The EMBO Journal Vol.18 No.8 pp.2229–2240, 1999

Role of the essential yeast protein PSU1 in transcriptional enhancement by the ligand-dependent activation function AF-2 of nuclear receptors

Claudine Gaudon, Pierre Chambon and Regine Losson[1]

Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/ULP, College de France, BP 163, 67404 Illkirch Cedex, France

[1]Corresponding author

Nuclear receptors (NRs) can function as ligand-inducible transregulators in both mammalian and yeast cells, indicating that important features of transcriptional control have been conserved throughout evolution. We report here the isolation and characterization of an essential yeast protein of unknown function, PSU1, which exhibits properties expected for a co-activator/mediator of the ligand-dependent activation function AF-2 present in the ligand-binding domain (LBD, region E) of NRs. PSU1 interacts in a ligand-dependent manner with the LBD of several NRs, including retinoic acid (RARα), retinoid X (RXRα), thyroid hormone (TRα), vitamin D3 (VDR) and oestrogen (ERα) receptors. Importantly, both in yeast and *in vitro*, these interactions require the integrity of the AF-2 activating domain. When tethered to a heterologous DNA-binding domain, PSU1 can activate transcription on its own. By using yeast reporter cells that express PSU1 conditionally, we show that PSU1 is required for transactivation by the AF-2 of ERα. Taken together these data suggest that in yeast, PSU1 is involved in ligand-dependent transactivation by NRs. Sequence analysis revealed that in addition to a highly conserved motif found in a family of MutT-related proteins, PSU1 contains several α-helical leucine-rich motifs sharing the consensus sequence LLxΦL (x, any amino acid; Φ, hydrophobic amino acid) in regions that elicit either transactivation or NR-binding activity.
Keywords: co-activator/ERα/LxxLL motif/MutT domain/RARα

Introduction

Nuclear receptors (NRs) represent a large family of ligand-inducible transcriptional regulators that control complex developmental and homeostatic events in vertebrates by binding as homodimers or heterodimers to cognate DNA response elements present in target genes. NRs display a modular structure, with five to six distinct regions (denoted A–F; Figure 1A). The N-terminal A/B region contains an autonomous activation function (AF-1), the highly conserved region C encompasses the DNA-binding domain (DBD) and the C-terminal E region contains the ligand-binding domain (LBD), a dimerization surface and a ligand-dependent transcriptional activation function, AF-2 (for reviews see Gronemeyer, 1991; Mangelsdorf *et al*., 1995; Chambon, 1996). The core of the AF-2 activation domain (AF-2 AD) has been characterized in the C-terminal part of the E region and corresponds to the conserved amphipatic α-helix H12 of the LBD. Upon binding to agonistic ligand, this helix is thought to fold back over the LBD, thus contributing to the interaction surface(s) for transcriptional intermediary factors (TIFs, also denoted co-activators or mediators; Beato and Sanchez-Pacheco, 1996; Chambon, 1996; Horwitz *et al*., 1996; Glass *et al*., 1997; Moras and Gronemeyer, 1998; Torchia *et al*., 1998). Although the mechanisms of action for most of these co-activators are unknown, the recent finding that several possess histone acetyltransferase activities suggests that chromatin remodelling by histone acetylation is a critical, albeit apparently insufficient (Wong *et al*., 1997), step in NR-mediated gene activation (see Torchia *et al*., 1998 and references therein).

Although the yeast *Saccharomyces cerevisiae* does not possess endogenous NRs, it has been shown that a number of NRs, including the oestrogen receptor (ERα), glucocorticoid receptor (GR), retinoic acid (RA) receptors (RARs and RXRs), thyroid hormone receptor (TR) and vitamin D3 receptor (VDR) can function as ligand-dependent transactivators in yeast (Metzger *et al*., 1988; Schena and Yamamoto, 1988; McDonnell *et al*., 1989; Hall *et al*., 1993; Heery *et al*., 1993). As in vertebrates (Gronemeyer, 1991; Chambon, 1996 and references therein), the AF-1 and AF-2 of ERα activate transcription independently and synergistically in yeast (Metzger *et al*., 1988, 1992, 1995; White *et al*., 1988; Pierrat *et al*., 1992). These AFs function in a promoter-context-dependent manner in both animal and yeast cells (Tora *et al*., 1989; Berry *et al*., 1990; Metzger *et al*., 1992, 1995). Thus, the transactivation properties of NRs in yeast are in many respects similar to those in mammalian cells, suggesting that some important features of the mechanism(s) by which these AFs stimulate transcription have been conserved during evolution.

The characterization of yeast factors that mediate transactivation by NRs might therefore be helpful to identify functionally related factors in vertebrates. Supporting this view, the yeast SWI/SNF complex, which is involved in chromatin remodelling (reviewed in Burns and Peterson, 1997), has been reported to interact both functionally and physically with the GR (Yoshinaga *et al*., 1992), and subsequently to be conserved in mammalian cells (Wang *et al*., 1996 and references therein). Two human homologues of the SWI2/SNF2 subunit have been characterized: hSNF2α or hbrm and hSNF2β or BRG1 (Muchardt and Yaniv, 1993; Chiba *et al*., 1994). Both can interact in yeast with the LBD/AF-2 of ERα in the presence of oestrogen, but not anti-oestrogens (Ichinose *et al*., 1997). They can also cooperate with ERα, RARα and GR in transcriptional activation (Muchardt and Yaniv, 1993;

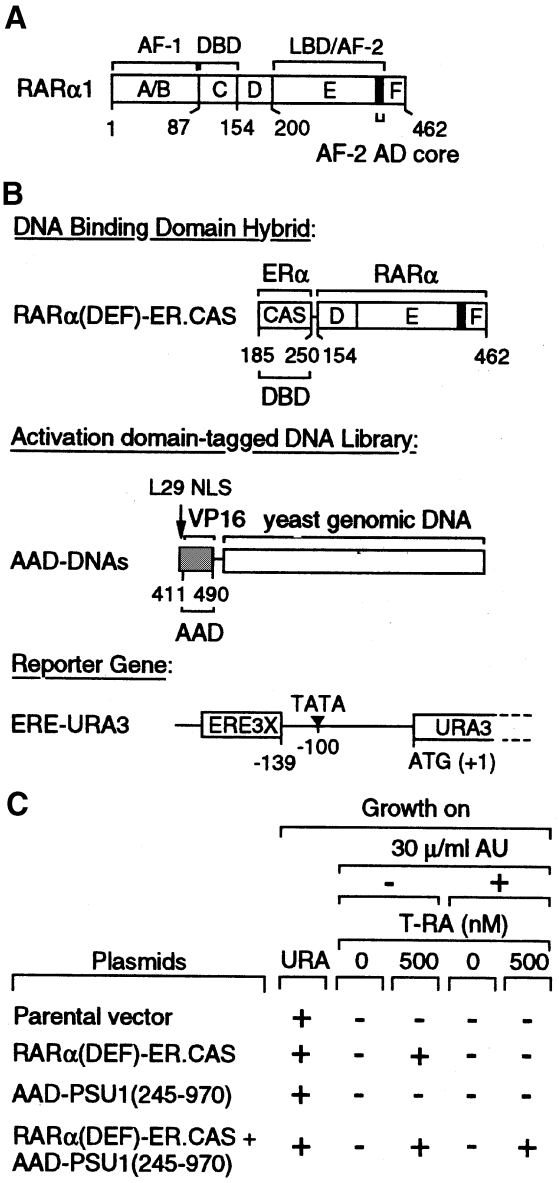


Fig. 1. A two-hybrid screen for yeast proteins that interact with RARα identified PSU1. (**A**) Schematic representation of RARα. Indicated are the various regions of the receptor (denoted A–F) that are differentially conserved among members of the NR family. The transactivation domains (AF-1 and AF-2), DBD and LBD are indicated. The filled bar represents the core motif of the AF-2 activating domain. Numbers refer to amino acid positions. (**B**) A schematic representation of the chimeric receptor RARα(DEF)–ER.CAS used as a bait in the yeast two-hybrid screen is shown. Below, the VP16 acidic activation domain (AAD)-tagged *S.cerevisiae* genomic DNA library is represented; the AAD tag also includes codons specifying the nuclear localization signal (NLS) of the yeast ribosomal protein L29. Transcription of the integrated *URA3*-based reporter gene shown further below is regulated by three oestrogen response elements (ERE3X) in the yeast reporter strain PL3. (**C**) Expression of AAD–PSU1(245–970) DNA complements the growth defect of PL3 cells expressing RARα(DEF)-ER.CAS in the presence of 500 nM T-RA and 30 μg/ml azauracil (AU). Plasmids expressing the indicated fusion proteins were introduced into the yeast reporter strain PL3. Transformants were grown on medium containing uracil (URA), and spot tested on URA-negative medium ± 500 nM T-RA and ± 30 μg/ml 6-azauracil (AU) as indicated. Plates were incubated at 30°C for 3 days. '+', wild-type growth; '–', no growth.

Chiba *et al*., 1994), indicating that, like their yeast counterpart, the mammalian SWI/SNF complexes may participate in the NR signalling pathway. The yeast ADA/SAGA histone acetyltransferase complexes (Grant *et al*., 1997, 1998) represent additional examples of co-activators that have been reported to be important for the AF-2 activity of ERα and RXRα in yeast (vom Baur *et al*., 1998). Interestingly, two mammalian complexes have recently been isolated that are structural and functional homologues of the yeast ADA/SAGA complexes: the human P/CAF and GCN5 complexes (Ogryzko *et al*., 1998). Both possess histone acetyltransferase activity (Martinez *et al*., 1998; Ogryzko *et al*., 1998) and share a subunit, ADA2, that can enhance transactivation by the GR when overexpressed in transfected cells (Henriksson *et al*., 1997), suggesting that, like the yeast ADA/SAGA complex, the human P/CAF and/or GCN5 complex is involved in NR-dependent gene activation.

In the present study, we report the cloning of a yeast MutT domain-containing protein, which fulfils the criteria anticipated for a co-activator/mediator of the ligand-dependent activation function AF-2 of NRs: it interacts, both *in vivo* and *in vitro*, with the LBDs of several NRs in an agonist- and AF-2-integrity-dependent manner, harbours an autonomous activation function and is crucial for the AF-2 activity of ERα in yeast. This protein is identical to PSU1 which was originally identified in a yeast screen for suppressors of a respiratory deficient pet mutant and shown to be essential for cell viability (A.A.Tzagoloff, unpublished data; DDBJ/EMBL/GenBank accession number L43065).

