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Biochemistry

Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells

(steroid receptor associated protein/dihydrotestosterone)

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ABSTRACT **The androgen receptor (AR) is a member of the steroid receptor superfamily that plays an important role in male sexual differentiation and prostate cell proliferation. Mutations or abnormal expression of AR in prostate cancer can play a key role in the process that changes prostate cancer from androgen-dependent to an androgen-independent stage. Using a yeast two-hybrid system, we were able to isolate a ligand-dependent AR-associated protein (ARA_{70}), which functions as an activator to enhance AR transcriptional activity 10-fold in the presence of 10^{-10} M dihydrotestosterone or 10^{-9} M testosterone, but not 10^{-6} M hydroxyflutamide in human prostate cancer DU145 cells. Our data further indicated that ARA_{70} will only slightly induce the transcriptional activity of other steroid receptors such as estrogen receptor, glucocorticoid receptor, and progesterone receptor in DU145 cells. Together, these data suggest that AR may need a specific coactivator(s) such as ARA_{70} for optimal androgen activity.**

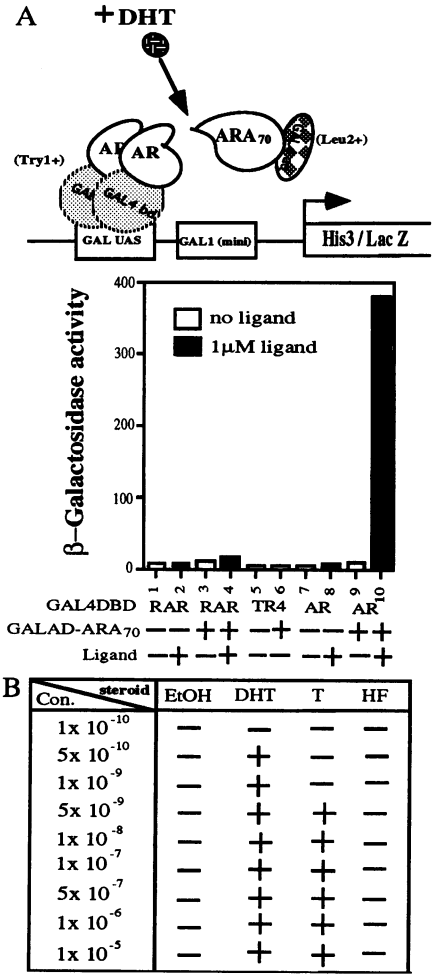
Androgen receptor (AR) is a transcriptional factor that belongs to the member of the steroid receptor superfamily based on its structural similarity (1, 2). Members of this family are characterized by three major structure regions: a variable amino-terminal domain, a highly conserved cysteine-rich DNA binding domain, and a carboxyl-terminal ligand-binding domain. When bound to androgens and androgen-responsive element, AR can up or down regulate the expression of androgen target genes through a complicated process that may require other adaptors or coactivators (3).

In addition, a fundamental issue in the steroid hormone regulation is the question of how specific transcription can be achieved *in vivo* when several receptors, such as AR, glucocorticoid receptor, and progesterone receptor can recognize the same DNA sequence (4, 5). It has been speculated that some accessory factors may selectively interact with the AR to determine the specificity of AR target gene activation.

To understand further the mechanism of androgen–AR function, we decided to use a yeast two-hybrid system to isolate AR-associated proteins that may contribute the activation of AR. We report here the isolation of the first ligand-dependent AR-associated protein, ARA_{70}, which may function as a specific coactivator to enhance the transcriptional activity of AR in prostate DU145 cells.

MATERIALS AND METHODS

Yeast Two-Hybrid Screening. A fusion protein (GAL4AR) containing GAL4 DNA binding domain (GAL4DBD) and carboxyl terminus of AR was used as bait to screen for His synthetase gene (His3) positive clones from 3×10^6 transformants of MATCHMAKER human brain library. Mating tests (6) further confirmed that two of the initial 41 potentially positive clones react firmly with AR fusion proteins. ARA_{70} was further characterized for androgen effects in the yeast two-hybrid system. The transformed yeast Y190 cells were selected



