Direct recruitment of transcriptional corepressors to estrogen receptors (ER) is thought to contribute to the tissue-specific effects of clinically important ER antagonists. Here, we present the crystal structures of two affinity-selected peptides in complex with antagonist-bound ERα ligand-binding domain. Both peptides adopt helical conformations, bind along the AF2 coregulator interaction surface and mimic corepressor (CoRNR) sequence motif binding. Peptide binding is weak in a wild-type context but significantly enhanced by removal of ER helix 12. This region contains a previously unrecognized CoRNR motif that is able to compete with corepressors for binding to AF2 thereby providing a structural explanation for ER’s poor ability to directly interact with classical corepressors. Furthermore, the ability of other sequence motifs to mimic corepressor binding raises the possibility that coregulators do not necessarily require CoRNR motifs for direct recruitment to antagonist-bound ER.

The biological significance of corepressor recruitment by nuclear receptors (NRs) in gene repression is well documented (reviewed in (1)). Known NR corepressors have been isolated in complexes together with histone deacetylases, which facilitate gene repression through deacetylation of histone tails (2). Even though the components of several complexes have been identified, less is known about the processes controlling their recruitment by NRs. Unlike most NRs, the estrogen receptor (ER) is unusual in that it does not appear to be repressed in the absence of hormone and, consequently, the importance of NR corepressors in ER-mediated transcriptional signalling remains controversial. However, a number of recent studies have demonstrated that both agonist and antagonist-bound ERs are able to recruit a variety of proteins that can repress its activity (3). Differential coregulator recruitment is also known to contribute to the tissue specific effects of selective ER modulators (SERMs), a therapeutically-important class of ER ligands that exhibit characteristics of both estrogens and anti-estrogens depending on the tissue (4,5).
interact with the extended LXXXIXXXL CoRNR consensus motifs of NR corepressors.

Affinity-selected peptides that recognize SERM bound ERs have been isolated from both random (11,12) and focused (13) peptide libraries. These studies have revealed a variety of hydrophobic sequence motifs that act as highly specific conformational probes and are good predictors of a particular ligand’s biological effects (14). Such motifs also provide information regarding potential ligand-specific ER-coregulator interaction sites (11,15,16).

McDonnell and co-workers have reported the isolation and identification of a number of short peptides using phage display that specifically recognize 4-hydroxytamoxifen (OHT)-bound ER (12,20). This study focuses on two such peptides: the OHT-specific αβV peptide (SPGSREWFKDMLS) was isolated from a random peptide library and contains a novel interaction motif (12). A second peptide, bT1 (hereafter referred to as CoRNRER box; sequence DAFQLRQLILRGLQDD) was isolated from a focused library based on the corepressor consensus motif (20).

To understand the structural basis for the interaction between ER and SERM-specific motifs, we have crystallized and solved the structure of SERM-bound ERαLBD in complex with both an affinity-selected CoRNR-box peptide and a tamoxifen (OHT)-specific peptide. This study extends previous structural information on corepressor binding to NRs and provides novel insights into the specific recognition of the antagonist-bound state of ER.

EXPERIMENTAL PROCEDURES

**Materials** – 4-hydroxy-tamoxifen, 17β-estradiol and raloxifene were purchased from Sigma-Aldrich (Poole, UK). Biotinylated and crystallization-grade peptides (>95% purity) were purchased from Thermo Electron (Ulm, Germany).

** Constructs** – Human ERα and ERβ cDNA cloned into VP16 expression vector (Clontech) were used as templates for mutagenesis. Mutations were introduced using the QuickChange XL site-directed mutagenesis kit (Stratagene). Generation of the domain deleted ERα and ERβ constructs and the ERαD351Y, E542A, G442H and E443A and ERβE448A have been described previously (11,15). Introduction of amino acid changes were made by using the following primers: ERαL359R (sense primer- 5’GAGCTGGTTCAATGAGAAACTGGGCG AAGAGG and antisense- 5’CCTCTTCGCCAGTTTCTCATGTGAACC AGCTC), ERαK362A (sense primer- 5’CATGATCACTGGGCAGGAGGTGC AAGCTTTGTGG and antisense- 5’CCACAAAGCCTGACCCCTCGCCGCC AGTTGATCATG), ERαL372A (sense primer- 5’CTTTGTGAGTTGACCCCATGATCAG GTCCAC and antisense- 5’GTGGAACCTGTACATGCC GGGTCAATCCACAAAG), ERαL379A (sense primer- 5’GATCAGGTCCACCTTGACAATGTGCG TGGCTAG and antisense- 5’CTAGCCAGCCACATCTGCAAGGTA CCTGATACG and antisense- 5’GCTGTACAGTATGCTTCATTGTGTTA CTCATG GCC), ERαG521A (sense primer- 5’GGCCATGAGTAACAAAGCAATGGAGC ATCTGTACAGC and antisense- 5’GCTGTACAGTATGCTTCATTGTGTTA CTCATG GCC), ERαH524A (sense primer- 5’GTAACAAAGCCATGAGGCAGC ACTTGACAGC and antisense- 5’GCACTTCATGCTAGTACGTGCCCTCCATG CTTTTGTTAC).

