Functional *in Vivo* Interaction between the Amino-Terminal, Transactivation Domain and the Ligand Binding Domain of the Androgen Receptor†

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ABSTRACT: The ligand binding domain (LBD) and the amino-terminal, transactivation domain (TAD) of the androgen receptor (AR) were separately linked to the GAL4 DNA binding domain (DBD) and to the GAL4(TAD). Resulting constructs were tested in the yeast two-hybrid system for protein−protein interactions. In the presence of androgen [methyltrienolone (R1881) or dihydrotestosterone (DHT)] a transcriptionally active complex was formed, reflecting an association between the AR(LBD) and the AR(TAD). No interactions were found in the presence of low-affinity ligands like estradiol (E2), promegestone (R5020), or progesterone (Pg). Use of the Thr-868-Ala mutated AR(LBD) in the assay resulted not only in a clear AR TAD−LBD interaction in the presence of R1881 and DHT but also in the presence of E2, Pg, and R5020, corresponding to the alteration in ligand specificity induced by the mutation. Coexpression of the fusion protein Gal4(DBD)AR(LBD) and the separate AR(TAD) also gave rise to the formation of a transcriptionally active complex. No interactions were found between two AR LBDs at the low-expression level of the two components. However, LBD−LBD interaction was detectable by application of a high-expression vector for GAL4(TAD)AR(LBD), albeit at high ligand concentrations. To substantiate the observation of the AR LBD−TAD interaction, CHO cells were cotransfected with expression plasmids for a truncated AR, which lacks the TAD [AR(DBD)(LBD)], and for the separate AR(TAD). This resulted in stimulation of a MMTV-LUC reporter gene in the presence of R1881 but not in the absence of hormone. This finding indicates that, like in the yeast system, in mammalian cells, TAD−LBD interactions are of importance for AR activation. In the mammalian system, a maximal AR TAD−LBD interaction was obtained at approximately 10-fold higher ligand concentrations than required for full-length AR activation. In the presence of low-affinity ligands, the AR TAD−LBD interaction as measured by transcriptional activation was considerably weaker than the activity of the full-length AR. From the present results a concept of hormone-dependent AR activation is proposed, which requires a functional, direct or indirect intramolecular interaction between the TAD and the LBD.

The androgen receptor (AR)[1] is a member of the steroid receptor superfamily of ligand-dependent transcription factors (Tsai & O'Malley, 1994; Beato et al., 1995; Mangelsdorf et al., 1995). It mediates androgen-induced physiological responses, which are essential for the development and maintenance of the male phenotype (Mooradian et al., 1987). Consistent with the conserved structural and functional organization of the steroid receptor family members, several, apparently separate, functional domains have been characterized: an amino-terminal, transactivation domain (TAD), a highly conserved DNA binding domain (DBD), and a moderately well conserved carboxyl-terminal, ligand binding domain (LBD) (Jenster et al., 1991; Simental et al., 1991).

Upon androgen binding, the AR can interact with its cognate androgen response elements (AREs), which are commonly located in the promoter regions of androgen target genes. Presumably, this AR(ligand)−ARE complex stabilizes the transcription preinitiation complex, in this way augmenting gene transcription by RNA polymerase II. Alternatively, it tethers an RNA polymerase II holoenzyme complex to the promoter (Tjian & Maniatis, 1994; Koleske & Young, 1995).

Steroid receptor binding sites commonly exhibit a palindromic structure, to which receptor homodimers can bind. One of the dimerization interphases is found in the D-(distal) box of the DNA binding domain (DBD), as proven for glucocorticoid receptor (GR) and estrogen receptor (ER) homodimers by crystallographic analyses (Luisi et al., 1991; Schwabe et al., 1993). Because of the high level of DBD sequence conservation, an important function of the D-box in dimerization seems applicable to all steroid hormone receptors.

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[1] Abbreviations: LBD, ligand binding domain; TAD, transactivation domain; DBD, DNA binding domain; AR, androgen receptor; GR, glucocorticoid receptor; ER, estrogen receptor; PR, progesterone receptor; ARE, androgen response element; TAU, transactivating unit; R1881, methyltrienolone; DHT, dihydrotestosterone; T, testosterone; E2, estradiol; Pg, progesterone; R5020, promegestone; CPA, cyproterone acetate; OH-F, hydroxyflutamide; ICI, bicalutamide; LUC, luciferase; PCR, polymerase chain reaction; SEM, standard error of the mean.

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Analysis of ER dimer formation as demonstrated in gel retardation experiments with two receptor size variants, as well as the dissection of the nuclear uptake mechanism of the progesterone receptor (PR), indicated a ligand-inducible dimerization interphase present within the LBD (Kumar & Chambon, 1988; Guiochon-Mantel et al., 1989). These findings coincided with the identification of a 22 amino acid region within the ER LBD, required for receptor homodimer formation and high-affinity DNA binding (Fawell et al., 1990; Lees et al., 1990). Sequence alignment among the steroid receptor family members revealed this region to be associated with a conserved heptad repeat of hydrophobic residues (Wurtz et al., 1996).

Little is known about direct or indirect, intra- or intermolecular interactions between steroid receptor domains with different functions. Recently, we defined in transient transfection assays the presence of two separate TADs within the amino-terminal region of the AR (Jenster et al., 1995). One of these TADs (TAU-1, amino acid residues 100−370) was found to be active in the presence of the ligand-bound LBD, whereas in a truncated AR, which lacks the LBD, the region 360−485 (TAU-5) functions as a constitutively active transactivator of comparable strength. These observations suggested to us the occurrence of interactions between the TAD region and the LBD of the AR. This hypothesis was in accordance with androgen dissociation kinetics and *in vitro* AR degradation rates, which indicates that the AR amino-terminal region stabilizes the receptor by slowing the rate of ligand dissociation and AR degradation (Zhou et al., 1995).

In this study we present an *in vivo* approach based upon yeast and mammalian protein−protein interaction assays, in which we investigated the occurrence of a functional interaction between the LBD and TAD of the human AR.

MATERIALS AND METHODS

Materials. Methyltrienolone (R1881) and promegestone (R5020) were purchased from NEN (Boston, MA). Cyproterone acetate was a gift from Schering (Berlin, Germany), hydroxyflutamide from Schering USA (Bloomfield, NJ), and ICI 176.334 (bicalutamide) from Zeneca Ltd. (Macclesfield, U.K.). All other steroids were purchased from Steraloids (Wilton, NH).

Construction of Plasmids. Plasmids were constructed according to standard methods and, where denoted, blunt ended with Klenow DNA polymerase (Sambrook et al., 1989). All GAL4 fusion constructs, and constructs including a PCR amplification step for preparation, were sequenced to verify the correct reading frame and the absence of random mutations.

The glyceraldehyde-3-phosphate dehydrogenase promoter-based yeast expression vector pG1 (Schena et al., 1991) was kindly provided by Dr. Picard. We constructed the pG1 derivative pG1AR-I, containing the complete human AR coding region, by subcloning the 3.0 kb *Sal*I fragment from the mammalian hAR cDNA expression vector pSVARo (Brinkmann et al., 1989) into the homologous pG1 site. A low-expression level, corresponding to a low transactivating activity of pG1AR-I, prompted us to subject the 5′-untranslated region to PCR-mediated mutagenesis (Higuchi et al., 1988). Amplification was performed utilizing the forward primer 5′-CG**GGATCC**AAAAATGGAAGTGCAGTTAGGGCTGGGAAGGGTC-3′ (AR cDNA sequences are underlined), including an internal *Bam*HI site (boldface underlined), and the internal AR cDNA reverse primer 5′-GGAGCAGCTG**CTTAAG**CCGGGG-3′ (*Afl*II site boldface underlined). The amplified, *Bam*HI−*Afl*II digested product was subsequently exchanged with the corresponding pG1AR-I fragment, yielding plasmid pG1AR-II, which now contained a consensus yeast translation initiation region preceded by a 30 bp leader sequence.

pG1AR-L, containing the T868A substitution, was constructed by transferring the 1.3 kb *Kpn*I−*Pst*I fragment of the AR(T868A) cDNA expression vector pSVAR-L (Veldscholte et al., 1990) to the homologous sites in pBluescript II SK$^+$ (Stratagene, La Jolla, CA). The fragment was reexcised with *Kpn*I and *Sma*I and exchanged with the corresponding *Kpn*I−*Sal*I (blunt ended) pG1AR-II fragment.

The expression vector pG1AR-5, encoding the constitutively active AR(TAD)(DBD), was constructed by exchanging the *Rsr*II−*Xba*I (blunt ended) fragment from the previously described pSVAR-5 (Jenster et al., 1995) with the 1.7 kb *Rsr*II−*Sal*I (blunt ended) pG1AR-II fragment.

The androgen-inducible yeast expression vector pUCΔSS-26X containing a triple arranged 26 bp GRE(ARE) oligonucleotide, derived from the rat tyrosine aminotransferase gene, fused upstream to the yeast *CYC1* promoter region was provided by Dr. Picard (Schena et al., 1991).

