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# Differential interaction of steroid hormone receptors with LXXLL motifs in SRC-1a depends on residues flanking the motif

Maurice Needham[a,*], Stephanie Raines[a], Jane McPheat[a], Clare Stacey[a], Jonathan Ellston[a], Susan Hoare[b], Malcolm Parker[b]

[a] *AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*
[b] *Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK*

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**Abstract**

Steroid hormones induce the transcriptional activity of their cognate receptors by recruiting a variety of cofactors. One of these, steroid receptor co-activator-1 (SRC-1) interacts with the ligand binding domains of a number of different receptors by means of LXXLL motifs. We have investigated the relative interaction of four such motifs in SRC-1a using a yeast two-hybrid assay. We demonstrate that ERα, ERβ and ERβ2 preferentially interact with motif 2 while GR, AR, PPARα and PPARγ preferentially interact with motif 4. We show that the interactions depend not only on the LXXLL motif itself but also on residues flanking the motif. 

## 1. Introduction

Nuclear receptors stimulate transcription from target genes in response to hormonal ligands by recruiting co-activator proteins. The majority of nuclear receptors contain two activation domains, AF1 near the N-terminus and AF2 in the ligand binding domain [1–3]. AF2 is probably conserved by all members of the nuclear receptor superfamily and consists of residues in helix 3, 5 and 12 which appear to form a protein interaction surface [4–6]. The formation of this surface in the oestrogen receptor is dependent on the binding of an agonist, which induces the realignment of helix 12 [7,8].

Numerous proteins have been reported to interact with the ligand binding domains of receptors [9,10], but the roles of most of them have yet to be established. The best characterised co-activators are CBP/p300 [11–13], and the p160/RIP160 family of proteins [14,15], which is encoded by three distinct genes referred to as SRC1 [16], TIF2/GRIP1 [17,18] and pCIP/ACTR/AIB1/RAC3 [13,19,20]. Recently, the so-called TRAP [21,22] and DRIP [23] complexes have also been shown to potentiate transcription by nuclear receptors and are related to a number of other cofactor complexes including a mediator complex [24,25].

The p160 co-activators appear to bind to most, if not all of the nuclear receptors in a ligand-dependent manner by means of an amphipathic helix, which contains an LXXLL motif. Three of these motifs are quite well conserved in both sequence and in relative spatial positioning in all family members [26,27], but the SRC-1a isoform contains an additional motif at the C-terminus.

Co-crystallisation studies of ligand binding domains of a number of nuclear hormone receptors have been carried out with either peptides comprising individual motifs, or with an SRC-1 fragment containing motifs 1 and 2. These studies indicate that the LXXLL motifs form a two-turn amphipathic α-helix which binds in a hydrophobic cleft composed of residues from helices 3, 4, 5, and 12. In addition, as originally proposed for PPARγ [28], a conserved lysine in helix 3 and a glutamate in helix 12 form a charge clamp, which stabilises

* Corresponding author. Fax: +44-1625-516-667.

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the conformation of the helix by forming capping interactions with each end [8,28,29]. The relative affinity of different motifs with AF2 surfaces from different receptors seems to vary with, for example, motif 2 in SRC-1 being preferentially used for binding to the oestrogen receptor α (ERα) [30–32]. What determines this specificity has yet to be elucidated, however, it is most likely to be due to factors such as the spatial arrangement of the helical domain motifs in the receptor interaction domain or the effect of residues that flank the LXXLL motifs.

We have investigated the LXXLL preference for a number of nuclear receptors using a yeast two-hybrid approach. We demonstrate that motif 2 preferentially interacts not only with the ERα, but also ERβ and ERβ2. In contrast, motif 4 was found to show the greatest interaction with the peroxisome proliferator activated receptors, PPARα and PPARγ, the androgen receptor (AR) and the glucocorticoid receptor (GR), indicating a role for SRC-1a as a co-activator of these receptors. By generating chimeric motifs, we have also demonstrated that the differential interaction depends not on the LXXLL motif itself, but on residues N-terminal to the motif.