B

Con. \ steroid	EtOH	DHT	T	HF
$1x\ 10^{-10}$	−	−	−	−
$5x\ 10^{-10}$	−	+	−	−
$1x\ 10^{-9}$	−	+	−	−
$5x\ 10^{-9}$	−	+	+	−
$1x\ 10^{-8}$	−	+	+	−
$1x\ 10^{-7}$	−	+	+	−
$5x\ 10^{-7}$	−	+	+	−
$1x\ 10^{-6}$	−	+	+	−
$1x\ 10^{-5}$	−	+	+	−

FIG. 1. Identification of ARA_{70} that interacts with human AR in a ligand-specific manner. (*A*) The interaction of ARA_{70} with AR, retinoic acid receptor (RAR), and TR4 orphan receptor by liquid assay in the yeast Y190. The liquid assay was performed as described (6). The transformed yeast cells were cultured in the medium containing 10^{-6} M *trans*-retinoic acid (lanes 1–4) or 10^{-6} M DHT (lanes 7–10) without leucine and tryptophan. Data represent an average of two independent transformations. (*B*) The effects of DHT, T, and HF on the interaction of ARA_{70} with AR by plate nutritional selection in the yeast Y190.

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Abbreviations: AR, androgen receptor; DHT, dihydrotestosterone; MMTV, mouse mammary tumor virus; CAT, chloramphenicol acetyltransferase; HF, hydroxyflutamide; RACE, rapid amplification of cDNA ends; DBD, DNA-binding domain; HBD, hormone-binding domain; ARE, androgen response element.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. L49399).

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  1 Met Asn Thr Phe Gln Asp Gln Ser Gly Ser Ser Ser Asn Arg Glu Pro Leu Leu Arg Cys Ser Asp Ala Arg Arg Asp Leu Glu Leu Ala
 77 ATG AAT ACC TTC CAA GAC CAG AGT GGC AGC TCC AGT AAT AGA GAA CCC CTT TTG AGG TGT AGT GAT GCA CGG AGG GAC TTG GAG CTT GCT

 31 Ile Gly Gly Val Leu Arg Ala Glu Gln Gln Ile Lys Asp Asn Leu Arg Glu Val Lys Ala Gln Ile His Ser Cys Ile Ser Arg His Leu
167 ATT GGT GGA GTT CTC CGG GCT GAA CAG CAA ATT AAA GAT AAC TTG CGA GAG GTC AAA GCT CAG ATT CAC AGT TGC ATA AGC CGT CAC CTG

 61 Glu Cys Leu Arg Ser Arg Glu Val Trp Leu Tyr Glu Gln Val Asp Leu Ile Tyr Gln Leu Lys Glu Glu Thr Leu Gln Gln Gln Ala Gln
257 GAA TGT CTT AGA AGC CGT GAG GTA TGG CTG TAT GAA CAG GTG GAC CTT ATT TAT CAG CTT AAA GAG GAG ACA CTT CAA CAG CAG GCT CAG

 91 Gln Leu Tyr Ser Leu Leu Gly Gln Phe Asn Cys Leu Thr His Gln Leu Glu Cys Thr Gln Asn Lys Asp Leu Ala Asn Gln Val Ser Val
347 CAG CTC TAC TCG TTA TTG GGC CAG TTC AAT TGT CTT ACT CAT CAA CTG GAG TGT ACC CAA AAC AAA GAT CTA GCC AAT CAA GTC TCT GTG

121 Cys Leu Glu Arg Leu Gly Ser Leu Thr Leu Lys Pro Glu Asp Ser Thr Val Leu Leu Phe Glu Ala Asp Thr Ile Thr Leu Arg Gln Thr
437 TGC CTG GAG AGA CTG GGC AGT TTG ACC CTT AAG CCT GAA GAT TCA ACT GTC CTG CTC TTT GAA GCT GAC ACA ATT ACT CTG CGC CAG ACC

151 Ile Thr Thr Phe Gly Ser Leu Lys Thr Ile Gln Ile Pro Glu His Leu Met Ala His Ala Ser Ser Ala Asn Ile Gly Pro Phe Leu Glu
527 ATC ACC ACA TTT GGG TCT CTC AAA ACC ATT CAA ATT CCT GAG CAC TTG ATG GCT CAT GCT AGT TCA GCA AAT ATT GGG CCC TTC CTG GAG