Results

Isolation of yeast PSU1 as a RARα-interacting protein

The yeast two-hybrid system (Fields and Sternglanz, 1994) was used to identify yeast proteins that interact with the LBD/AF-2-containing region DEF of RARα in the presence of all-*trans* retinoic acid (T-RA). A chimeric receptor consisting of the DBD of ERα (ER.CAS, ERα aa 185–250) fused to the LBD of RARα [RARα(DEF)-ER.CAS, Figure 1B; Heery *et al*., 1993], was expressed in the PL3 yeast strain, which contains a URA3 reporter gene under the control of three oestrogen response elements (ERE-URA3) (Figure 1B; Pierrat *et al*., 1992). Activation of this reporter in the presence of T-RA was sufficient to allow yeast growth on medium lacking uracil but not in the presence of 6-azauracil (AU), an inhibitor of the URA3 gene product (Figure 1C). The strain was then transformed with a library of yeast genomic DNA fragments, fused to the acidic activation domain (AAD) of the VP16 protein (Figure 1B; vom Baur *et al*., 1998). Approximately 2×10^5 yeast transformants containing both plasmids were recovered, and plated at a multiplicity of 10 onto Ura$^-$ plates containing 500 nM T-RA and 30 μg/ml AU. Twenty-eight AU-resistant yeast clones were isolated; seven were classified as positive when retested in another version of the two-hybrid system using a LexA–RARα(DEF) fusion protein (or an unfused LexA as a control), together with a reporter gene driven by a GAL1 promoter containing LexA-binding sites (Vojtek *et al*., 1993). Among these clones, two isolates of a single

'fused' DNA [designated AAD–PSU1(245–970), see below] were identified (Figure 1C) and further characterized.

DNA sequence analysis revealed an open reading frame (ORF) of 726 amino acids fused inframe with the VP16 AAD. Upon searching the DDBJ/EMBL/GenBank, this ORF was found to be identical to the C-terminal residues 245–970 of PSU1, an essential protein of unknown function that was originally identified as a suppressor protein of the respiratory deficiency of a yeast pet mutant (A.A.Tzagoloff, unpublished data; DDBJ/EMBL/GenBank accession number L43065; Figure 2A). A second database search with the entire PSU1 amino acid sequence revealed the presence of a MutT domain between residues 129 and 158 (Figure 2A and C). This ~30 amino acid conserved sequence has been found in a number of prokaryotic, viral and eukaryotic enzymes that hydrolyse derivatives of nucleoside diphosphates (Koonin, 1993; Bessman *et al*., 1996; Figure 2C; also see Discussion). Downstream of the MutT domain, PSU1 contains other noteworthy features (Figure 2A and B), including: (i) a cluster of glutamine (Q) residues (11 Q over 32 residues) from position 294 to 326; (ii) a proline (P)-rich region (24% in 104 residues) from position 327 to 430; (iii) a central region with a high content of serine (S) residues (17% over 421 amino acids) from position 433 to 853; and (iv) a C-terminal asparagine (N)-rich region (22% in 105 residues) from position 857 to 961, all of which are common features of transcriptional regulators (Tjian and Maniatis, 1994). Ten copies of a short α-helical leucine-rich motif (thereafter referred to as HLM) have also been identified in PSU1 (Figure 2D). The motif is repeated three times between residues 270 and 500 and seven times within the last 240 amino acids of PSU1 (Figure 2A and B). The major features of this repeated motif, from which the consensus sequence Sx(E,D)LLxΦL(K,R,H) can be derived (x being any residue and Φ being a hydrophobic residue), include three invariant leucine residues flanked by acidic and basic residues (Figure 2D). Note that a putative bipartite nuclear localization signal is also present between residues 450 and 465 of PSU1 (Figure 2A).

Ligand-dependent interactions between PSU1 and the LBD of several members of the nuclear receptor superfamily in yeast and in vitro

Full-length PSU1 was fused to the ERα DBD (residues 176–282) and the resulting hybrid protein (DBD–PSU1; Figure 3A) was assayed for interaction with full-length RARα fused to the VP16 AAD (AAD–RARα1; Figure 3A) in the yeast PL3 strain in the presence or absence of T-RA. Activation of the URA3 reporter gene was determined by measuring the specific activity of the URA3 gene product, orotidine 5′-monophosphate decarboxylase (OMPdecase; Figure 3A). When expressed alone or in the presence of AAD, DBD–PSU1 activated the URA3 reporter 5-fold above the level of unfused DBD (Figure 3A; see below), indicating that PSU1 can activate transcription on its own when tethered to DNA. In the presence of AAD–RARα1, a 2- to 3-fold constitutive activation and a further 6-fold stimulation upon addition of T-RA was observed (Figure 3A), showing that PSU1 and RARα functionally interact with each other in a ligand-enhanced manner in

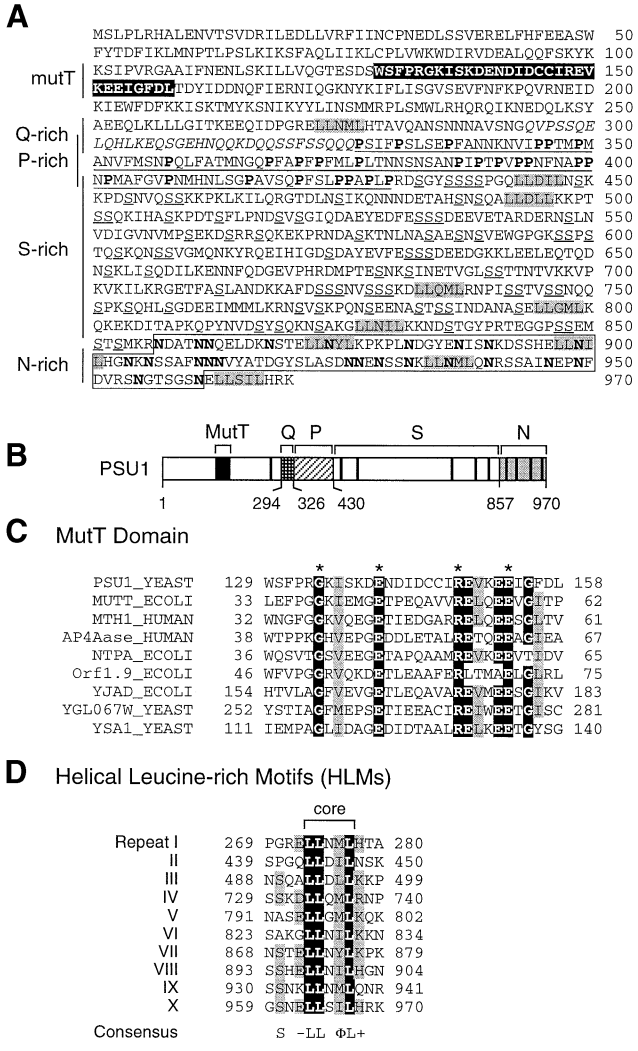


Fig. 2. PSU1 is a MutT domain-containing protein. (**A**) The amino acid sequence and structural features of PSU1 are shown. Note an amino acid change at position 425 (P instead of L) compared with the DDBJ/EMBL/GenBank sequence (accession number L43065). The MutT domain is indicated by a black box. Italicized sequences denote the cluster of glutamine residues. Underlined sequences indicate the proline-rich region. The abundant serine residues in the central region of the protein are underlined. The asparagine-rich region is boxed. Leucine-rich repeats are shaded. (**B**) Schematic representation of PSU1. Indicated are the MutT domain, the regions rich in particular amino acids (see text) and the leucine-rich motifs (vertical bars). (**C**) Alignment of the PSU1 MutT domain with eight other MutT sequences. The sequences were aligned using both the program of CLUSTALW and manual adjustment. The numbers refer to amino acid positions in the corresponding proteins. Stars indicate invariant residues. Identical amino acid residues in >75% of the proteins are shown in white-on-black. Amino acid residues conserved in >75% of the proteins are shaded. DDBJ/EMBL/GenBank accession Nos: *Escherichia coli* MutT (8-oxo-dGTPase; P08337), human MTH1 (8-oxo-dGTPase; P36339), human AP4Aase (diadenosine tetraphosphatase; P50583), *E.coli* NTPA (dATP pyrophosphorylase; P24236), *E.coli* Orf1.9 (GDP-mannose mannosyl hydrolase; L11721), *E.coli* YJAD (NADH pyrophosphatase; P32664), *S.cerevisiae* YGL067W (hypothetical 43.5 kDs protein; P53164), *S.cerevisiae* YSA1 or YBR0907 or YBR111C (hypothetical 26.1 kDd protein; Q01976). (**D**) Alignment of the helical leucine-rich motifs (HLMs) present in PSU1. Invariant amino acids are shown in white-on-black. Residues conserved in >50% of the repeats are shaded. The consensus represents the most prevalent amino acid residue at each position when >50% of the positions are occupied by a single amino acid or a single class of residue: –, negatively charged (E, D); Φ, hydrophobic (L, I, V, M, F, W, Y); +, positively charged (K, R, H).

yeast cells. This interaction was specific to the LBD/AF-2 of RARα, as no increase in the reporter gene activity was detected with AAD hybrid proteins containing either the N-terminal AB region of RARα, which harbours the transcriptional activation function AF-1 [AAD–RARα(1–87); Figure 3A], or the CD region [AAD–RARα(80–211); Figure 3A], whereas co-expression of DBD–PSU1 with AAD–RARα(DEF) resulted in a ~6-fold T-RA-dependent increase in OMPdecase activity [AAD–RARα(154–462); Figure 3A].

To investigate whether PSU1 could interact with the LBD/AF-2s of other NRs, AAD fusion proteins containing the DE/F region of TRα, VDR, RXRα or ERα were co-expressed in the PL3 yeast with either DBD or DBD–PSU1. In combination with DBD–PSU1 (but not DBD; data not shown), each of the VP16 AAD fusion proteins stimulated expression of the URA3 reporter in the presence of the cognate ligand (Figure 3B), indicating that PSU1 can also interact with the LBD/AF-2s of TRα, VDR, RXRα and ERα in a ligand-dependent manner. Interestingly, synthetic retinoids that are less efficient than T-RA at stimulating the AF-2 activity of RARα in yeast (BMS453 and BMS411; M.Gehin, V.Vivat, J-M.Wurtz, R.Losson, P.Chambon, D.Moras and H.Gronmeyer, submitted) were also less active than T-RA in inducing interaction between RARα and PSU1 (Figure 3C). Similarly, the anti-oestrogen hydroxytamoxifen (OHT; Figure 3D), which does not induce the AF-2 activity of ERα in animal and yeast cells (Berry *et al*., 1990; Metzger *et al*., 1992), failed to induce an interaction between the DEF region of ERα and PSU1 (Figure 3D). Thus, specific agonist-induced conformational changes in the LBD structure are required for both activation by the AF-2 and interaction with PSU1.

