bound in a similar orientation to that observed with $E_2$ and DES. Examination of the binding cavity of ERβ shows that both of the conservative amino acid changes between ERα and ERβ (Leu384 → Met and Met421 → Ile) impinge on the ligand and also subtly alter the size and shape of the binding cavity [14]. However, at this stage, with only one representative structure, the origins of GEN's selective effect are not clear. Further studies of both the ER isoforms in complex with a variety of ligands will be required to fully explain the structural determinants of selective binding.

## 3. Interaction with coactivators

A variety of nuclear factors termed coactivators serve as intermediaries between liganded NRs and the general transcription machinery [3]. p160 coactivators, such as steroid receptor coactivator 1 (SRC-1) and transcription intermediary factor 2 (TIF-2), are recruited by NRs in a ligand- and AF2-dependent through a distinctive common signature motif termed the NR-box which comprises the core consensus sequence LxxLL (where L is leucine and x is any amino acid) [22].

The coactivator recruitment site has been mapped by both mutagenesis and X-ray crystallography. These studies with ER, and other NRs, demonstrate that the coactivator binding site is perfectly proportioned to bind the LxxLL core consensus motif of the NR-box and alterations in either partner abrogate this interaction [5,10,13,23–26]. The site itself comprises a shallow, hydrophobic groove, about 10 Å in length and 6 Å wide, that is formed by residues between H3, H4, H5 and H12 (Fig. 2A). The NR-box modules bind in a helical conformation so that all the three leucines of the LxxLL motif are in contact with the LBD. The peptide conformation is stabilised by a so-called 'charge clamp' [5] with N- and C-capping interactions provided by a glutamic acid residue from H12 and a lysine located at the C-terminal end of H3 respectively.