181 Lys Arg Gly Cys Ile Ser Met Pro Glu Gln Lys Ser Ala Ser Gly Ile Val Ala Val Pro Phe Ser Glu Trp Leu Leu Gly Ser Lys Pro
617 AAG AGA GGC TGT ATC TCC ATG CCA GAG CAG AAG TCA GCA TCC GGT ATT GTA GCT GTC CCT TTC AGC GAA TGG CTC CTT GGA AGC AAA CCT

211 Ala Ser Gly Tyr Gln Ala Pro Tyr Ile Pro Ser Thr Asp Pro Gln Asp Trp Leu Thr Gln Lys Gln Thr Leu Glu Asn Ser Gln Thr Ser
707 GCC AGT GGT TAT CAA GCT CCT TAC ATA CCC AGC ACC GAC CCC CAG GAC TGG CTT ACC CAA AAG CAG ACC TTG GAG AAC AGT CAG ACT TCT

241 Ser Arg Ala Cys Asn Phe Phe Asn Asn Val Gly Gly Asn Leu Lys Gly Leu Glu Asn Trp Leu Leu Lys Ser Glu Lys Ser Ser Tyr Gln
797 TCC AGA GCC TGC AAT TTC TTC AAT AAT GTC GGG GGA AAC CTA AAG GGC TTA GAA AAC TGG CTC CTC AAG AGT GAA AAA TCA AGT TAT CAA

271 Lys Cys Asn Ser His Ser Thr Thr Ser Ser Phe Ser Ile Glu Met Glu Lys Val Gly Asp Gln Glu Leu Pro Asp Gln Asp Glu Met Asp
887 AAG TGT AAC AGC CAT TCC ACT ACT AGT TCT TTC TCC ATT GAA ATG GAA AAG GTT GGA GAT CAA GAG CTT CCT GAT CAA GAT GAG ATG GAC

301 Leu Ser Asp Trp Leu Val Thr Pro Gln Glu Ser His Lys Leu Arg Lys Pro Glu Asn Gly Ser Arg Glu Thr Ser Glu Lys Phe Lys Leu
977 CTA TCA GAT TGG CTA GTG ACT CCC CAG GAA TCC CAT AAG CTG CGG AAG CCT GAG AAT GGC AGT CGT GAA ACC AGT GAG AAG TTT AAG CTC

331 Leu Phe Gln Ser Tyr Asn Val Asn Asp Trp Leu Val Lys Thr Asp Ser Cys Thr Asn Cys Gln Gly Asn Gln Pro Lys Gly Val Glu Ile
1067 TTA TTC CAG TCC TAT AAT GTG AAT GAT TGG CTT GTC AAG ACT GAC TCC TGT ACC AAC TGT CAG GGA AAC CAG CCC AAA GGT GTG GAG ATT

361 Glu Asn Leu Gly Asn Leu Lys Cys Leu Asn Asp His Leu Glu Ala Lys Lys Pro Leu Ser Thr Pro Ser Met Val Thr Glu Asp Trp Leu
1157 GAA AAC CTG GGC AAT CTG AAG TGC CTG AAT GAC CAC TTG GAG GCC AAG AAA CCA TTG TCC ACC CCC AGC ATG GTT ACA GAG GAT TGG CTT

391 Val Gln Asn His Gln Asp Pro Cys Lys Val Glu Glu Val Cys Arg Ala Asn Glu Pro Cys Thr Ser Phe Ala Glu Cys Val Cys Asp Glu
1247 GTC CAG AAC CAT CAG GAC CCA TGT AAG GTA GAG GAG GTG TGC AGA GCC AAT GAG CCC TGC ACA AGC TTT GCA GAG TGT GTG TGT GAT GAG

421 Asn Cys Glu Lys Glu Ala Leu Tyr Lys Trp Leu Leu Lys Lys Glu Gly Lys Asp Lys Asn Gly Met Pro Val Glu Pro Lys Pro Glu Pro
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451 Glu Lys His Lys Asp Ser Leu Asn Met Trp Leu Cys Pro Arg Lys Glu Val Ile Glu Gln Thr Lys Ala Pro Lys Ala Met Thr Pro Ser
1427 GAG AAG CAT AAA GAT TCC CTG AAT ATG TGG CTC TGT CCT AGA AAA GAA GTA ATA GAA CAA ACT AAA GCA CCA AAG GCA ATG ACT CCT TCT

