Transcriptional activation by estrogen receptor (ERα) and steroid receptor coactivator (SRC1) involves distinct mechanisms in yeast and mammalian cells

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Abstract

Steroid receptors activate transcription in yeast cells via interactions with endogenous coactivators and/or basal factors. We examined the effects of mutations in the ligand binding domain on the transcriptional activity of ERα in yeast. Our results show that mutations in Helix 3 (K366A) and Helix 12 (M547A, L548A) disrupt transcriptional activity of ERα in yeast, as previously observed in mammalian cells. However, replacement of a conserved tyrosine residue in Helix 12 with alanine or aspartate (Y541A and Y541D), which renders ERα constitutively active in mammalian cells, had only a weak stimulatory effect on ligand-independent reporter activation by ERα in yeast. Two-hybrid interaction experiments revealed that a Y541A mutant expressed in yeast was capable of ligand-independent binding to a mammalian coactivator, suggesting that there is a subtle difference in how this mutant interacts with mammalian and yeast cofactors. We also show that the ligand-dependent activities of ERα and progesterone receptor (PR) in yeast cells were strongly enhanced by the human p160 protein steroid receptor coactivator (SRC1), but not by CREB-Binding Protein (CBP) or the p300/CBP associated factor (P/CAF). Although the SRC1 activation domains AD1 and AD2 are functional in yeast, deletion of these sequences only partially impaired SRC1 coactivator function in this organism; this is in contrast to similar experiments in mammalian cells. Thus SRC1 sequences involved in recruitment of CBP/p300 and Co-Activator-Associated Arginine Methyltransferase (CARM-1) in mammalian cells are not essential for its function in yeast, suggesting that SRC1 operates via distinct mechanisms in yeast and mammalian cells.

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Introduction

The molecular machinery involved in gene transcription is well conserved among the eukaryotes (Guarante & Bermingham-McDonogh 1992). Yeast and mammalian cells share homologous RNA polymerases, basal factors, mediator proteins, chromatin remodelling factors and chromatin modifying enzymes. This has facilitated functional studies of mammalian transcription factors in the genetically tractable yeast organism. The nuclear receptors (including steroid, retinoid, vitamin D and thyroid receptors) activate reporter gene transcription in the presence of cognate ligands in yeast cells (McEwan 2001). The ligand binding domains (LBDS) of ERα (Pierrat et al. 1992) and progesterone receptor (PR) (Mak et al. 1989, Meyer et al. 1992) containing the AF2 transactivation function have been shown to mediate ligand-dependent reporter activation in yeast. The LBD of ERα also contains a minor activation domain (AF2-α) that binds mammalian TAFII30 (Jacq et al. 1994). This sequence was originally identified as a transcriptionally active domain in yeast (Pierrat et al. 1994), although its molecular target in that organism is unknown. As in mammalian cells, the AF1 transactivation domain located within the N-terminus of steroid and retinoid receptors is constitutively active in yeast cells when separated from the LBD (Pierrat et al. 1992, Metzger et al. 1988, 1992, 1995, Heery et al. 1993). Synergistic interactions between AF1 and AF2 domains have
also been observed for retinoic acid receptor heterodimers in yeast (Heery et al. 1993, 1994, Hall et al., 1993, Allegretto et al. 1993). Mutations in Helix 3 and Helix 12 that disrupt ERα transcriptional activity (Danielian et al. 1992, Henttu et al. 1997) result in loss of coactivator recruitment via LXXLL motifs (Heery et al. 1997). Thus, nuclear receptor sequences necessary for cofactor recruitment in mammalian cells are also necessary for transcriptional activity in yeast cells.

Homologs of nuclear receptor coactivators such as p160s, PGC1 and TRAP220 appear to be absent from yeast, although other components of coactivator complexes are conserved in this organism e.g. GCN5, SRB/Med proteins. Similarly, CBP and p300 have no close sequence homologs in yeast, although they share conserved functional domains, such as the bromodomain and a histone acetyltransferase domain, with a number of yeast proteins. In addition, a cysteine/histidine rich region of CBP/p300 shows partial homology with the yeast coactivator ADA2p (Guarente & Bermingham-McDonogh 1992). Genetic screens for yeast proteins required for the function of steroid receptors have identified HSP90 (Picard et al. 1990), SWI2/SWI3 proteins (Yoshinaga et al. 1992, Wallberg et al. 2000), GCN5, ADA proteins (vom Baur et al. 1998, Anafi et al. 2000) PSU1 (Gaudon et al. 1999), SPT6 (Baniahmad et al. 1995), RSP5 (Imhof et al. 1996) and several other cofactors (McEwan 2001). In this study we assess the effects of mutations in Helices 3 and 12 on the transcriptional activity of ERα in yeast. We also compare the abilities of the mammalian coactivators SRC1, CBP and P/CAF to enhance the activities of steroid receptors in yeast, and define sequences in SRC1 responsible for its activity in this organism.