Binding assays between PSU1 and various NR LBDs were also performed *in vitro*. Purified *E.coli*-expressed glutathione *S*-transferase (GST)-receptor LBD fusion proteins were attached to glutathione–Sepharose beads and subsequently mixed with *in vitro* synthesized ^{35}S-labelled PSU1 in the presence or absence of ligand. The matrix-associated PSU1 protein was eluted and analysed using SDS–PAGE and autoradiography. In agreement with the yeast two-hybrid data, a ligand-dependent interaction was observed between PSU1 and the LBDs of RARα, TRα, VDR, RXRα and ERα (Figure 3E).

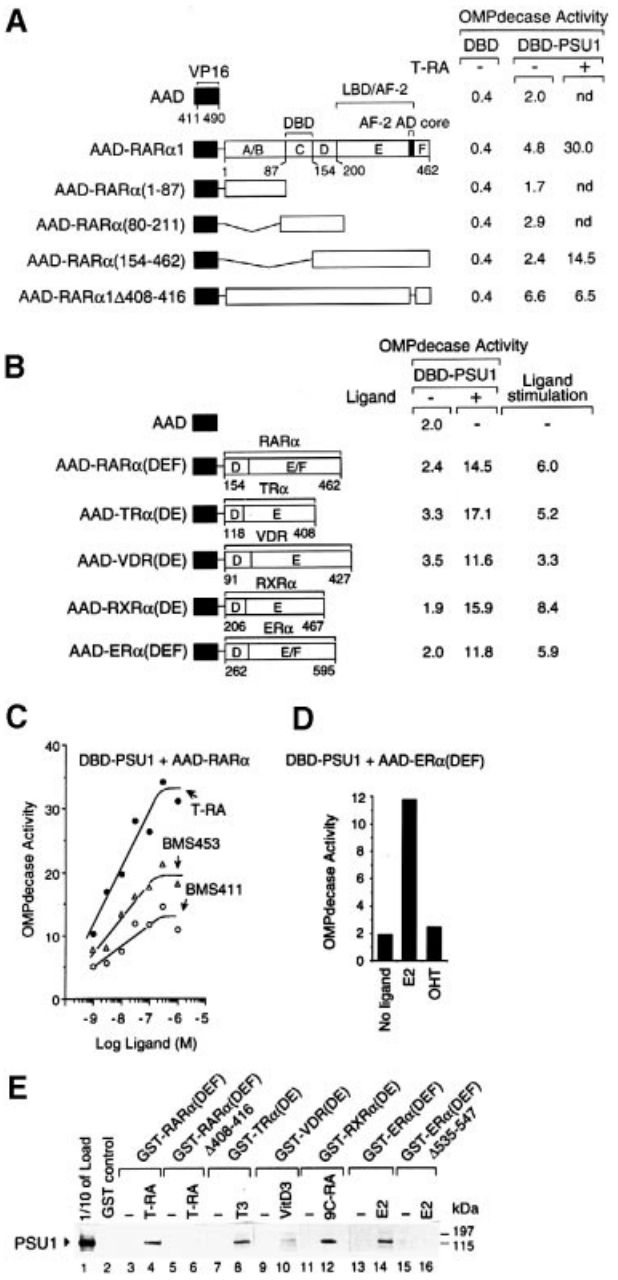


Fig. 3. Ligand-dependent interaction between PSU1 and various nuclear receptors. (**A**) The LBD/AF-2 of RARα is sufficient for mediating a ligand-dependent interaction with PSU1 in yeast. Schematic representations of the AAD–RARα fusions are displayed on the left. These chimera were expressed in the yeast reporter strain PL3 together with either unfused DBD or DBD–PSU1. Transformants were grown in liquid medium containing uracil in the presence (+) or absence (–) of 500 nM T-RA. Extracts were prepared and assayed for OMPdecase activity, which is expressed in nmol substrate/min/mg protein. The values (± 20%) are the average of at least three independent transformants. Expression of the fusion proteins was confirmed by Western blotting (data not shown). (**B**) PSU1 interacts with the LBD/AF-2 of TRα, VDR, RXRα and ERα in a ligand-dependent manner in yeast. The indicated AAD fusions were assayed for interaction with DBD–PSU1 in the yeast strain PL3 grown in the presence (+) or absence (–) of the cognate ligand (500 nM T-RA for RARα, 5 μM T3 for TRα, 5 μM vitamin D3 for VDR, 500 nM 9C-RA for RXRα, 500 nM E_2 for ERα). OMPdecase activities are expressed as in (A). (**C**) RARα-specific antagonists are less efficient than T-RA in inducing interaction between PSU1 and RARα. DBD–PSU1 was co-expressed with AAD–RARα in the yeast strain PL3. Transformants were grown in the absence or presence of T-RA (●), BMS411 (○) or BMS453 (△) at the indicated concentrations and treated as in (A). (**D**) No interaction is seen between PSU1 and ERα in the presence of the AF-2 antagonist OHT. DBD–PSU1 was co-expressed with AAD–ERα(DEF) in the presence or absence of 500 nM E2 or 10 μM OHT. OMPdecase activities are expressed as in (A). (**E**) PSU1 binds *in vitro* to the LBDs of RARα, TRα, VDR, RXRα and ERα in a ligand-and AF-2-integrity-dependent manner. *In vitro* ^{35}S-labelled PSU1 was incubated in a batch assay with 'control' GST (lane 2) or GST fusions containing the indicated LBDs (lanes 3–16) bound to glutathione–Sepharose beads in the presence (+) or absence (–) of the cognate ligand (1 μM T-RA for RARα, 5 μM T3 for TRα, 5 μM vitamin D3 for VDR, 1 μM 9C-RA for RXRα, 1 μM E_2 for ERα). After extensive washing, the bound PSU1 protein was resolved on SDS–PAGE and visualized by autoradiography. Lane 1 represents 1/10 the amount of input labelled PSU1, the position of which is indicated by an arrow. Numbers on the right indicate the positions of the molecular mass markers in kDa.

Differential requirements of the conserved residues of the AF-2 AD core motif of RARα and ERα for interaction with PSU1

The ligand dependency of the interactions of PSU1 with the NR LBDs suggests that a ligand-induced conformational change is required to generate the receptor–PSU1 interaction surface. As the same ligand-induced receptor transconformation is apparently also required for the AF-2 activity of RARα and ERα (see above), several receptor mutants carrying mutations in the core motif of the AF-2 activation domain (AF-2 AD core/helix 12 of the LBD) were tested for their ability to interact with PSU1 in the presence or absence of ligand. In contrast to wild-type RARα, a mutant bearing an internal deletion of the AF-2 AD core motif, which still binds T-RA (Durand *et al*., 1994; Tate *et al*., 1996), showed no ligand-dependent interaction, whereas some (~3-fold) constitutive interaction remained (compare AAD–RARα with AAD–RARαΔ408–416; Figure 3A). Similarly, addition of T-RA did not enhance the direct binding of PSU1 to GST-RARα(DEF)Δ408–416 *in vitro* (Figure 3E, lanes 3–6), indicating that the core of the AF-2 AD of RARα is essential for ligand-induced, but not ligand-independent, interaction with PSU1. When compared with the wild-type RARα(DEF), the point mutants RARα(DEF)L409A/I410A, RARα(DEF)M413A/L414A and RARα(DEF)-E412Q/E415Q, in which the conserved hydrophobic and acidic residues of the AF-2 AD core were replaced with Ala and Gln residues, respectively, were also impaired in their ability to interact with PSU1 in the presence of T-RA in yeast (Figure 4A). Note, however, that the interaction was reduced only 2- to 3-fold by these AF-2 AD core mutations (Figure 4A), whereas equivalent mutations in the hydrophobic residues of the ERα AF-2 AD core (L539A and M543A/L544A) generated receptors which, similar to the mutant lacking the AF-2 AD core (Δ535–547), completely lost their ability to interact with PSU1 in the presence of oestradiol in yeast (Figure 4B), as well as *in vitro* [see GST-ERα(DEF) and GST-ERα(DEF)Δ535–547; Figure 3E]. Thus, even if RARα and ERα both require an intact AF-2 AD core for ligand-dependent interaction with PSU1, there are some differences in the contributions of the conserved residues of their AF-2 AD core to PSU1 binding.

PSU1 contains two transcriptional activation domains and two nuclear receptor interaction domains

Because the PSU1 protein can activate transcription on its own when fused to an heterologous DNA-binding domain (Figure 3A), it should contain at least one sequence that functions as an autonomous AD. Various deletion mutants of PSU1 were fused to the ERα DBD and assayed for transactivation in the yeast reporter strain PL3 (Figure 5A and C). Full-length PSU1 enhanced OMPdecase activity 7-fold in this assay, whereas amino acids 243–326 of PSU1 activated the reporter gene ~25-fold above the level of unfused DBD, and the C-terminal 165 amino acids of PSU1 stimulated ~13-fold (Figure 5A). In contrast, no increase in reporter gene activity was observed with DBD fusions bearing the N-terminal PSU1 residues 1–242 or portions of PSU1 from position 327 to 707 (Figure 5A), whereas a relatively weak (~3-fold) stimulation was detected with residues 708–805 (Figure 5A). Thus, it appears that PSU1 contains two potent autonomous ADs, termed AD1 (residues 243–326) and AD2b (residues 806–970), in addition to a less potent AD which immediately precedes AD2b (designated AD2a; residues 708–805; Figure 5C). As AD2a and AD2b are contiguous domains, they may represent subdomains of a larger activation domain (AD2) extending from position 708 to the end of the PSU1 protein (Figure 5C).

To map the nuclear receptor interaction domain(s) (NID) in PSU1, the various DBD–PSU1 fusion proteins listed in Figure 5A were assayed for interaction with the LBD of RARα and ERα in the yeast reporter strain PL3. Residues 436–502 interacted with both RARα and ERα in a ligand-dependent manner (Figure 5B), indicating that this region of PSU1 contains a NID, termed NID1 (Figure 5C). Interestingly, residues 708–805 and 806–970, which include the activation domains AD2a and AD2b, respectively, also exhibited some ligand-dependent interaction with both RARα and ERα (Figure 5B), but the interaction potential of the AD2a region was weak relative to that of the AD2b-containing region (Figure 5B). No additional NID could be detected within the other portions of PSU1 (Figure 5B). Thus, in addition to a N-terminal NID1 domain, PSU1 appears to contain two other C-terminal NIDs, hereafter designated NID2a and NID2b, which apparently overlap the AD2a and AD2b activation domains (Figure 5C).

