Structure and mechanism of the oestrogen receptor

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Abstract
We have determined the structures of the oestrogen receptor ligand-binding domain in complex with a range of ligands and with fragments of co-regulator proteins. These structures provide insights into the structural mechanisms underlying the receptor’s complex pharmacological properties and how the conformation of the receptor modulates its ability to recruit co-regulators that are necessary for transcriptional activation.

Introduction
The oestrogen receptor (ER) is responsible for mediating the physiological effects of its ligand, the steroid hormone 17-β-oestradiol (E2). In addition to its involvement in controlling adolescence and reproductive processes, it also participates in health preservation, including the maintenance of bone density and cholesterol levels. Due to its importance, the receptor is also an intensively studied therapeutic target. Selective ER modulators (SERMs) have been used successfully for fighting breast cancer and osteoporosis, besides avoiding the harmful side effects that are associated with natural oestrogen replacement [1,2].

The ER is a ligand-activated transcription factor and a member of the steroid hormone nuclear receptor (NR) superfamily, all of which share the same structural features, such as having distinct DNA- and hormone-binding domains [3]. Hormone binding initiates receptor conformational changes, which allows the ER to interact with a specific DNA-response element and other components of cellular transcription machinery, such as co-regulators that result in the activation or repression of target genes [4]. The recruitment of co-regulators is associated with regions of the protein called activation functions (AF1 and AF2 in the case of ER). Figure 1 summarizes the domain organization of the receptor.

Structures of the ER ligand-binding domain (LBD) in complex with a range of ligands and with fragments of co-regulator proteins have been determined. Here, we briefly summarize how these structures have provided a mechanistic understanding of co-activator recruitment and the receptor’s complex pharmacological properties.

Key words: activation function 2, ligand binding, steroid receptor, transcription factor.

Structural analysis of ER: mechanistic basis for antagonism
The ER binds a variety of ligands including agonists such as E2 and diethylstilbestrol (DES), SERMs such as raloxifene (RAL) and tamoxifen [5,6], full antagonists such as ICI 164384 and partial agonists such as genistein. In all cases, the receptor forms a dimer, with distinctive conformations associated with each group of compounds as shown in Figure 2. Key differences in receptor conformation in the presence of different ligands suggest a structural basis for antagonism.

The position adopted by helix 12 (H12) is different in the various structures. In the E2 structure, H12 lies across the ligand-binding cavity, but in the RAL structure the protruding side chain of RAL forces the displacement of H12, which binds instead along a shallow groove formed by the helices H3 and H5. There are also slight changes in the positioning of the ligand and the packing of residues within the cavity. This groove is part of a region on the surface known as AF2, an important co-activator-binding site. Because H12 is displaced in the RAL structure, AF2 is incorrectly formed and hence co-activator binding is blocked.

In the complex between ICI 164384 and the ER LBD [7], the ligand side chain prevents H12 from adopting both of the positions that are described above. Consequently, H12 is not associated with the rest of the LBD, and is not seen in the X-ray diffraction electron density map. This disordered conformation may lead to full antagonism, either through disruption of the AF1 site, or through destabilizing the receptor structure leading to degradation in the cell.

In the complex between genistein and the ER LBD [8], H12 adopts a quasi-agonist position, inhibiting the AF2 groove. At the same time, the helix is also in contact with the ligand-binding cavity entrance. It is thought that H12 could be displaced to the agonist position to accommodate co-activator binding, and overcoming this energy barrier may account for the partial agonistic behaviour of genistein. Such behaviour is seen in the structures of ER in complex with epiestriol in the presence and...
**Figure 1** | Domain structure representation of human ERα and ERβ isoforms

Domains (labelled A–F), amino acid sequence numbering, AF1 and AF2, and percentage homology between the two isoforms in different regions, including the DNA-binding domain (DBD) and LBD, are shown.

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<th>hERα</th>
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<td>224</td>
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<tr>
<td>18%</td>
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<td>254</td>
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<tr>
<td>DBD (97%)</td>
<td>Hinge (30%)</td>
<td>LBD (47%)</td>
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**Figure 2** | ER LBD monomer in complex with different agents

The ER LBD monomer is a three-layered α-helical sandwich and the ligand lies buried within a predominantly hydrophobic cavity. The structure of a representative monomer is shown in complex with (A) E2, (B) raloxifene, (C) ICI 164384 and (D) genistein.

absence of co-activator peptide (A.C.W. Pike, unpublished results).

**ER isoforms: ERα and ERβ**

The recent discovery of the β-isoform has added a further new level of complexity to understanding the receptor’s pharmacology. The relative populations of the two isoforms vary greatly between different types of tissue and, at the present time, it is clear that they perform different biological functions [9–12]. The domain organization of the two isoforms is compared in Figure 1. The LBDs have a moderately high sequence identity (47%) but the overall
three-dimensional arrangement is identical [5,8]. There are many questions concerning how each isoform modulates and reacts to different compounds in various tissues and what role heterodimerization may have. The structure of the LBD alone is not sufficient to explain these differences, some of which arise because of the essential lack of AF1 region in the β isoform.

**Co-activator recruitment**

Many co-activators are known, but the majority binds the ER at AF2 [13]. All that do this contain multiple copies of a signature LXXLL motif (NR-box) through which the interaction occurs. Short LXXLL-motif–containing peptides have been used to mimic ER–co-activator interactions, and mutagenesis and crystallization studies involving them have provided a valuable structural insight [6,14–17]. These revealed the important interactions required to stabilize the peptide in the hydrophobic groove formed by the helices H3, H4, H5 and H12. The NR-boxes bind in an α-helical conformation and are stabilized by a ‘charge clamp’ involving residues Lys362 and Glu542 as shown in Figure 3. Furthermore, the structure of the ER binding E2 in complex with rat TIF2 (transcriptional intermediate factor 2) NR-box III has revealed the groove can also accommodate LXXφL motifs (where φ is a large hydrophobic residue) [14].

**AF2 Co-activator differentiation**

Studies using phage-display technology by creating a ‘focused’ combinatorial peptide library showed that LXXLL motifs could be placed into three classes [18,19]. These classes were distinguished by their different LXXLL-motif-flanking residues and different interaction patterns towards various ERα-mutants, isoforms and also other NRs. This study demonstrated that in addition for the LXXLL motif to function as an AF2-docking unit, it appears that sequences flanking the core motif are also important in receptor selectivity. Together with studies such as surface plasmon resonance (SPR), which have allowed binding kinetics and affinity data of these peptide interactions to be obtained [20], this will provide a tool in determining how competing co-activators or co-repressors are recruited by the receptor at AF2, leading to its resultant transcriptional activity.

**AF1 and oestrogen response elements (EREs)**

At the present time, many factors that underlie how the biological effect of a SERM is determined are not fully understood. For example, it is known that ER transcription is largely mediated through regions AF1 and AF2 in ERα (although in ERβ, AF1 activity is insignificant), but how these regions communicate with one another is unclear.
In addition, there is a need to understand how the ER binds to specific DNA sequences called EREs, how different ERE sequences affect ER-binding affinity and transcriptional activity [24], and also what the effect of different co-activator and isoform populations in different tissues has on ER transactivation.

Structural studies on the LBD of ER have revealed much about the mechanisms of agonist and antagonist binding and co-activator recruitment. Structures of larger receptor constructs, in complex with larger portions of co-activators, co-repressors and/or DNA will be required to make the next steps in unravelling the detailed molecular mechanisms that underlie the broad range of ER function.

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References