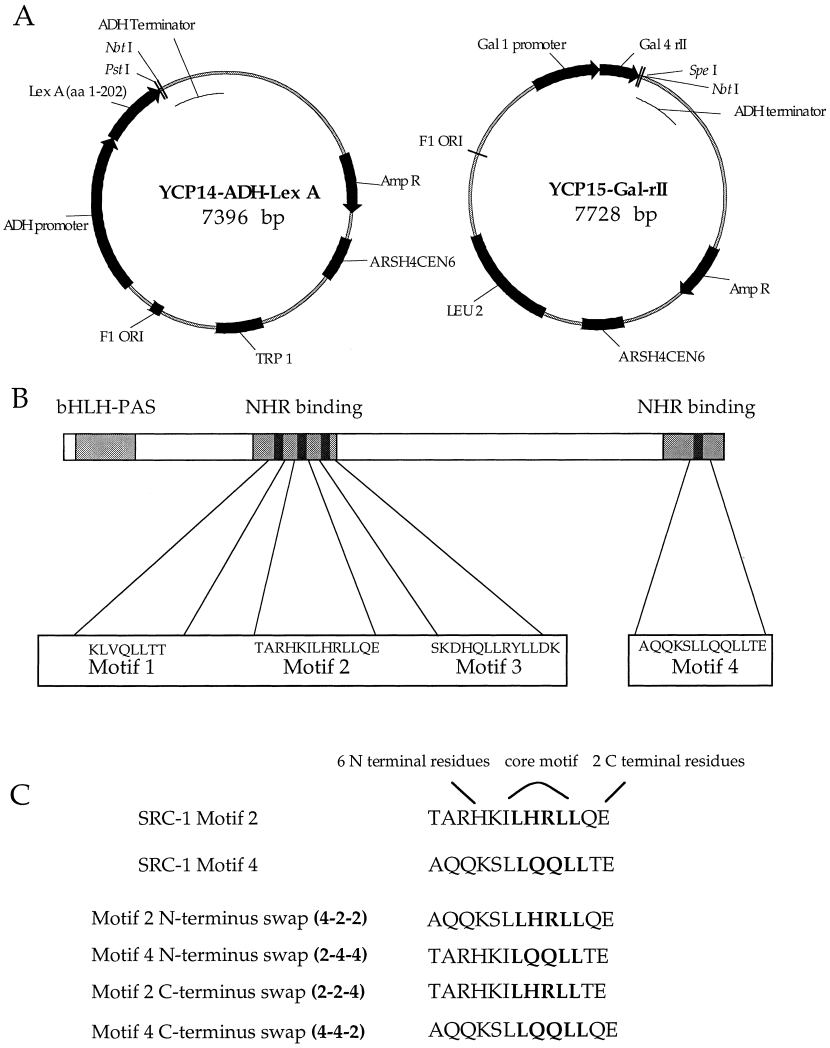


Fig. 1. (A) Yeast two-hybrid expression vectors were used to investigate the interaction between nuclear receptors and LXXLL co-activator motifs. SRC-1a LXXLL motifs together with flanking N- and C-terminal residues, were cloned in frame using Pst I and Not I directly downstream of the LexA DNA binding domain in YCP14 ADH LexA. Nuclear receptor ligand binding domains were fused in frame to the Gal 4 region II activation domain using the SpeI and NotI sites in the YCP15-Gal-rII vector. (B) A schematic representation of SRC-1a illustrating the positions of the 4 LXXLL motifs. Motifs 1, 2 and 3 are conserved several of the co-activator family members including SRC-1e, whereas motif 4 is only present in SRC-1a. (C) A summary of the hybrid LXXLL motifs made by fusing N- and C-terminal residues from SRC-1a motif 2 and motif 4. Hybrids consisted of the six N-terminal residues from M4 combined with the core LXXLL motif and C-terminal residues from M2 (4-2-2), the six N-terminal residues from M2 combined with the core LXXLL motif and C-terminal residues from M4 (2-4-4), the six N-terminal residues and core LXXLL motif from M2 combined with the C-terminal residues from M4 (2-2-4), or the six N-terminal residues and the core LXXLL motif from M4 combined with and C-terminal residues from M2 (4-4-2).

## 2. Materials and methods

### 2.1. Yeast two-hybrid expression constructs

Transactivation and DNA binding domain constructs were generated using the yeast shuttle vectors YCP14 ADH LexA and YCP15-Gal-rII (see Fig. 1). These vectors were derived from pRS314 and pRS315, respectively [33]. Briefly the ADH1 promoter/terminator fragment (BamHI) and the LexA (1–202) fragment (HindIII/NotI) were sub-cloned into pRS314 to generate YCP14 ADH LexA. The Gal 4 region II (HindIII/PstI) and the Gal I promoter fragment (SacI/HindIII) and a polylinker sequence (SpeI/NotI) were cloned in to pRS415 to generate YCP15-Gal-rII.

Double stranded oligonucleotides encoding individual SRC-1a LXXLL motifs together with flanking N-terminal and C-terminal residues, were cloned in frame using Pst and Not 1 directly downstream of the LexA DNA binding domain (DBD) in YCP14 ADH LexA.

**Motif 1**; (sense strand) 5′ GAAACTAGTG CAGCTTTTGACAACAACTTGAGC 3′.
**Motif 2**; 5′ GACAGCACGGCATAAAATTCTACACCGGCTCTTACAGGAGTGAGC 3′
**Motif 3**; 5′ GTCAAAAGACCATCAGCTCCTACGCTATCTTTTAGATAAATGAGC 3′.
**Motif 4**; 5′ GCAGGCCCAGCAGAAGAGCCTCCT TCAGCAGCTACTGACTGAATAAGC 3′

SRC-1a motif 2 and 4 chimeric constructs were made by ligating double stranded oligonucleotides encoding the individual LXXLL motifs to Pst/Not 1 digested YCP14 ADH LexA. For each LXXLL motif, the sequence of the flanking amino acid residues was modified. The six N-terminal amino acids of LXXLL motif 2 were exchanged with the equivalent residues from LXXLL motif 4 and vice-versa. An equivalent swap was also carried out for the two residues located C-terminal to the helical motifs (Fig. 1C).

Expression of the LexA fusion (amino acids 1–202) is under the control of the alcohol dehydrogenase (ADH) promoter [34]. The plasmid also contains a selectable marker for growth in tryptophan deficient medium.

Nuclear receptor ligand binding domains were amplified by the polymerase chain reaction from receptor cDNAs using the following primers;

**ERα** 5′ primer, 5′ GGGACTAGTTCTGCTGGAGACATGAGAGCT 3′
**ERα** 3′ primer, 5′ ATCGCGGCCGCTCAGACTGTGGCAGGGAAACC 3′
**ERβ** 5′ primer, 5′CAGACTAGTAAGGCCAAGAGAAGTGGCGGCCAC 3′
**ERβ** 3′ primer, 5′ GAATGCGGCCGCTCACTGAGACTGTGGGTTCTGGGA 3′
**ERβ2** 5′ primer, 5′ CAGACTAGTAAAGCCAAGAGAAACGGTGGGCAT 3′
**ERβ2** 3′ primer, 5′ GAATGCGGCCGCTCACTGAGACTGTAGGTTCTGGGA 3′
**GR** 5′ primer, 5′ CAGACTAGTCAGCAGGCCACTACAGGAGTCTCA 3′
**GR** 3′ primer, 5′ GAATGCGGCCGCTCACTTTTGATGAAACAGAAG 3′
**AR** 5′ primer, 5′ TCCACTAGTGAGGCTTCCAGCACCACCACCAGCCCC 3′
**AR** 3′ primer, 5′ GAACGCGGCCGCTCACTGGGTGTGCAAATAGATGGG 3′
**PPARα** 5′ primer, 5′ TCCACTAGTGTGGAGACCGTCACGGAGCTCACG 3′
**PPARα** 3′ primer, 5′ GAATGCGGCCGCTCAGTACATGTCCCTGTAGATCTC 3′
**PPARγ** 5′ primer 5′ TCCCCCGGGTCTCTCATAATGCCATCAGGTTTGGGCGG 3′
**PPARγ** 3′ primer 5′ GCTCTAGAAGGGAAATGTTGGCAGTGGCTCAGGACTCT 3′

The polymerase chain reaction products were cleaved with SpeI and NotI and fused in frame to the Gal 4 region II activation domain (amino acids 768–881) using the YCP15-Gal-rII vector (see Fig. 1). The PPARγ PCR product was cloned into pCR-Blunt (InvitrogenBV, De Schelp 12,9351 NV Leek, The Netherlands) then excised from this vector using SpeI and NotI and subcloned into YCP15-Gal-rII as above. Human ERβ and Rat ERβ2 cDNAs were kind gifts of Dr. Nigel Brooks (AstraZeneca Central Toxicology Laboratories UK).