**Materials and methods**

**Plasmid constructs**

The reporter p3ERE- lacZ (also termed pRLΔ21U3 ERE), consisting of minimal URA3 promoter flanking the E.coli lacZ gene, has been described previously (Metzger et al. 1995). cDNAs encoding full length SRC1e, SRC1a (Kalkhoven et al. 1998), CBP (Sheppard et al. 2001) and P/CAF (Yang et al. 1996) were subcloned into Yep20 (TRPI selectable) or Yep30 (HIS3 selectable) vectors, which are modified versions of Yep10 and Yep90, respectively (Heery et al. 1993, 1994) containing multiple cloning sites within a PGK promoter cassette and a consensus Kozak’s sequence. The mouse ERα wild type and mutant cDNAs were cloned into the Yep90 (HIS3 selectable) vector. The construct DBD-PR consists of the progesterone receptor LBD (amino acids 633–933) fused in frame with the human ERα DNA binding domain (DBD) in the vector pBL1 (HIS3 selectable) (Le Douarin et al. 1995, 2001). The SRC1 deletion mutant series was generated by PCR, introducing flanking restriction sites, and a Kozak’s sequence and initiation codon at the 5’ end of each PCR fragment, before subcloning into the Yep20 vector. SRC1 mutants containing the ΔAD1 deletion (amino acids 901–950) were PCR amplified using the pSG5-hSRC1eΔAD1 construct as a template (Sheppard et al. 2001). The DBD-SRC1 series used to map the transactivation domains of SRC1 in yeast were also generated by PCR and subcloned in frame with the DBD in the pBL1 vector. All PCR constructs were fully sequenced.

**Yeast reporter assays**

Yeast W303 or BY4704 cells were cotransformed with p3ERE-lacZ reporter and cDNA expression vectors encoding wild type or mutant ERα. For coactivator activity assays, yeast cells containing the reporter were cotransformed with expression plasmids encoding ERα or DBD-PR in combination with various coactivators expression plasmids, or empty vector controls, and maintained on selective media. For yeast two-hybrid assays, yeast cells were cotransformed with Yep90 ERα and pASV3 (VP16) (Le Douarin et al. 2001) or pASV3-mouse SUG1 (VP16-SUG1) (vom Baur et al. 1996). Co-transformants were grown in liquid culture for 16 h in the presence of 10⁻⁶ M 17-β-estradiol (E2), progesterone (R5020) or vehicle (ethanol) as required. For mapping the activation domains of SRC1, yeast cells were cotransformed with reporter and the DBD-SRC1 series or empty vector pBL1 controls. Reporter β-galactosidase activity in yeast cell-free extracts (prepared by the glass bead method) was measured quantitatively as described previously (Le Douarin et al. 2001). In experiments where cell growth arrest was induced by ligands, cells were cultured to late
log phase prior to addition of the ligand, and reporter activity was assessed after a further 8 h. Reporter activity is expressed as units of specific activity (nmoles/mg/min).

