Evidence for an Anti-parallel Orientation of the Ligand-activated Human Androgen Receptor Dimer*

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Domain interactions of the human androgen receptor (AR) dimer were investigated using a protein-protein interaction assay in which the NH2- and carboxyl-terminal regions of human AR were fused to the Saccharomyces cerevisiae GAL4 DNA-binding domain and herpes simplex virus VP16 transactivation domain to produce chimeric proteins. Transcriptional activation of a GAL4 luciferase reporter vector up to 100-fold was greater than Fos/Jun leucine zipper binding, indicating stable AR interaction between AR NH2-terminal residues 1–503 and steroid-binding domain residues 624–919 that was specific for and dependent on androgen binding to the steroid-binding domain and was inhibited by anti-androgen binding. Deletion mutagenesis within the NH2-terminal region indicated transactivation domain residues 142–337 were not required for dimerization, whereas deletions near the NH2 terminus (141–150) or NH2-terminal to the DNA-binding domain (159–499) reduced or eliminated the AR interaction, respectively. An NH2/NH2-terminal interaction was also observed, but no interaction was detected between ligand-free or bound steroid-binding domains. The results indicate that high affinity androgen binding promotes interactions between the NH2-terminal and steroid-binding domains of human AR, raising the possibility of an androgen-induced anti-parallel AR dimer.

The androgen receptor (AR) is a ligand-activated transcription factor that requires high affinity androgen binding to initiate a series of molecular events leading to specific gene activation required for male sex development. In its unliganded state, AR resides in the cytoplasm (1), where it rapidly degrades (2) and is regulated by a cytoplasmic DNA homologue (3). High affinity androgen binding slows AR degradation in a concentration-dependent manner, accounting at least in part for the physiological differences between the biologically active androgens (4). Androgen binding activates a bipartite nuclear targeting signal (5) and triggers receptor dimerization and acquisition of DNA binding that involves distal regions of the AR (6). Once activated, AR binds androgen response elements that resemble the simple consensus glucocorticoid response element (7) or more distinct, specific complex response elements (8–10). Little is known, however, about transcription factors that interact with AR during gene activation or the role of AR phosphorylation (11). That AR is crucial for specific gene regulation required for male sex development is demonstrated by an abundance of AR gene mutations that result in different degrees of impaired male sex development characteristic of the androgen insensitivity syndrome (12–15).

Steroid receptor dimerization is well documented (16–19) and apparently does not have a strict requirement for ligand or DNA binding, particularly with the estrogen (ER) (20–24) and progesterone receptors (25, 26). The glucocorticoid receptor forms ligand-dependent homodimers independent of DNA binding (27). A mutant progesterone receptor lacking the steroid-binding domain fails to dimerize in solution but activates a reporter gene, suggesting that receptor dimerization mediated through the steroid-binding domain is not a requirement for DNA binding and that dimerization after DNA binding is mediated by the DNA-binding domain (28). Direct evidence for receptor dimerization was revealed in electron micrographs showing dumbbell shaped glucocorticoid receptor monomers with globular NH2-terminal and steroid-binding domains and four-leaf clover shaped dimers. It was not established, however, whether dimer orientation was parallel or anti-parallel (29). Additional protein-protein interactions and alterations in DNA structure are indicated by increased gene activation following cooperative dimer binding to tandem hormone response elements (30). ER dimerization involves hydrophobic interactions between the steroid-binding domains (31) and, for thyroid hormone receptor α, regions outside the steroid-binding domain (32). In addition, a leucine zipper-like structure in the thyroid receptor ligand-binding domain mediates heterodimerization with the retinoic acid receptor (33).

Numerous recent studies have taken advantage of a protein-protein interaction assay developed originally in yeast (34) and later adapted for mammalian cells (35) that relies on the coexpression of two fusion proteins, each containing a protein or protein region coupled to a transcription factor functional domain. Stable protein-protein interactions bring together DNA binding and transactivation functions that regulate a reporter gene. Using this assay, we demonstrate an androgen-dependent interaction between the AR NH2- and carboxyl-terminal domains that raises the possibility of an anti-parallel oriented AR dimer.