We have noted above that PSU1 contains several highly conserved α-helical leucine-rich motifs (HLMs), that share

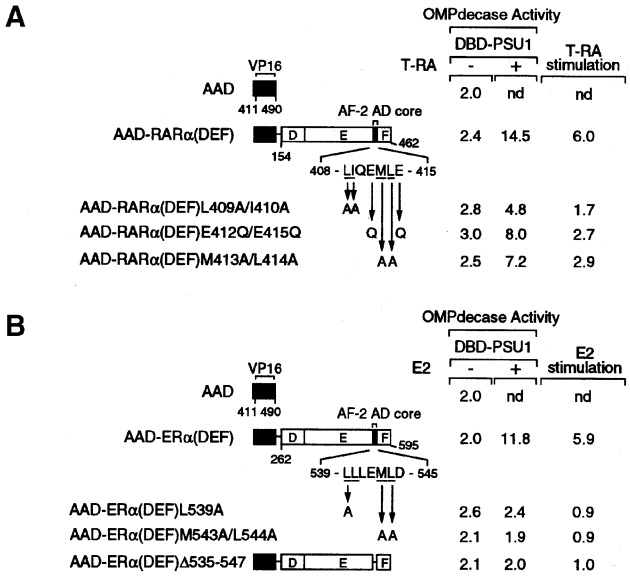
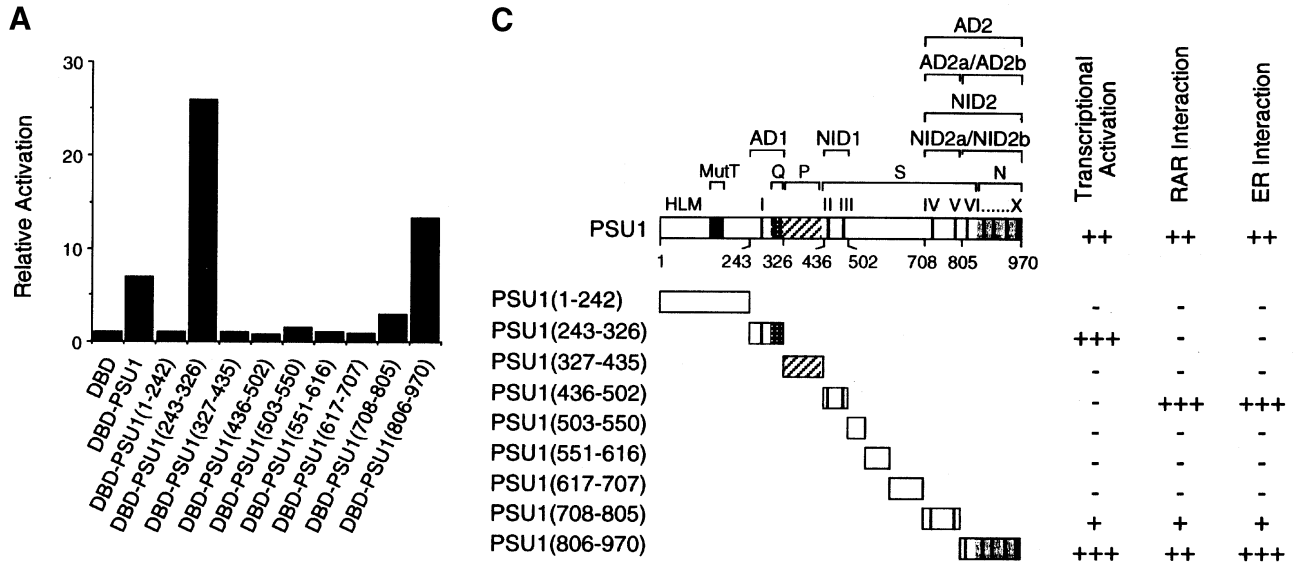


Fig. 4. The integrity of the AF-2 AD core motif is important for the ligand-dependent interaction of the RARα and ERα LBDs with PSU1. (**A**) Point mutations in the AF-2 AD core of RARα impair interaction with PSU1. The indicated mutants of RARα fused to the VP16 AAD were assayed for interaction with DBD–PSU1 in the yeast reporter strain PL3 grown in the presence or absence of 500 nM T-RA. OMPdecase activities are expressed in nmol substrate/min/mg protein. (**B**) Deletion or point mutations in the AF-2 AD core of ERα abolish interaction with PSU1. The indicated mutants of ERα fused to the VP16 AAD were assayed for interaction with DBD–PSU1 in the yeast reporter strain PL3 grown in the presence or absence of 500 nM E2. OMPdecase activity is expressed as in (A). In (A) and (B), expression of the AAD fusion proteins was confirmed by Western blotting using the antibody 2GV4 against VP16 (data not shown).



B

Ligand	OMPdecase Activity AAD -	OMPdecase Activity AAD-RARα(DEF) -	OMPdecase Activity AAD-RARα(DEF) +	T-RA stimulation	OMPdecase Activity AAD-ERα(DEF) -	OMPdecase Activity AAD-ERα(DEF) +	E2 stimulation
DBD	0.4	0.4	0.4	1.0	0.4	0.4	1.0
DBD-PSU1	2.0	2.4	14.5	6.0	2.0	11.8	5.9
DBD-PSU1(1-242)	0.1	0.1	0.1	1.0	0.1	0.1	1.0
DBD-PSU1(243-326)	17.8	19.8	18.0	0.9	17.5	15.6	0.9
DBD-PSU1(327-435)	0.5	0.5	0.5	1.0	0.5	0.6	1.2
DBD-PSU1(436-502)	0.3	0.5	12.7	25.4	0.4	22.9	57.2
DBD-PSU1(503-550)	0.7	0.9	1.3	1.4	0.8	1.0	1.2
DBD-PSU1(551-616)	0.3	0.3	0.3	1.0	0.3	0.4	1.3
DBD-PSU1(617-707)	0.5	0.4	0.4	1.0	0.5	0.5	1.0
DBD-PSU1(708-805)	1.3	1.3	3.4	2.6	1.3	2.7	2.1
DBD-PSU1(806-970)	6.6	7.0	37.8	5.4	6.7	133.7	19.9
DBD-PSU1L495A/L496A	1.3	1.5	13.4	8.9	1.6	13.5	8.4
DBD-PSU1(1-707)	0.4	0.4	6.6	16.5	0.9	8.2	9.2
DBD-PSU1(1-707)L495A/L496A	0.3	0.6	0.7	1.1	0.7	0.4	0.6

D

Ligand	OMPdecase Activity AAD -	OMPdecase Activity AAD-RARα(DEF) -	OMPdecase Activity AAD-RARα(DEF) +	T-RA stimulation	OMPdecase Activity AAD-ERα(DEF) -	OMPdecase Activity AAD-ERα(DEF) +	E2 stimulation
DBD	0.4	0.4	0.4	1.0	0.4	0.4	1.0
DBD-PSU1(269-281)/HLM I	0.9	0.8	1.2	1.5	0.6	0.7	1.2
DBD-PSU1(440-451)/HLM II	0.6	1.5	1.2	0.8	0.6	0.9	1.5
DBD-PSU1(489-499)/HLM III	0.9	1.0	11.9	11.9	1.0	18.8	18.8
DBD-PSU1(729-740)/HLM IV	2.2	1.3	1.9	1.5	1.3	2.1	1.6
DBD-PSU1(789-802)/HLM V	0.8	0.9	0.8	0.9	0.5	2.1	4.2
DBD-PSU1(820-838)/HLM VI	0.4	0.4	0.5	1.2	0.6	1.1	1.8
DBD-PSU1(868-879)/HLM VII	0.7	1.0	1.6	1.6	1.3	1.7	1.3
DBD-PSU1(889-907)/HLM VIII	0.2	0.2	0.3	1.5	0.2	0.2	1.0
DBD-PSU1(930-941)/HLM IX	0.8	0.8	2.1	2.6	0.9	25.6	28.4
DBD-PSU1(959-970)/HLM X	0.8	0.7	0.7	1.0	0.8	1.4	1.7
DBD-PSU1(489-499)L495A/L496A	0.5	0.5	0.6	1.2	0.5	0.7	1.4

Fig. 5. PSU1 contains several transcriptional ADs and NIDs. (**A**) Mapping of the ADs of PSU1. Various regions of PSU1 were fused to the ERα DBD and assayed for transactivation in the yeast reporter strain PL3. Transcription of the reporter gene was determined by measuring OMPdecase activity and is represented relative to the activity displayed by unfused DBD. The mean of at least three independent experiments are shown. In all cases, expression of the DBD fusions was confirmed by Western blotting using the antibody F3 against the F region of ERα (data not shown). (**B**) Mapping of the NIDs of PSU1. The indicated DBD–PSU1 fusions were assayed for two-hybrid interaction with AAD–RARα(DEF) and AAD–ERα(DEF) in the yeast strain PL3 grown in the presence (+) or absence (–) of the cognate ligand (500 nM T-RA for RARα, 500 nM E2 for ERα). OMPdecase activity is expressed in nmol substrate/min/mg protein. (**C**) PSU1 structural and functional features. The various PSU1 constructs are denoted. Symbols are as in Figure 2B. Constructs that scored positive or negative for transactivation or NR interaction are identified on the right by '+' and '–' signs, respectively. (**D**) The ability of the helical leucine-rich motifs (HLMs) of PSU1 to transactivate and/or to interact with the LBDs of RARα and ERα. DBD–HLM fusions were co-expressed in PL3 with either AAD or AAD fusion receptors, as indicated to the right. Transformants were treated as in (B).

the consensus core sequence LLxΦL (Figure 2A and C). This sequence is present as a single copy in AD1 (repeat I; residues 269–280), is repeated in AD2a/NID2a (repeats IV and V; residues 729–740 and 791–802) and is present five times in AD2b/NID2b (repeats VI to X; Figures 2A and 5B). NID1 also contains two HLMs (repeats II and III; residues 439–450 and 488–499). In contrast, no similar motif was found in PSU1 regions that did not exhibit any activation or receptor interaction activity (Figure 5C), suggesting that HLM sequences may be critical for these functions. To determine whether these HLMs had some activation and/or NR-binding activity on their own, each of them was fused to the ERα DBD and tested for transcriptional activity in yeast (Figure 5D). In the presence of unfused AAD, none of the DBD–HLM fusions significantly increased the reporter gene expression above the level of unfused DBD, except DBD–HLM IV, which activated transcription as efficiently as the AD2a-containing DBD–PSU1(708–805) fusion (Figure 5B and D). Thus, transactivation by AD1 and AD2b must involve sequence features other than HLMs, acting either alone or in combination with these HLMs, whereas AD2a may transactivate through HLM IV. In this respect, it is noteworthy that, in addition to HLMs, AD1 and AD2b contain a preponderance of glutamine and asparagine residues, respectively (Figure 5C).