**Western blots**

Yeast cells expressing recombinant proteins were cultured to late log phase in a selective liquid medium (15 ml). Cell pellets were resuspended in a buffer containing 1 M sucrose, 25 mM EDTA, 50 mM dithiothreitol (in a final volume of 1 ml). Sphaeroplasts were prepared by incubating 100 µl of the cell slurry with 1 unit Zymolyase 20T (Seikagaku America, Falmouth, MA, USA) for 1 h at 37 °C. Sphaeroplasts were harvested by low speed centrifugation, resuspended in sample buffer, boiled for 10 min, and aliquots of the cell-free extracts were separated on 6% or 12% acrylamide gels by SDS-PAGE, followed by transfer to nitrocellulose. For transfer of larger proteins such as CBP, high glycine buffer (300 mM) was used. Western blotting was carried out using standard procedures. Recombinant ERα expressed in yeast was detected using a 1:5000 dilution of a monoclonal antibody raised against human ERα (anti-F region mouse monoclonal, a gift from P Chambon) which cross-reacts with mouse ERα. Controls for the western blot include cell-free extract from yeast expressing human ERα (1–595) and transiently transfected COS-1 cells over-expressing mouse ERα (1–599) (shown in Fig. 1F), or SRC1e (Fig. 2C). Polyclonal antisera purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) were as follows; SRC1 C20 (sc-6096), SRC1 M20 (sc-6098), CBP A22 (sc-369), CBP C20 (sc-583) and P/CAF (sc-6300). The polyclonal antibodies were used at a dilution of 1:500. For detection of CBP, best results were obtained using both anti-CBP antibodies in combination. Peroxidase-linked anti-goat, antimouse or anti-rabbit secondary antibodies were used to detect the proteins using ECL Plus detection kits (Amersham, Buckinghamshire, UK).

**Results**

**Point mutations in Helix 3 and Helix 12 of the LBD disrupt the function of ERα in yeast**

Previous studies revealed that mutation of amino acids such as L543, L544, M547 or L548 in Helix 12, or K366 in Helix 3, leads to loss of cofactor binding in vitro and reduced transcriptional activity by mouse ERα in mammalian cells (Danielian et al. 1992). In contrast, replacement of Y541 in Helix 12 with alanine or acidic residues did not perturb ligand-dependent activity, but resulted in a strong ligand-independent activation of ERα (White et al. 1997). These amino acids are comprised within the LBD AF2 surface that accommodates recruitment of coactivators via LXXLL amphipathic α-helices (Heery et al. 1997). The importance of key residues in Helix 3 and Helix 12 for coactivator binding has been highlighted by the structure of the LBD of PPARγ in complex with a portion of the SRC1 nuclear receptor interaction domain (Nolte et al. 1998), and the ERα LBD complexed with LXXLL peptides (Shiau et al. 1998). The amino acids K366 and E542, which are highly conserved throughout the nuclear receptor family, appear to form a charged clamp that locks the LXXL α-helix into the AF2 channel (Nolte et al. 1998).

To characterise the effects of similar mutations on the transcriptional activity of ERα in yeast cells, reporter activation by ERα mutants was assessed. The domain structure and relative positions of amino acids mutated in this study are depicted in Fig. 1A. As shown in Fig. 1B, mutation of the conserved hydrophobic residues M547 and L548 to alanine resulted in almost complete loss of the ligand-dependent activity of ERα, as previously observed in mammalian cells (Danileian et al. 1992). Consistent with this, we have previously shown that a VP16-ERα construct containing these mutations failed to bind to LXXL peptides or full-length coactivators in yeast cells (Heery et al. 1997). Similarly, mutation of K366 to alanine resulted in a substantial loss of ligand-dependent activity of ERα, as demonstrated by comparison of the ligand dose-dependent reporter activation of ERα with that of ERα K366A (Fig. 1C). These results indicate that the AF2 surface is important for transcription in both mammalian and yeast cells, and suggests that yeast proteins mediating ERα activity may contact AF2 in a similar fashion to mammalian coactivators.

Our experiments revealed that ligand-dependent reporter activation by the ERα mutants Y451D, Y541E and Y541F was very similar to that observed for wild type ERα, although we noted a reproducible 1.7-fold increased level of reporter activation by Y541A, compared to wild type or other Y541 mutants (Fig. 1D). Reporter activation
H M SHEPPARD and others · ERα / SRC1 function in yeast

Mouse ERα

AF1

DBD

H3

H12

LBD (AF2)

A

B

C

D

E

F

G

β-Galactosidase Activity

ERα

ERα ML547/Aaa

No Ligand

E2

β-Galactosidase Activity

Log Ligand (nM)