**Cell culture and Transient transfections** – Mammalian two-hybrid experiments were performed as described in (15). HuH7 (human liver) cells were maintained in DMEM high glucose (Gibco, Invitrogen) supplemented with 10% FBS and 2mM L-glutamine. For transient transfection, cells were seeded into 24-wells plates 24h before transfection in phenol red free media supplemented with 10% DCC stripped FBS and 2mM L-glutamine. Cells were transfected using lipofectamin 2000 as per manufacturer’s instructions (Invitrogen Corp.). After transfection, cells were treated with ligands for 16h before assaying luciferase and β-galactosidase activity.

**Surface Plasmon Resonance (SPR)** – Measurements were performed using a Biacore X instrument and streptavidin-coated sensor chips. All experiments were carried out at 25°C in 50 mM Tris pH7.4, 150 mM NaCl, 1 mM EDTA, 0.05% Tween 20. 50-120RU of biotinylated αβV peptide (biotin-
SGSGPGSREWFKDML) was immobilized onto the chip surface. Qualitative binding experiments were performed by flowing liganded ERαLBDs (1µM dimeric concentration) over the sensor chip for 2min at 5µl min⁻¹. For the competition experiments, OHT-liganded ERαΔH12 was pre-incubated with CoRNR₅ box peptide (DAFQLRQLILRGLQDD) at the desired molar ratio for 30 min prior to injection of the protein-peptide mixture over the αβV sensor chip. Pre-incubation with an LXXLL-containing peptide (EKHKILHRLLQDS) was used as a control.

Crystallography – A truncated ERαLBD (ERαΔH12; residues 305-533) mutant was used to facilitate crystallization. OHT- and RAL-liganded ERαΔH12 LBD was prepared as previously described (15). Peptide complexes were assembled by incubating protein with a 1.5-fold molar excess of peptide followed by concentration using ultrafiltration. Additional peptide was added to obtain a final peptide / LBD molar ratio of 3:1. Initial screening of crystallization conditions was performed at 19°C in a 300nl, 96-well sitting-drop format using a Mosquito® liquid-handling robot (TTP Labtech, UK). A single crystal of the ERαΔH12-RAL-CoRNR box complex was grown from a 300nl drop containing an equal mixture of protein (7mg/ml) and reservoir solution of 0.35M (NH₄)₂SO₄, 0.7M Li₂SO₄, 0.07M tri-sodium citrate pH5.6. Crystals of the ERαΔH12-OHT-αβV complex were grown in hanging drops comprising equal volumes of protein (10mg/ml) and reservoir solution of 2.5% (v/v) PEG 550 monomethylether, 2.5% (w/v) PEG 20000, 0.06M calcium acetate, 0.1M Tris pH 8.5.

Crystals were cryoprotected by passing through a mother liquor solution supplemented with 25-30% (v/v) ethylene glycol prior to vitrification in liquid nitrogen. X-ray diffraction data were recorded on a Quantum-4 CCD detector at 100K at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) and were processed using the HKL suite of programs (17). The structures were solved by molecular replacement with AMoRe (18) using the coordinates of ERαΔH12-OHT (15); PDB code: 2BJ4) as a search model. The peptides were clearly visible in the initial sigmaa-weighted electron density maps. Model building was performed with QUANTA (Accelrys, San Diego) and the complexes were refined with REFMAC (19) using appropriate NCS and TLS restraints. Data collection and refinement statistics are given in Table 1.