EXPERIMENTAL PROCEDURES

Materials—The following reagents were purchased. Monkey kidney COS-1 and Chinese hamster ovary (CHO) cells were from the American Type Culture Collection (Rockville, MD); α minimum essential medium and pregestoned protein molecular weight standards were from Life Technologies, Inc.; Dulbecco’s modified essential medium with high glucose with or without phenol red was from JRH Biosciences; fetal calf serum was from Hyclone Laboratories (Logan, UT), and bovine calf

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1 The abbreviations used are: AR, androgen receptor; ER, estrogen receptor; CHO cells, Chinese hamster ovary cells; PCR, polymerase chain reaction; DHT, dihydrotestosterone; R1881, methyltrienolone; hydroxyflutamide, a,a,a-trifluoro-2-methyl-4-nitro-m-1-3-toluidide.
serum was from Irvine Scientific (Santa Ana, CA); AR PG-21 rabbit polyclonal AR anti-peptide antiserum was from Affinity Bioreagents, Inc. (Neshanic Station, NJ); [3H]methyltrienolone (17α-methyl- 

|H1R881, 80 Ci/mmol was from DuPont NEN; hydroxyflutamide was provided by R. O. Neri (Schering Corporation, Bloomfield, NJ); deep vent polymerase, T4 DNA ligase, and restriction enzymes were from New England Biolabs (Beverly, MA); Sequenase was from U. S. Biochemical Corp.; diethylaminoethyl dextran was from Pharmacia Biotech Inc.; α-tau-ferin was from Analytical Luminescence; cell lysis buffer was from Ligand Pharmaceuticals (San Diego, CA); phenylmethylsulfonyl fluoride and general chemicals were from Sigma; ECL Western blotting detection kit was from Amersham Corp.; and Immobilon-P was from Millipore (Bedford, MA).

Expression Plasmids—Eukaryotic expression vectors pGALO containing the DNA-binding domain of the Saccharomyces cerevisiae GAL4 protein (amino acid residues 1–147) and pNLVP coding for the transcriptional activation domain of the herpes simplex virus VP16 protein (residues 411–456) were previously described (36) and kindly provided by Gordon Tomaselli, Johns Hopkins University. Expression vectors contain the SV40 promoter, nuclear targeting signals, and a 3′ multiple cloning site. Control plasmid pGAL-Fos contains the Fos leucine zipper region (amino acid residues 137–216) and pVP-Jun, the Jun leucine zipper domain (amino acid residues 1–147) and pVP-A1 were constructed by deleting sequence coding for carboxy-terminal residues 504–919 using KpnI/XhoI followed by ligation of the filled ends. NH2-terminal deletion and insertion mutants pCMVhAR14–150, pCMVhAR142–337, pCMVhAR339–499, and pCMVhARGln66 previously described (4) were used to clone the corresponding deletions into the pGAL-A1 and pVP-A1 vectors or into the full-length pGAL-hAR and pNLVP vectors using unique sites in the AR coding sequence to exchange the deletion fragment or, in the case of the A14–150 deletion, using PCR to include the Ndel site at the initiation codon. pGAL-D-H and VPD-H comprise the GAL4 DNA-binding and carboxy-terminal AR fragment from pCMVhAR (1), and the Ndel/XhoI fragments of the pGALO or pNLVP expression vectors. pGAL-A1 (A1 comprising human AR amino acids 1–503) and pVP-A1 were constructed by deleting sequence coding for carboxy-terminal residues 504–919 using KpnI/XhoI followed by ligation of the filled ends. NH2-terminal deletion and insertion mutants pCMVhAR14–150, pCMVhAR142–337, pCMVhAR339–499, and pCMVhARGln66 previously described (4) were used to clone the corresponding deletions into the pGAL-A1 and pVP-A1 vectors or into the full-length pGAL-hAR and pNLVP vectors using unique sites in the AR coding sequence to exchange the deletion fragment or, in the case of the A14–150 deletion, using PCR to include the Ndel site at the initiation codon. pGAL-H (human AR amino acids 624–919; 624–627 from exon C, 628–919 as a positive control. Expression vectors containing wild-type or mutant AR chimeric expression plasmids using DEAE-dextran (38). Cells were maintained in Dulbecco’s modified essential medium with 4.5 g/liter glucose and t-glutamine with 10% bovine calf serum and, for cells transfected with plasmids expressing the AR steroid-binding domain, supplemented with 0.1 μM dihydrotestosterone. 48 h after transfection, cells were washed in phosphate-buffered saline and harvested in 0.1 ml of 2% SDS, 10% glycerol, and 10 mM Tris, pH 6.8. β-Mercaptoethanol (4%) and bromphenol blue (1%) were added, and cells were boiled for 5 min. 30-μl aliquots were analyzed by SDS-PAGE containing 8% acrylamide gels as described previously using AR anti-peptide rabbit antiserum AR32 (40) or AR PG-21 (41) and the ECL immunoblotting detection kit.