Expression of the Gal 4 region II activation domain/NR LBD fusions is under the control of the Gal-1 promoter [35]. The plasmid also contains a selectable marker for growth in leucine deficient medium.

### 2.2. Yeast two hybrid interaction studies

Interactions between nuclear hormone receptors and coactivators were detected using an in vivo assay based on the yeast two-hybrid system. "Bait" and "prey" fusion vectors containing co-activator motifs and nuclear hormone receptor LBDs were co-transformed into the Mey 132 2 Lex Lac Z yeast reporter strain. This *S. cerevisiae* strain has the genotype Mat a, leu 2–3, 112, ura 3–52, rme1, trp1, his4 and contains a stably integrated Ura 3 selectable marker and a stably integrated Lac Z reporter gene under the control of two tandem LexA operators. 3 ug of each plasmid DNA was transformed with 50 ug of denatured Herring Testes carrier DNA (Clontech Laboratories Inc., Palo Alto, CA, USA) using a high efficiency lithium acetate transformation method. Transfectants were cul-

tured in $Ura^-$, $Leu^-$, $Trp^-$ selection medium, and the resultant colonies were incubated with ligand for various periods of time (see results), before being assayed for β-galactosidase activity. All clones were assayed using the β-galactosidase substrate CPRG (Boehringer Mannheim Ltd, East Sussex, UK). 100 ul of a cocktail containing 10 ul 50 mM CPRG, 7 ul Z buffer (600 M $Na_2HPO_4$, 400 mM $NaH_2PO_4$, 100 mM KCl, 10 mM $MgSO_4$, 500 mM β Mercapto-ethanol), 1 ul 20% SDS and 82 ul $H_2O$ was added to 100 ul of each yeast clone, and the optical density of the reaction was recorded at 570 nm. Data analysis was carried out by calculating the mean optical density reading and standard deviation value of four representative clones for each interaction, in the presence and absence of ligand.

### 2.3. Antibodies and western analysis

Soluble protein extracts were prepared from yeast transfectants using the Urea/SDS method (Clontech Laboratories. Inc.). The protein concentration of extracts was determined using a Bradford Assay (Bio-Rad Laboratories, Hercules, CA, USA). 6 ug of each extract was run on 4–20% Tris–glycine denaturing gels (Novex Experimental Technology, San Diego, CA, USA) Gels were electroblotted onto immobilon P membranes (Millipore Corporation, Bedford, MA, USA) using a Novex transfer apparatus. Immunoblots were probed with a primary antibody specific for either the LexA DBD (Clontech Laboratories) or the Gal 4 AD (Clontech laboratories) (see Section 3). An HRP-conjugated rabbit anti-mouse IgG (Santa Cruz Biotechnology, USA) was used for detection. Signals were visualised using ECL reagent (Amersham Life Science, Bucks, and UK).

### 2.4. GST pull-down assays

Recombinant cDNAs in the pSP65 or pSG5 vector were transcribed and translated in vitro in reticulocyte lysate (Promega) in the presence of [$^{35}$S]methionine (Amersham International) according to the manufacturer's instructions. GST fusion proteins were induced and purified as described earlier [14]. [$^{35}$S]-Labelled proteins were incubated with GST fusion proteins in NETN buffer (20 mM Tris pH 8.0, 1 mM EDTA, 0.5% NP40, 100 mM NaCl) containing $10^{-6}$ M E2, in the absence or presence of peptides. In peptide inhibition assays, individual peptides consisting of 14-mers encompassing motifs 1–4 in SRC-1a, namely M1, YSQTSHKLLQLLTT; M2, LTERHKILHRLLQE; M3, ESKDHQLLRYLLDK; M4, QAQQKSLLQQLLTE were added to the GST-binding reactions immediately before the ligand. Samples were subsequently washed and separated on 10% SDS-polyacrylamide gels. The bound proteins were visualised by fluorography.

## 3. Results

### 3.1. LXXLL motifs in SRC-1 exhibit differential ligand-dependent binding to the ER

To identify potential determinants for high affinity binding of SRC-1a to nuclear receptors we first examined the ability of individual LXXLL motifs to interact with human ERα (aa 281–595), human ERβ (aa 190–477), and rat $ER\beta_2$ (aa 198–485) in a yeast two hybrid assay. The four LXXLL motifs, fused to the LexA DBD were tested for their interaction with the ligand binding domains of the receptors, fused to the Gal-4-rII activation domain (Fig. 1) by determining their ability to stimulate transcription of the β-galactosidase reporter gene in the presence and absence of 17β oestradiol ($10^{-7}$ M). The use of centromeric yeast plasmids which are maintained at 1 or 2 copies/cell in transfected yeast cells have allowed semi-quantitative comparisons of the strength of the interactions between the LXXLL motifs and the receptor LBDs.

The interaction of all three receptors was greatest with motif 2, and entirely dependent on the presence of hormone. Motifs 1 and 4 supported weaker interactions but motif 3 failed to interact with any of the ER LBDs (Fig. 2).

We analysed the expression levels of the two fusion proteins to ensure that differences in the reporter gene activity in the different recombinant yeast strains reflected differences in the relative affinity of the motifs for the receptors, and were not merely due to variable expression levels of "bait" or "prey" proteins. The LexA/LXXLL motif fusion proteins were measured by western blotting using a monoclonal antibody specific for the LexA DNA binding domain. No significant variation was seen in the expression levels of the individual LexA/LXXLL motif fusion proteins (Fig. 3). The expression level of the ERα LBD/gal-4 fusion proteins, quantitated using a gal 4 monoclonal antibody, were also similar in the different yeast strains. Thus, we conclude that the relative affinity of the four LXXLL motifs varies with M2 > M4 > M1 > M3.

The relative affinities of the four LXXLL motifs for human ERα was also examined in GST pull-down experiments using peptide inhibition assays. The receptor interacting domain of SRC-1 (residues 570–780), fused to GST was incubated with [$^{35}$S]-labelled ERα and translated in vitro. The interaction was entirely ligand-dependent ([31,36] and data not shown) and so the peptide inhibition assays were only performed in the presence of oestrogen. 14-mer peptides corresponding to M1, M2, M3 and M4 were used to compete the in

vitro interaction. The ability of the peptides to inhibit the interaction varied with M2 > M4 > M1 > M3 (Fig. 4). These results parallel those obtained in the yeast two hybrid experiments and confirm that motif 2 has a higher affinity for ERα than other motifs.