Analysis – Coordinates were superposed in QUANTA and secondary structure assignment was carried out using DSSP. The peptide binding interfaces were analyzed with the help of the Protein interfaces PISA server at the European Bioinformatics Institute (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html). Figures 2 and 3A-C were generated using the program PyMol (Delano Scientific, San Carlos, http://pymol.sourceforge.net/).

RESULTS AND DISCUSSION

Corepressor motifs bind to a common site in ER — Previous studies have reported the isolation and identification of a number of short peptides using phage display that specifically recognize 4-hydroxytamoxifen (OHT)-bound ER (11,12,20). The OHT-specific αβV peptide was isolated from a random phage display peptide library (12) whereas the CoRNR₅ box peptide was isolated from a focused library based on the corepressor consensus motif (20). While both peptides were affinity selected using OHT-bound ERs, they exhibit differing abilities to interact with full-length ERs in a mammalian two-hybrid assay (Fig. 1B). In comparison, the CoRNR₅ motif displayed a robust interaction with full-length ER in the presence of OHT comparable to E2-dependent LXXLL motif binding (Fig. 1B). In comparison, the CoRNR₅ box peptide provoked a much weaker reporter response. Removal of helix H12 from full-length ER (ERαΔH12) significantly enhanced binding of both peptides and had a dramatic effect on the CoRNR₅-motif interaction (Fig. 1C). Furthermore, real-time binding analysis between ER-LBD and an immobilized αβV peptide using surface plasmon resonance (SPR) demonstrated that, in the context of the LBD alone, no interaction occurs unless H12 is removed (Fig. 1D). Competition studies using SPR indicated that both peptides target similar binding sites on the LBD surface as pre-incubation of the LBD with one peptide reduced the binding to a peptide sensor chip (Fig. 1E).
Previous studies by a number of groups have concluded that regions outside the LBD contribute to corepressor binding by NRs (10, 21-23). We also performed extensive domain deletion experiments as well as single-site mutations to further investigate the architecture of the corepressor binding site (Fig. 5C and Supplemental Material). Domain mapping experiments suggest that the presence of ER’s N-terminal AB domain is also required for interaction as constructs lacking this region exhibit no apparent binding to either CoRNR, H12 ER or αβV peptide in our M2H assay. As removal of H12 potentiates corepressor binding (Fig. 1, C-E), the role of the AB domain appears to be indirect, presumably through an effect on the positioning of H12 in the intact receptor that alters the accessibility of the AF2 binding site in the SERM-bound state. Interestingly, even though structural evidence does not exist, there is data indicating a functional communication between distant receptor N-terminal domains and the LBD (24).

Structure Determination — Initial co-crystallization trials with peptide and either OHT-liganded ERα- or ERβLBD did not yield any crystals suitable for structural studies. Based on the SPR observations that minimal peptide binding to the isolated LBD occurs in the presence of H12, an H12-truncated ERαLBD (ERαΔH12) mutant, in which 21 amino acids at the C-terminus of the LBD encompassing the H11-12 loop and H12 were removed, was used to facilitate crystallization of the LBD/peptide complexes. The resulting structures of antagonist-bound ERαLBD in complex with either the CoRNRER box (DAFQLRQLLRLGLQDD) or the OHT-specific αβV (SPGSREWFKDMLS) peptides were solved by molecular replacement and refined to resolutions of 2.55Å and 2.1Å respectively (Table 1).

Structure of ERαΔH12-RAL-CoRNRER box complex —The overall structure of the ΔH12 LBD and the conformation of raloxifene (RAL; Fig. 1C) is identical to that observed in the full-length ERαLBD-RAL complex (8). The CoRNRER box peptide is bound along the AF2 groove between H3 and H5 as an extended, 3.5-turn amphipathic α-helix (Fig. 2). The peptide, which is 20Å in length, occupies the entire AF2 cleft with its N-terminus extending into the position normally occupied by H12 in its agonist orientation. The canonical Leu / Ile residues of the CoRNRER motif (Leu5 (L+1), Ile9 (I+5), Leu13 (L+9) lie along one face of the helix and are buried at the interface between the LBD and peptide (Fig. 2B). Contacts with the LBD are almost exclusively non-polar in character. The N-terminus of the peptide is packed against RAL’s antagonist side chain. L+1, I+5 and L+9 form a hydrophobic keel that anchors the peptide in the AF2 groove. L+1 contacts Leu354, Trp383 and the terminal piperidine ring of RAL’s side chain. The central I+5 makes contact with Ile358, Val376 and Leu379. L+9 is bound in a shallow depression formed by the side chains of Ile358, Phe367, Leu372 and Val376. These van der Waals interactions are complemented by two polar interactions: Lys362 is hydrogen bonded to the main chain carbonyl group of Gly12 and provides electrostatic stabilization to the C-terminal end of the CoRNRER box. In addition, Glu380 participates in a salt bridge with the side chain of Arg6 (Fig. 2B).