β-Galactosidase Activity

vector

ERα

ERα Y541A

ERα Y541D

ERα Y541E

ERα Y541F

vector

ERα

ERα Y541A

ERα Y541D

ERα Y541E

ERα Y541F

BS kDa

Control

β-Galactosidase Activity

ERα + VP15

ERα + VP15-SUG1

Y541A + VP15

Y541A + VP16-SUG1

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by Y541A and Y541D in the absence of ligand was 6-fold and 3.5-fold greater, respectively, than that observed for wild type ERα whereas Y541E and Y541F showed no substantial increase in ligand-independent reporter activation (Fig. 1E). The expression of wild type and mutant proteins at similar levels was confirmed by western blotting (Fig. 1F). Thus, in contrast to similar assays in mammalian cells (White et al. 1997), Y541 mutants do not have strong constitutive transcriptional activity in yeast cells, but rather show a weak increase in ligand-independent reporter activation. To examine whether the Y541 mutants retained their ability to interact with a mammalian coactivator in a constitutive manner, we performed yeast two-hybrid assays. Whereas the interaction of wild type ERα with VP16-SUG1 was almost entirely dependent on ligand, the Y541A mutant showed a very high level of reporter activation (800-fold over basal) when coexpressed with VP16-SUG1 in the absence of ligand (Fig. 1G), indicating constitutive interaction of these proteins in yeast. The mouse SUG1 protein has been shown to bind ERα and other NRs via AF2 (Lee et al. 1995, vom Baur et al. 1996). Thus, while the Y541A mutation stimulates ligand-independent interaction with a mammalian coactivator in yeast, it does not result in constitutive activity of the mutant ERα, suggesting that recruitment of yeast coactivators is not substantially affected by this mutation. Our results imply a subtle difference in the way yeast and mammalian cofactors interact with ERαLBD.

**SRC1 proteins, but not CBP or P/CAF, enhance steroid receptor activity in yeast**

cDNAs encoding human SRC1a, human SRC1e, human P/CAF and mouse CBP (depicted schematically in Fig. 2A) were cloned into the yeast expression vector Yep20. The yeast strain W303 (or BY4705) was cotransformed with plasmids p3ERE-lacZ (Metzger et al. 1995) and Yep90 mouse ERα. Transforms containing these plasmids were then further transformed with Yep20 empty vector, or Yep20 containing a cDNA encoding a mammalian coactivator. Reporter activity was assessed after overnight liquid culture in the presence or absence of E2, as outlined in Materials and methods. As shown in Fig. 2B, yeast cells expressing ERα alone showed a strong induction of reporter activity in the presence of ligand, whereas negligible reporter activity was observed in its absence. Co-expression of ERα with P/CAF or CBP did not substantially enhance or decrease the reporter activity, either with or without ligand (Fig. 2B). However coexpression of human SRC1a enhanced the reporter activity approximately 4-fold in the presence of ligand. In addition, a strong increase in ligand-independent activity was observed, although this level of reporter activity remained 10-fold lower than that induced by E2 (Fig. 2B). Similar ligand-independent enhancement of ERα activity by SRC1 isoforms has been observed in transiently transfected mammalian cells (Sheppard et al. 2001, White et al. 1997, Kalkhoven et al. 1998 Bevan et al. 1999). Interestingly, coexpression of the human SRC1e isoform with ERα strongly enhanced the ligand-independent reporter activity, but had only a modest effect on the ligand-induced activity (Fig. 2B). Analysis of cell morphology in transformants co-expressing ERα and SRC1e in the presence of ligand revealed that the majority of cells were atypical elongated cells or pseudohyphal structures (Fig. 2E). Similar phenotypes were previously observed in cells undergoing growth arrest in liquid media, due to expression of VP16–ERα fusion
proteins in the presence of ligand (Gilbert et al. 1993). Cells expressing SRC1a were also found to have atypical cell growth in the presence of ligand, albeit to a lesser extent, and similar results were obtained in both the W303 and BY4705 strains (data not shown). Thus, reporter activation by ERα/SRC1 proteins (in particular SRC1e) in the presence of ligand is likely to be underestimated in these clones due to deleterious effects on cell growth by transcriptional interference or ‘squelching’. No squelching effects were observed in yeast cell-free extracts expressing ERα with CBP or P/CAF, or due to expression of SRC1 proteins in the absence of ERα (data not shown).

The expression of full length ERα, CBP and SRC1 proteins in yeast cell-free extracts was confirmed by western blotting (Fig. 2C). However, we were unable to detect expression of full-length P/CAF in yeast extracts using a commercially available anti-P/CAF antibody, although we did detect expression of LexA-tagged P/CAF constructs using an anti-LexA antibody, and these proteins also failed to enhance ERα activity (data not shown).