### 3.2. LXXLL motif 4 binds preferentially to the ligand binding domains of the GR, AR, PPARα and PPARγ

We next investigated the ability of SRC-1a LXXLL motifs 1–4 to interact with the ligand binding domain of the GR (aa 501–777) in the presence and absence of dexamethasone. Recombinant yeast strains expressing the GR/gal4 activation construct and the LXXLL/LexA DNA binding domain constructs were incubated with or without dexamethasone ($10^{-4}$ M) for 16 hours. The GR exhibited a strong ligand-dependent interaction with LXXLL motif 4, but no detectable interactions were seen between the GR and LXXLL motif 1, 2 or 3 (Fig. 5).

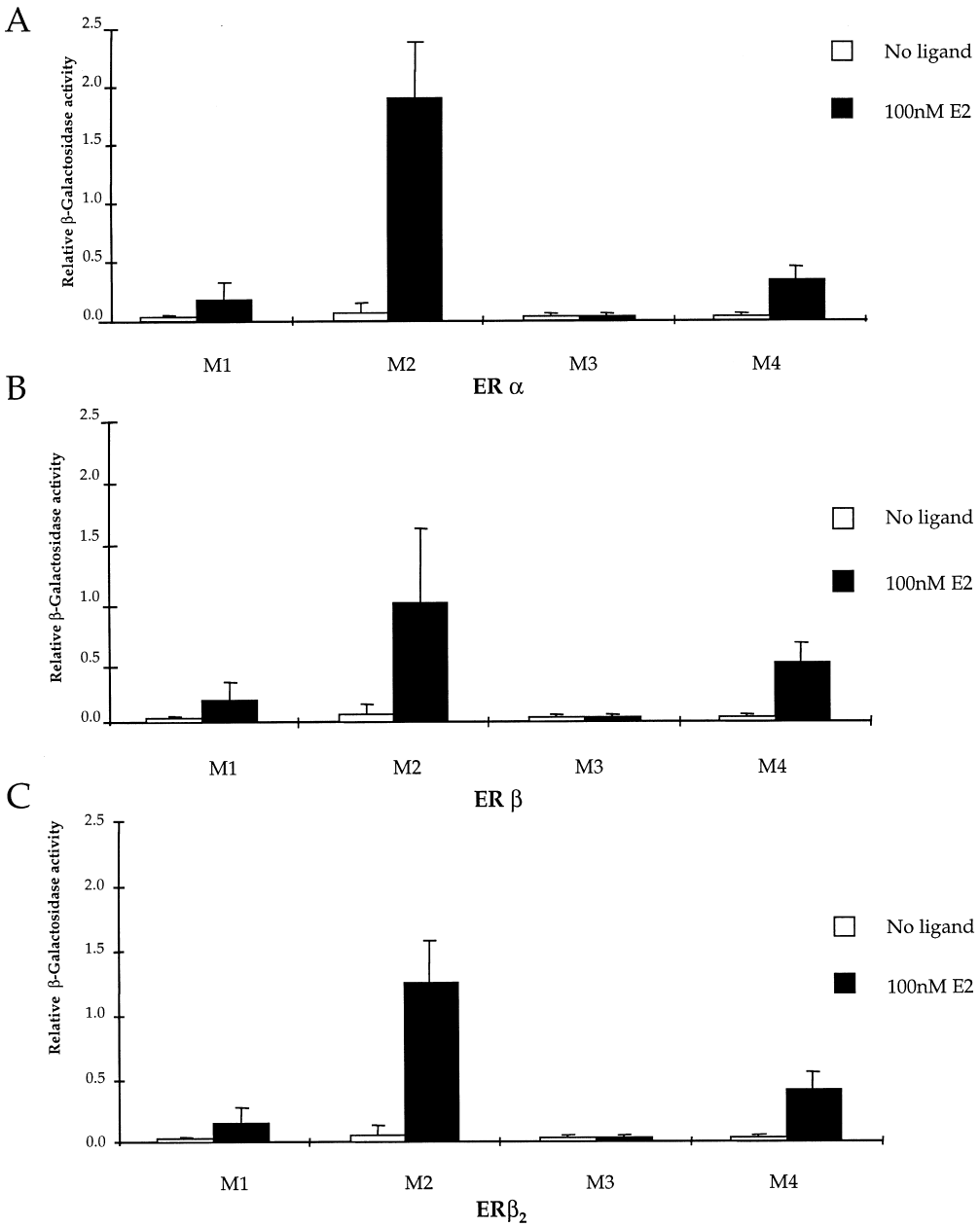


Fig. 2. Yeast two-hybrid interaction between the SRC-1a LXXLL motifs M1 (aa 627–640), M2 (aa 684–696), M3 (aa 743–755) and M4 (aa 1428–1441) with A) Human ERα (aa 281–595), B) Human ERβ (aa 190–477), C) Rat ERβ2 (aa 198–485). Recombinant yeast strains were incubated with estradiol (100 nM) for 5 hours, before being assayed for β-galactosidase activity. The error bars are the standard deviation value of the mean of four representative clones for each interaction.

Similar experiments were performed with the ligand binding domain of the AR (aa 459–735) in the presence and absence of dihydrotestosterone ($10^{-6}$M). Like the GR, the AR exhibited a strong interaction with LXXLL motif 4, but no detectable interaction with motifs 1, 2 or 3 (Fig. 6 ).

Finally, PPARα (aa 281–468) and PPARγ (aa 176–479) were also tested for their interaction with the SRC-1a motifs in the presence and absence of synthetic thiazolodinedione ligands. The strongest ligand-dependent interaction was observed between PPARα and LXXLL motif 4 or between PPARγ and LXXLL motif 4. PPAR γ also interacted weakly with motifs 2 and 3, but there was no detectable interaction with motif 1 (Fig. 7).

### 3.3. Chimeric LXXLL motif/receptor interactions

To investigate the molecular basis for the specificity of the LXXLL motifs for different receptors, we generated chimeric motifs in which we swapped either the N- or the C-terminal residues flanking the core LXXLL motif, and tested them for their ability to interact with ERα (aa 281–595) and GR (aa 501–777).