Comparison of the ER CoRNRER complex with the structure of PPARαLBD bound to antagonist and the ID2 CoRNR box motif of SMRT (10) reveals that the general principles of corepressor motif binding to the AF2 region of NRs are conserved. Nonetheless, several notable differences are apparent due to the differing surface topology of the AF2 regions of these two NR LBDs (Fig. 3A). The SMRT ID2 helix is shorter than the ER motif and is severely distorted at its N-terminus so as to maintain favourable packing contacts with PPARα’s AF2 cleft. In particular, differences in the amino acid composition of the H5/6 junction dictate that the Leu+1 (Leu685) residue of the SMRT motif binds closer to the LBD surface. In addition, whereas the bulkiy side chain of RAL protrudes from the ligand-binding cavity and interacts with L+1 of the CoRNRER motif, the bound antagonist in the PPAR-SMRT structure contributes very little to the immediate CoRNR binding surface.

Role of H12 in CoRNR-box binding — The inhibitory properties of H12 on CoRNR box binding to ER (Fig. 1C) are readily apparent when one compares the interaction modes of these two elements. Both peptides interact with the LBD in a similar fashion to that observed for
H12 in complexes of the intact ERαLBD bound to SERM AF2 antagonists such as RAL and OHT (Fig. 3B). A structure-based, sequence alignment clearly highlights the similarity between the affinity-selected CoRNR-box motif and the ERα sequence in the vicinity of H12 (Fig. 3D). The CoRNR<sup>ER</sup>-box motif’s Leu<sup>1</sup>, Ile<sup>5</sup> and Leu<sup>9</sup> perfectly mimic the equivalent interactions made by H12’s Leu536, Leu540 and Leu544. This observation may also explain the apparent inability of ER to bind CoRNR-box sequences found in bona fide NR corepressors such as N-CoR and SMRT (13,25). In effect, ER possesses its own, highly effective CoRNR-box surrogate within H12 that preferentially occupies the AF2 site in the presence of SERMs and passive antagonists. Consequently, H12 would need to be displaced from AF2 before any CoRNR-box mediated, corepressor binding could occur. Nonetheless corepressors are understood to play a significant role in the biological effects of ER antagonists. SERM-bound ERα has been shown to be associated with N-CoR/SMRT in vivo (5,26,27), however, based on our study it seems highly unlikely that such associations are directly mediated through the AF2 region. Importantly, both N-CoR and SMRT have been isolated as part of multiprotein complexes (28,29) and are more likely to be recruited to ERα via indirect mechanisms that require additional factors.

**H12 length as a predictor of corepressor binding** — Examination of the sequences in the vicinity of H12 suggests that this mechanism to resist corepressor binding may be quite common within the NR superfamily (Fig. 4). NRs that exhibit poor corepressor binding, such as RXR, have a H12 sequence that resembles the CoRNR consensus motif and are able to adopt a relatively long amphipathic helix that, like ER, would be able to occlude the entire AF2 binding site (‘CoRNR-’ class in Fig. 4). In case of DAX-1 and SHP there is strong support for a CoRNR-box corepressor-independent repression (reviewed in (30,31). In contrast, NRs that exhibit good corepressor binding, such as TR, PPAR, LXR and RAR (10,22,32-34) exhibit much less similarity to the CoRNR-box sequence. Critically, these NRs all possess a H12 sequence that is incompatible with the formation of a long AF2-blocking helix due to the presence of a proline residue that restricts the length of H12 (‘CoRNR+’ class). The resultant shorter H12 would constitute no barrier to corepressor binding as CoRNR-box containing corepressors are more likely to be able to displace H12 and bind along the AF2 groove of these NRs. Structural data to support such a mechanism to resist corepressor binding is limited as there are relatively few examples of crystal structures of antagonist-bound NRs, other than with ER, in which H12 is observed to occupy the AF2 cleft. Nonetheless, in the structure of the RXR / RAR heterodimer (35), H12 of both partners lies in the antagonist position but RXR’s helix is considerably longer and buries 20% more accessible surface area than that of RAR. Consequently, corepressors are more likely to be able to displace RAR’s H12 and preferentially bind along its AF2 groove within this heterodimer. Notably, AR, GR, MR and PR contain an intermediate H12 length that do not harbor a complete CoRNR box like sequence and would allow corepressor binding. In light of recent reports describing interactions (“direct” or “indirect”) between NCoR/SMRT and these steroid receptors, it would be interesting to reinvestigate the possibility of direct recruitment to LBD (23,36-39). The role of sequences C-terminal to H12 is unknown but long extensions may influence both H12 stability and coregulator access.