In the presence of AAD–RARα(DEF) and AAD–ERα(DEF), a ligand-dependent activation was observed with the DBD fusion bearing the HLM III repeat of NID1 (Figure 5D). HLM V of NID2a interacted with the liganded ERα, but not with RARα (Figure 5D). Similarly to NID2b, HLM IX interacted with both receptors in a ligand-dependent manner, but showed a strong preference for ERα over RARα (Figure 5D). The other HLMs of PSU1 (HLM I, HLM II, HLM VI–VIII, HLM X) did not significantly interact with RARα and ERα (Figure 5D). Thus, the NR-binding activities of NID1 and NID2b may be due to HLM III and HLM IX, respectively, whereas HLM V may account for most of the ERα-binding activity of NID2a.

Mutations in the HLM III repeat of NID1 and a C-terminal deletion that deleted both NID2a and NID2b, were used to assess the relative contributions of these NIDs to the NR-binding function of PSU1. Point mutations in the core sequence of HLM III (LLDLL) replacing the leucine residues at position 4 and 5 with alanine residues, yielded a mutated repeat that failed to interact with RARα and ERα [compare DBD–PSU1(789–899)L495A/L496A with DBD–PSU1(789–899); Figure 5D]. However, the same mutations in PSU1 generated a mutant protein PSU1L495A/L496A that interacted with the liganded LBDs of RARα and ERα as efficiently as wild-type PSU1 (Figure 5B), suggesting that the NIDs of PSU1 are functionally redundant. This redundancy was supported by the observation that a C-terminal deletion of NID2a/AD2a and NID2b/AD2b did not reduce the NR-binding activity of PSU1, whereas the transactivation activity was abolished [compare DBD–PSU1 with DBD–PSU1 (1–707); Figure 5B]. In contrast, when the HLM III mutations L495A and L496A were introduced in the context of the truncated mutant PSU1(1–707), binding to RARα and ERα was eliminated completely [compare DBD–PSU1 (1–707) with DBD–PSU1(1–707)L495A/L496A; Figure 5B). Thus, mutation of all NIDs is required in PSU1 to abrogate NR binding.

PSU1 is required for ligand-dependent activation by the AF-2 of ERα in yeast

To further investigate the involvement of PSU1 in NR-mediated transactivation in yeast, we focused our analysis on the AF-2 of ERα, which has been reported to efficiently activate chimeric oestrogen-responsive URA3 promoters in yeast (Metzger *et al*., 1992; Pierrat *et al*., 1992). The transcriptional activity of this AF-2 was tested in the presence of a reduced level of PSU1. To this end, a yeast reporter strain expressing PSU1 under the control of a conditional promoter was generated using the plasmid shuffle method (Materials and methods; Figure 6A). Δpsu1 PL3 cells carrying the centromeric plasmid pRS314-Met/HA-PSU1, in which a hemaglutinin (HA) epitope-tagged PSU1 is expressed under the control of the methionine-repressed Met25 promoter (Kerjan *et al*., 1986; legend to Figure 6A), were transformed with a high-copy-number plasmid YEp90-PGK/ERα(CDEF) (Pierrat *et al*., 1992) expressing, under the control of the PGK promoter, an N-terminally truncated ERα derivative that contains the ligand-dependent transactivation function AF-2 [ERα(CDEF), formerly called HEG19; Berry *et al*., 1990; Figure 6A]. Transformants were grown in the presence or absence of methionine for four generations. Activation of the URA3 reporter by the ERα AF-2 was then determined by measuring OMPdecase activity in cell free extracts from cells exposed to various concentrations of oestradiol (E2). Under these growth conditions, addition of methionine caused an ~2-fold reduction in growth rate (data not shown), but did not change the expression level of the ERα(CDEF) receptor proteins as evaluated by immunodetection (Figure 6B), indicating that depleting PSU1 had no effect on the activity of the PGK promoter used to express the ERα derivative. In contrast, a dramatic (10- to 20-fold) reduction in the ligand-dependent transcriptional activity of ERα(CDEF) was observed in cells grown in the presence of methionine compared with cells

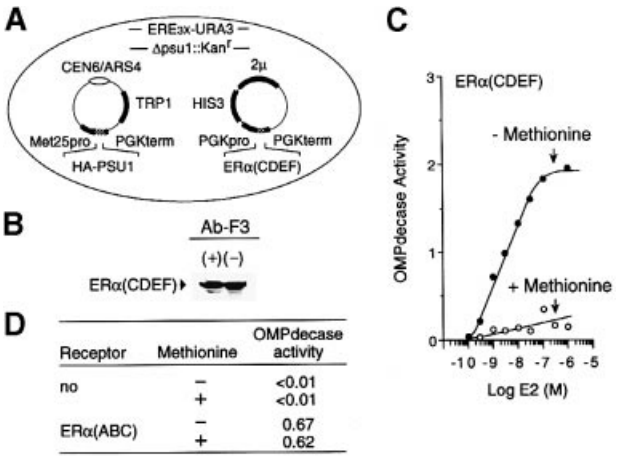


Fig. 6. PSU1 depletion effect on the AF-2 activity of ERα. (**A**) An haploid Δpsu1 PL3 reporter strain that contains the centromeric plasmid PRS314-Met/HA-PSU1 expressing an HA epitope-tagged PSU1 under the control of the methionine-repressed Met25 promoter was transformed with the yeast episomal plasmid YEp90-PGK/ERα(CDEF) expressing the AF-2-containing ERα(CDEF) derivative under the control of the PGK promoter. (**B**) Western blot showing the expression levels of the ERα(CDEF) protein in cells grown in the presence (+) or absence (–) of 1 mM methionine. Yeast cell extracts (30 μg) were fractionated by SDS–PAGE, electrotransferred to a nitrocellulose filter and probed with the ERα-specific mAb F3. ERα(CDEF) is indicated by an arrow. (**C**) The AF-2 activity of ERα is reduced in the presence of methionine. Transformants were grown in the absence or presence of 1 mM methionine for four generations and were exposed to the indicated concentrations of E2 for an additional overnight. OMPdecase activities determined on each cell-free extract are expressed in nmol substrate/min/mg protein. (**D**) PSU1 depletion has no effect on the transcriptional activity of the AF-1-containing ERα(ABC) construct. Δpsu1 PL3 cells carrying PRS314-Met/HA-PSU1 were transformed with YEP90 or YEp90-ERα(ABC). Transformants were grown in the absence (–) or presence (+) of 1 mM methionine. OMPdecase activity is expressed as in (C).

grown in its absence (Figure 6C). Thus, the transcriptional activity of the ligand-dependent activation function AF-2 of ERα is dependent upon PSU1 in yeast. Note that no effect of PSU1 depletion was observed on the transcriptional activity of the C-terminally truncated ERα derivative [ERα(ABC), formerly called HE15; Pierrat *et al*., 1992], that contains the AF-1 activation function, but does not interact with PSU1 (Figure 6D; data not shown).

Discussion

PSU1, a yeast MutT-domain-containing protein involved in transcription

PSU1 was initially identified in a genetic screen for suppressors of the respiratory deficiency of a yeast pet mutant and shown to encode an essential protein (A.A.Tzagoloff, unpublished data; DDBJ/EMBL/GenBank accession number L43065). In this study, we report that PSU1 is a MutT-domain-containing protein that functions in transcription.

The MutT domain is a highly conserved sequence motif that was originally identified in nucleoside triphosphatases from diverse species that are related to the MutT antimutator protein of *E.coli* (Koonin, 1993). MutT is involved in the GO system that protects cells from the mutagenic effect of the oxidatively damaged base 8-oxoguanine (for a review see Nash *et al*., 1996). This modified base can pair with adenine as well as cytosine, and thus has the

potential to induce G:C to T:A transversions (Shibutani *et al*., 1991). Bacterial and mammalian cells both possess MutT proteins that specifically degrade 8-oxo-dGTP and 8-oxo-GTP, which are otherwise misincorporated into DNA and RNA, respectively (Nash *et al*., 1996; Taddei *et al*., 1997). NMR structural analysis of the *E.coli* MutT protein has revealed that the MutT domain is part of the nucleotide-binding and active site of MutT (Abeygunawardana *et al*., 1995 and references therein). However, this conserved domain is not unique to the MutT enzyme. Other enzymes have recently been identified that contain a MutT domain, but do not possess the nucleoside triphosphate pyrophosphohydrolase activity described for MutT (reviewed in Bessman *et al*., 1996). Among these enzymes are a Ap4A tetraphosphatase, a GDP-mannose mannosyl hydrolase, an NADH pyrophosphatase and a diphosphoinositol polyphosphate phosphohydrolase (termed DIPP; Safrany *et al*., 1998). Although apparently distinct in their selection of substrates, members of the MutT family are in fact closely related in that, DIPP excepted, they all hydrolyse derivatives of nucleoside diphosphates (Bessman *et al*., 1996). The MutT conserved domain might therefore corresponds to a signature sequence for nucleoside diphosphate hydrolases. Thus, PSU1 may possess a similar enzymatic activity.

PSU1 is a large protein of 970 amino acids with a predicted molecular mass of 109 kDa. In this respect, PSU1 differs from the other members of the MutT family which are relatively small proteins made up of ~130 to 350 amino acid residues. In addition to the MutT domain, PSU1 contains several regions rich in either glutamine, proline, serine or asparagine residues, which are common features of transcriptional regulators (Tjian and Maniatis, 1994). That PSU1 may represent the first member of the MutT family to be involved in transcription is supported by several lines of evidence: (i) PSU1 can activate transcription on its own in yeast, when fused to a heterologous DNA-binding domain; (ii) PSU1 contains two major autonomous activation domains, AD1 and AD2b, that include the glutamine- and asparagine-rich regions, respectively, in addition to a less potent AD (AD2a) which immediately precedes AD2b; and (iii) PSU1 physically and functionally interacts with ligand-activated nuclear receptors (see below).