A series of chimeric LXXLL motifs were generated, firstly by substituting the six N-terminal residues flanking motif 2 with those of motif 4 to generate M4-2-2 (where 4-2-2 refers to the N-terminal residues of motif 4, LXXLL core motif 2 and the C-terminal residues of motif 2, respectively). Conversely, the N-terminal residues flanking motif 4 were replaced with those of motif 2 to generate M2-4-4. Finally, the C-terminal residues were switched to generate M2-2-4 and M4-4-2 (Fig. 1). All chimeric motifs were fused to the LexA DNA binding domain in YCP14 ADH LexA and tested for their ability to interact with the ligand-binding domains of the ER and GR using the yeast two-hybrid interaction assay.

We found that the interaction of M4-2-2 and M2-4-4 with ER was negligible compared with the native motifs suggesting that the N-terminal residues are necessary for the interaction between the ER and motifs 2 and 4 (Fig. 8). On the other hand, swapping the C-terminal residues in M2-2-4 and M4-4-2 had no effect on the interaction suggesting that the C-terminal residues were not essential. Similarly, the ability of motif 4 to interact with the GR was dependent on the presence of specific N-terminal residues but not C-terminal residues (Fig. 8). The importance of the N-terminal residues that flank motif 4 is supported by the observation that M4-2-2 exhibits a weak but significant interaction with GR (approximately 10 fold induction with dexamethasone), whereas M2-4-4 does not interact with GR. The motif 4 chimera M4-4-2 supports a strong interaction with the GR indicating that the C-terminal residues were not essential for the interaction. These results indicate that the specificity of

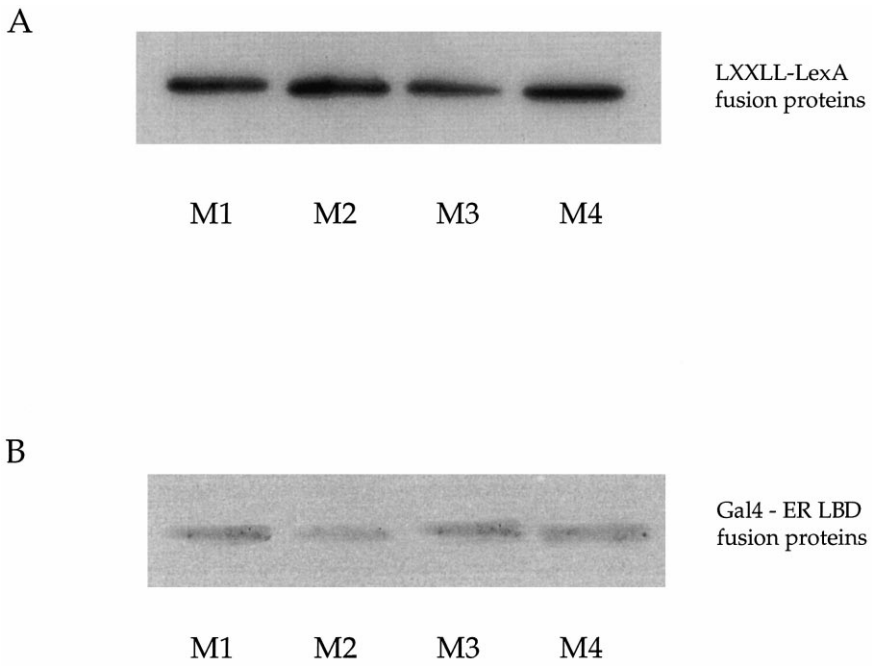


Fig. 3. Western blot analysis of recombinant yeast strains used in the ER/LXXLL motif 2-hybrid analyses. Soluble protein extracts were prepared from yeast transfectants and electrophoresed on Tris–glycine denaturing gels. Immunoblots from these gels were hybridised with (A) LexA antibody to detect LexA/LXXLL fusion protein, (B) Gal 4 antibody to detect Gal-4r-II/ERLBD fusion proteins.

the interaction between the GR and SRC-1a in part resides in the N-terminal residues that flank the core LXXLL motif 4.

## 4. Discussion

Transcriptional activation by nuclear receptors is achieved by the recruitment of co-activator proteins upon ligand binding. One co-activator interaction surface, located on the LBD of receptors, is composed of hydrophobic residues from helices 3, 5 and 12 flanked by a lysine residue in helix 3 and a glutamic residue in helix 12 [6,29,36]. Previous work has demonstrated that the recruitment of the p160 family of co-activators is dependent upon the integrity of a short hydrophobic motif, LXXLL or NR box, three of which are conserved in individual family members [26,27,37]. Recently, a number of receptors have been shown to exhibit preferential binding to distinct motifs [29–32,38]. Here we demonstrate that not only ERα but also human ERβ and rat ERβ2 bind with highest affinity to motif 2 while GR, AR, PPARα and PPARγ preferentially bind to motif 4, found only in SRC-1a.

Sequence alignment indicates that the degree of conservation of a particular LXXLL motif in the different p160 co-activators is greater than that between different motifs within any one p160 protein. Since such conservation sometimes extends beyond the minimal LXXLL sequence, it is conceivable that residues flanking the LXXLL motif may confer preferential binding of particular motifs to different receptors. Our results