481 Arg Ile Ala Asp Ser Phe Gln Val Ile Lys Asn Ser Pro Leu Ser Glu Trp Leu Ile Arg Pro Pro Tyr Lys Glu Gly Ser Pro Lys Glu
1517 AGA ATT GCT GAT TCC TTC CAA GTC ATA AAG AAC AGC CCC TTG TCG GAG TGG CTT ATC AGG CCC CCA TAC AAA GAA GGA AGT CCC AAG GAA

511 Val Pro Gly Thr Glu Asp Arg Ala Gly Lys Gln Lys Phe Lys Ser Pro Met Asn Thr Ser Trp Cys Ser Phe Asn Thr Ala Asp Trp Val
1607 GTG CCT GGT ACT GAA GAC AGA GCT GGC AAA CAG AAG TTT AAA AGC CCC ATG AAT ACT TCC TGG TGT TCC TTT AAC ACA GCT GAC TGG GTC

541 Leu Pro Gly Lys Lys Met Gly Asn Leu Ser Gln Leu Ser Ser Gly Glu Asp Lys Trp Leu Leu Arg Lys Lys Ala Gln Glu Val Leu Leu
1697 CTG CCA GGA AAG AAG ATG GGC AAC CTC AGC CAG TTA TCT TCT GGA GAA GAC AAG TGG CTG CTT CGA AAG AAG GCC CAG GAA GTA TTA CTT

571 Asn Ser Pro Leu Gln Glu Glu His Asn Phe Pro Pro Asp His Tyr Gly Leu Pro Ala Val Cys Asp Leu Phe Ala Cys Met Gln Leu Lys
1787 AAT TCA CCT CTA CAG GAG GAA CAT AAC TCC CCC CCA GAC CAT TAT GGC CTC CCT GCA GTT TGT GAT CTC TTT TCC TGT ATG CAG CTT AAA

601 Val Asp Lys Glu Lys Trp Leu Tyr Arg Thr Pro Leu Gln Met STOP
1877 GTT GAT AAA GAG AAG TGG TTA TAT CAG ACT CCT CTA CAG ATG TGA
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FIG. 2. cDNA sequences and deduced amino acid sequences of ARA_{70}. Sequence data have been deposited into GenBank under accession no. L49399. Sequence of ARA_{70} was confirmed by dideoxy method from positive clones isolated from yeast two-hybrid system and RACE-PCR. The nucleotide sequences and the deduced amino acid sequences are numbered on the left. Different amino acids between ARA_{70} and RFG are boxed.

for growth on plates with 20 mM 3-aminotriazole and serial concentrations of androgens but without histidine, leucine, or tryptophan. The liquid assay was performed as described (6). The transformed yeast cells were cultured in the medium containing 10^{-6} M *trans*-retinoic acid (Fig. 1*A*, lanes 1–4) or 10^{-6} M dihydrotestosterone (DHT) (Fig. 1*A*, lanes 7–10) without leucine and tryptophan. GAL4-DBD-TR4 bait was constructed from TR4 orphan receptor without known ligand.

The Far Western Assay. AR–N/DBD and AR–DBD/hormone binding domain (HBD) were expressed, with calculated molecular weight of 66 and 43K, respectively, as poly-histidine fusion protein by inserting the amino-terminal or carboxyl-terminal fragments into the pET 14b (Novagen). Proteins were separated on 10% SDS-polyacrylamide gel. *In vitro* transcribed/translated ^{35}S-labeled ARA_{70} was diluted into hybridization buffer, and the filters were hybridized overnight at 4°C in the presence of 1 μM DHT. After three washings with hybridization buffer, filters were dried and exposed for autoradiography.