PSU1, a transcriptional co-activator/mediator for the ligand-dependent activation function AF-2 of nuclear receptors in yeast

Vertebrate nuclear receptors can stimulate transcription in yeast in the presence of their cognate ligand through their ligand-dependent transcriptional activation function AF-2 (see Introduction for references). This suggests that at least some of the basic features of the mechanisms that mediate these effects have been conserved across eukaryotes. In this report, we show that in yeast, the presence of PSU1 is critical for the activity of the activation function AF-2 of ERα. PSU1 interacts in yeast with the LBD/AF-2 of ERα in the presence of oestradiol, but not in the presence of the anti-oestrogen hydroxytamoxifen. This interaction is direct, and importantly, can be abolished, both in yeast and *in vitro*, by point mutations within the AF-2 AD core motif (helix 12 of the LBD) of ERα. Furthermore, PSU1 has an autonomous transactivation activity. Thus, PSU1 fulfils the requirements of a co-activator/mediator of the AF-2 of ERα in yeast.

That PSU1 is also a co-activator for the AF-2s of other NRs (RARα, RXRα, TRα and VDR) is strongly suggested by the ability of the corresponding LBDs to interact with PSU1 in a ligand-dependent manner. In the case of the RARα LBD, this interaction was also demonstrated to be dependent on the AF-2 AD core motif. However, in contrast to the case of ERα, point mutations in the hydrophobic residues of the RARα AF-2 AD core motif reduced, but did not abolish, PSU1 binding. This result is reminiscent of previous data on putative mammalian mediators that revealed differential requirements of the conserved residues of the AF-2 AD core motif for interaction with various NRs (vom Baur *et al*., 1996; Thenot *et al*., 1997). Whether this reflects a differential involvement of additional regions in the receptor LBD remains to be determined, but this possibility is supported by structural studies showing that upon ligand binding, the NR LBD undergoes a conformational change that brings the AF-2 AD core amphipatic α-helix 12 into a new receptor environment, thus creating a composite surface(s) for co-activator/mediator interaction which encompasses helix 12 and parts of helices 3, 4 and 5 (Wurtz *et al*., 1996; Darimont *et al*., 1998; Nolte *et al*., 1998 and references therein).

The ADs and nuclear receptor interaction domains of PSU1 contain HLMs related to those found in mammalian NR co-activators

PSU1 contains 10 short α-helical leucine-rich motifs, referred to as HLMs, that share the consensus core sequence LLxΦL. Only regions that elicit activation and/or NR-binding activity contain HLMs. A single HLM is present in AD1. There are two HLMs in nuclear receptor interaction domain 1 (NID1), whereas AD2a/NID2a and AD2b/NID2b contain two and five HLMs, respectively. Interestingly, a number of mammalian NR co-activators have been shown to contain similar helical leucine-rich motifs, some of which are responsible for their specific NR binding, whereas others interact with downstream targets (Heery *et al*., 1997; Li *et al*., 1997; Torchia *et al*., 1997; Voegel *et al*., 1997; McInerney *et al*., 1998). Three functionally redundant motifs sharing the LxxLL consensus sequence, also termed the NR box motif (Le Douarin *et al*., 1996), were found in the NIDs of NR co-activators belonging to the p160 family, TIF2, SRC-1, p/CIP and RAC3 (for review see Moras and Gronemeyer, 1998). Each of these motifs can independently mediate NR binding (Heery *et al*., 1997; Torchia *et al*., 1997; Voegel *et al*., 1997). Mutation in any one of the three motifs of TIF2 does not abrogate NR interaction (Voegel *et al*., 1997). In many other AF-2 co-activators, single or repeated LxxLL sequences have also been identified and shown to be important for their binding to liganded NRs (Le Douarin *et al*., 1996; Heery *et al*., 1997). In this respect, it is noteworthy that the amino acid sequence LLDLL of the HLM III of PSU1 NID1, which showed strong ligand-dependent binding to RARα and ERα, also fits the LxxLL consensus. In contrast, the LLNML core sequence of the HLM IX of NID2b, which interacted strongly with liganded ERα, but only weakly with liganded RARα, is an imperfect LxxLL motif. Note, however, that

this HLM IX of PSU1 fits the LxxML sequence, which has been reported to be the hydrophobic surface through which the helix 12/AF-2 AD core of ERα (Figure 4B) contacts helix 3 and helix 5 in the ERα LBD–raloxifen complex in a configuration remarkably similar to that of the helix formed by the hydrophobic residues of the NR box 2 of TIF2/GRIP1 with the agonist-bound TR LBD (Brozozowski *et al*., 1998; Darimont *et al*., 1998). Moreover, that a certain flexibility in the NR box motif LxxLL might be tolerated is supported by the recent identification of a related FxxLL motif in the mouse NR-interacting protein NSD1, that has been shown to be required and sufficient for NR binding (Huang *et al*., 1998).

In addition to the LxxLL NR box motifs, the p160 co-activators contain related leucine-rich motifs in their activation domain AD1, which is believed to transactivate via a recruitment of CBP/p300 (Torchia *et al*., 1997; Voegel *et al*., 1997; McInerney *et al*., 1998). Mutational analysis of the AD1 of TIF2 revealed that the leucine residues of the LLEQL sequence, which is closely related to the LLxΦL consensus, are critical for both interaction with CBP and transactivation by AD1, whereas the motif on its own is a very poor transactivator and binds CBP only weakly (Voegel *et al*., 1997). CBP/p300, which can also associate with liganded NRs as well as with many other transcription factors (CREB, Jun, STATs and bHLH factors; see Shikama *et al*., 1997 for review), contains LxxLL motifs in both the NR- and p/CIP-interacting domains (Torchia *et al*., 1997). Thus, these short helical leucine-rich motifs may serve as interaction motifs to interact with ligand-activated receptors and/or to recruit targets that are involved in transcriptional activation. The presence of similar HLMs in the NIDs and ADs of PSU1 suggests an evolutionary conservation of these motifs across eukaryotes.

Possible function(s) of PSU1 in yeast

In the present study, PSU1 has been shown to exhibit a number of functional and sequence features that are shared with various mammalian NR co-activators, thus suggesting that PSU1 may play a role in activator-dependent stimulation of transcription in yeast. Because PSU1 is an essential gene that has been originally identified in a genetic screen for suppressors of the respiratory deficiency of a pet mutant (A.A.Tzagoloff, unpublished data), it might be required for the function of transcriptional activators that affect one or more essential genes, and for the function of activators that regulate expression of genes required for respiratory growth.

As in mammalian cells, a number of yeast co-activators/mediators have been identified that function through an effect on chromatin structure and/or through an interaction with the general transcriptional machinery (reviewed in Guarente, 1995; see also Introduction). Some of these transcriptional cofactors have been reported to be essential for cell viability. These include TAF_{II}s, which associate with TATA-binding protein (TBP) to form TFIID (for a review, see Verrijzer and Tjian, 1996), and SRB proteins, which associate with the C-terminal domain (CTD) of the large subunit of RNA polymerase II to form the holoenzyme complex (reviewed in Bjorklund and Kim, 1996). The essential chromatin-remodelling complex RSC may also be involved in transcriptional activation (Cairns *et al*., 1996; Cao *et al*., 1997). Whether PSU1 is associated with either of these complexes or is part of a novel complex will be determined by isolating proteins to which it possibly binds. In this respect, it is interesting to note the recent identification of PSU1 in a two-hybrid screen for proteins that interact with the essential splicing factor Yjr022W of *S.cerevisiae* (Fromont-Racine *et al*., 1997). Although the biological relevance of this two-hybrid interaction has not yet been demonstrated, it argues for a role of PSU1 in splicing, and indicates together with our present observations, that PSU1 could be involved in linking transcription and splicing. Interestingly, these two processes have previously been established to be intimately associated by virtue of a direct interaction of splicing factors with the CTD of the RNA polymerase II large subunit (Yuryev *et al*., 1996; McCracken *et al*., 1997). Whether PSU1 can function as a bridge between NRs and the general transcriptional machinery in yeast remains to be determined.

So far, no mammalian PSU1 homologue has been reported. However, because the AF-2 AD of ERα functions in yeast cells through interaction with PSU1, it is not unreasonable to assume that in mammalian cells, this nuclear receptor also transactivates through a mammalian PSU1 homologue. Furthermore, using the PSU1 amino acid sequence to screen the databases for PSU1-related genes, we identified expression sequence tags similar to PSU1 from three plant species (*Arabidopsis thaliana*, DDBJ/EMBL/GenBank accession number AB006704; *Oryza sativa*, D39512; and cotton AI054538), *Drosophila* (AA802328 and AA390813) and *Caenorhabditis elegans* (Z82269), providing additional support for a conservation of PSU1 in higher eukaryotes (data not shown). We are currently screening mammalian cDNA libraries for suppressors of the inability of ERα to transactivate the URA3 reporter gene in a PSU1-depleted mutant, which should facilitate the identification of a PSU1 homologue(s).

Materials and methods

Yeast strains, transformations and media

Saccharomyces cerevisiae strain PL3 strain [*MATα leu2-Δ1 ura3-Δ1 his3-Δ200, trp1::(ERE)$_3$-URA3*] was as described elsewhere (Pierrat *et al*., 1992). The reporter strain L40 [*MAT***a** *his3-Δ200 trp1-901 leu2-3,112 ade2 LYS::(LexAop)4-HIS3 URA3::(LexAop)8-LacZ*] was a gift from S.M.Hollenberg (Vojtek *et al*., 1993). The *Δpsu1* deletion strain was generated in the PL3 (α) background according to the PCR-based disruption method described by Wach *et al*. (1994). Because PSU1 is essential for cell growth (A.A.Tzagoloff, unpublished data; DDBJ/EMBL/GenBank accession number L43065), we first created a PSU1/*Δpsu1 PL3* diploid strain containing one wild-type PSU1 allele and one null mutant allele in which the coding sequence of PSU1 was replaced with that of the kanamycin resistance (*Kan*^{r}) gene (Wach *et al*., 1994). This heterozygous strain was transformed with a URA3-marked plasmid carrying PSU1 and was subsequently subjected to a tetrad analysis. All four spores were viables, the Kan^r phenotype segregated 2^+:2^-, and all Kan^r spores were Ura$^+$ as expected. For the plasmid shuffle assay, a Kan^r Ura$^+$ spore was transformed with a TRP1-marked centromeric plasmid PRS314-Met/HA-PSU1 expressing a HA epitope-tagged PSU1 under the control of the methionine-repressed Met25 promoter (Kerjan *et al*., 1986). Transformants were placed on medium containing 5-fluoroorotic acid (5-FOA), a compound that prevents the growth of cells expressing URA3, thereby selecting for the loss of the URA3-marked PSU1 plasmid (Boecke *et al*., 1984). Yeast transformation was carried out by the lithium acetate procedure (Gietz *et al*., 1995). Standard media were used for growth (Rose *et al*., 1990).