**Structure of ERαΔH12/OHT-αβV peptide complex** — Despite bearing little sequence homology to the CoRNR-box consensus sequence, the OHT-specific αβV motif acts as an effective structural mimic of a corepressor motif and binds along ER’s AF2 groove in a similar fashion (Fig. 2A). The αβV peptide adopts a compact two-turn helix with a non-helical, N-terminal extension that sits atop the dimethylamino headgroup of OHT’s basic side chain (Fig. 3C, 5A). Whereas the CoRNR<sup>ER</sup>-box peptide uses three hydrophobic Leu / Ile residues to bind to AF2, the αβV motif utilizes only two. Phe8 and Leu12, which are characteristic of the αβV class of motif (16), occupy analogous spatial positions to CoRNR<sup>ER</sup> box I+5 and L+9 residues. Trp7 packs against the face of Phe8, clamping the phenylalanine into a deep pocket in the AF2 groove (Fig. 5A). This structural feature appears to be important for the binding of the motif and explains why this position is always occupied by an aromatic residue (16). The helical nature of the αβV motif is further stabilized by the side chains of Pro2, Trp7 and
Met11 which interlock and form a hydrophobic stack that interacts with the H3 edge of the binding groove (Fig. 5A). Analysis of other SERM-specific peptides reveals that the majority contain a CoRNR-like consensus sequence enriched with bulky hydrophobic residues replacing the canonical Leu/Ile residues (14).

αβV’s ability to adopt a shorter, compact α-helical structure compared to the CoRNRER motif may account for its ability to efficiently compete with H12 for the AF2 site in full-length ER (Fig. 1B). Furthermore, the structure of the αβV complex suggests that direct contact with OHT’s protruding side chain contributes to the peptide’s observed ligand specificity and provides a molecular basis for SERM-specific coregulator interactions. This ligand-dependent interaction is supported by mutagenesis data which shows that either replacement of residues at the N-terminus of αβV that are in direct contact with OHT’s terminal dimethylamino moiety, or mutation of the ER side chain involved in the positioning of the OHT side chain (Asp351) abolishes peptide interaction (Fig. 5B). The ERα mutation D351Y is the only naturally-occurring receptor mutation found in a tumor cell-line responsive to OHT-stimulated growth (40). This residue is suggested to be able to regulate both estrogenic and antiestrogenic properties of OHT-complexed ER (41,42). While the precise contribution of this residue to ER ligand pharmacology is not clear, it has been suggested that, by affecting the positioning of OHT’s basic side chain, this may provide an extra coactivator interaction site (41). An alternative explanation of the regulatory properties attributed to this residue is that its mutation leads to the disruption of a potential αβV-like corepressor interaction surface.

To further investigate the specificity determinants of the novel αβV motif, we performed alanine scanning mutagenesis and evaluated binding to full-length ER using a M2H interaction assay (Fig. 5B). Replacements of residues that lie on the hydrophobic face of the αβV helix (Trp7, Phe8, Met11, Leu12) abolish binding. Similarly, mutations that disrupt the AF2 surface (L358R, L379R, L372R) or remove the ‘charge clamp’ lysine (K362A) also abolished binding of both the CoRNRER and αβV peptides (Fig. 5C). Intriguingly, mutation of Asp351 has a differential effect on peptide binding and significantly enhances the interaction of the CoRNRER-box containing motif whilst abolishing α/βV binding (Fig. 5C).