Transient Transfection Assay. The DU145 cells were plated in 60-mm diameter Petri dish at a density of 3.5×10^5 cells/dish. The expression plasmids were transfected into cells by the calcium

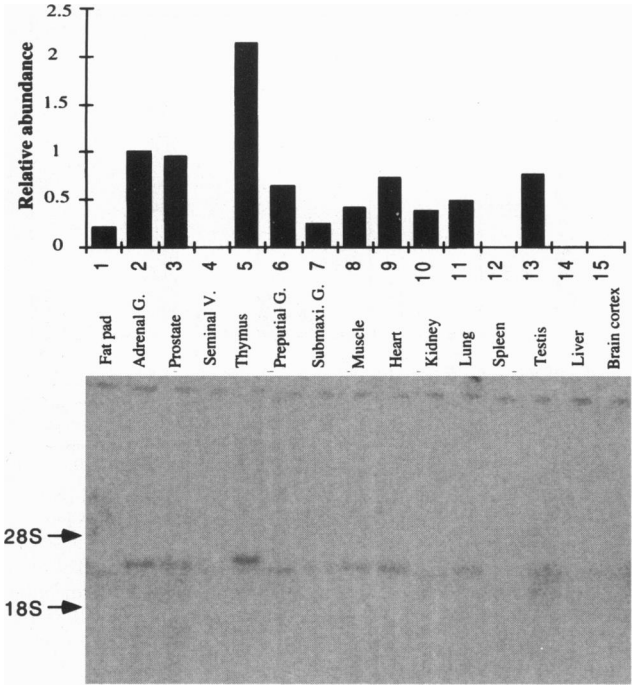

FIG. 3. Northern blot analysis of ARA_{70} mRNA levels in different mouse tissues. Total RNA (25 μg) from different mouse tissues was fractionated on denaturing gel, transferred to membrane, and hybridized against an ARA_{70}-specific cDNA probe. mRNA species migrating ~3600 bp was detected. A β-actin probe was used as control for equal loading (data not shown)

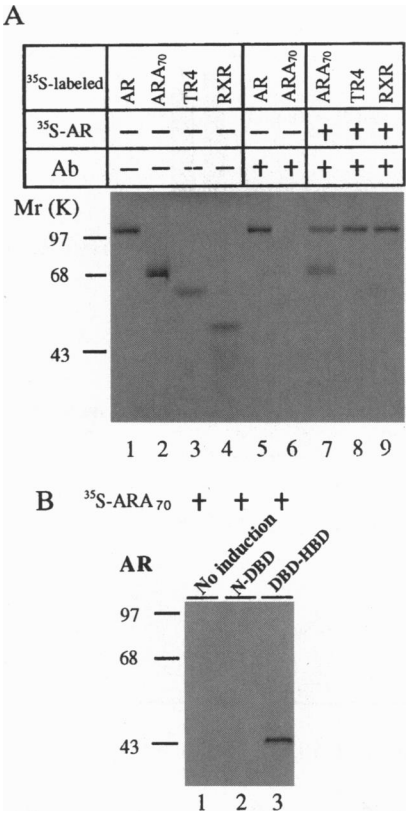

FIG. 4. Specific interaction of ARA_{70} with AR. (*A*) Immunoprecipitation of AR and ARA_{70}. The amount of *in vitro* transcribed/translated AR, ARA_{70}, RXR, and TR4 orphan receptors were shown from lanes 1 to 4. The polyclonal anti-AR antibody, CW2, was used for immunoprecipitation. Twenty μl of protein A/G-Sepharose beads were applied to precipitate the protein–antibody complex. (*B*) The Far Western assay. The bacterically expressed AR-N/DBD and AR-DBD/HBD and no induction BL21 extract were electroblotted onto nitrocellulose and processed for Far Western assay, as described (11). *In vitro* transcribed/translated ^{35}S-labeled ARA_{70} was diluted into hybridization buffer, and the filters were hybridized overnight at 4°C in the presence of 1 μM DHT. After three washings with hybridization buffer, filters were dried and exposed for autoradiography

phosphate method (7). In each transfection, 3.5 μg of reporter mouse mammary tumor virus chloramphenicol acetyltransferase (MMTV-ARE-CAT or MMTV-ΔARE-CAT) and 1.5 μg of pSG5hAR and various amounts of pSG5ARA_{70} were co-transfected into the DU145 cells. Relative CAT activity was calculated by the quantification of phosphorimager. The cytomegalovirus–β-galactosidase construct was used as an internal control, and the relative CAT activity was normalized by the β-galactosidase activity.