Plasmids

Details on individual plasmid constructs, which were all verified by sequencing, are available upon request. Receptor cDNAs used in this study correspond to human RARα1, ERα and VDR, mouse RXRα and chicken TRα (DDBJ/EMBL/GenBank database). RARα(DEF)-ER.CAS, ERα(CDEF)/HEG19 and ERα(ABC)/HE15 were expressed from the yeast HIS3 multicopy vector YEp90 (Pierrat *et al*., 1992; Heery *et al*., 1993). DBD and AAD fusion proteins were expressed from the yeast multicopy plasmids pBL1 and pASV3, respectively (Le Douarin *et al*., 1995). All these plasmids express inserts under the control of the phosphoglycerate kinase (PGK) promoter. pBL1 contains the HIS3 marker and directs the synthesis of epitope (region F of ERα)-tagged ERα DBD fusion proteins. pASV3 contains the LEU2 marker and a cassette expressing a nuclear localized VP16 AAD, preceding a polylinker and stop codons in all reading frames. All inserts cloned into pBL1 and pASV3 were obtained by PCR and verified by sequencing. The LexA fusion protein LexA–RARα(DEF) was expressed from a derivative of the TRP1 pBTM116 plasmid (Vojtek *et al*., 1993; vom Baur *et al*., 1996). For the complementation test of the lethality phenotype of the Δpsu1 mutation, PSU1 was expressed from pRS316-PGK/PSU1, a derivative of the URA3-marked centromeric plasmid pRS316 (Sikorski and Hieter, 1989), in which the coding sequence of PSU1 was cloned into the *Bam*HI site of the polylinker of the yeast PGK expression cassette, previously cloned as a *Cla*I fragment into the unique *Cla*I site of the polylinker of pRS316. The TRP1-marked centromeric plasmid pRS314-Met/HA-PSU1, which expresses an HA epitope-tagged PSU1 under the control of the Met25 promoter, was constructed by inserting a *Bam*HI–*Bgl*II fragment encoding PSU1 into the *Bam*HI site of the polylinker of the Met25-HA cassette cloned as a *Cla*I (ends blunted) fragment from plex9-3H (Tirode *et al*., 1997) into pRS314 (Sikorski and Hieter, 1989), previously digested by *Kpn*I–*Sac*I (ends blunted). For *in vitro* binding assays the indicated cDNAs were fused to GST in the pGEX2T plasmid (Pharmacia; vom Baur *et al*., 1996). For expression of ^{35}S-labelled PSU1, the coding sequence of PSU1 DNA was inserted into a modified version of the pet15b vector (Novagen) and coupled transcription/translation was performed using T7 RNA polymerase with the TNT lysate system (Promega).

Two-hybrid screening

A yeast genomic VP16 fusion library was constructed by limited *Sau*3AI digestion of genomic DNA and subsequent insertion of fragments into a modified LEU2 pASV3 vector (vom Baur *et al*., 1998). The library was introduced by LiAc transformation into the PL3(α) reporter strain expressing RARα(DEF)-ER.CAS from the HIS3 YEp90. Approximately 2×10^5 yeast transformants were selected on His$^-$ Leu$^-$ plates and replated at a multiplicity of 10 onto Ura$^-$ His$^-$ Leu$^-$ plates containing 500 nM T-RA and 30 μg/ml AU. Twenty-eight clones were isolated. Library plasmids were rescued in *E.coli* strain HB101 (leuB$^-$) and introduced into the yeast reporter strain L40 expressing LexA–RARα(DEF) or LexA. Clones that grew on His$^-$ Leu$^-$ Trp$^-$ plates containing 500 nM T-RA were considered positive for interaction with RARα(DEF).

Transactivation assays

Yeast PL3 transformants were grown exponentially for about five generations in minimal medium supplemented with uracil and the required amino acids. When necessary, medium was supplemented with appropriate ligands. Yeast cell-free extracts were prepared and assayed for OMPdecase activity as described previously (Pierrat *et al*., 1992).

Antibodies

Monoclonal antibodies (mAbs) B10 and F3 are directed against the B and F regions of human ERα, respectively. mAb 2GV4 is directed against VP16 (see Le Douarin *et al*., 1995 and references therein).

In vitro binding assays

The assay was performed as described previously (vom Baur *et al*., 1996). Briefly, GST or GST fusion proteins were expressed in *E.coli* and purified on glutathione–Sepharose (Pharmacia). Purified proteins were quantified by Coomassie staining after SDS–PAGE separation and by Bradford protein assay. Glutathione–Sepharose beads were equilibrated with binding buffer (BB: 50 mM Tris–HCl, pH 8.0, 100 mM NaCl, 0.3 mM DTT, 10 mM $MgCl_2$, 10% glycerol, 0.1% NP-40), loaded with equimolar amounts of GST or GST fusions in the presence of 300 μg/ml insulin and washed. Appropriate ligands or carrier (ethanol) and ^{35}S-labelled PSU1 (10 μl) were added to each reaction. Incubation was carried out at 4°C for 16 h with gentle agitation. After three washes with 200 μl BB buffer beads were resuspended in 25 μl SDS-loading buffer, boiled for 5 min and proteins analysed by SDS–PAGE. The amount of bound PSU1 was quantified using a phosphoimager (Fuji BAS2000).

Acknowledgements

We are grateful to S.M.Hollenberg for the generous gifts of yeast strain L40 and plasmid pBTM116. We thank A.Wach and P.Philippsen for the gift of KanMX gene disruption plasmids, F.Tirode for the gift of the Met25-HA expression cassette and Bristol-Myers-Squibb for providing the compounds BMS411 and BMS453. Special thanks also to J.-M.Garnier for construction of the yeast genomic VP16 fusion library, M.Cervino for technical assistance, S.Vicaire and D.Stephan for sequencing all isolated library clones and constructs, O.Poch for sequence alignment, and our colleagues of the yeast and retinoid groups for helpful discussions. This work was supported by the Groupement de Recherches et d'Etudes sur les Genomes (GREG47/95), the Centre National de la Recherche Scientifique, the Institut National de la Sante et de la Recherche Medicale, the Centre Hospitalier Universitaire Regional, the Association pour la Recherche sur le Cancer, the College de France, the Fondation pour la Recherche Medicale (FRM), the Human Frontier Science Program and Bristol-Myers-Squibb.

References

Abeygunawardana,C., Weber,D.J., Gittis,A.G., Frick,D.N., Lin,J., Miller,A.-F., Bessman,M.J. and Mildvan,A.S. (1995) Solution structure of the MutT enzyme, a nucleoside triphosphate pyrophosphohydrolase. *Biochemistry*, **34**, 14997–15005.

Beato,M. and Sanchez-Pacheco,A. (1996) Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr. Rev.*, **17**, 587–609.

Berry,M., Metzger,D. and Chambon,P. (1990) Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J.*, **9**, 2811–2818.

Bessman,M.J., Frick,D.N. and O'Handley,S.F. (1996) The MutT Proteins or 'nudix' hydrolases, a family of versatile, widely distributed 'housecleaning' enzymes. *J. Biol. Chem.*, **271**, 25059–25062.

Bjorklund,S. and Kim,Y.-J. (1996) Mediator of transcriptional regulation. *Trends Biochem. Sci.*, **21**, 335–337.

Boecke,J.D., Lacroute,F. and Fink,G.R. (1984) A positive selection for mutants lacking orotidine-5′-monophosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. *Mol. Gen. Genet.*, **197**, 345–346.

Brozozowski,A.M. *et al.* (1998) Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, **389**, 753–758.

Burns,L.G. and Peterson,C.L. (1997) Protein complexes for remodeling chromatin. *Biochim. Biophys. Acta*, **1350**, 159–168.

Cairns,B.R. *et al.* (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell*, **87**, 1249–1260.

Cao,Y., Cairns,B.R., Kornberg,R.D. and Laurent,B.C. (1997) Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. *Mol. Cell. Biol.*, **17**, 3323–3334.

Chambon,P. (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J.*, **10**, 940–954.

Chiba,H., Muramatsu,M., Nomoto,A. and Kato,H. (1994) Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila brahma* are transcriptional coactivators cooperating with the oestrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.*, **22**, 1815–1820.

Darimont,B.D., Wagner,R.L., Apriletti,J.W., Stallup,M.R., Kushner,P.J., Baxter,J.D., Fletterick,R.J. and Yamamoto,K.R. (1998) Structure and specificity of nuclear receptor interaction. *Genes Dev.*, **12**, 3343–3356.

Durand,B., Saunders,M., Gaudon,C., Roy,B., Losson,R. and Chambon,P. (1994) Activation function 2 (AF-2) of retinoic acid receptor and 9-*cis* retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *EMBO J.*, **13**, 5370–5382.

Fields,S. and Sternglanz,R. (1994) The two-hybrid system: an assay for protein–protein interactions. *Trends Genet.*, **10**, 286–292.

Fromont-Racine,M., Rain,J.-C. and Legrain,P. (1997) Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens. *Nature Genet.*, **16**, 277–282.

Gietz,R.D., Schiestl,R.H., Willems,A.R. and Woods,R.A. (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. *Yeast*, **11**, 355–360.

Glass,C.K., Rose,D.W. and Rosenfeld,M.G. (1997) Nuclear receptor coactivators. *Curr. Opin. Cell Biol.*, **9**, 222–232.

Grant,P.A. *et al*. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.*, **11**, 1640–1650.

Grant,P.A., Schietz,D., Pray-Grant,M.G., Steger,D.J., Reese,J.C., Yates,J.R.,III and Workman,J.L. (1998) A subset of TAF_{II}s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell*, **94**, 45–53.

Gronemeyer,H. (1991) Transcription activation by oestrogen and progesterone receptors. *Annu. Rev. Genet.*, **25**, 89–123.

Guarente,L. (1995) Transcriptional coactivators in yeast and beyond. *Trends Biochem. Sci.*, **20**, 517–521.