Androgen binding was determined in COS and CHO cells using a whole cell binding assay (38) for vectors comprised of exons D–H (residues 624–919) (42). COS cells in 12-well (105 cells/well) or 35-mm (0.25 × 106 cells/plate for Scatchard plot analysis) tissue culture plates were transiently transfected using DEAE-dextran with 1 μg of pGAL or VP16 fusion plasmids containing wild-type or LNCap AR steroid-binding domain. Cells were maintained for 48 h in Dulbecco’s modified essential medium with 4.5 g/liter glucose and t-glutamine media containing 10% bovine calf serum and labeled for 2 h at 37 °C with 5 nM [3H]R1881 in serum-free, phenol red-free medium in duplicate. For Scatchard analysis, cells were incubated with 0.05–4 nM [3H]R1881 in the presence and the absence of a 100-fold excess of unlabeled R1881 for 2 h at 37 °C. Aliquots of free [3H]R1881 were taken, and the cells were washed in phosphate-buffered saline and collected in SDS sample buffer for scintillation counting. Nonspecific binding was determined by parallel incubations in the presence of a 100-fold excess unlabeled R1881. Labeling medium was removed, and cells were washed twice with phosphate-buffered saline and harvested in 0.2 ml of 2% SDS, 10% glycerol, and 10 mM Tris, pH 6.8. Radioactivity was determined by scintillation counting.

GAL-D-H and VPD-H comprise the GAL4 DNA-binding and carboxy-terminal AR fragment from pCMVhAR (1), and the Ndel/XhoI fragments of the pGALO or pNLVP expression vectors. pGAL-A1 (A1 comprising human AR amino acids 1–503) and pVP-A1 were constructed by deleting sequence coding for carboxy-terminal residues 504–919 using KpnI/XhoI followed by ligation of the filled ends. NH2-terminal deletion and insertion mutants pCMVhAR14–150, pCMVhAR142–337, pCMVhAR339–499, and pCMVhARGln66 previously described (4) were used to clone the corresponding deletions into the pGAL-A1 and pVP-A1 vectors or into the full-length pGAL-hAR and pNLVP vectors using unique sites in the AR coding sequence to exchange the deletion fragment or, in the case of the A14–150 deletion, using PCR to include the Ndel site at the initiation codon. pGAL-H (human AR amino acids 624–919; 624–627 from exon C, 628–919 as a positive control. Expression vectors containing wild-type or mutant AR chimeric expression plasmids using DEAE-dextran (38). Cells were maintained in Dulbecco’s modified essential medium with 4.5 g/liter glucose and t-glutamine with 10% bovine calf serum and, for cells transfected with plasmids expressing the AR steroid-binding domain, supplemented with 0.1 μM dihydrotestosterone. 48 h after transfection, cells were washed in phosphate-buffered saline and harvested in 0.1 ml of 2% SDS, 10% glycerol, and 10 mM Tris, pH 6.8. β-Mercaptoethanol (4%) and bromphenol blue (1%) were added, and cells were boiled for 5 min. 30-μl aliquots were analyzed by SDS-PAGE containing 8% acrylamide gels as described previously using AR anti-peptide rabbit antiserum AR32 (40) or AR PG-21 (41) and the ECL immunoblotting detection kit.