In previous studies we reported that, in mammalian cells, the ligand-independent effect of SRC1 on ERα is mediated by the interaction of the AF1 domain of ERα with the glutamine-rich (Q-rich) domain of SRC1 (Sheppard et al. 2001, Bevan et al. 1999). To test whether this was also the case in yeast, we transformed BY4705 with a construct consisting of the LBD of the progesterone receptor fused to the DBD of the ERα (DBD-PR). The steroid receptors ERα and PR show no differences in their interactions with p160s, CBP/p300 or P/CAF in reporter assays in mammalian cells, or in vitro. As shown in Fig. 2D, DBD-PR gave a moderate but reproducible enhancement of reporter activity in yeast in the presence of the agonist ligand R5020. This level of reporter activation is approximately 4–5-fold lower than ERα on the same reporter (Fig. 2B) or full length PR on a PRE-driven reporter gene (data not shown). This reflects the loss of synergistic interaction with the AF1 domain, as reported previously for other steroid receptors (Pierrat et al. 1992, Heery et al. 1993). As observed with full length ERα, the co-expression of P/CAF or CBP proteins did not have a substantial effect on the ligand-dependent or ligand-independent reporter activities. However, a strong stimulation of ligand-dependent reporter activity was observed in cotransformants expressing either SRC1a or SRC1e (Fig. 2D). In contrast to the full length ERα, no ligand-independent activity was observed with DBD-PR, due to the absence of the AF1 domain. In addition, the overall reporter activity saturates at a lower level, and we did not observe squelching effects using the DBD-PR construct.

**Sequences required for SRC1 coactivator activity in yeast**

To determine which domains of SRC1 are required for its ability to enhance transcriptional activity of the PR AF2 in yeast, we constructed a series of SRC1 deletion mutants in the vector Yep20 (Fig. 3A). We then assayed reporter activity due to DBD-PR in cells co-expressing the SRC1 mutants. As shown in Fig. 3B, C-terminal truncations up to amino acid 980 increased the ligand-dependent reporter activity approximately 2–5-fold over wild type. This may result from an inhibitory effect of the C-terminus on SRC1.

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**Figure 2** (A) Schematic representation showing domain structure of mammalian coactivators SRC1a, SRC1e, CBP and P/CAF. Functional domains indicated include the basic Helix-Loop-Helix (bHLH) and Per-Arnt-Sim (PAS) domains, LXXLL motifs (LXM1–3 in CBP), activation domains (AD1 and AD2), glutamine rich region (Q-rich), cysteine/histidine rich regions (CH1–3), SRC1 interaction domain (SID), bromodomain (bromo) and histone acetyltransferase (HAT) domain. (B) Reporter β-galactosidase activity in cell-free extracts from yeast cells co-expressing mouse ERα with mammalian coactivator proteins as indicated, in the presence or absence of ligand. (C) Western blot using specific antibodies to detect mammalian proteins in yeast cell-free extracts as indicated. The amount of cell-free extract loaded (10, 20, or 40 µl) is indicated. The control for SRC1 western blots is cell-free extract of transiently transfected COS-1 cells over-expressing SRC1e. The antibodies M20 and C20 are specific for SRC1e and SRC1a, respectively. The approximate molecular weights are indicated. (D) Reporter β-galactosidase activity in cell-free extracts from yeast cells co-expressing DBD-PR with mammalian coactivator proteins as indicated, in the presence or absence of ligand. (E) Phase contrast images (magnification 100X) showing morphology of yeast cells co-transformed with Yep90 ERα and Yep20, Yep20 SRC1a or Yep20 SRC1e.
interaction with endogenous coactivators, or another unknown mechanism. Note, that while we did not observe the drastic cell growth arrest phenotype, we cannot rule out the possible squelching effects in cells expressing proteins containing the C-terminal domain of SRC1e. In any case, the highest activity was observed with SRC1 1–980, indicating that the AD2 domain and the Q-rich region are not critical for the ability of SRC1 to enhance PR activity in yeast. In contrast, however, truncation at amino acid 900, which deletes AD1, resulted in a decrease in the ligand-dependent reporter activity to a level lower than that achieved with full length SRC1. This indicates an involvement of the AD1 domain in achieving the maximal activity, although the reporter activity was still markedly (8–10-fold) higher than that due to DBD-PR alone. The result contrasts with studies in mammalian cells indicating a requirement for the AD1 domain and CBP recruitment (Sheppard et al. 2001, Li et al. 2000, Kim et al. 2001). Further truncation of SRC1 up to amino acid 780, resulted in a dramatic reduction in activity to a level similar to that obtained in the absence of exogenous coactivator (Fig. 2D & 3B). These results suggest that the sequence between 780 and 900, in addition to the AD1 region, may mediate interactions with yeast cofactors and thus contribute to SRC1 coactivator function in yeast.