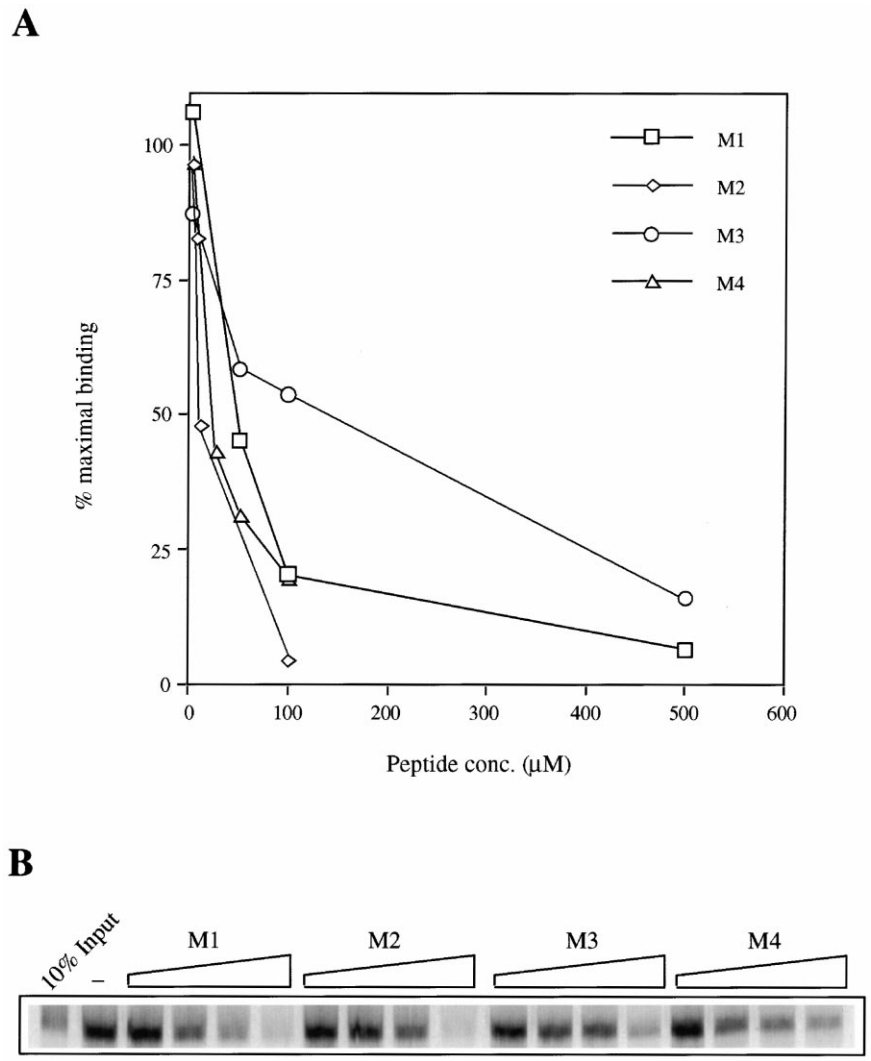


Fig. 4. Differential inhibition of mERα-SRC-1 interaction in vitro by LXXLL motif containing peptides. (A) Effect of increasing concentrations of peptides (14 mers) encompassing M1–M4 on the amount of in vitro translated [$^{35}$S]methionine-labelled mER bound to GST-SRC-1 (550–780) as determined in a pull-down assay shown in (B). Bound, labelled proteins were eluted, separated on 10% SDS- polyacrylamide gels and detected by fluorography. The input lane represents 10% of the total volume of the lysate used in each reaction. Data from one representative experiment are presented but two independent experiments were performed.

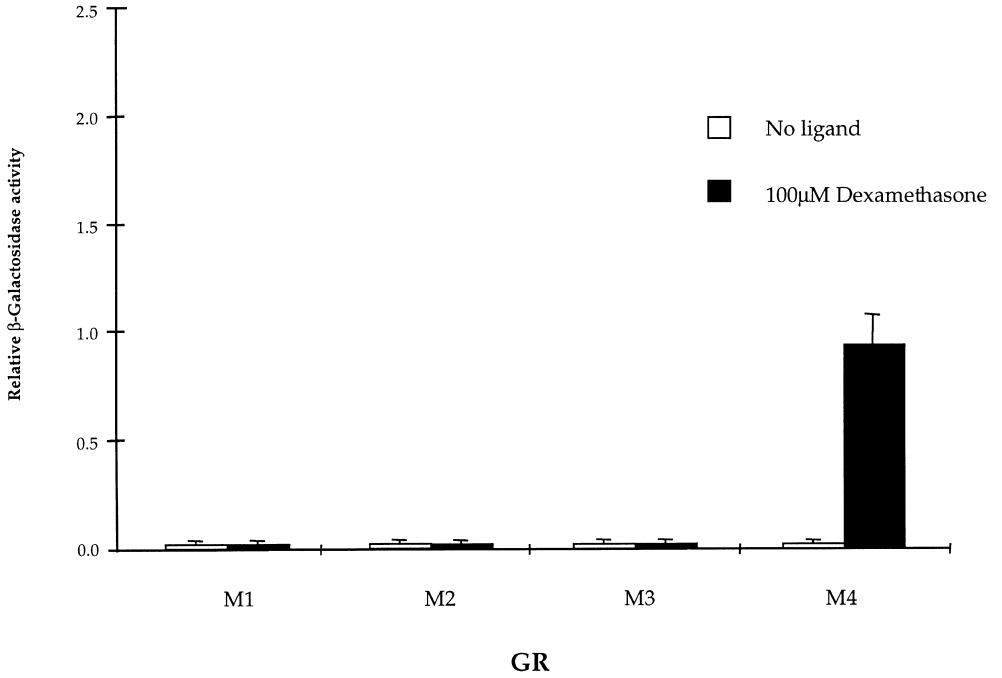


Fig. 5. Yeast two-hybrid interaction between the SRC-1a LXXLL motifs M1 (aa 627–640), M2 (aa 684–696), M3 (aa 743–755) and M4 (aa 1428–1441) and human GR LBD (aa 501–777). Recombinant yeast strains were incubated with dexamethasone (100 μM) for 16 h, before being assayed for β-galactosidase activity. The error bars are the standard deviation value of the mean of four representative clones for each interaction.