RESULTS AND DISCUSSION

Identification of the Androgen Receptor-Specific Associated Protein, ARA_{70}. To further understand the mechanism of androgen-AR action, we applied a yeast two-hybrid system using the GAL4AR fusion protein as bait to isolate a cDNA encoding ARA_{70}, which interacts specifically with AR. In this system, yeast will survive when GAL4AR is co-expressed with ARA_{70} in the presence of DHT (Fig. 1 *A* and *B*). Neither GAL4AR nor ARA_{70} was active when ARA_{70} was expressed alone or when ARA_{70} was co-expressed with GAL4RAR or GAL4TR4 (8) (GAL4 fusion proteins with two other members of the steroid receptor superfamily) (Fig. 1*A*). These data, therefore, clearly suggest that ARA_{70} can interact specifically with AR in the yeast cells.

We then tested whether the interaction of ARA_{70} with AR in yeast was ligand-dependent. As shown in Fig. 1*B*, DHT (5×10^{-10} M) can promote the interaction between ARA_{70} and GAL4AR. Testosterone (T), a less potent androgen in the prostate, can also promote this interaction at higher concentrations (5×10^{-9} M). Hydroxyflutamide (HF), an antiandrogen used in the treatment of prostate cancer, had no activity even at very high concentration (10^{-5} M).

The RACE-PCR technique (8, 9) was then used to clone the full-length ARA_{70} cDNA that encodes a protein of 614 amino acids with a calculated molecular weight of 70 K (Fig. 2). A search of GenBank indicated that ARA_{70} shares 99% homology (three different amino acids in the coding region) with one identified cDNA clone (RET-fused gene, RFG) isolated from human thyroid (10). Although the biological functions of RFG are mostly unknown, the expression of RFG in thyroid tumor may suggest some potential roles of RFG in thyroid carcinogenesis (10).

The Tissue Distribution of ARA_{70}. Northern blot analysis in mouse indicated that ARA_{70} is expressed as an mRNA of ~3600 bp in many tissues, including prostate, testis, adrenal gland, and thymus (Fig. 3). The relative expression of ARA_{70} in the following mouse tissues, using adrenal gland as 100%, are: testis, 77%; prostate, 97%; preputial gland, 64%; thymus, 214%; submaxillary gland, 24%; muscle, 41%; heart, 73%; kidney, 37%; lung, 49%; fat pad, 20%; seminal vesicle, spleen, liver, and brain cortex, undetectable. Among the cell lines (LNCaP, MCF-7, Chinese hamster ovary, HeLa, and DU145) we tested, the human prostate cancer cell line, DU145, proved to be the only cell line that did not express ARA_{70} (data not shown) and therefore was chosen for further functional studies.

The *in Vitro* Interaction Between AR and ARA_{70}. To further confirm that the interaction that occurred in yeast cells is due to a direct interaction between AR and ARA_{70}, we applied an *in vitro* immunoprecipitation assay with an anti-AR antibody (CW2). We demonstrated that CW2 can co-precipitate the AR and ARA_{70} when *in vitro* transcribed/translated full-length