Concluding remarks — The two structures presented here demonstrate that the AF2 region of ER is, in principle, capable of interacting with coregulator proteins that recognize the SERM-bound conformational state of the receptor. However, the internal CoRNR-box motif within ERα’s H12 serves as an effective ‘corepressor surrogate’ and provides a considerable barrier to binding. In addition, this study shows that amino acid sequences, other than the classical CoRNR box, can bind to the ER’s corepressor site. These observations raise the possibility that cofactors with the binding characteristics of αβV may exist in vivo and contribute to the effects of SERMs as this interaction motif, in contrast to CoRNR-box containing factors, is readily recruited to full-length antagonist-bound ER.

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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID code 2JFA (CoRNRER complex) & 2JF9 (αβV complex))

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3 The abbreviations used are: AF, activation function; CoR, corepressor; E2, 17β-estradiol; ER, estrogen receptor; LBD, ligand-binding domain; NR, nuclear receptor; OHT, 4-hydroxytamoxifen; RAL, raloxifene; SERM, selective estrogen receptor modulator; SPR, surface plasmon resonance.

ACKNOWLEDGEMENTS

ACWP and TP are funded by a Wellcome Trust Career Development Award to ACWP (grant number: 064803). NH, ET and JÅG are supported by grants from the Swedish Research Council, the Swedish Cancer Society and the European Network of Excellence CASCADE.

FIGURE LEGENDS

FIGURE 1. Affinity selected peptides bind to overlapping sites on ER LBD. A, Chemical structures of SERM ligands used in this work. The antagonistic side chains of 4-hydroxy-tamoxifen (OHT) and raloxifene (RAL) are shaded in grey. B, Interaction of GAL-DBD tagged peptides with VP16-tagged ERα. Data presented as % activity with the LxxLL peptide interaction with wild-type ER in the presence of E2 set to 100%. C, Comparison of GAL-DBD tagged CoRNRER box peptide interaction with full-length and H12-truncated (ERαΔH12) ERα. Data presented as % activity with the peptide interaction with wild-type ER in the presence of OHT set to 100%. D, Interaction of αβV peptide with ER LBD using surface plasmon resonance (SPR). Sensorgrams shown were obtained from injection of 1µM wild-type or H12-truncated (ΔH12) ERα-LBD, liganded to either E2 or OHT, over a surface immobilized with αβV peptide. E, Competitive binding studies using SPR. Sensorgrams obtained from injection of 1µM ERαΔH12-OHT over an αβV surface. ERαΔH12 was incubated with increasing molar ratios of CoRNR peptide prior to injection.
FIGURE 2. Peptides bind along AF2 site. A, overall structure. ERαΔH12 is shown schematically in grey. Binding modes of CoRNRER box (purple) and αβV (green) peptides are highlighted. B, detailed view of CoRNRER peptide binding site between H3 and H5. Peptide is coloured cyan. For clarity, some side chains have been omitted. Peptide residues are labelled in black with one-letter amino acid codes. LBD residues that are buried on CoRNRER-box binding are coloured green. Hydrogen bonds are drawn as dotted lines.

FIGURE 3. Structural mimicry of motif binding to AF2. The orientation of the CoRNRER-box peptide (purple) is shown in comparison with: A, SMRT ID2 CoRNR-box motif (green) from antagonist-bound PPARα complex (PDB code: 1KKQ (10)). B, ERα H12 (green) in its antagonist conformation (PDB code: 3ERT (7)). C, αβV peptide (green). D, structure-based sequence alignment of corepressor motifs and ERα H12. Boxed regions indicate key contact points with AF2 binding groove. The lengths of the various helical elements are depicted above the alignment.

FIGURE 4. Helix H12 sequence as a predictor of corepressor (CoR) binding to NRs. Comparison of the amino acid sequences of a range of NRs in the vicinity of H12. Sequences are shown beginning at the proline residue (red) that typically defines the N-terminal extent of H12. The core region of H12 is highlighted. The corepressor CoRNR consensus motif is boxed. NRs that are known to be poor binders of corepressors show a strong similarity between the sequence of H12 and the CoRNR motif. Sequences are subdivided into two classes based on H12 length (“CoRNR−” and “CoRNR+”). Sequences indicated by asterisks have a C-terminal extension of either 20 amino acids (*) or more than 30 amino acids (**).