The GAL4(DBD$_{1-147}$) two-hybrid cloning vector pGBT9 and the parental GAL4(TAD$_{768-881}$) cloning vectors pGAD424, or the high level expression derivative pACT2 (all from Clontech, Palo Alto, CA), were used to generate GAL4 fusion protein constructs. The GAL4-AR(TAD$_{3-494}$) fusion constructs were prepared by integration of the blunt-ended 1.5 kb *Mlu*I−*Acc*651 fragment of the previously described pSVAR-3 (Jenster et al., 1995) into the *Sma*I site of pTZ19 (Pharmacia, Uppsala, Sweden). An additional *Bam*HI site was introduced into the resulting vector pTZ19NAR by addition of a *Bam*HI linker to the blunt-ended *Eco*RI site in the polylinker. The AR(TAD) fragment was excised with *Bam*HI and cloned in frame into the corresponding sites in pGBT9 and pGAD424, yielding pGAL4(DBD)AR(TAD) and pGAL4(TAD)AR(TAD), respectively. This procedure resulted in the insertion of eight amino acid residues, intermediate to the chimeric substituents (PEFPGIPR and IEFPGIPR, respectively). GAL4-AR(LBD$_{652-910}$) fusion constructs were generated by integration of the blunt-ended 0.9 kb *Tth*III−*Sma*I fragment from pSVARo into the *Sma*I site of pTZ19. The AR(LBD) fragment was excised from the resulting plasmid pTZ19CAR, with *Bam*HI and cloned in frame into the homologous sites in pGBT9, pGAD424, and pACT2, yielding the plasmids pGAL4(DBD)AR(LBD), pGAL4(TAD)AR(LBD), and pACT-GAL4(TAD)AR(LBD), respectively. Additional amino acids, intermediate to the chimer substituents, are PEFPGIP, IEFPGIP, and ICMAYPYDVPDYASLGGHMAMEAPGIP, respectively. The latter includes the HA epitope (YPYDVPDYA) and flanking sequences present in pACT2.

The derivatives with the T868A substitution in the AR(LBD) were constructed by exchanging the 0.45 kb *Eco*RI fragment from pTZ19CAR with the corresponding pSVAR-L fragment. The fragment was reexcised and subcloned in frame into pGBT9 and pGAD424 via *Bam*HI and *Pst*I compatible ends, yielding the plasmids pGAL4(DBD)AR(LBD-L) and pGAL4(TAD)AR(LBD-L), respectively. pACT-

GAL4(TAD)AR(LBD-L) was constructed by exchanging the internal 0.5 kb *Eco*RI fragment in pACT-GAL4(TAD)AR-(LBD) with the corresponding fragment in pSVAR-L. Consequently, all GAL4-AR(LBD-L) chimeric derivatives exhibit identical amino acid additions between the GAL4 and AR parts, as compared to the wild-type GAL4-AR(LBD) chimeras.

pAR(TAD_{3-494}) was generated by exchanging the *Acc*651 (blunt ended)−*Eco*RI fragment of pGAD424 with the 1.5 kb *Bam*HI (blunt ended)−*Eco*RI fragment of pTZ19NAR. The resulting plasmid pAR(TAD) now contained the AR-(TAD) fragment cloned in frame to the SV40 large T antigen nuclear localization signal in pGAD424.

The mammalian expression plasmid pSVAR(TAD_{1-494}) was obtained by modification of pSVAR-12 (Jenster et al., 1993): the 1.3 kb *Xba*I−*Eco*RI fragment, encoding the AR DBD and LBD, was deleted, and the remaining vector, containing the AR TAD, was religated by a *Xba*I−*Eco*RI linker. For the expression plasmid pSVAR(DBD)(LBD-L), a 0.5 kb *Eco*RI−*Eco*RI fragment from pSVAR-L was exchanged with the same fragment in the previously described plasmid pSVAR(DBD)(LBD) (pSVAR-104; Jenster et al., 1995).

Yeast Strains and Growth. AR transactivation activity was measured in the multiple protease-deficient *Saccharomyces cerevisiae* strain BJ2168 (*MATa, pep4−3, pcr1−407, prb-1122, ura3−52, trp1, leu2*) (kindly provided by Dr. Picard). Two-hybrid protein−protein analysis was performed in strain Y190 (*MATa, ura3−52, his3−Δ200, ade2−101, trp1−901, leu2−3, leu2−112, GAL4Δ, GAL80Δ, URA3::GAL-lacZ, cyh^r, LYS2::GAL-HIS3*), which was purchased from Clontech. Yeast cells were grown either in standard YEPD medium (1% w/v yeast extract, 2% w/v peptone, and 2% w/v dextrose, pH 5.8) or the appropriate selective minimal medium (0.67% w/v yeast nitrogen base without amino acids and 2% w/v dextrose, pH 5.8) supplemented to the nutritional requirements of the yeast transformants. All yeast transformations were carried out according to the lithium acetate method (Gietz et al., 1992).

β-Galactosidase Assay. β-Galactosidase activity, indicative of AR transactivation activity, was assessed by cotransfecting *S. cerevisiae* BJ2168 with the appropriate AR expression vector and the androgen-inducible yeast reporter vector pUCΔSS-26X. β-Galactosidase activity of the GAL4 chimers expressed in strain Y190 involves the activation of an integrated UAS_{GAL1}-lacZ reporter gene and depends on the *in vivo* functional reconstitution of GAL4 due to interactions between AR fragments fused to separate GAL4 TAD and DBD domains.

A saturated culture of yeast transformants of either strain, propagated in 1−5 mL of the appropriate selective medium, was diluted to an A_{600} of 0.2 in the same selective medium supplemented with the respective AR ligands. Subsequently, the yeast cells were incubated to midlog phase (A_{600} approximately 1.0; 6−8 h). To 1.0 mL of Y190 culture (spun down and resuspended to a final volume of 0.1 mL in minimal medium) or 0.1 mL of BJ2168 culture we added 0.7 mL of Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 0.27% v/v β-mercaptoethanol, pH 7.0). The cells were permeabilized by addition of 50 μL of $CHCl_3$ and 50 μL of 0.1% SDS and vortexed for 30 s. The reactions were started with the addition of 0.16 mL of prewarmed β-galactosidase substrate *o*-nitrophenyl β-galactoside (0.4% w/v in 0.1 M phosphate buffer, pH 7.0) at 30 °C. The reactions were quenched after development of the yellow reaction product (1 h or less), by addition of 0.4 mL of 1 M Na_2CO_3. Absorbance at 420 nm (A_{420}) was read, and β-galactosidase activity was calculated using the equation:

$$\beta\text{-galactosidase (U)} = \frac{1000A_{420}}{tVA_{600}}$$

where t = reaction time (min) and V = volume of yeast culture added to Z-buffer (mL).

Immunoblot Analysis of Proteins Expressed in Yeast. AR and GAL4-AR chimer expression was assessed by immunoblot analysis of yeast extracts. For this, 50 mL of YEPD medium was inoculated with 5 mL of a saturated culture of yeast transformants, propagated in the appropriate selective medium containing 2% dextrose, and grown until an A_{600} value of 0.4−0.6. The cultures were poured into 100 mL centrifuge tubes, which were halfway filled with ice, and harvested by centrifugation (1000*g*) for 5 min at 4 °C. The pellet was successively washed in 50 mL of ice-cold H_2O, collected by centrifugation, and resuspended into 100 μL of ice-cold S-buffer [20 mM Tris-HCl (pH 8.0), 50 mM ammonium acetate, 2 mM EDTA, 0.5 mM bacitracin, 0.5 mM leupeptin, 2 mM PMSF] per 7.5 A_{600} units of cells. The cell suspension was transferred to a 1.5 mL microcentrifuge tube containing 100 μL of glass beads [acid-washed glass beads; 425−600 μm, (Sigma, St. Louis, MO)] and 100 μL of ice-cold 20% TCA per 7.5 A_{600} units of cells. The cells were disrupted by vigorous vortexing for 10 min at 4 °C, after which the supernatant was withdrawn. The second aliquot was obtained after the glass beads were washed with 500 μL of an ice-cold, 1:1 mixture of 20% TCA and S-buffer. The combined samples were pelleted by centrifugation at 14 000 rpm for 10 min at 4 °C and resuspended into 10 μL of loading buffer [120 mM Tris-HCl (pH 6.8), 8 mM EDTA, 3.5% (w/v) SDS, 14% (v/v) glycerol, 1 mM PMSF, 5% (v/v) β-mercaptoethanol] per A_{600} units of cells. The samples were successively boiled for 10 min and centrifuged at 14 000 rpm for 10 min, after which 25 μL of the supernatant was used for SDS−PAGE and Western blotting. The blot was blocked with 5% non-fat dry milk and incubated with either the polyclonal antiserum SP197 (recognizing AR amino acid residues 1−20; Kuiper et al., 1993), a monoclonal antibody against the GAL4 TAD (Clontech, Palo Alto, CA), or a monoclonal antibody against GAL4 DBD (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were visualized by chemiluminescence using the "Renaissance" Western blotting kit (DuPont/NEN, Boston, MA).

CHO Cell Culture, Transfection, and LUC Assay. Chinese hamster ovary (CHO) cells were maintained in Dulbecco's modified Eagle's DME/F12 culture medium, supplemented with 5% dextran-coated charcoal-treated fetal calf serum. For transfection experiments, CHO cells were plated at a density of 1.5×10^5 cells/well (10 cm^2) and grown for 24 h. Transfections were performed according to the calcium phosphate coprecipitation method as described previously (Veldscholte et al., 1992a,b). Cells were transfected with AR expression plasmids pSVARo (25 ng), pSVAR-L (25 ng), pSVAR(TAD) (1 μg), pSVAR(DBD)(LBD) (60 ng), or pSVAR(DBD)(LBD-L) (60 ng), or a combination of these constructs, and 500 ng of the MMTV-LUC reporter plasmid

(kindly provided by Dr. Dijkema, Organon, Oss, The Netherlands). Carrier DNA (pTZ19) was added in each case to a total of 5 μg per well. After transfection, the cells were washed and AR ligands were added. Following 24 h incubation, cells were harvested for the luciferase (LUC) assay, as described previously (Kuil et al., 1995).