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RESULTS

Expression of GAL4-AR and VP16-AR Fusion Proteins—Intermolecular interactions within the AR dimer were investigated using a protein-protein interaction assay in mammalian cells. Fusion protein expression vectors contained partial AR sequence cloned in frame and carboxy-terminal to the herpes simplex virus VP16 protein transactivation domain (shown schematically in Fig. 1). Activation of the GAL4-luciferase reporter vector results when regions of AR interact stably after expression of the chimeras in CHO cells. GAL-Fos and VP-Jun vectors expressing Fos and Jun leucine zipper fusion proteins (36) were expressed as a positive control.

GAL-D-H and VPD-H comprise the GAL4 DNA-binding domain of the herpes simplex virus VP16 protein transactivation domain (shown schematically in Fig. 1). Activation of the GAL4-luciferase reporter vector results when regions of AR interact stably after expression of the chimeras in CHO cells. GAL-Fos and VP-Jun vectors expressing Fos and Jun leucine zipper fusion proteins (36) were expressed as a positive control.
was indistinguishable from full-length wild-type AR; however, these fragments would be expected to have increased ligand dissociation rates based on results with AR deletion mutants lacking the NH₂-terminal domain (4). Expression levels were similar for the two constructs and were approximately 10-fold greater in COS cells than in CHO cells (data not shown).

GAL-A1 and VP-A1 comprise the GAL4 DNA-binding and VP16 transactivation domains, respectively, fused NH₂-terminal to AR NH₂-terminal residues 1–503 (A1), and express at similar levels as 90–95-kDa proteins on immunoblots using an AR anti-peptide antibody, shown for VP-A1 in Fig. 2 (lane 1). Deletions within the NH₂-terminal region resulted in correspondingly smaller proteins expressed either in CHO and COS cells (Fig. 2, lanes 2–4), whereas expansion of the glutamine repeat from 21 to 66 residues resulted in a slightly larger peptide (Fig. 2B, lane 5). All were smaller than the 120-kDa full-length, wild-type AR (Fig. 2, lane 6).

Androgen-dependent Interaction between the AR Steroid-binding and NH₂-terminal Domains—In the absence of androgen, luciferase activity was negligible when cotransfecting GALD-H with VP-A1 (Fig. 3). Treatment with 1 nM DHT-induced luciferase activity greater than that observed with the Fos/Jun leucine zipper chimeras relative to background activity (Fig. 3). Similar results were obtained using COS cells for transfection (data not shown); however, CHO cells were used in subsequent experiments due to lower background activity. No significant transactivation was observed in CHO cells in the presence of androgen when either plasmid was transfected with the other parent plasmid lacking AR sequence (Fig. 3). The results indicate an androgen-dependent stable interaction between the AR steroid-binding domain and the NH₂-terminal region.

Only a 2-fold induction of luciferase activity was observed using the reciprocal chimeras GAL-A1 and VPD-H (Fig. 3), suggesting a preferential orientation for fusion protein interaction. GAL-A1 alone activated luciferase expression (Fig. 3) presumably resulting from linking the AR transactivation domain to the GAL4 DNA-binding domain, thus increasing background activity in the NH₂-carboxy-terminal interaction using this construct. Cotransflecting GALD-H and VPD-H both containing the AR steroid-binding domain failed to activate the reporter vector in the presence or absence of androgen (Fig. 3).

Transfecting GAL-A1 with VP-A1 increased activation 4–5-fold over the activity of GAL-A1 alone (Fig. 3), indicating a ligand-independent interaction between the AR NH₂-terminal domains. The high transactivation activity induced by GAL-A1 with VP-A1 was likely due in part to the presence of three transactivation domains, two from the AR NH₂-terminal domains and one from VP-16. The lower fold induction by the NH₂-NH₂-terminal interaction may therefore reflect a weaker interaction than that observed for the androgen-induced NH₂-carboxy-terminal interaction (4–5 versus 59-fold; Fig. 3).

Steroid Specificity and Concentration Dependence—Steroid specificity of the GALD-H and VP-A1 interaction was investigated by determining transcriptional activation in the presence of androgens, the antiandrogens hydroxyflutamide and cyproterone acetate, and other steroids. The strongest transcriptional activation was observed at 1 nM DHT and between 1 (Fig. 4) and 10 nM R1881 or testosterone. Hydroxyflutamide up to 1 µM failed to activate luciferase activity (Fig. 4).