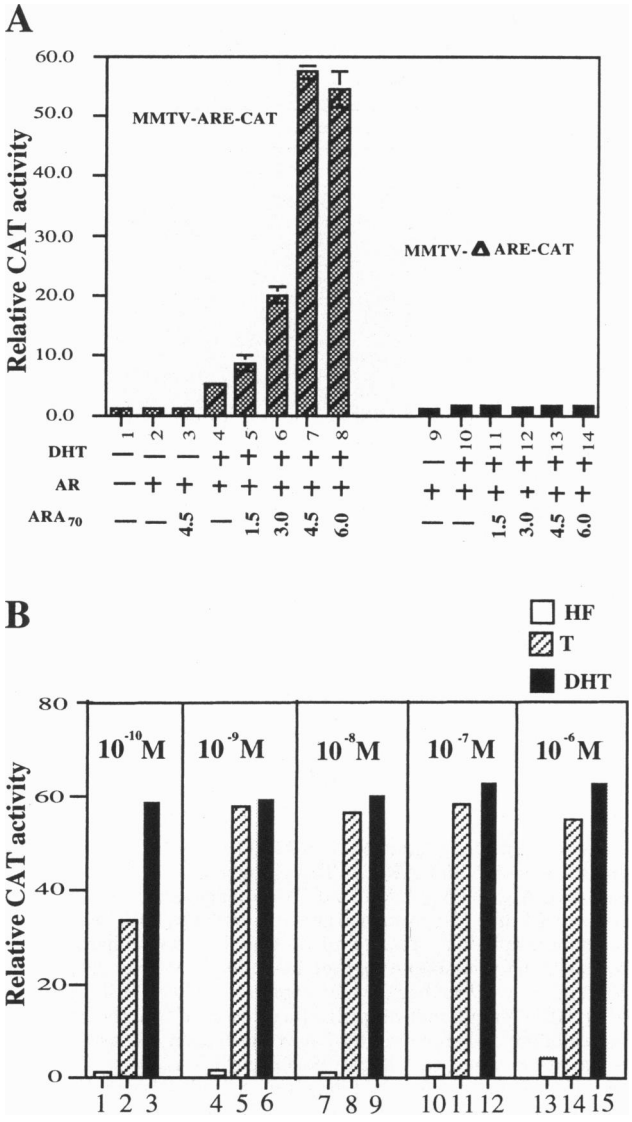

FIG. 5. (*A*) Effect of ARA_{70} on the AR transcription activity in the MMTV-CAT reporter with or without ARE DNA fragment (MMTV-ARE-CAT or MMTV-ΔARE-CAT). The increased amounts of ARA_{70} (1.5, 3, 4.5, and 6 μg) (lanes 5–8) were cotransfected with 1.5 μg of pSG5AR in DU145 cells in the absence or presence of 10^{-8} M DHT for CAT assay. (*B*) Effects of DHT, T, and HF on the interaction of ARA_{70} and AR in DU145 cells. The fixed amount of MMTV-ARE-CAT (3.5 μg) were cotransfected with 4.5 μg of ARA_{70} and 1.5 μg of pSG5AR in DU145 cells in the absence or presence of different DHT/T/HF for CAT assay. Relative CAT activity was calculated by the quantification of phosphorimager. The cytomegalovirus–β-galactosidase construct was used as an internal control, and the relative CAT activity was normalized by the β-galactosidase activity. All data were the average from results of four independent experiments.

human AR and ARA_{70} were incubated with it in the lysate mixture (Fig. 4*A*, lane 7). This precipitation is specific, as CW2 did not precipitate the ARA_{70} in the absence of AR (Fig. 4*A*, lane 6) and CW2 did not precipitate two other proteins (RXR and TR4 orphan receptors) incubated with AR (Fig. 4*A*, lanes 8 and 9). A Far Western assay also demonstrated that ARA_{70} can bind to immobilized AR peptide containing DNA binding domain and hormone binding domain (AR–DBD/HBD), but not the BL21 protein lysate or the AR peptide containing the amino-terminal and DNA binding domain of AR (AR–N/DBD) (Fig. 4*B*). Together, these data indicate that the association is due to a direct interaction between AR and ARA_{70}.

Stimulation of the Transcriptional Activity of AR by ARA_{70}. DU145 cells were co-transfected with ARA_{70} and AR under

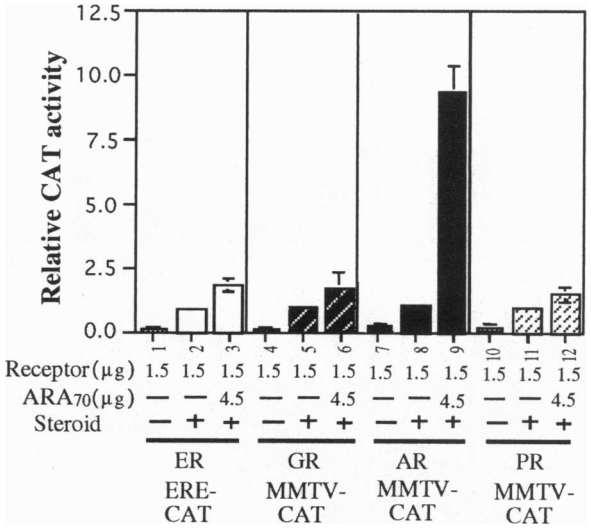

FIG. 6. Effect of ARA_{70} on other steroid receptors. DU145 cells were transfected with varies receptors and reporters (3.5 μg), including glucocorticoid receptor/MMTV-CAT, PR/MMTV-CAT, ER/ERE-CAT, AR/MMTV-CAT in the presence of 10^{-8} M DEX, progesterone, 17β-estradiol (E2), DHT, respectively.