Immunoaffinity Purification and Immunoblot Analysis of Proteins Expressed in CHO Cells. For analysis of protein products of the various AR expression plasmids, CHO cells were plated at 1.5×10^6 cells/175 cm^2 and transiently transfected, using the calcium phosphate precipitation method, with pSVARo (20 μg), pSVAR(TAD) (20 μg), pSVAR-(DBD)(LBD) (80 μg), or a combination of pSVAR(TAD) and pSVAR(DBD)(LBD) (20 and 80 μg, respectively). After 48 h cytosol was prepared. For this, cells were washed once in ice-cold PBS and harvested by scraping in 1 mL of ice-cold buffer A [10 mM sodium phosphate, 1.2 mM EDTA, 12 mM 1α-thioglycerol, 10 mM DTT, 10% (v/v) glycerol, 10 mM Na_2MoO_4, 0.6 mM PMSF, 0.25 mM leupeptin, 0.5 mM bacitracin (pH 7.4)]. The cells were lysed by three cycles of freeze−thawing (freezing in liquid nitrogen, thawing at 10 °C) and centrifuged for 10 min at 400 000*g*. The supernatant was used for immunoprecipitation with either the monoclonal antibody F39.4.1 (directed against AR amino acid residues 301−320; Zegers et al., 1991) or F52.24.4 (directed against amino acid residues 593−612) as described previously (Veldscholte et al., 1992a,b). For the immunoprecipitation of AR(DBD)(LBD) with F52.24.4, 300 μL of cytosol was used, and NaCl was added to a final concentration of 0.5 M. Immunoprecipitation of the full-length AR and AR(TAD) was done with 300 μL and 100 μL cytosol samples, respectively. Subsequent to SDS−PAGE and Western blotting, the membrane was blocked with 5% non-fat dry milk and incubated with the polyclonal antisera SP197 or SP066 (directed against AR amino acid residues 892−910) (Zegers et al., 1991). The proteins were visualized by the chemiluminescence method.

RESULTS

Expression and Transactivating Activity of the Wild-Type and Mutated Androgen Receptors in S. cerevisiae. To evaluate the transactivating properties of the wild-type AR in yeast, the complete protein coding sequence was inserted into the yeast expression vector pG1, yielding the expression plasmid pG1AR-II (see Materials and Methods). Similar expression vectors were made for AR-L (pG1AR-L) and AR-(TAD)(DBD) (pG1AR-5). AR-L contains the T868A substitution in the AR(LBD), which alters the ligand specificity of the receptor, as previously shown in ligand binding and transactivation experiments in mammalian cells (Veldscholte et al., 1990). AR(TAD)(DBD) lacks the LBD and is constitutively active in mammalian cells. In mammalian cells, full-length AR and AR(TAD)(DBD) use different, largely separated TADs (TAU-1 and TAU-5, respectively), both located within the amino-terminal domain (Jenster et al., 1995).

Protein expression was assessed by immunoblot analysis of extracts of strain BJ2168, transiently transfected with either pG1AR-II, pG1AR-L, or pG1AR-5, using the AR-specific polyclonal antibody SP197. The antibody detected immunoreactive proteins of the appropriate lengths [110 kDa for full-length AR and AR-L and 80 kDa for AR(TAD)-(DBD)] (Figure 1A). The expression level of AR(TAD)-(DBD) was slightly lower than that of the full-length proteins.

Next, the transactivating activities of the three different proteins were determined. As depicted in Figure 1B, in the absence of ligand, neither wild-type AR nor AR-L was active. In contrast, AR(TAD)(DBD) was very efficient in activation of the reporter gene. Addition of dihydrotestosterone (DHT; 10^{-6} M), however, activated both full-length AR and AR-L. The level of activation was approximately 3-fold lower than AR(TAD)(DBD) activity, which, as expected, was essentially not affected by the addition of DHT to the culture medium. Our data indicated that both TAU-1 and TAU-5 were active in yeast, albeit that TAU-5 activity was more prominent under the conditions tested.

In a subsequent series of experiments, dose−response curves to different ligands were determined for full-length AR and AR-L. For most ligands, in the yeast system an approximately 10-fold higher concentration was required than in mammalian cells to obtain maximal activity (Figure 1C). This might be due to limited permeability of the different ligands in yeast or due to differences in active transport mechanisms (Kralli et al., 1995). Progesterone (Pg) seemed relatively more active in yeast than the other steroids tested. The activation of full-length AR and AR-L by the androgenic compounds R1881 and DHT was comparable (see Figure 1C). Approximately 100-fold more ligand was required for strong activation of the full-length, wild-type AR by the progestins Pg and R5020 as compared to activation of AR-L. For estradiol- (E2-) dependent activation of wild-type AR and AR-L the difference was small. So, although the yeast system was slightly less selective, in general these findings were in agreement with those previously found in HeLa cells transiently transfected with a wild-type AR or AR-L expression vector (Veldscholte et al., 1990) and those observed in CHO cells, as presented below. In conclusion, the yeast system was found to be suitable for the study of AR functioning.

Transactivating Activity of GAL4−Androgen Receptor Chimeric Proteins in S. cerevisiae. A yeast two-hybrid protein−protein interaction assay, as originally described by Fields and Song (1989), was used to study associations between the different functional AR domains. The applied two-hybrid system involved the activation of an integrated UAS_{GAL1}-lacZ reporter gene. One set of constructs expressed chimeric AR(TAD) (amino acid residues 3−494) proteins, fused to the GAL4(DBD) or the GAL4(TAD). Similar vectors were constructed for the AR(LBD) (amino acid residues 652−910) linked to GAL4(DBD) or GAL4(TAD).

We first assayed whether all components were properly expressed in Y190 yeast cells. To this end, lysates of yeast cells were analyzed by Western blotting, using antibodies directed against the GAL4 part of the fusion proteins, and, in selected cases, against the AR parts. The results are shown in Figure 2A. Immunoblotting with the antibody against the GAL4(TAD) showed a comparable expression level of the GAL4(TAD)AR(TAD) and GAL4(TAD)AR(LBD) chimeric proteins (lanes 1 and 2). Using the antibody against the GAL4(DBD), GAL4(DBD)AR(LBD) could be visualized (lane 4) but not GAL4(DBD)AR(TAD) (lane 3), indicating a lower expression level of the latter. However, GAL4-(DBD)AR(TAD) was detectable with an antibody against the AR(TAD) (lane 6). The GAL4(DBD)AR(TAD) expres-