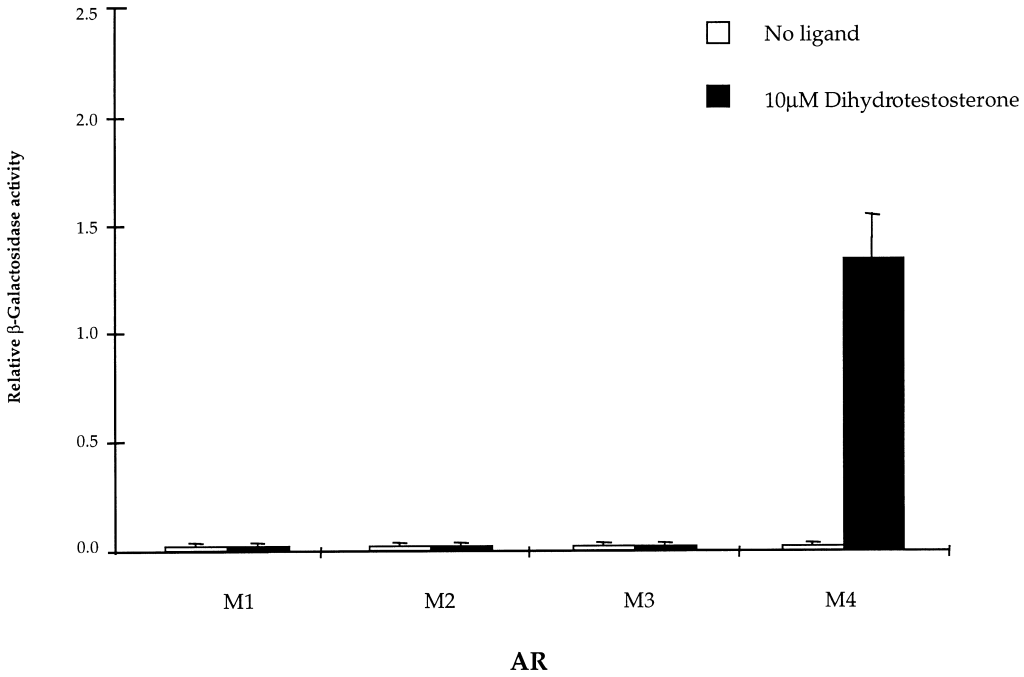


Fig. 6. Yeast two-hybrid interaction between the SRC-1a LXXLL motifs M1 (aa 627–640), M2 (aa 684–696), M3 (aa 743–755) and M4 (aa 1428–1441) and human AR LBD (aa 459–735). Recombinant yeast strains were incubated with dihydrotestosterone (1 μM) for 16 hours, before being assayed for β-galactosidase activity. The error bars are the standard deviation value of the mean of four representative clones for each interaction.

with chimeric LXXLL motifs support this view and suggest that residues, N-terminal to the core motif, contribute to high affinity binding. Similar conclusions were drawn for the preferential binding of motif 2 to ERα [36] and residues adjacent to motif 2 were also shown to modulate its affinity with TRβ [29], although the relative contribution of N- and C-terminal residues was not assessed.

While the crystal structures of a number of LBDs and SRC-1 fragments or peptides encompassing a LXXLL motif indicate the position of the helical motif in a cleft on the surface of the receptor, it is unclear where the residues flanking the motif are located. It is conceivable that the basic residues adjacent to motif 2 might be accommodated by a shallow groove between H5 and H12 in TRβ [29]. However, the residues are disordered in the structure of the agonist bound receptors [8,29] suggesting that they are unlikely to form stable interactions with residues of LBD in equilibrium binding. One possibility is that the adjacent residues may play a role in structuring the LXXLL motif to allow docking with a specific LBD. This hypothesis is supported by our observation that switching the N-terminal residues of motif 2 and 4 generates 2 hybrid motifs neither of which can interact with the ER LBD (see Fig. 8). Alternatively, the flanking residues may be involved in long range recognition of surface features of ER, which are not necessarily in the proximity of the coactivator docking site.

Microinjection studies implicated residues C-terminal to SRC-1 motif 2 as specificity determinants for ER binding, [38] although we were unable to confirm

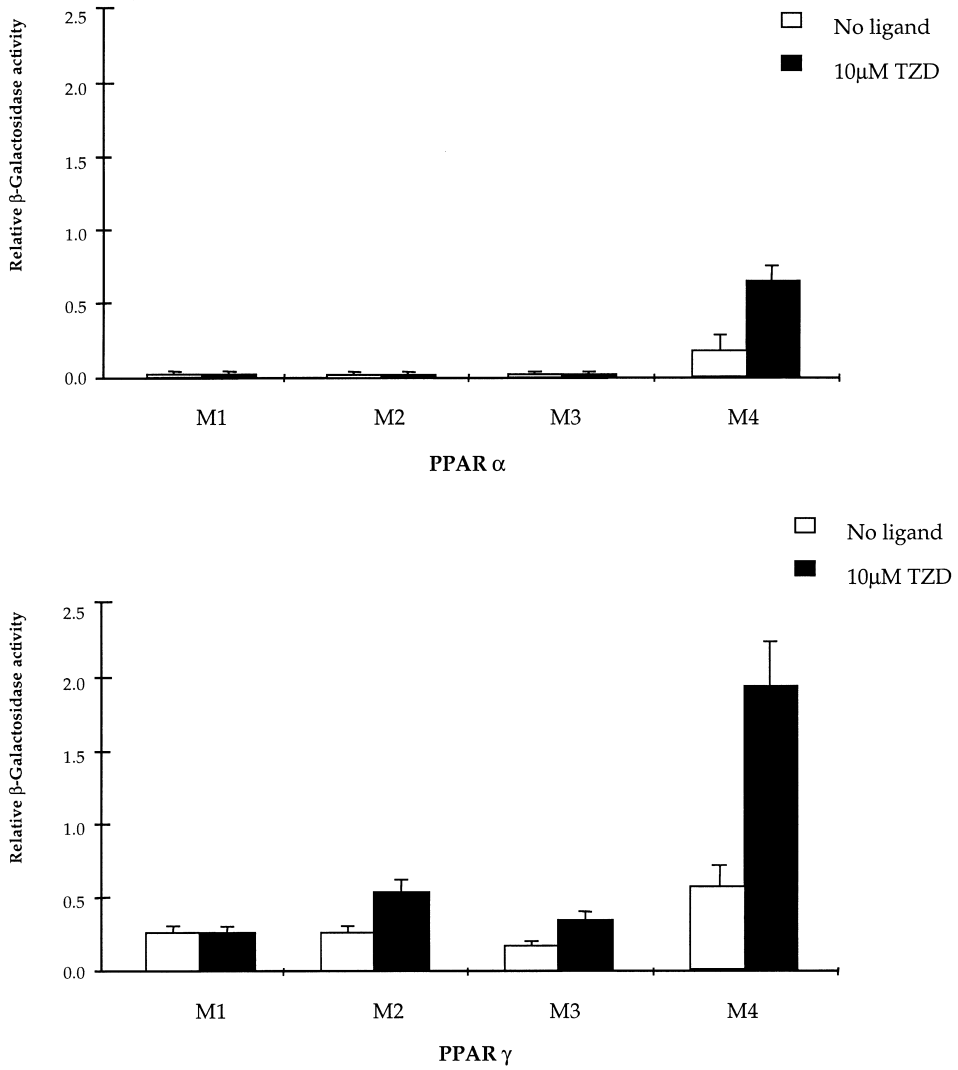


Fig. 7. Yeast two-hybrid interaction between the SRC-1a LXXLL motifs M1 (aa 627–640), M2 (aa 684–696), M3 (aa 743–755) and M4 (aa 1428–1441) and A) Human PPARα LBD (aa 281–468), B) Human PPARγ LBD (aa 176–479). Recombinant yeast strains were incubated with a TZD (10 μM) for 16 h, before being assayed for β-galactosidase activity. The error bars are the standard deviation value of four representative clones for each interaction.