Because hydroxyflutamide is a potent antiandrogen, we investigated whether it would disrupt the NH₂-carboxy-terminal interaction in the presence of androgen. Increasing concentrations of hydroxyflutamide between 0.2–1 µM inhibited androgen-induced gene activation (Fig. 4). Estradiol, progesterone, and cyproterone acetate failed to induce luciferase activity and at 0.5 µM inhibited transcriptional activation induced by 1 nM DHT (Fig. 5). The results indicate that the NH₂-carboxy-terminal interaction induced by androgens is blocked by moderate affinity ligands such as hydroxyflutamide, progesterone, and estradiol, paralleling the activation and inhibition properties of these ligands with wild-type, full-length AR.

Activity of the LNCaP AR Mutant—It was shown previously that the mutant AR in the androgen-dependent human prostate cancer cell line, LNCaP, contains a single base mutation in the steroid-binding domain that changes threonine 877 to alanine that results in increased affinity for hydroxyflutamide with concomitantly increased agonist activity (43–45). We inserted the LNCaP Thr-877→Ala mutation into GALD-H and tested for the NH₂-terminal/steroid-binding domain interaction. Whole cell binding assays using [3H]R1881 showed similar binding affinities for GALD-H and GAL-LNCaPD-H (data not shown). In the presence of 1 nM DHT, greater than 100-fold induction of luciferase activity indicated an NH₂-carboxy-terminal interaction similar to wild-type AR (Fig. 4). At 1 µM, hydroxyflutamide-induced luciferase activity almost 10-fold and was a less active inhibitor of androgen-induced complex formation, with luciferase activity remaining approximately 10-fold (Fig. 4). This result is in agreement with the known agonist activity of hydroxyflutamide acquired by the LNCaP mutation (43–45). Estradiol and progesterone similarly induced luciferase activity by GAL-LNCaPD-H and VP-A1, whereas no detectable activity was observed with these steroids with the wild-type GALD-H and VP-A1 chimeras (Fig. 5). Cyproterone acetate did not induce luciferase activity of the wild-type or LNCaP mutant chimeras. All three ligands, estradiol, progesterone, and cyproterone acetate, at 0.5 µM were less...
active inhibitors of DHT-induced luciferase activity of GAL-LNCaPD-H and VP-A1 relative to wild-type GALD-H and VP-A1 chimeras (Fig. 5). Thus, although the magnitude of the interaction was 5–10-fold less than that observed with DHT, the NH2-/carboxyl-terminal interaction induced by hydroxyflutamide, estradiol, and progesterone with the threonine 877 to alanine mutation correlated with the agonist activities of these ligands with the full-length LNCaP mutant AR.

**NH2-terminal Deletions Define the Dimerization Domain**

To characterize the dimerization region within the AR NH2-terminal domain, PCR mutagenesis was used to create deletions within VP-A1 (shown schematically in Figs. 6 and 7) for cotransfection with GALD-H, GAL-LNCaPD-H, and GAL-A1. Expression levels of the deletion mutants were similar as determined by immunoblotting (see Fig. 2). Deletion of the AR transactivation region (VP-A1Δ142–337) resulted in 14-fold stimulation of luciferase activity (Fig. 6), representing a significant decrease relative to the wild-type sequence. Because the AR transactivation domain likely contributes to luciferase induction, it is possible that the decrease reflects deletion of the AR activating region rather than a decrease in dimerization.

Deletion immediately NH2-terminal to the DNA-binding domain (VP-A1Δ339–503) abolished luciferase activity (Fig. 6) even after a 5-fold increase in plasmid concentration (data not shown). Deletion near the NH2 terminus (VP-A1Δ14–150) also inhibited transcriptional activation, with 2–5-fold induction of luciferase activity using a 5-fold higher plasmid DNA concentration (Fig. 6). Expansion of the NH2-terminal polymorphic glutamine repeat from 21 (residues 58–78 in wild-type AR) to 66 glutamine residues (VP-A1Gln66) had little effect on transcriptional activation of luciferase (Fig. 6). Expansion of the glutamine repeat is associated with spinal/bulbar muscular atrophy (Kennedy’s disease) (46), and thus, the amplified repeat does not interfere with this aspect of AR dimerization, in agreement with its wild-type level of activation.2 Results using the NH2-terminal deletion and insertion mutants with the GAL-LNCaPD-H mutant were essentially identical to those with wild-type GALD-H fusion protein (data not shown).