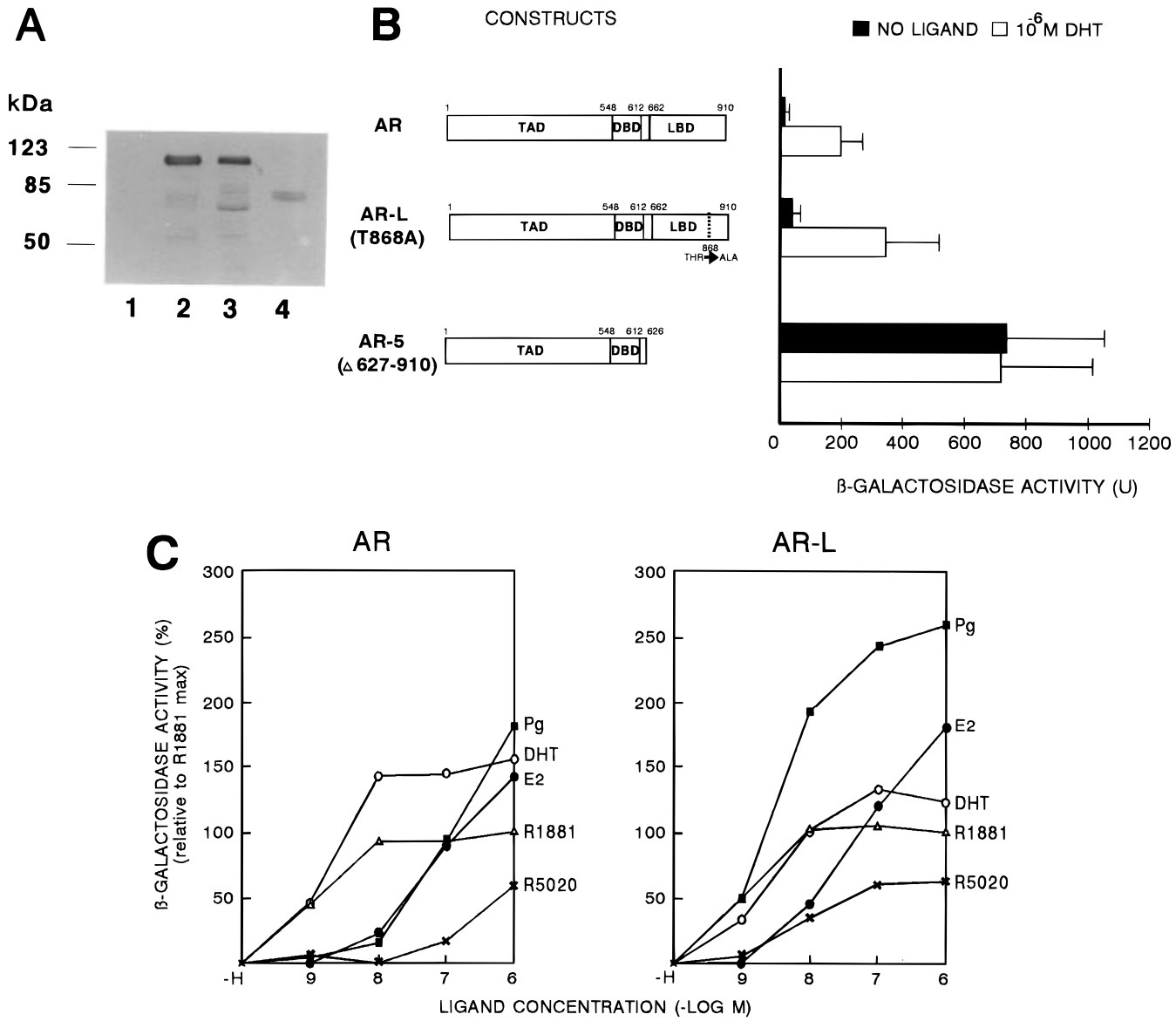

FIGURE 1: Protein expression and transcriptional activity of wild-type and mutant ARs in *S. cerevisiae* BJ2168. (A) Expression levels of wild-type AR (lane 2), the mutant AR-L (lane 3), and the mutant AR-5 (Δ627−910) (lane 4) were tested in cell-free extracts of yeast, transformed with the respective plasmids, by immunoblotting with the polyclonal antiserum SP197 (against amino acid residues 1−20 of the AR) as described in Materials and Methods. Lane 1 represents the mock-transformed yeast. Molecular mass standards (kDa) were run on a parallel lane. (B) β-Galactosidase activity was determined in yeast transformed with the indicated AR expression plasmid and the androgen-inducible reporter plasmid UCΔSS-26X and incubated without hormone or in the presence of DHT (10^{-6} M). β-Galactosidase values represent the mean (±SEM) of three separate determinations. (C) Dose−response curves of activation of the wild-type AR and mutant AR-L in the presence of R1881, DHT, E2, Pg, or R5020. β-Galactosidase activity in the presence of 10^{-6} M R1881 was set at 100%. Values represent the mean of three separate determinations.

sion level was slightly lower than that of the GAL4(TAD)-AR(TAD) chimeric protein (lane 7; see also lane 1).

Next, we investigated whether any of these chimeric proteins could activate GAL1 promoter-driven LacZ expression, if expressed individually (see Figure 2B). Apparently, no significant β-galactosidase activity was observed in yeast carrying either one of the AR(LBD) fusion proteins in the absence or presence of DHT. Not surprisingly, expression of GAL4(DBD)AR(TAD) markedly increased reporter gene expression. The transactivating activity of the GAL4(DBD)-AR(TAD) chimeric protein was found to be approximately 20% of wild-type GAL4 activity, as measured under the same conditions. No significant β-galactosidase activity was observed in yeast cells carrying GAL4(TAD)AR(TAD). This fusion protein lacks UAS binding potential and consequently fails to localize to the GAL1-lacZ upstream promoter sequences.

In Vivo Ligand-Dependent Androgen Receptor LBD−TAD Interaction in S. cerevisiae. We next assessed in the two-hybrid system the putative tethering of the GAL4(TAD)-AR(TAD) chimer to the UAS_{GAL1}-bound GAL4(DBD)AR-(LBD). In the absence of DHT, no significant β-galactosidase activity was observed in lysates of Y190 cells, which coexpressed both fusion proteins (see Figure 3). However, DHT (10^{-6} M) markedly induced transcription of the LacZ reporter gene. Similar results were obtained, using a construct containing LBD-L(T868A) in the GAL4(DBD)-AR(LBD) chimer (data not shown). Cotransfection of either one of the chimeric constructs with the basic GAL4 vectors, lacking AR sequences, did not result in significant β-galac-

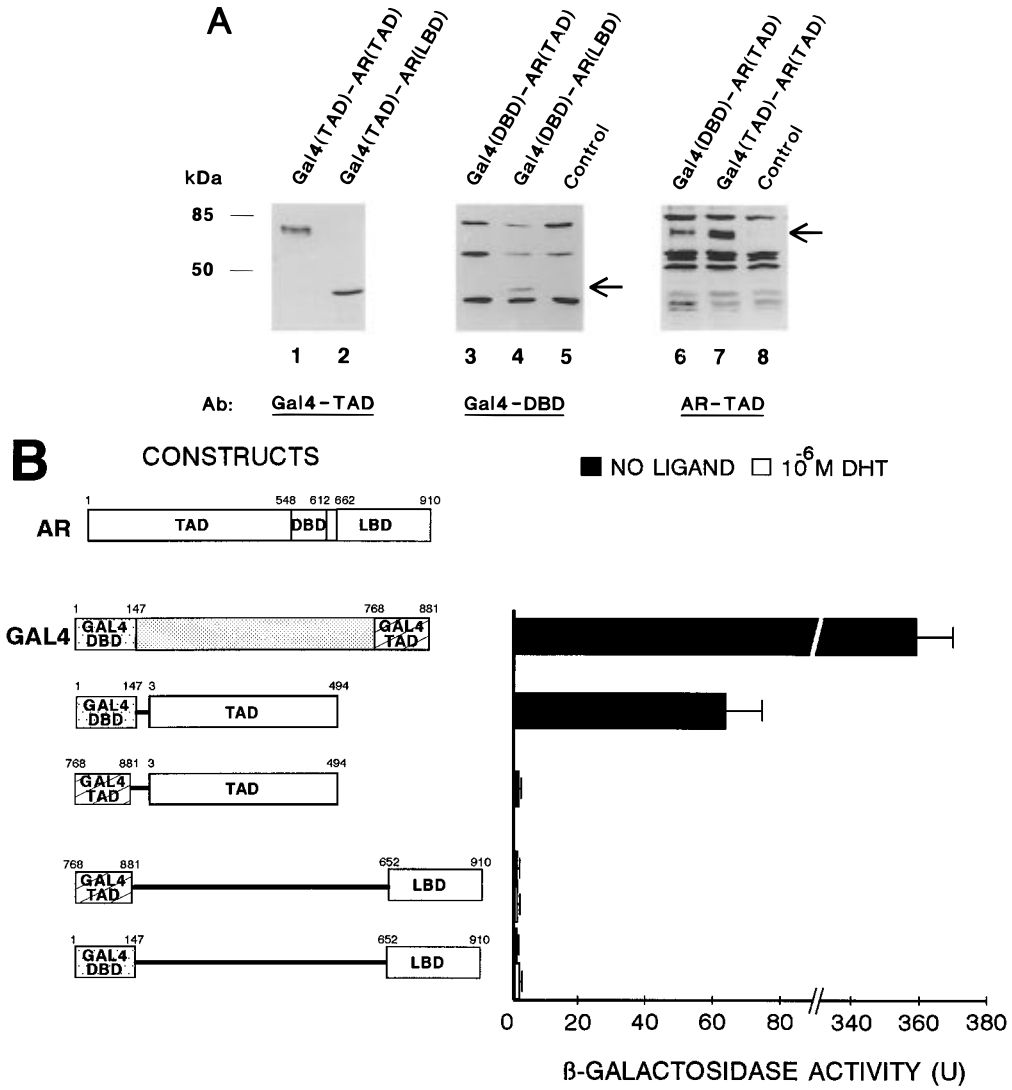

FIGURE 2: Protein expression and transcriptional activity of separate GAL4 fusion proteins in *S. cerevisiae* Y190. (A) Expression levels of the various GAL4 fusion proteins (indicated above the lanes) were determined in cell-free extracts of yeast, transformed with the respective expression plasmids, by immunoblotting with antisera recognizing respectively the GAL4 transcription activation domain (TAD) (lanes 1 and 2), the GAL4 DNA binding domain (DBD) (lanes 3−5), or the AR-TAD (lanes 6−8) as described in Materials and Methods. Lanes 5 and 8 represent mock-transformed yeast. Arrows indicate specific GAL4 fusion protein bands. Molecular mass standards (kDa) were run on a parallel lane. (B) β-Galactosidase activity as determined in yeast strain Y190, containing the integrated UAS_{GAL1}-lacZ reporter gene, and transformed with the expression plasmids for GAL4 or GAL4 fusion proteins. Yeast cells were incubated in the absence or presence of DHT (10^{-6} M). β-Galactosidase values represent the mean (±SEM) of three separate determinations.

tosidase activity (data not shown). Taken together, these results indicate an *in vivo* androgen-dependent association of the amino-terminal TAD and the LBD of the AR. Coexpression of GAL4(DBD)AR(LBD) and GAL4(TAD)-AR(LBD) did not result in the formation of a functionally active complex under the conditions tested.

To evaluate the ligand specificity, and thus obtain additional evidence for the physiological significance of the AR TAD−LBD interaction, the dose−response curves to a variety of ligands were determined (Figure 4). Experiments were done with both wild-type LBD and LBD from AR-L. Both R1881 and DHT were capable of inducing functional wild-type and AR-L LBD−TAD interactions with a similar affinity, although the absolute transcriptional activity induced by the AR-L interaction was slightly higher (data not shown). In the protein−protein interaction assay, identical ligand concentrations were required to obtain similar levels of reporter gene activity as compared to the full-length AR (or AR-L) (see also Figure 1C).

The progestins Pg and R5020 (almost completely) failed to induce the wild-type TAD−LBD interaction, whereas a small induction of β-galactosidase activity was observed in response to the highest E2 concentration tested (10^{-6} M). However, E2, R5020, and, very efficiently, Pg readily induced the AR-L TAD−LBD interaction. Summarizing, these findings clearly point out the ligand dependency of the functional AR LBD−TAD interaction *in vivo*. Furthermore, the agonistic activity of E2, Pg, and R5020 on induction of the AR-L LBD−TAD interaction substantiated the physiological importance of the observations. Interestingly, high concentrations of the ligands Pg, R5020, and E2 were not sufficient to establish wild-type AR LBD−TAD interaction, although the full-length, wild-type receptor could be activated by these ligands (compare Figures 1C and 4). This apparent discrepancy might be contributed to differences in interaction kinetics between separate and linked protein domains and a low-affinity ligand.