Hall,B.L., Smit-McBride,Z. and Privalsky,M.L. (1993) Reconstitution of retinoid X receptor function and combinatorial regulation of other nuclear hormone receptors in the yeast *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **90**, 6929–6933.

Heery,D.M., Zacharewski,T., Pierrat,B., Gronemeyer,H., Chambon, P and Losson,R. (1993) Efficient transactivation by retinoic acid receptors in yeast requires retinoid X receptors. *Proc. Natl Acad. Sci. USA*, **90**, 4281–4285.

Heery,D.M., Kalkhoven,E., Hoare,S. and Parker,M.G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature*, **387**, 733–736.

Henriksson,A., Almlof,T., Ford,J., McEwan,I.J., Gustafsson,J.-A. and Wright,A.P.H. (1997) Role of the Ada adaptor complex in gene activation by the glucocorticoid receptor. *Mol. Cell. Biol.*, **17**, 3065–3073.

Horwitz,K.B., Jackson,T.A., Bain,D.L., Richer,J.K., Takimoto,G.S. and Tung,L. (1996) Nuclear receptor coactivators and corepressors. *Mol. Endocrinol.*, **10**, 1167–1177.

Huang,N., vom Baur,E., Garnier,J.-M., Lerouge,T., Vonesch,J.-L., Lutz,Y., Chambon,P. and Losson,R. (1998) Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *EMBO J.*, **17**, 3398–3412.

Ichinose,H., Garnier,J.-M., Chambon,P. and Losson,R. (1997) Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene*, **188**, 95–100.

Kerjan,P., Cherest,H. and Surdin-Kerjan,V. (1986) Nucleotide sequence of the *Saccharomyces cerevisiae MET25* gene. *Nucleic Acids Res.*, **14**, 7861–7871.

Koonin,E.V. (1993) A highly conserved sequence motif defining the family of MutT-related proteins from eubacteria, eukaryotes and viruses. *Nucleic Acids Res.*, **21**, 4847.

Le Douarin,B., Pierrat,B., vom Baur,E., Chambon,P. and Losson,R. (1995) A new version of the two-hybrid assay for detection of protein protein interactions. *Nucleic Acids Res.*, **23**, 876–878.

Le Douarin,B., Nielsen,A.L., Garnier,J.-M., Ichinose,H., Jeanmougin,F., Losson,R. and Chambon,P. (1996) A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors. *EMBO J.*, **15**, 6701–6715.

Li,H., Gomes,P.J. and Chen,J.D. (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc. Natl Acad. Sci. USA*, **94**, 8479–8484.

Mangelsdorf,D.J. *et al*. (1995) The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835–839.

Martinez,A., Kundu,T.K., Fu,J. and Reinberg,R.G. (1998) A human SPT3-TAF_{II}31-GCN5-L acetylase complex distinct from the transcription factor IID. *J. Biol. Chem.*, **273**, 23781–23785.

McCracken,S., Fong,N., Yankulov,K., Ballantyne,S., Pan,G., Greenblatt,J., Patterson,S.D., Wickens,M. and Bentley,D.L. (1997) The C-terminal domain of mRNA polymerase II couples mRNA processing and transcription. *Nature*, **385**, 357–361.

McDonnell,D.P., Pike,J.W., Drutz,D.J., Butt,T.R. and O'Malley,B.W. (1989) Reconstitution of the vitamin D-responsive Osteocalcin transcription unit in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **9**, 3517–3523.

McInerney,E.M. *et al.* (1998) Determinants of coactivator LXXLL motif specificity in nuclear receptor coactivation. *Genes Dev.*, **12**, 3357–3368.

Metzger,D., White,J.H. and Chambon,P. (1988) The human oestrogen receptor functions in yeast. *Nature*, **334**, 31–36.

Metzger,D., Losson,R., Bornert,J.-M., Lemoine,Y. and Chambon,P. (1992) Promoter specificity of the two transcriptional activation functions of the human oestrogen receptor in yeast. *Nucleic Acids Res.*, **20**, 2813–2817.

Metzger,D., Ali,S., Bornert,J.-M. and Chambon,P. (1995) Characterization of the amino-terminal transcriptional activation function of the human estrogen receptor in animal and yeast cells. *J. Biol. Chem.*, **270**, 9535–9542.

Moras,D. and Gronemeyer,H. (1998) The nuclear receptor ligand-binding domain: structure and function. *Curr. Opin. Cell Biol.*, **10**, 384–391.

Muchardt,C. and Yaniv,M. (1993) A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.*, **12**, 4279–4290.

Nash,H.M., Bruner,S.D., Scharer,O.D., Kawate,T., Addona,T.A., Spooner,E., Lane,W.S. and Verdine,G.L. (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Curr. Biol.*, **6**, 968–980.

Nolte,R.T. *et al.* (1998) Ligand binding and coactivator assembly of the peroxysome proliferator-activated receptor γ. *Nature*, **395**, 137–144.

Ogryzko,V.V., Kotani,T., Zhang,X., Schiltz,R.L., Howard,T., Yang,X.-J., Howard,B.H., Qin,J. and Nakatani,Y. (1998) Histone-like TAFs within the PCAF histone acetylase complex. *Cell*, **94**, 35–44.

Pierrat,B., Heery,D.M., Lemoine,Y. and Losson,R. (1992) Functional analysis of the human estrogen receptor using a phenotypic transactivation assay in yeast. *Gene*, **119**, 237–245.

Rose,M.D., Winston,F. and Hieter,P. (1990) *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Safrany,S.T., Caffrey,J.J., Yang,X., Bembenek,M.E., Moyer,M.B., Burkhart,W.A. and Shears,S.B. (1998) A novel context for the 'MutT' module, a guardian of cell integrity, in a diphosphoinositol polyphosphate phosphohydrolase. *EMBO J.*, **17**, 6599–6607.

Schena,M. and Yamamoto,K.R. (1988) Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. *Science*, **241**, 965–967.

Shibutani,S., Takeshita,M. and Grollman,A.P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.

Shikama,N., Lyon,J. and La Thangue,N.B. (1997) The p300/CBP family: integrating signals with transcription factors and chromatin. *Trends Cell Biol.*, **7**, 230–236.

Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 18–27.

Taddei,F., Hayakawa,H., Bouton,M.-F., Cirinesi,A.-M., Matic,I., Sekiguchi,M. and Radman,M. (1997) Counteraction by MutT protein in transcriptional errors caused by oxidative damage. *Science*, **278**, 128–130.

Tate,B.F., Allenby,G., Perez,J.R., Levin,A.A. and Grippo,J.F. (1996) A systematic analysis of the AF-2 domain of human retinoic acid receptor α reveals amino acids critical for transcriptional activation and conformational integrity. *FASEB J.*, **10**, 1524–1531.

Thenot,S., Henriquet,C., Rochefort,H. and Cavailles,V. (1997) Differential interaction of nuclear receptors with the putative human transcriptional coactivator hTIF1. *J. Biol. Chem.*, **272**, 12062–12068.

Tirode,F., Malaguti,C., Romero,F., Attar,R., Camonis,J. and Egly,J.-M. (1997) A conditionally expressed third partner stabilizes or prevents the formation of a transcriptional activator in a three-hybrid system. *J. Biol. Chem.*, **272**, 22995–22999.

Tjian,R. and Maniatis,T. (1994) Transcriptional activation: a complex puzzle with few easy pieces. *Cell*, **77**, 5–8.

Tora,L., White,J., Brou,C., Tasset,D., Webster,N., Scheer,E. and Chambon,P. (1989) The human oestrogen receptor has two independent nonacidic transcriptional activation functions. *Cell*, **59**, 447–487.

Torchia,J., Glass,C. and Rosenfeld,M. (1998) Co-activators and co-repressors in the integration of transcriptional responses. *Curr. Opin. Cell Biol.*, **10**, 373–383.

Torchia,J., Rose,D.W., Inostroza,J., Kamei,Y., Westin,S., Glass,C.K. and Rosenfeld,M.G. (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature*, **387**, 677–684.

Verrijzer,C.P. and Tjian,R. (1996) TAFs mediate transcriptional activation and promoter selectivity. *Trends Biochem. Sci.*, **21**, 338–342.

Voegel,J.J., Heine,M.J.S., Tini,M., Chambon,P. and Gronemeyer,H. (1997) The coactivator TIF2 contains three nuclear receptor binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO J.*, **17**, 507–519.

Vojtek,B.A., Hollenberg,S.M. and Cooper,J.A. (1993) Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell*, **74**, 205–214.

vom Baur,E. *et al.* (1996) Differential ligand-dependent interactions

between the AF-2 activating domain of nuclear receptors and putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J.*, **15**, 110–124.

vom Baur,E., Harbers,M., Um,S.-J., Benecke,A., Chambon,P. and Losson,R. (1998) The yeast Ada complex mediates the ligand-dependent activation function AF-2 of nuclear receptors. *Genes Dev.*, **12**, 1278–1289.

Wach,A., Brachat,A., Pohlmann,R. and Philippsen,P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae. Yeast*, **10**, 1793–1808.

Wang,W., Xue,Y., Zhou,S., Kuo,A., Cairns,B.R. and Crabtree,G.R. (1996) Diversity and specialization of mammalian SWI/SNF complexes. *Genes Dev.*, **10**, 2117–2130.

White,J.H., Metzger,D. and Chambon,P. (1988) Expression and function of the human estrogen receptor in yeast. *Cold Spring Harbor Symp. Quant. Biol.*, **LIII**, 819–828.

Wong,J., Shi,Y.B. and Wolffe,A.P. (1997) Determinants of chromatin disruption and transcriptional regulation investigated by the thyroid hormone receptor: hormone-regulated chromatin disruption is not sufficient for transcriptional activation. *EMBO J.*, **16**, 3158–3171.

Wurtz,J.M., Bourguet,W., Renaud,J.P., Vivat,V., Chambon,P., Moras,D. and Gronemeyer,H. (1996) A canonical structure for the ligand binding domain of nuclear receptors. *Nature Struct. Biol.*, **3**, 87–94.

Yoshinaga,S., Peterson,C., Herskowitz,I. and Yamamoto,K. (1992) Roles of SWI1, SWI2 and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science*, **258**, 1598–1604.

Yuryev,A., Patturajan,M., Litingtung,Y., Joshi,R.V., Gentile,C., Gebara,M. and Corden,J.L. (1996) The C-terminal domain of the largest subunit of RNA polymerase interacts with a novel set of serine/arginine-rich proteins. *Proc. Natl Acad. Sci. USA*, **93**, 6975–6980.

Received December 18, 1998, revised and accepted February 24, 1999