The NH2/NH2-terminal interaction was investigated using the VP-A1 deletion mutants described above. Inhibition was observed after deleting transactivation domain residues 142–337 and to a lower extent by deletion of residues 339–499 or 14–150 (Fig. 7). Expanding the glutamine repeat to 66 residues as described above slightly enhanced the interaction. Thus, different regions appear to be involved in the NH2/NH2-terminal interaction than in NH2-terminal interaction with the androgen-bound steroid-binding domain.

**Smaller Domains within the Interacting Regions**

Insertion of shorter regions of AR coding sequence into GAL4 and VP16, such as NH2-terminal fragments 1–150 or 339–503 linked to VP16, failed to activate the reporter plasmid when cotransfected with GALD-H and analyzed in the presence of androgen (data not shown). Similarly, subdividing the steroid-binding domain into regions encoded by exons D–E (residues 624–780) and F–H (residues 774–919) resulted in no reporter vector

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2 E. M. Wilson, unpublished data.
activation. The results suggest that the sites of interaction localize to larger domains that may involve concerted actions within the NH2-terminal and entire steroid-binding domains, with the latter required for androgen binding.

**DISCUSSION**

The objective of the present study was to establish whether a direct interaction occurs between the NH2-terminal and steroid-binding domains in androgen-induced AR dimer formation. Domain interactions were analyzed by reporter gene activation using fusion proteins that linked the NH2- and carboxyl-terminal regions of human AR to the GAL4 DNA-binding or VP16 transactivation domains. The results support previous evidence that *in vivo* dimerization of human AR is mediated through direct intermolecular interactions between the androgen-bound steroid-binding domain and the NH2-terminal region. The dependence on androgen binding and inhibition by an antiandrogen and other steroids parallels properties of native AR and raises the possibility of an androgen-activated, anti-parallel AR dimer.

Although the present data do not rule out that the ligand-induced NH2-/carboxyl-terminal interaction occurs intramolecularly, previous studies using baculovirus-expressed AR fragments support a ligand-induced intermolecular interaction. Androgen-dependent dimerization was observed between NH2-terminal plus DNA-binding domain and DNA plus steroid-binding domain fragments (6). However, as the DNA-binding domain is implicated in receptor dimerization through the so-called D box region (47–49) with the other monomer (50, 51), the DNA-binding domain could have accounted for the observed AR dimerization. In the present study, the AR DNA-binding domain, which itself dimerizes,3 was excluded from the chimeric proteins, indicating an additional dimerization interface between the NH2-terminal and androgen-bound steroid-binding domain of AR. Further support for AR NH2- and carboxyl-terminal interactions comes from kinetic studies where the dissociation rate of bound androgen slows about 5-fold by the presence of the NH2-terminal domain despite no change in equilibrium dissociation constant (4). Crystallographic data of glucocorticoid receptor/DNA interactions (50) and the asymmetric dimer proposed for the vitamin D receptor (53) suggest a ligand-activated anti-parallel dimer may be the active conformation for other members of the steroid receptor family.

The two regions of the AR NH2-terminal domain required most for carboxyl-terminal interaction were immediately NH2-

3 Marschke, K. B., Tan, J. A., Kupfer, S. R., Wilson, E. M., and French, F. S. (1995) *Endocrine* 3, 819–825.
terminal to the DNA-binding domain and near the NH2 terminal. Lack of a direct role of the more centrally positioned transactivation domain might allow this region to remain accessible for transcription factor interaction. In our unpublished studies and the work of others (54), deletion of the transactivation domain creates a strong dominant negative AR inhibitor, suggesting that loss of the transactivation domain does not interfere with receptor dimerization. It is interesting, therefore, that this region or the region NH2-terminal to the DNA-binding domain (residues 142–337 and 339–499, respectively) may be involved in an interaction between the NH2-terminal domains and may reflect an association that occurs in the unliganded receptor that could contribute to suppression of activation in the absence of ligand. Shown is a representative of three independent experiments.