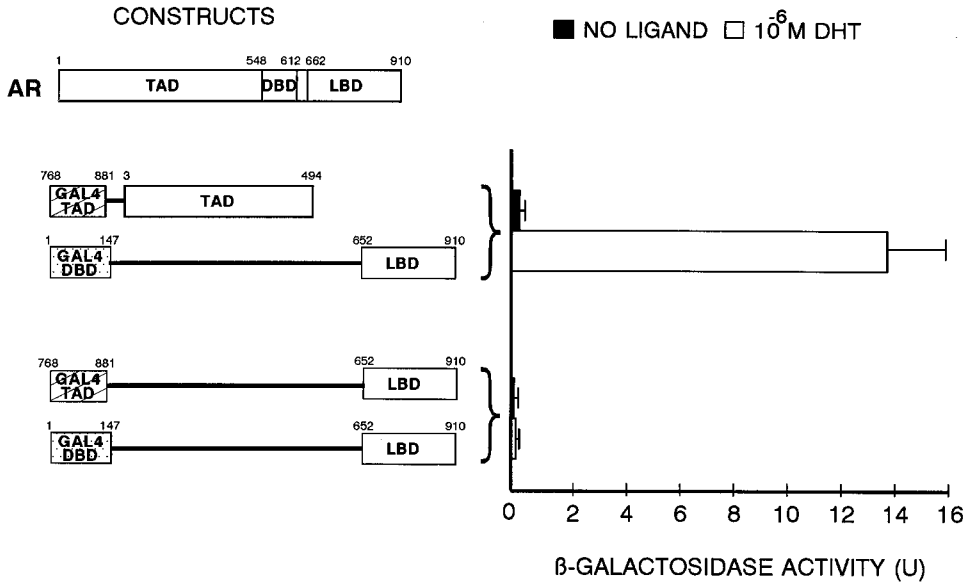

FIGURE 3: Transcriptional activity of coexpressed GAL4 fusion proteins in *S. cerevisiae* Y190. Yeast strain Y190, containing the integrated UAS_{GAL1}-lacZ reporter gene, was transformed with the expression plasmids encoding the indicated GAL4 fusion proteins. β-Galactosidase activity was determined after incubation in the absence or presence of DHT (10^{-6} M). β-Galactosidase values represent the mean (±SEM) of three separate determinations.

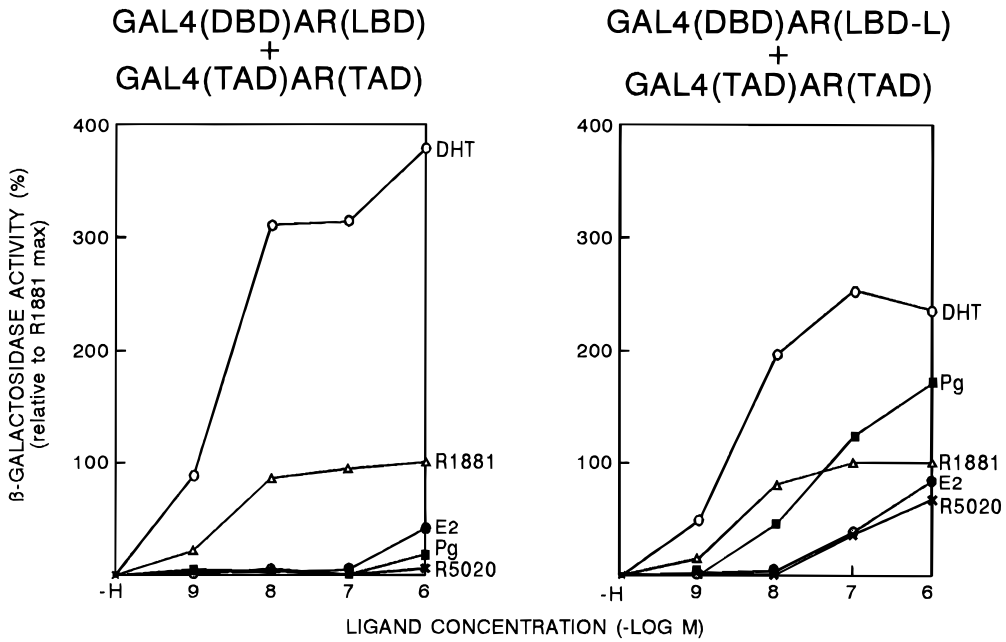

FIGURE 4: Effects of various ligands on transcriptional activity of coexpressed GAL4(TAD)AR(TAD) and GAL4(DBD)AR(LBD) or GAL4-(DBD)AR(LBD-L) fusion proteins in *S. cerevisiae* Y190. The yeast strain, containing the integrated UAS_{GAL1}-lacZ reporter gene, was transformed with the expression plasmids, encoding the indicated GAL4 fusion proteins. β-Galactosidase activity was determined upon incubation with increasing concentrations of either R1881, DHT, E2, Pg, or R5020. β-Galactosidase activity in the presence of 10^{-6} M R1881 was set at 100%. Values represent the mean of three separate determinations.

Because the GAL4(DBD)AR(TAD) fusion protein showed intrinsic transactivating properties (Figure 2), we addressed the question whether AR(TAD) by itself was active in the two-hybrid system, if coexpressed with GAL4(DBD)AR-(LBD). As illustrated in Figure 5, AR(TAD) and GAL4-(DBD)AR(LBD) were inactive when expressed separately. In contrast, in the coexpression system, a clear DHT-induced β-galactosidase activity could be observed. The activity was less than the GAL4(TAD)AR(TAD) fusion protein activity (approximately 50%) (Figure 3). This indicates that both AR(TAD) and GAL4(TAD) might contribute to the activity in GAL4(TAD)AR(TAD).

In Vivo Ligand-Dependent Androgen Receptor LBD−LBD Interaction in S. cerevisiae. The high intrinsic transactivating activity of GAL4(DBD)AR(TAD) (see Figure 2) excluded a reliable study of interactions between two AR TADs in the yeast two-hybrid system. This high background problem could not be overcome by overexpression of GAL4(TAD)-AR(TAD), because high concentrations of this protein turned out to be toxic to the yeast cells.

A similar approach was applied to assess a putative interaction between two AR LBDs. As illustrated in Figure 3, such a functional association could not be observed with

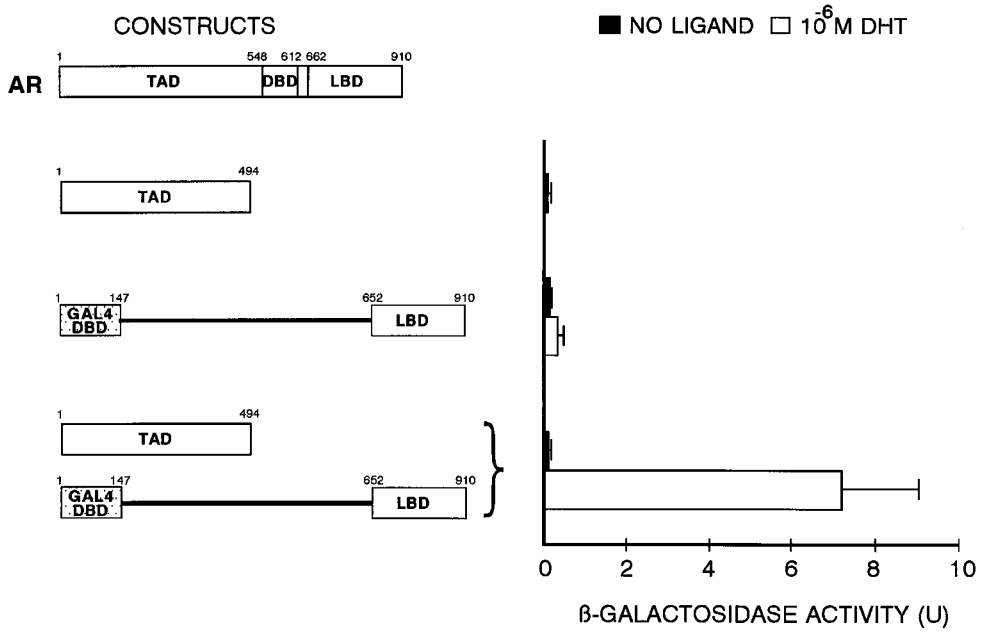

FIGURE 5: Transcriptional activity of coexpressed AR(TAD) and GAL4(DBD)AR(LBD) proteins in *S. cerevisiae* Y190. β-Galactosidase activity was determined in yeast strain Y190, containing the integrated UAS_{GAL1}-lacZ reporter gene, and transformed with the expression plasmid(s) encoding the indicated proteins. β-Galactosidase values were measured upon incubation in the absence or presence of DHT (10^{-6} M) and represent the mean (±SEM) of three separate determinations.