To determine whether the bHLH-PAS domain and N-terminal sequences of SRC1 are important in yeast, we constructed the mutant SRC1 626–970, which retains only the central nuclear receptor interaction domain (NID) and the adjacent AD1 domains. As shown in Fig. 3B, this construct maintains a strong coactivator activity (almost 2-fold greater than full length SRC1e). A similar result was obtained in mammalian cells in which the minimal SRC1 construct was equally potent as the full length SRC1 (Sheppard et al. 2001).

Deletion of the AD1 region (Helix A amino acids 901–950, see below) in the context of the full-length protein SRC1e (SRC1e-ΔAD1) resulted in only a 40% reduction in the ability of SRC1 to enhance AF2 activity (Fig. 3C). A similar result was obtained on comparing 1–1100 and 1–1100ΔAD1 (data not shown). Taken together, our data suggest that several surfaces on SRC1, including AD1 and the sequence between 780–900, mediate interactions with endogenous yeast cofactors resulting in enhanced ligand-dependent transcription by ERα or PR LBDs.
SRC1 activation domains (AD1 and AD2) are functional in yeast

Two regions of SRC1 have been shown to be involved in transcriptional activation of reporter genes in mammalian cells when fused to a heterologous DBD (Onate et al. 1995, Kalkhoven et al. 1998, Sheppard et al. 2001). These regions are AD1 (926–970) and AD2, which functions by binding the methyltransferases CARM1 and PRMT1 (Chen et al. 1999). As shown in Fig. 4A, a construct consisting of SRC1 amino acids 867–990 fused to the ERα DBD produced a very high level of reporter activity in yeast, indicating that the AD1 region is also a potent transcriptional activation domain in this organism. Similarly, the C-terminal sequences from SRC1e (1241–1399) and SRC1a (1241–1441), which contain the AD2 domain, are also strong activators in yeast, albeit at a level of activity 5 fold lower than AD1. These results suggest that yeast cofactors bind to sequences within the 2 major activation domains of SRC1.

To map the sequence responsible for the transcriptional activity of SRC1 867–990 more precisely, we generated a series of DBD-SRC1 fusion proteins spanning the AD1 region. As shown in Fig. 4B, the minimal sequence having transcriptional activity was contained within amino acids 926–940 of SRC1. This region contains Helix A of the AD1 domain (Sheppard et al. 2001, Demarest et al. 2002), which is not sufficient on its own to bind CBP or activate transcription in mammalian cells (Sheppard et al. 2001). Thus, our results highlight a difference in the way SRC1 AD1 domain functions in yeast and mammalian cells, consistent with the absence of a sequence homolog of CBP in yeast.

Discussion

The structure of the human ERα LBD in complex with peptides derived from GRIP1 NR box 2 motif shows that K362, M543 and L544 (the human equivalents of mouse K366, M547 and L548) are involved in contacts with the GRIP1 LXXLL peptides (Shiau et al. 1998). In contrast, Y537 (the human equivalent of mouse Y541) does not appear to be in close contact with the LXXLL core motif sequence. However, sequences flanking the core motifs are known to be important for differential interactions of coactivators with steroid receptors and other NRs (Needham et al. 2000, Coulthard et al. 2003), although such sequences have yet to be observed in LBD/coactivator peptide crystal structures. Thus, it remains to be established how replacement of Y541 with alanine or acidic residues enhances ligand-independent binding of coactivators in mammalian cells. Our results show the Y541A mutation has only a very weak effect on the ligand-independent activity of ERα in yeast (Fig. 1D, Fig. 1E). Nonetheless, we observed a strong ligand-independent interaction of ERα Y541A with the mammalian cofactor SUG1 in yeast cells (Fig.
have established that the sequences containing AD1 and AD2, which function in mammalian cells by recruiting CBP/p300 and CARM1, respectively, are also potent transactivation domains in yeast (Fig. 4A). However further mapping of the AD1 region revealed that the sequence required for activity (926–940; termed Helix A) is coincident with, but not identical to, the CBP recruitment domain (926–970; Helix A and Helix B) (Fig. 4B). Interestingly, a study of the sequences required for the function of the N-terminal transactivation domain (AF1) of ERα also concluded that the surfaces required for AF-1 activity are not identical in yeast and mammalian cells (Metzger et al. 1995).