The affinity of the NH2-/carboxyl-terminal interaction appears to be similar to that observed for Fos-Jun leucine zipper binding. When the Fos/Jun leucine zipper regions were fused to progesterone receptor, agonist-induced progesterone receptor dimerization persisted through the receptor dimerization domain, suggesting that ligand-induced receptor dimerization was of equal or greater affinity than the Fos/Jun leucine zipper interaction (55). Leucine zipper motifs are often involved in transcription factor dimerization resulting in efficient DNA binding (56). A heptad repeat of hydrophobic amino acid residues in the steroid-binding domain of mouse ER resembles a leucine zipper, is conserved among the family of steroid receptors, and is implicated in dimerization and high affinity estrogen binding (20). Like ER (57), other steroid receptors appear to have two dimerization interfaces: a constitutive region in the DNA-binding domain and a stronger, hormone-dependent region in the hormone-binding domain that may be involved in stable dimer formation required for high affinity DNA binding. The carboxyl-terminal end of the thyroid hormone receptor was also implicated in receptor dimerization (58, 59). Human AR and ER differ in the length of their NH2-terminal domains, i.e. 559 amino acid residues in AR versus 185 in ER. It is noteworthy, therefore, that a transcriptionally inactive AR deletion mutant AR507–919, lacking all but 52 NH2-terminal amino acid residues (4), dimerizes and binds DNA independent of ligand binding (6) as observed with full-length ER (20–23) but not full-length AR (6). The androgen-independent dimerization of this AR deletion mutant suggests two forms of DNA-binding homodimers: one for the AR deletion mutant AR507–919 and perhaps ligand free ER and another for androgen-bound full-length AR and perhaps ligand-bound ER. A parallel dimer capable of binding DNA may form constitutively through interactions between the DNA-binding domains if no extended NH2-terminal region interferes, in the case of ER and the AR deletion mutant. However, the active configuration requiring ligand binding for full-length AR might be anti-parallel and depends on the presence of the NH2-terminal domain. This hypothesis is supported by studies using glucocorticoid recep-

FIG. 5. Effects of estradiol, progesterone, and cyproterone acetate on the GALD-H and GAL-LNCaP-D-H interaction with VP-A1.

VP-A1 and GALD-H containing wild-type or LNCaP mutant AR sequence were cotransfected into CHO cells with the reporter vector, G5E1bLuc, as described under “Experimental Procedures” and incubated with 0.5 µM 17β-estradiol (E), progesterone (P), and cyproterone acetate (CA) in the absence or the presence of 1 nM DHT as indicated. Shown are optical units and fold induction relative to the activity determined in the absence of ligand. Shown is a representative of three independent experiments.
tor deletion mutants, where deletion of the NH₂-terminal domain changed the contact points within the dimer in cross-linking studies and reduced the specificity of DNA binding (60).

Androgen-induced conformational effects on full-length AR that might establish the anti-parallel dimer may be required for DNA binding that results in transcriptional activation.

**Fig. 6.** Effect of AR NH₂-terminal deletions on VP-A1 interaction with GALD-H. Several mutants with portions of the AR NH₂-terminal domain deleted, including VP-A1Δ14–150 (5 µg), VP-A1Δ142–337 (1 µg), VP-A1Δ339–499 (1 µg), VP-A1Gln66 (1 µg), or VP-A1 (1 µg), were cotransfected with GALD-H (1 µg). The expanded glutamine repeat replaces 21 Gln residues with 66 Gln residues identified in a patient with spinal/bulbar muscular atrophy (52). Relative luciferase activities are shown for GALD-H cotransfected in CHO cells with wild-type and deletion mutants of VP-A1 in the absence and presence of 1 nM DHT. Amino acid residues deleted from AR are indicated by Δ. Shown are optical units and fold induction relative to activity in the absence of DHT. GAL-Fos cotransfected with VP-Jun was a positive control and the parent vector VP16 lacking AR sequence cotransfected with GALD-H served as negative controls. The data shown are representative of three independent experiments.

**Fig. 7.** Effect of AR NH₂-terminal deletions on the VP-A1/GAL-A1 interaction. The VP-A1 mutant vector DNAs described in legend to Fig. 6 were cotransfected with GAL-A1, and luciferase activity was determined as described in the legend to Fig. 6. The data shown are representative of three experiments.
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