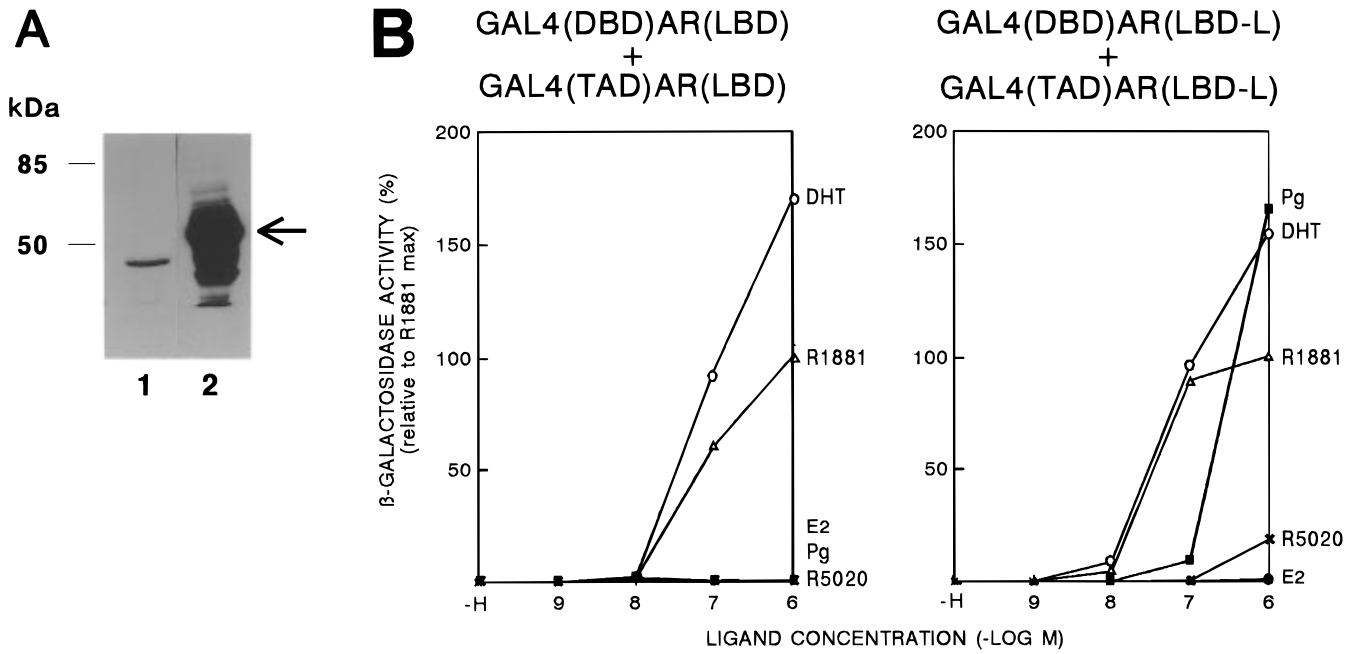

FIGURE 6: GAL4(TAD)AR(LBD) protein expression levels and transcriptional activity of ligand-dependent LBD−LBD interaction in *S. cerevisiae* Y190. (A) Expression levels of GAL4(TAD)AR(LBD) in cell-free extracts of yeast, either transformed with the low- (lane 1) or high- (lane 2) expression vector. Proteins were visualized by immunoblotting with the monoclonal antibody against GAL4 TAD as described in Materials and Methods. Molecular mass standards (kDa) were run on a parallel lane. (B) The yeast strain Y190, containing the integrated UAS_{GAL1}-lacZ reporter gene, was transformed with the indicated low-expression plasmids, encoding the GAL4(DBD) fusion proteins, and the high-expression plasmids, encoding the GAL4(TAD) fusion proteins. β-Galactosidase activity was determined upon incubation with increasing concentrations of either R1881, DHT, E2, Pg, or R5020. β-Galactosidase activity in the presence of 10^{-6} M R1881 was set at 100%. Values represent the mean of three separate determinations.

low-expression vectors. Evidently, putative LBD dimers would be constituted of a mixture of transcriptionally inactive GAL4(DBD)AR(LBD) and GAL4(TAD)AR(LBD) homodimers and transcriptionally active GAL4(DBD)AR(LBD)/GAL4(TAD)AR(LBD) heterodimers. We hypothesized that overexpressing the GAL4(TAD)AR(LBD) chimer would favor its tethering to the UAS_{GAL1}-bound GAL4(DBD)AR(LBD), thereby more efficiently competing with the formation of transcriptionally inactive GAL4(DBD)AR(LBD) homodimers at the promoter site. An AR(LBD) cDNA fragment was cloned in the high-expression vector pACT2 (see Materials and Methods). Western blot analysis of the chimeric protein showed a 20−50-fold higher expression as compared to the originally applied vector (Figure 6A). The chimeric protein migrated slightly slower than the comparable protein expressed from pGAD424, due to the presence of the HA epitope and flanking amino acid residues in this construct (see Materials and Methods).

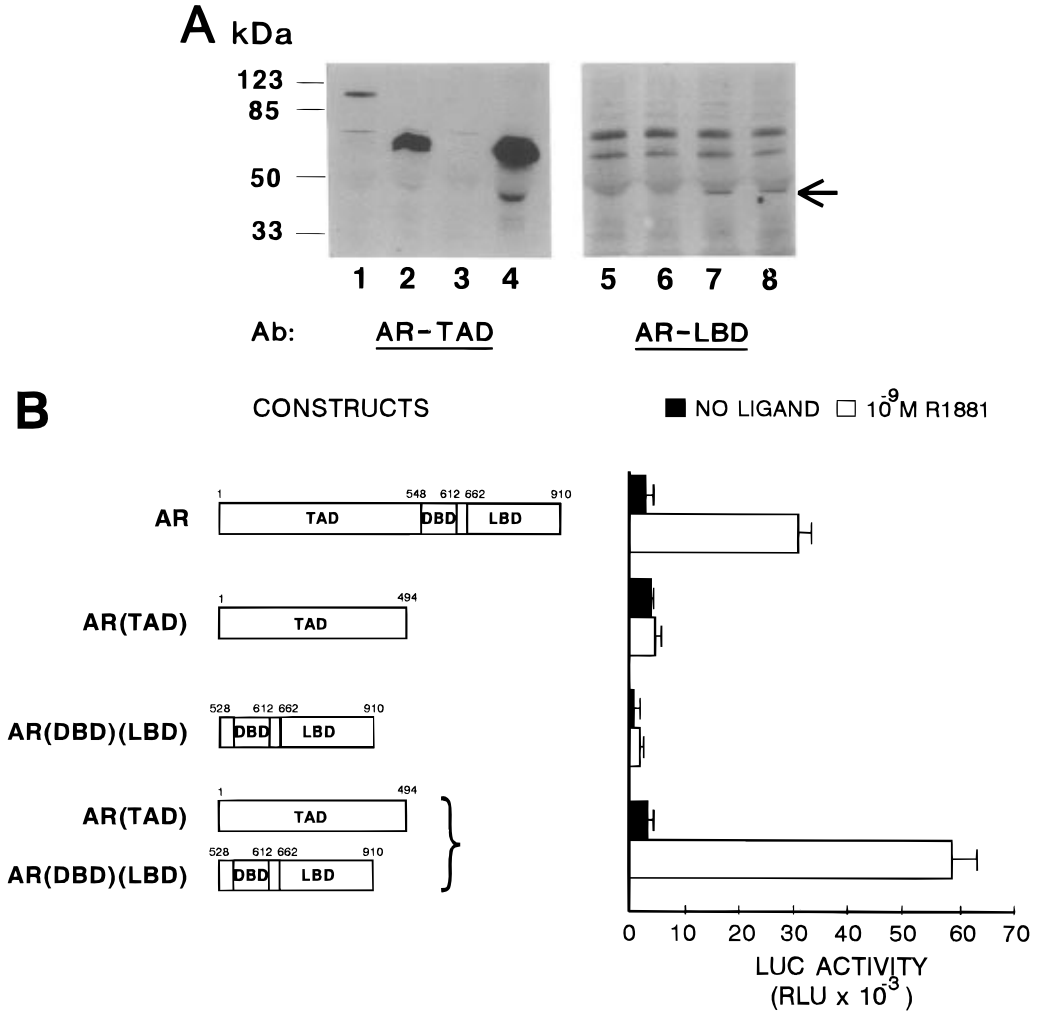

FIGURE 7: Protein expression and transcriptional activity of the AR fragments AR(TAD) and AR(DBD)(LBD), as compared to full-length AR in transiently transfected CHO cells. (A) Cytosols of transfected CHO cells were immunoprecipitated with antibody F39.4.1 and immunoblotted with antiserum SP197 [both against AR(TAD)] (lanes 1−4) or immunoprecipitated with antibody F52.24.4 [against AR-(DBD)] and immunoblotted with antiserum SP66 [against AR(LBD)] (lanes 5−8). Lanes: 1 and 5, full-length AR; 2 and 6, AR(TAD); 3 and 7, AR(DBD)(LBD); 4 and 8, AR(TAD) + AR(DBD)(LBD). The arrow indicates the specific AR(DBD)(LBD) fragment in lanes 7 and 8. Molecular mass standards (kDa) were run on a parallel lane. (B) LUC expression was determined in CHO cells transiently cotransfected with the indicated AR expression plasmid(s) and the reporter plasmid MMTV-LUC. After transfection, cells were incubated without hormone or with R1881 (10^{-9} M) for 24 h. Values of a representative experiment are shown and represent the mean (±SEM) of triplicate determinations.