these observation in our yeast assays. Nevertheless, it is tempting to speculate that the same principle of long-range recognition might be applicable. Thus, it is conceivable that coactivator recruitment depends on two steps. First flanking residues may direct the core motif to a broad area of the receptor, which encompasses the co-activator interaction surface, whereupon polarity, imposed by K362 and E542, direct the formation and docking of the LXXLL helix in one orientation due to the dipole intrinsic to helical structure. Specific hydrophobic and electrostatic interactions between the motif and the receptor would then result in stable interaction of the coactivator with the receptor.

We have found that GR, AR, PPARα and PPARγ binds with highest affinity to motif 4 present in SRC-1a, this is consistent with observations recently made by Stallcup and co-workers [30]. This motif is found in only one isoform of SRC-1, namely SRC-1a suggesting that it maybe recruited selectively compared with other p160 co-activators. Nevertheless, exogenously expressed SRC-1e and TIF-2 [32] have been shown to potentiate the transcriptional activity of a

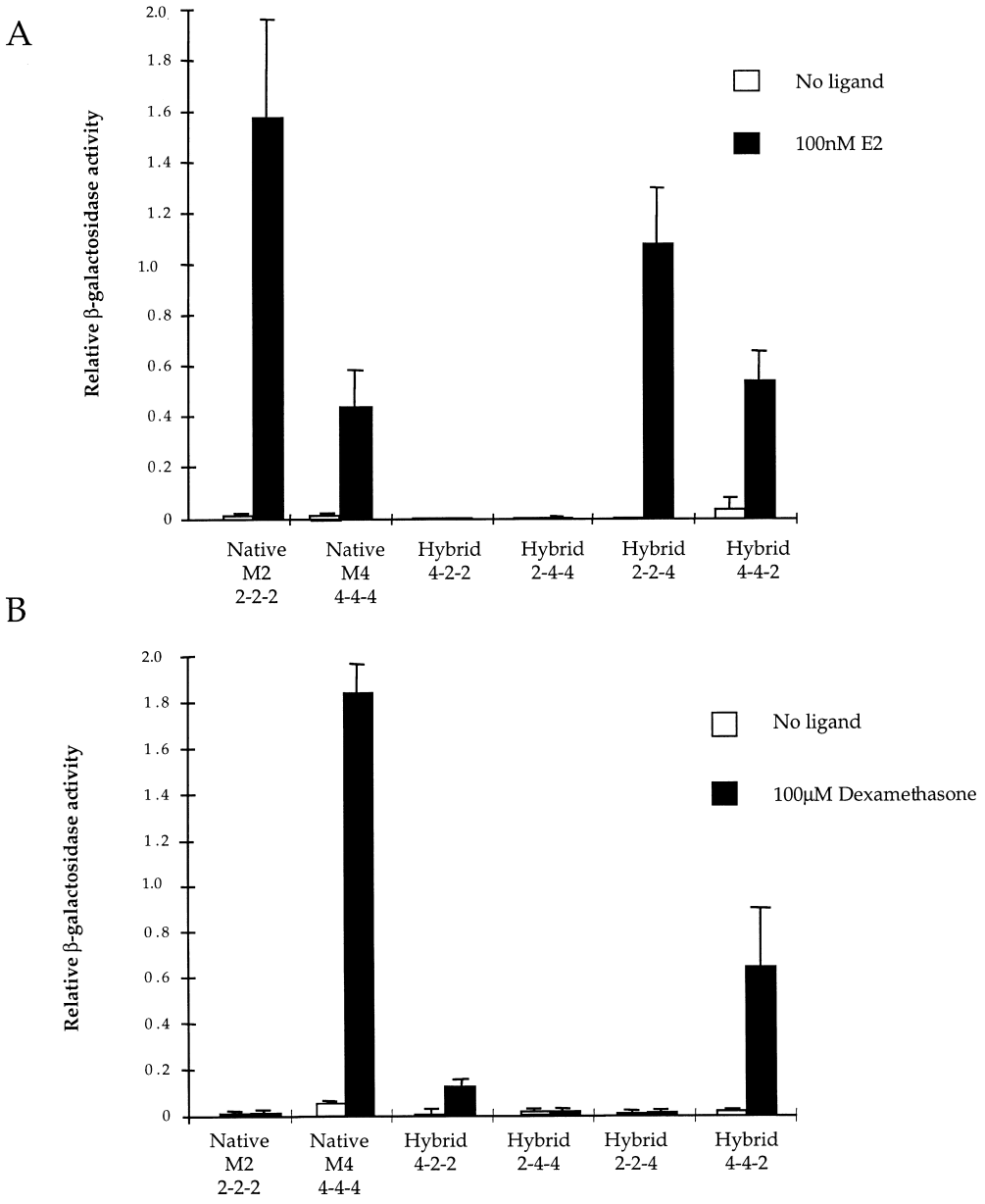


Fig. 8. Yeast two-hybrid interaction between M2/M4 chimeric motifs and (A) Human ERα LBD (aa 281–595). (B) Human GR LBD (aa 501–777). Recombinant yeast strains were incubated with (A) Estradiol (100 nM) for 5 hours, or with (B) Dexamethasone (100 μM) for 16 hours, before being assayed for β-galactosidase activity. The error bars are the standard deviation value of the mean of four representative clones for each interaction.

number of receptors in mammalian cells. Although these receptors bind with highest affinity to motif 4, it seems likely that they can bind to other motifs, perhaps two motifs binding in a co-operative fashion to each AF 2 surface in a receptor dimer.

The recruitment of individual coactivators will depend on both their relative affinities for receptor and their cellular concentration. In cell lines, the mRNAs for SRC-1a and SRC-1e are expressed in a ratio of approximately 1:2, but their relative protein concentration is unknown. Therefore, it is difficult to determine whether SRC-1a is selectively recruited to activate this subset of receptors, or whether it serves a specific function.

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