eukaryotic promoter control. Ligand-free AR has minimal MMTV-ARE CAT reporter activity with or without the presence of ARA_{70} (Fig. 5*A*, lanes 2 and 3). Addition of DHT results in a 6-fold increase of AR activity (Fig. 5*A*, lanes 2 *versus* 4). Furthermore, this transcriptional activity can be increased to 58 (± 3.2)-fold (mean ± SEM; $n = 4$) by the co-transfection of ARA_{70} cDNAs in a dose-dependent manner (Fig. 5*A*, lanes 5–8). The induced activity reached a plateau at 4.5 μg of co-transfected ARA_{70} cDNA and the additional ARA_{70} beyond 4.5 μg (up to 6 μg) did not affect the induced activity of AR in DU145 cells. To rule out any indirect effects on the basal activity of the MMTV-ARE-CAT reporter, we removed the ARE DNA fragment from our reporter (MMTV-ΔARE-CAT). The results showed that ARA_{70} induced no activity on this reporter in the presence or in the absence of DHT (Fig. 5*A*, lanes 9–14).

We also replaced ARA_{70} with another nuclear orphan receptor-associated protein, TR4AP, in the AR:MMTV-ARE CAT reporter assay and found that this protein had no effect in our assay (data not shown). Furthermore, when we replaced DU145 cells with Chinese hamster ovary cells, which express a relative abundance of ARA_{70}, we found that although the exogenously transfected ARA_{70} did not show a dramatic effect on induction of AR transcriptional activity, the transfection of antisense ARA_{70} did partially block the AR transcriptional activity (data not shown). Together, these data strongly suggest that stimulation of AR transcriptional activity by ARA_{70} occurs through a specific ligand-bound AR and that the relative amount of AR *versus* ARA_{70} in cells may also play an important role for the activation of AR.

The effect of ARA_{70} on transactivation of AR bound to different concentrations of T, DHT, and HF in DU145 cells was also tested. Whereas 10^{-10} M DHT might maximize induced transcriptional activity of AR, T needed a 10-fold higher concentration (10^{-9} M) for maximum activity (Fig. 5*B*). HF induced only a few activity at a pharmacological concentration (10^{-6} M). These results are consistent with the data generated from yeast cells (Fig. 1*B*) and previous reports (12, 13), which indicated that DHT is a more potent androgen in the prostate. In fact, the greater potency of DHT to modulate the interaction between AR and ARA_{70} may actually provide another reason why DHT is a more potent androgen in prostate.

The enhancement of AR transcriptional activity from 6-fold to 58-fold by ARA_{70} may expand androgen activity in the

prostate that androgen-AR alone cannot reach. Since we detected ARA_{70} in AR-positive LNCaP prostate cancer cells but not in AR-negative DU145 cells, it will be important to determine if the expression of ARA_{70} and its ability to interact properly with androgen-AR change during the progression of prostate cancer from an androgen-dependent to an androgen-independent state.

ARA_{70} Functions As a Specific Activator to Enhance the Transcriptional Activity of AR. We also examined the effect of ARA_{70} on the transcriptional activity of several other steroid receptors through their cognate DNA response elements. As shown in Fig. 6, while ARA_{70} can induce the transcriptional activity of AR up to 10-fold, ARA_{70} can only slightly enhance (up to 2-fold) the transcription activity of other steroid receptors, such as glucocorticoid receptor (GR), progesterone receptor (PR), and estrogen receptor (ER). These results clearly indicate that ARA_{70} is a more specific coactivator for AR.

Several proteins have been demonstrated to interact with other steroid receptors in a ligand-dependent or ligand-independent manner (14–18). However, none of these proteins have been shown to enhance specifically AR-mediated transcriptional activity; therefore, it is likely that ARA_{70} has a different mechanism for interacting with AR.

In summary, our data demonstrated that ARA_{70} is the first identified ligand-dependent associated protein for AR that may function as a specific coactivator for inducing the transcriptional activity of AR in human prostate cells. Further studying the potential role of ARA_{70} may therefore help us to understand better the molecular mechanism of androgen action.

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