Y190 cells cotransfected with pGAL4(DBD)AR(LBD) and the high-expression vector pACT2-GAL4(TAD)AR(LBD) showed no significant β-galactosidase activity in the absence of DHT (see Figure 6B). However, increasing concentrations of DHT or R1881 clearly induced LacZ transcription. Similar results were obtained with AR-L LBD constructs. However, compared to the AR TAD−LBD interaction (Figure 3), AR(LBD) dimer formation required not only a much higher GAL4(TAD)AR(LBD) concentration but also a higher ligand concentration for a maximal response. The absolute level of maximal β-galactosidase activity measured for LBD−LBD interaction with the high-expression vector for GAL4(TAD)AR(LBD) was comparable to the level measured for TAD−LBD interaction using a low-expression GAL4(TAD)AR(TAD) vector (Figure 3). For wild-type AR-(LBD), no interactions were found with the low-affinity ligands E2, Pg, and R5020. In contrast, Pg, was clearly able to stimulate the LBD-L/LBD-L interaction, reflecting the alteration of ligand specificity in LBD-L.

In Vivo Ligand-Dependent AR TAD−LBD Interaction in CHO Cells. The interaction between the AR TAD and LBD was also examined in a mammalian cell protein−protein interaction system. To this end, two expression vectors were constructed, encoding the AR(TAD) and the AR(DBD)-(LBD), respectively (see Materials and Methods).

Protein expression was assessed by immunoaffinity purification and Western blot analysis of cytosol of CHO cells, transiently transfected with expression plasmids encoding full-length AR, AR(TAD), and AR(DBD)(LBD), using AR-specific antibodies. Figure 7A shows the immunodetection of proteins of the appropriate length for the full-length AR and the receptor fragments AR(TAD) and AR(DBD)(LBD) (110, 65, and 48 kDa, respectively). Although the expression levels differed markedly, the various proteins could be clearly visualized. Expression levels of the receptor fragments AR-(DBD)(LBD) and especially AR(TAD) were high, as compared to full-length AR expression (compare lanes 1, 2, and 4 with 5, 7, and 8, respectively).

To study protein−protein interactions, expression plasmids encoding AR(TAD) and AR(DBD)(LBD) were transiently transfected to CHO cells, together with the AR-responsive reporter plasmid MMTV-LUC. LUC activity was measured in cells incubated in the presence and in the absence of 10^{-9} M R1881 (Figure 7B). Separate expression of AR(TAD)

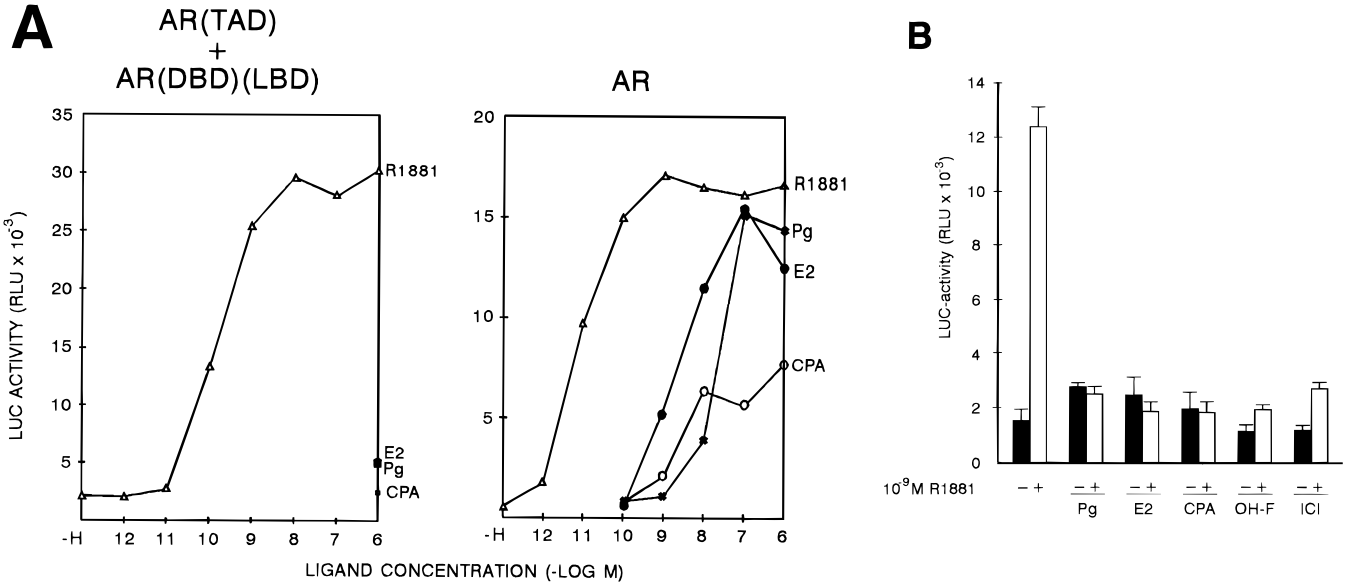

FIGURE 8: Transcriptional activity of the full-length AR and seperate AR(TAD) and AR(DBD)(LBD) fragments in the presence of various ligands. LUC expression was determined in CHO cells transiently cotransfected with the indicated AR expression plasmid(s) and the reporter plasmid MMTV-LUC. After transfection, cells were incubated for 24 h without hormone (−H) or in the presence various ligands. (A) Dose−response curves of R1881, Pg, E2, and cyproterone acetate (CPA) with wild-type AR and separate AR(TAD) and AR(DBD)-(LBD) proteins. (B) For determination of steroid specificity of AR(TAD) and AR(DBD)(LBD) interaction, the cells were incubated in the presence of Pg (10^{-6} M), E2 (10^{-6} M), CPA (10^{-6} M), hydroxyflutamide (OH-F, 10^{-6} M), and bicalutamide (ICI, 10^{-6} M), respectively, either in the absence (−) or in the presence (+) of 10^{-9} M R1881. Values of a representative experiment are shown and represent the mean (±SEM) of triplicate determinations.

or AR(DBD)(LBD) did not result in ligand-induced LUC activity. However, coexpression of AR(TAD) and AR-(DBD)(LBD) resulted in R1881-induced LUC activity to a similar level as found for the full-length AR (Figure 7B). These results extend the observations made in the yeast system and indicate that a direct or indirect interaction between the AR(TAD) and AR(DBD)(LBD), which results in the formation of a transcriptionally active complex, can also occur in mammalian cells. In the experiment shown, a high-expression level of AR(TAD) was used, but functional complex formation was also observed at lower AR(TAD) levels, albeit less efficiently (data not shown). In the AR-(TAD) expression construct, a nuclear localization signal is lacking. As a consequence, AR(TAD) has to be transported to the nucleus by complex formation with AR(DBD)(LBD) or by diffusion, which might be less efficient (Jenster et al., 1993).

In a next series of experiments, we determined the dose−response curves to several ligands on the formation of a functional complex between AR(TAD) and AR(DBD)(LBD) (Figure 8A). R1881 induced LUC activity in a dose-dependent manner. Similar activities were observed with DHT or T (data not shown). The maximal response was reached at a 10-fold higher ligand concentration, as compared to the dose−response curve for the full-length AR (Figure 8A).

The full-length AR could be (partially) activated by high concentrations of E2, Pg, or the antiandrogen cyproterone acetate (CPA). However, these low-affinity ligands were unable to stimulate the formation of a transcriptionally active complex in cells cotransfected with the AR(TAD) and AR-(DBD)(LBD) expression plasmids. The absence of induction of LUC activity was not due to a loss of hormone binding by AR(DBD)(LBD), because all compounds, including hydroxyflutamide and bicalutamide, could inhibit the LUC activity induced by R1881 (Figure 8B).

In order to investigate the effect of the altered steroid specificity of the mutant AR-L on the interaction of AR-(TAD) and AR(DBD)(LBD), the T868A mutation was introduced into the AR(DBD)(LBD) expression plasmid. As expected, stimulation of the MMTV-LUC reporter was induced by R1881, DHT, and T in CHO cells (Figure 9 and data not shown). However, as found for wild-type AR(LBD) constructs, maximal reporter gene activity was detected at somewhat higher ligand concentrations, as compared to the full-length AR. CPA, E2, and Pg were able to induce LUC activity; however, bicalutamide still acted as a full antagonist (Figure 9 and data not shown). These results paralleled the activating and inhibitory properties of these ligands with the full-length AR-L in CHO cells, albeit at higher ligand concentrations.

DISCUSSION

In this study we assessed the *in vivo* association between the AR TAD and the AR LBD, leading to the formation of a transcriptionally active complex. Experiments were performed in both yeast and mammalian protein−protein interaction systems. The functional interaction was strictly hormone dependent and could be blocked by antiandrogens. The TAD−LBD association was also observed in the AR mutant T868A (AR-L). This mutant AR was originally found in the prostate cancer cell line LNCaP and can also be activated by estrogens, progestins, and several antiandrogens (Veldscholte et al., 1990). The data collected indicate that the TAD−LBD interaction is of physiological relevance, because it can occur at low ligand concentrations and at low expression levels of the various components. AR LBD−LBD interactions could be measured in the yeast two-hybrid system at high ligand concentrations and at high-expression level of the GAL4(TAD)AR(LBD) chimeric protein. Our findings are summarized in the schematical representation depicted in Figure 10. We propose an