The structure of the CBP/ACTR interface has recently been reported and shows that the AD1 and SID domains each contain three α-helices (Demarest et al. 2002). The structure confirms that Helix A makes intimate hydrophobic contacts with leucine residues within the CBP SID domain. The potent transcriptional activity of Helix A in yeast suggests that it mediates strong interactions with yeast nuclear proteins. Thus, short amphipathic α-helices, such as those in the AD1 and SID domains, and LXXLL motifs appear to be functionally conserved protein–protein interaction modules in eukaryotic nuclear proteins. It will be of interest to identify the yeast proteins that bind Helix A.

Our results revealed that a mutant SRC1e lacking residues 901–950, which deletes most of the AD1 sequence including Helix A, retained approximately 60% of the coactivator activity compared to wild type SRC1e (Fig. 3C). This is in contrast to our results in mammalian cells, in which a ΔAD1 mutation abrogated the ability of SRC1 proteins to enhance transactivation by ERα (Sheppard et al. 2001), and suggests that the Helix A is not the major determinant required for SRC1 function in yeast. Thus, other SRC1 sequences (including amino acids 780–900) appear to be involved in contacting yeast proteins to enhance transcription of the reporter gene. The increased reporter activation achieved using SRC1 mutants deleting the C-terminus up to amino acid 980 may indicate a repressive action of the C-terminus of SRC1. A similar mechanism has been invoked to explain the differential potency of SRC1a and SRC1e isoforms in mammalian cells (Kalkhoven et al. 1998).

Although CBP is required for the transcriptional activity of ERα in mammalian cells and in vitro we have shown that direct interaction of full-length CBP with steroid and retinoid receptors is relatively weak, as the three LXXLL motifs present in CBP (LXM 1–3) have low affinity for steroid and retinoid receptors (Sheppard et al. 2001, Heery et al. 2001). Consistent with this, we report here that we did not detect any enhancement of ERα activity due to co-expression with CBP in yeast. The CBP/p300 associated factor P/CAF, which is related to the yeast GCN5 histone acetyltransferase, has been reported to interact weakly with the DBD of steroid and retinoid receptors in GST pulldown experiments, but without a requirement for ligand (Blanco et al. 1998). However, P/CAF did not enhance the activity of ERα or PR AF2 in our yeast reporter assays. Previous studies have shown that P/CAF and CBP sequences can enhance transcription of reporter genes when tethered to promoters in yeast, suggesting they are at least partly functional in this organism. Therefore the failure of P/CAF and CBP to enhance steroid receptor activity in our experiments may indicate a lack of interaction with the NRs, or a requirement for additional factors not present in yeast.

Studies in mammalian cells have shown that p160 proteins such as SRC1 are essential for maximal activity of ERα both in vivo and in vitro (Sheppard et al. 2001, Li et al. 2000, Kim et al. 2001). The ability of SRC1 proteins to stimulate steroid receptor activity in yeast (Fig. 2B & Fig. 2C) is intriguing, given the absence of close homologs of CBP/p300 in this organism. Our results
We have shown previously that a truncated SRC1 protein (626–970) which contains only CBP-binding and NR-binding domains enhances the activity of ERα to the same level as full length SRC1 proteins in reporter assays in transiently transfected COS-1 cells (Sheppard et al. 2001). The SRC1 626–970 protein is also a potent activator of ERα in yeast, giving a 2-fold higher reporter activity than wild type SRC1 (Fig. 3B). The dispensability of bHLH-PAS and AD2 domains for SRC1 function in these assays could have several explanations: (1) it may indicate differential requirement for cofactors on different promoters; (2) additional cofactors are recruited via alternative interactions e.g. via CBP/p300; (3) cofactor requirements are not identical for natural genes and plasmidic reporter genes, due to differences in chromatin status or (4) these domains are required for interactions with other transcription factors such as Stats, MEF2C, etc. Additionally, the relative expression levels of activators and cofactors may influence transcriptional efficacy.

In conclusion, this study and others (Metzger et al. 1995) indicate that ectopically expressed mammalian transcription factors may utilise distinct molecular mechanisms to engage the transcriptional machinery in yeast and mammalian cells. Further investigation will be required to determine the extent of the differences between these experimental models.

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