FIGURE 5. AF2 surface is required for CoR peptide binding. A, surface representation of αβV binding site. The molecular surface of ERαΔH12 is shown. Peptide and LBD residues that are important for binding are highlighted. B, alanine scan of αβV motif. Each position of the αβV peptide motif was replaced by alanine and peptide binding was measured in M2H system using full-length ERα in the presence of OHT. C, binding of ERα mutants to affinity-selected αβV and CoRNRER peptides in mammalian cells. Data are presented as % activity where peptide interaction with OHT-liganded wt receptor is set to 100%.
| Data collection                              | αβV complex | CoRNRE complex |
|---------------------------------------------|-------------|----------------|
| Beamline                                    | ID14-2      | ID14-4         |
| Wavelength (Å)                              | 0.933       | 0.976          |
| Space Group                                 | P321        | P321           |
| Unit Cell dimensions (Å)                    | a=b= 193.71 | a=b= 126.57    |
|                                             | c= 64.44    | c= 113.43      |
| Resolution range (Å)                        | 50 – 2.1    | 30 – 2.55      |
| Observed / unique reflections               | 154913 / 80788 | 926335 / 34500 |
| Multiplicity                                | 9.1 (7.4)a  | 5.4 (5.4)a     |
| Completeness                                | 100 (100)a  | 99.9 (100)a    |
| <I/σ>                                       | 11.6 (3)a   | 13.4 (2)a      |
| Rsym (I)                                    | 0.064 (0.414)a | 0.057 (0.463)a |

**Refinement**

| Resolution range (Å)                        | 50 – 2.1    | 30 – 2.55      |
| Reflections used (Rfree set)                | 76657 (4052) | 32733 (1735)   |
| Rcryst (Rfree)                              | 18.0 (19.8) | 19.3 (21.3)    |
| LBD Molecules per AU                        | 3           | 2              |
| Protein / Peptide / ligand atoms            | 5044 / 316 / 87 | 3507 / 211 / 68 |
| Water molecules / other                     | 296 / 30    | 82 / 24        |
| Rmsd bonds / angles                         | 0.012Å / 1.266° | 0.015Å / 1.56° |
| Mean B-factor (Å²)                          | 31 / 41 / 27 / 34 | 51 / 92 / 53 / 48 |
| %A,B,L (a,b,p,l)                            | 99.8 (0.2)  | 98.5 (1.5)     |

Values in parentheses are for highest resolution shell 2.18-2.10Å (αβV), 2.59-2.55Å (CoRNRE).

Mean temperature factors (including TLS contributions) for protein, peptide, ligand and solvent atoms respectively.

Percentage of residues located in most favoured (allowed) regions of the Ramachandran plot as defined using MOLPROBITY webserver (http://molprobity.biochem.duke.edu/).
Figure 1

A) Chemical structures of OHT and RAL.

B) Bar graph showing % activity with LxxLL, αβV, and CoRNR

C) Bar graph showing % activity with DMSO, E2, OHT, and RAL for ERα wt and ERαΔH12.

D) Graph showing time (s) vs. response (RU) for ΔH12/OHT, ΔH12/E2, E2/OHT.

E) Graph showing time (s) vs. response (RU) for WT with LxxLL (1:3), CoRNR (1:1), CoRNR (1:3), CoRNR (1:5).
Figure 3
Figure 4

![Diagram showing CoNMR sequences and their interactions](http://www.jbc.org/Downloaded from http://www.jbc.org/)

- **CoNMR +**
  - PLEIQEITY
  - PLIQEMY
  - PLEQEVI
  - PLLSEIW
  - PEMMAEII
  - PEMLAEI
  - PAMLVEII
  - PEMSEVI
  - LLYDLEELML
  - ERYDLLELM
  - FVYDLLELM
  - PMHKLLELM
  - EYNNLEELM
  - IDTELMELM
  - PGMAKIDNLQEML
  - PIGTVSDMDML
  - PIGDVDEAGDL

- **CoNMR −**
  - LXXXIIXXL

**H12 core**

- PPARs
- RARs
- TRs
- LXR
- AR
- GR
- MR
- PR

**Factors**

- ERα
- ERβ
- ERR
- LRH1
- RXR
- HNF4
- DAX1
- SHP

**CoNMR motif**
Figure 5
Structural insights into corepressor recognition by antagonist-bound estrogen receptors
Nina Heldring, Tanya Pawson, Donald McDonnell, Eckardt Treuter, Jan-Åke Gustafsson and Ashley C.W. Pike

J. Biol. Chem. published online February 5, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M611424200

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