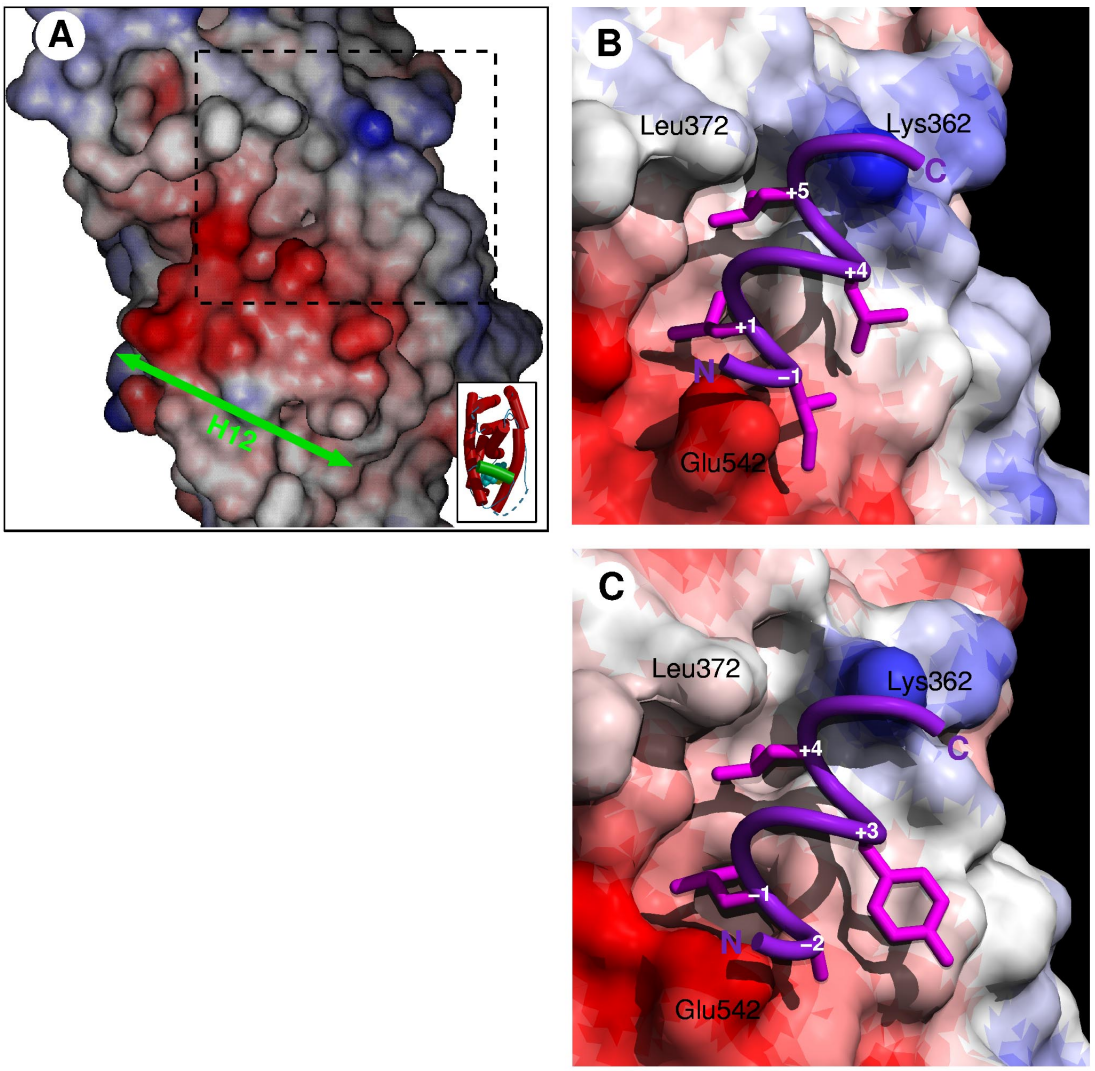


Fig. 2. View of the coactivator binding site of hERα-LBD. (A) Electrostatic surface representation of the NR-box binding face of the ERα-LBD-$E_2$ complex. The molecular surface of ERα-LBD is shown with regions of positive and negative potential coloured blue and red respectively. The inset at the lower right of the panel illustrates the approximate view of the LBD and the dashed box delineates the region of the surface that is shown in close-up in B and C. Close-up of the coactivator binding groove in the NR-box II (B) and NR-box III (C) peptide complexes with liganded ERα-LBD. The purple liquorice model represents the conformation adopted by the two 12-mer peptides derived from the box II (residues 685–696) and box III (residues 740–751) regions of the p160 coactivator rat TIF-2 [25]. For clarity, only those residues of the coactivator peptides that make contact with the LBD are shown. The peptide residues are numbered according to their positions relative to the consensus LxxLL motif (see text for details).

### *3.1. Box II*

The interactions made by peptides bearing the 'box II-like' sequence with NRs are well documented [5,10,13]. Shiau and co-workers have previously described the interactions made by a peptide derived from the box II region of GRIP1 with hERα-LBD complexed with DES [10]. We have also determined the structure of a complex between hERα-LBD and an identical peptide ($NH_2$-EKHKILHRLLQD-COOH), albeit derived from the NR-box II region of rat TIF2[1]

[1] TIF2 and GRIP1 are alternate designations for the same protein.

[25]. However, in our case the LBD was liganded with the aroylbenzothiophene core of RAL (essentially RAL *minus* its long sidechain substituent) which acts as an agonist. Despite the difference in bound ligand, the two structures are identical. The N-terminal end of the peptide interacts with the sidechain of Glu542 (H12) through hydrogen bonds between the carboxylate group and the mainchain amides of Ile689 and Leu690 (Fig. 2B). Similarly, the ε-amino group of Lys362 caps the C-terminal end of the peptide helix through a hydrogen bond with the mainchain carbonyl of Leu693. All three leucines of the LxxLL are in contact with the LBD — the leucines at the +1 and +5 positions of the peptide[2] project into two shallow pockets in the binding groove. The Ile at −1 and the +4 Leu lie on the periphery of the binding site. In contrast, the two spacer X residues make no interactions and project away from the LBD.

### 3.2. Box III

We have also recently solved the structure of hERα-LBD-$E_2$ in complex with a 12-mer peptide ($NH_2$-KENALLRYLLDK-COOH) derived from the NR-box III region of rat TIF2 to 2.4 Å resolution (unpublished results). Surprisingly the box III peptide binds in a different orientation to that observed in all NR-box/NR-LBD complexes determined to date (Fig. 2C). At first glance, the position adopted by the peptide looks identical to that observed in the box II complex. Both the hydrophobic pockets along the binding groove are occupied by leucine sidechains of the coactivator peptide. However, on closer examination, one can see that the peptide has 'corkscrewed' along the binding site by one residue towards Lys362. Consequently, residues at the −2 (Ala) and +3 (Tyr) positions lie on the edge of the groove and the completely buried leucines are contributed by the −1 and +4 peptide positions. In fact, both leucines that are buried in the box II complex are rotated out of the binding site and exposed to solvent. Therefore, for the box III peptide, the binding motif is LxxYL rather than the consensus LxxLL.