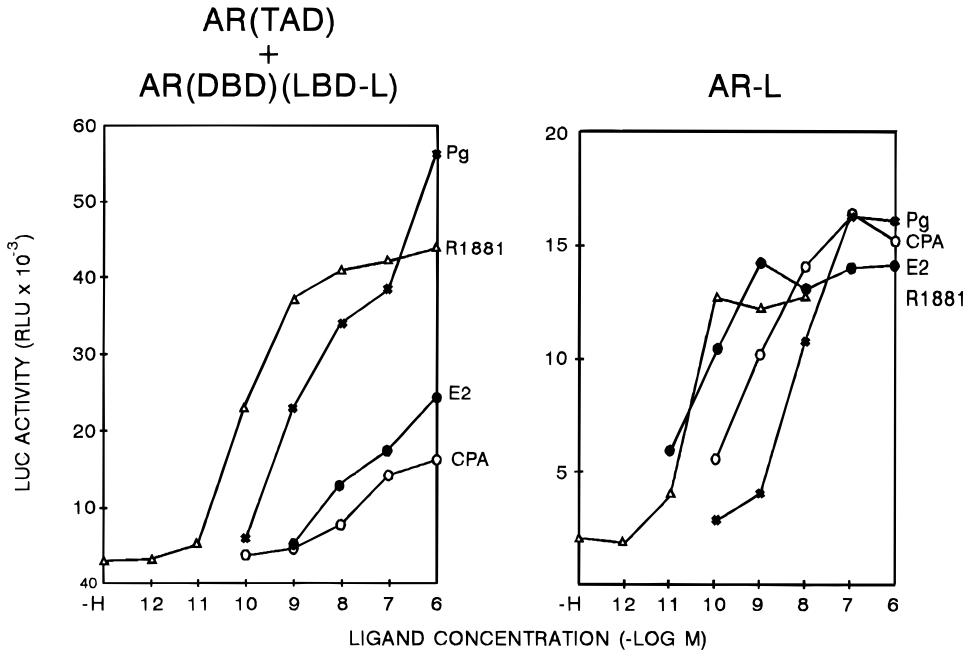

FIGURE 9: Dose−response curves of transcriptional activity of the mutant AR-L and separate AR(TAD) and AR(DBD)(LBD-L) in the presence of various ligands. LUC expression was determined in CHO cells transiently cotransfected with the indicated AR expression plasmid(s) and the reporter plasmid MMTV-LUC. After transfection, cells were incubated for 24 h without hormone (−H) or in the presence of R1881, Pg, E2, and CPA, respectively. Values of representative experiments are shown and represent the mean (±SEM) of triplicate determinations.

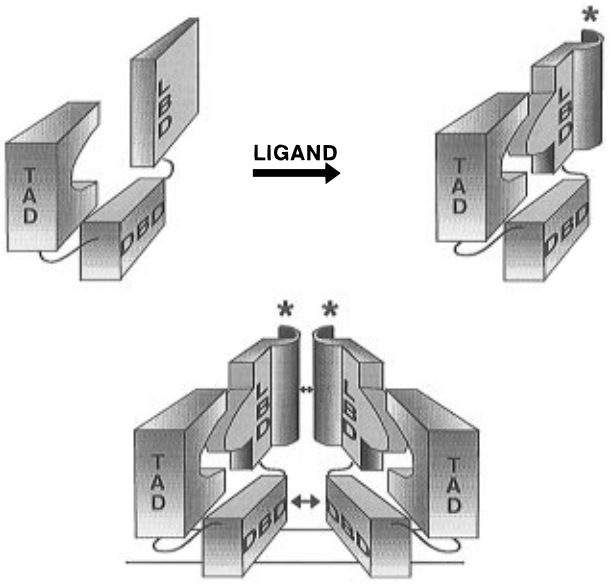

FIGURE 10: Model of functional interactions between AR domains. Binding of androgen induces conformational changes in the ligand binding domain (LBD) of the AR, leading to a transcriptionally functional interaction between the transcription activation domain (TAD) and ligand binding domain. Upon DNA binding, the DNA binding domains (DBD) and the ligand binding domains of the receptor dimer interact with their counterparts.

intramolecular, direct or indirect interaction between AR TAD and LBD. In addition to a DBD−DBD interaction, a weaker intermolecular LBD−LBD interaction is presumed in AR homodimers binding to the cognate DNA binding site. This concept is comparable to the ER dimerization model (Kraus et al., 1995) but differs in several aspects from a recently proposed model for the AR, which postulates an intermolecular interaction between TAD and LBD, resulting in the binding of AR homodimers in an antiparallel orientation to the DNA binding site, without LBD−LBD association (Wong et al., 1993; Langley et al., 1995).

Interactions between two LBDs have been described for several members of the steroid receptor family, especially the ER and the PR, using different experimental approaches (Kumar & Chambon, 1988; Guiochon-Mantel et al., 1989; Fawell et al., 1990). The *in vivo* experiments presented here extend these observations to the AR. However, a drawback of the two-hybrid system is the occurrence of a competition between the formation of transcriptionally inactive and active LBD−LBD complexes. Therefore, the strength of the LBD−LBD interaction might be underestimated.

The formation of LBD−LBD complexes might also affect the efficacy of TAD−LBD interactions. On the one hand, this could result in the underestimation of the strength of the TAD−LBD interaction, because of a competition between the formation of functionally inactive LBD−LBD complexes and active TAD−LBD complexes. On the other hand, it might be that ligand-dependent LBD−LBD formation is a prerequisite for TAD−LBD association. However, the observation that functional TAD−LBD association can take place at low concentrations of AR(TAD) or GAL4-(TAD)AR(TAD) and low ligand concentrations argues against interference of LBD−LBD with TAD−LBD complex formation. This would imply involvement of different regions in the AR LBD in LBD−LBD and TAD−LBD interactions and/or that TAD−LBD association is much stronger than LBD−LBD association.

Obviously, it would be of interest to identify the regions of AR TAD and AR LBD directly involved in the interaction. Unfortunately, mutations in the LBD, in general, lead to loss of ligand binding; mutations in the AR amino-terminal domain might affect the transactivating capacity. Effects of TAD mutations in TAD−LBD associations can be studied in a two-hybrid system, if AR(TAD) is hooked to a much stronger TAD, which largely excludes the contribution of AR(TAD) to the total transactivating activity of chimeric AR(TAD) constructs. In GAL4(TAD)AR(TAD), both TADs

seem to contribute equally to the total transactivating capacity of the chimeric protein. Previously, we showed that deletion of the regions 1−188 or 370−528 in the full-length AR hardly affected the transactivating activity and that almost full activity was retained within the 101−370 fragment (Jenster et al., 1995). We interpreted this as the presence of a ligand-dependent TAD (TAU-1) in this region, which interacts with the basal transcription machinery. It seems reasonable to presume that ligand-dependent TAU-1 activity depends on TAD−LBD interaction. In this concept, the 101−370 region contains not only TAU-1 but also the TAD−LBD interaction domain. Recently, it has been described that deletion of the 14−150 region or 339−499 region would affect the formation of a transcriptionally active complex of GAL4(DBD)AR(LBD) and VP16(TAD)AR-(TAD) (Langley et al., 1995). However, both deletions will hardly affect the transactivating capacity of the full-length AR (Jenster et al., 1995). Elucidation of this apparent discrepancy with the above postulated hypothesis requires further investigation.

In a recent study, interaction between the amino-terminal and the carboxyl-terminal domains of the ER was described (Kraus et al., 1995). It was supposed that this interaction was required for synergistic cooperation between a TAD in the amino-terminal domain (AF-1) and one in the LBD (AF-2). In contrast to the ER, evidence for an AF-2 in the AR LBD is lacking (Jenster et al., 1991; Simental et al., 1991). Our current knowledge favors the concept that the TAD−LBD interaction in the AR is required for a conformational change in the TAD, which enables TAU-1 to interact with the basal transcription initiation complex. In this regard, it would be of interest to find out whether or not functional TAD−LBD interactions occur for the GR and PR, which have been shown to contain a ligand-dependent transactivating domain (AF-2) in the LBD (Hollenberg & Evans, 1988; Danielian et al., 1992).

From our experiments it cannot be deduced whether the functional TAD−LBD interaction is direct or indirect. So far, *in vitro* experiments with purified AR fragments aimed at investigation of direct protein−protein contacts were unsuccessful (data not shown). Although this suggests that the TAD−LBD interaction is indirect, it cannot be excluded that we were unable to mimic *in vitro* the *in vivo* conditions for direct interaction. Indirect interaction might involve specific proteins, or more general bridging factors or coactivators, associating with AR segments (Halachmi et al., 1994; Cavailles et al., 1994; Onate et al., 1995; Yeh & Chang, 1996). If bridging factors are involved in TAD−LBD interaction, their function must be conserved in yeast proteins.

Essentially, TAD−LBD interaction can be intramolecular and intermolecular. However, kinetics of intramolecular interaction seem favorable to those of intermolecular association. In this model, it has to be assumed that the DBD, which links the TAD to the LBD, does not interfere with such an interaction. Because of the readily exchangeable domain structure of steroid receptors, the absence of this interference by steric hindrance is a real possibility.

Functional TAD−LBD association cannot be detected with the low-affinity ligands Pg, E2, and antiandrogens, even at ligand concentrations up to 10^{-6} M. However, competition with high-affinity ligands does take place, reflected in the inhibition of the formation of a functionally active complex, as studied in CHO cells. Antiandrogen-bound AR exhibits ligand-induced conformational changes that are distinct from those induced by androgens (Kuil et al., 1995). Whether the aberrant conformation of the antiandrogen-bound LBD results in the inhibition of a TAD−LBD interaction or in an association between both domains, which is transcriptionally nonproductive, remains to be determined. For the ER, antiestrogen-induced changes in the LBD of the ER differ from those induced by estrogens (Beekman et al., 1993) and result in a transcriptionally nonproductive association of the TAD and LBD (Kraus et al., 1995). Evidently, our results suggest a novel mechanism of antiandrogen action: blockade of a functional interaction between the TAD and LBD in the full-length AR.

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