This difference in binding orientation may represent a quirk of the sequence that flanks the core consensus LxxLL module. The NR-box III sequence is considerably more hydrophobic than the corresponding box II region of TIF2. Another explanation for the observed shift may reflect the need for correctly positioning the basic residue (−5) that precedes the LxxLL module. Mutagenesis studies suggest that the (−3 to −5) basic region of NR-box II is required for high affinity binding to ER [23]. The physiological significance of this altered binding mode is not clear but it illustrates the general principle that the binding groove of ERα can accommodate other sequence motifs apart from the characteristic LxxLL module. A further example of such a non-standard binding mode within the coactivator groove occurs in both RAL- and OHT-antagonised ER, where the LxxML motif on H12's hydrophobic surface (residues 540–544) mimics the interactions made by the coactivator peptides [8,10,14]. Therefore, it appears, at least in the case of ERα, that the coactivator binding groove can accommodate several different large hydrophobics at the +4 position of the consensus motif. In summary, the LxxϕL (where ϕ is a large hydrophobic residue) motif serves as a general interaction module between NR–LBDs and coactivators. Particular NR specificity is generated by the amino acid sequences that flank the LxxLL motif [13,23,25,26].

## 4. Structural basis of agonism and antagonism

Understandably, the integrity of the coactivator-binding groove is highly dependent on the orientation of H12. In turn, the alignment of H12 is highly sensitive to the nature of the bound ligand. Only those ER ligands that promote the positioning of H12 over the hormone binding cavity will act as full agonists. ER AF2 antagonists, such as RAL and OHT, are able to prevent the proper alignment of H12 through direct steric effects between their characteristic basic sidechain substituents and H12 [8,10]. Consequently, the recruitment site is incorrectly formed and such receptors are unable to interact with p160 coactivators.

The inability of certain ligands, such as GEN, to promote the correct positioning of H12 may be correlated to their partial agonist character. While GEN is of a similar size as $E_2$ and could potentially be accommodated within the ERβ's binding cavity without interfering with the alignment of H12, this is not the case. Perhaps the 'agonist' orientation of H12 is inherently more labile in ERβ and needs to be stabilised by interaction with coactivators. A recent study has demonstrated that coactivator binding to liganded ER can markedly decrease the rate of ligand dissociation [27]. Clearly, there are aspects of ligand binding to ER isoforms that we do not fully understand. For example, what are the structural changes that need to take place within the binding cavity for the correct alignment of H12?

Possibly the most surprising feature of the RAL- and OHT-complexed ER-LBDs is the observation that H12 is redirected by the AF2 antagonist's side chain substituent so that it binds along the coactivator recruitment site. The sidechains of Leu540, Met543 and

[2] The residues in the coactivator peptide are numbered so that the first leucine of the LxxLL motif represents the +1 position. Residues prior to the core consensus sequence are designated by negative numbers (−1 to −5) with the Ile immediately preceding the LxxLL given as the −1 position.

Leu544 on the inner hydrophobic surface of H12 mimic the interactions made by the three leucine residues of the NR-box LxxLL motif. The significance of this striking coincidence is given further weight by the well-documented observation that both the overall length of the sidechain substituent of AF2 antagonists, as well as the positioning of the basic group are critical for these compound's selective effects [28]. Is it possible that the partial agonist activity of RAL and OHT is in some way generated by the occlusion of the coactivator binding cleft? We are not aware of any studies that have addressed whether mutations in the recruitment site affect SERM's pharmacological profiles.

## 5. Future perspectives

Structural studies of NRs have had a considerable impact on our current understanding of the NR function. Initial studies shed light on the mechanisms of DNA response element recognition by NR DNA-binding domains. Subsequent structural analyses on NR-LBDs in a number of laboratories, such as those described here, have led to a better understanding of ligand recognition, conformational effects of receptor agonists and antagonists, interaction with NR coactivators and the structural basis of receptor agonism and antagonism.

Further advances in the understanding of NR-dependent transcription will, in part, require structural information on full-length receptors. In particular, knowledge of the three-dimensional relationships between the N-terminal (AF1), DNA- and ligand-binding domains in the intact receptor should provide some clues to receptor function. How, for example, do the AF1 and AF2 transactivation domains communicate in the DNA-bound receptor? Another fundamental question that remains unanswered is how NRs relay their ligation state to the basal transcription machinery. Given the inherent flexibility of these multidomain receptors, especially within the N-terminal AF1 transactivation domain, successful crystallisation will undoubtedly rely on the formation of stable complexes between full-length receptor and fragments of coactivators.

## Acknowledgements

We would like to thank Julia Walton for assistance with preparation and crystallisation of the ER:coactivator peptide complexes. We are indebted to our industrial collaborators Karo Bio and, in particular, the protein chemistry group under the leadership of Mats Carlquist, who provide us with material for structural studies. We also wish to thank Dr Stefan Nilsson (Karo Bio) and Dr Jan-Åke Gustafsson (Karolinska Institute) for useful discussion on the implications of our structural work on ER. This work was funded by Karo Bio AB and indirectly by the Biotechnology and Biological Sciences Research Council through support of the infrastructure of the Structural Biology Laboratory.

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