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Caveolin-1 Interacts with Androgen Receptor

A POSITIVE MODULATOR OF ANDROGEN RECEPTOR MEDIATED TRANSACTIVATION*

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Androgen receptor (AR) belongs to the steroid hormone nuclear receptor superfamily. It functions as an androgen-dependent transcriptional factor that regulates genes for cell proliferation and differentiation. Caveolin is a principal component of caveolae membranes serving as a scaffold protein of many signal transduction pathways. Recent results correlate caveolin-1 expression with androgen sensitivity in murine prostate cancer. Furthermore, immunohistochemical staining of patient specimens suggests that caveolin expression may be an independent predictor of progression of prostate cancer. In this study, we investigate the potential interactions between AR signaling and caveolin-1 and demonstrate that overexpression of caveolin-1 potentiates ligand-dependent AR activation. Conversely, down-regulation of caveolin-1 expression by a caveolin-1 antisense expression construct can down-regulate ligand-dependent AR activation. Association between these two molecules is also demonstrated by co-localization of AR with caveolin-rich, low-density membrane fractions isolated by an equilibrium sucrose gradient centrifugation method. Co-immunoprecipitation and glutathione *S*-transferase fusion protein pull-down experiments demonstrate that interaction between AR and caveolin-1 is an androgen-dependent process, offering further evidence for a physiological role of this interaction. Using a mammalian two-hybrid assay system, we determine that the NH_2 terminus region of caveolin-1 is responsible for the interaction with both the NH_2-terminal domain and the ligand-binding domain of AR.

Androgen receptor (AR)[1] belongs to the steroid hormone nuclear receptor superfamily. It functions as a ligand-dependent transcriptional factor that regulates genes for cell proliferation and differentiation. Similar to glucocorticoid and mineralocorticoid steroid receptors, AR remains in the cytoplasm until it is activated by ligand binding (1, 2). In a classic activation paradigm, AR is dissociated from the heat-shock protein chaperone complex upon ligand binding and dimerized AR translocates into the nucleus where transcriptional activation is initiated by binding to the cognate regulatory sequence on target genes. There is evidence that signal cross-talk between AR activation and growth factors such as epidermal growth factor, keratinocyte growth factor, and insulin-like growth factor-I (3–5) mediates signal transduction. Other nongrowth factor-related signal pathways reported to modulate AR include protein kinase A, which mediates ligand-independent AR activation (6, 7), and protein kinase C, which negatively regulates AR-dependent transcription (8). It is unclear how signaling by these nonsteroid growth factors or kinase pathways overlap with the AR activation pathway. Either they directly affect the assembly or transport of the transformed AR or they affect AR transcriptional activity by interacting with overlapping general transcriptional regulators.

Recent studies by Thompson and colleagues (9) suggest that expression of caveolin-1 may regulate androgen responsiveness in prostate cancer. They found, in samples from patients with prostate cancer, a positive correlation between expression of caveolin-1 and progression of the cancer (9). Using tumor cells derived from the mouse prostate reconstitution model (10, 11), these authors link the expression of caveolin-1 to androgen sensitivity in hormone-resistant metastatic prostate cancer (12). Moreover, immunohistochemical staining of caveolin-1 in tumor samples from patients who had undergone radical prostatectomy suggested that caveolin-1 immunostaining is an independent predictor of disease progression (13). Among 187 specimens from lymph node-negative cancers, 47 were found with caveolin-1 immunoreactivity, which correlates with a shorter interval to postsurgical recurrence.

Caveolin-1, a 21–24-kDa integral membrane protein, is a major component of the caveolae membrane structures, with a flask-shaped invagination that are enriched with cholesterol and glycosphingolipid as well as with lipid-modified signaling proteins. Caveolin-1 has been implicated as a principal structural scaffold for the oligomerization and organization of cytoplasmic signal complexes (14–16). Interaction with and modulation by caveolin-1 has been shown in many signal transduction pathways, including those regulated by receptor or soluble tyrosine kinases. Caveolin-1 has been shown to regulate the activity of phosphatidylinositol 3-kinase associated with receptor tyrosine kinase (17) and to associate with and regulate endothelial nitric-oxide synthase (18, 19), epidermal growth factor receptor (20), and insulin receptor (21). Targeted down-regulation of caveolin-1 expression in 3T3 cells results in hyperactivation of mitogen-activated p42/44 protein kinases as well as loss of anchorage-dependent cell growth (22, 23). However, the physiological consequences of caveolin-1 overexpression remain controversial. Interaction of caveolin-1 with many

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[1] The abbreviations used are: AR, androgen receptor; GST, glutathione transferase; DHT, dihydrotesteosterone; ARE, androgen-responsive element; DBD, DNA-binding domain; LBD, ligand-binding domain; HA, hemagglutinin antigen; CMV, cytomegalovirus; PAGE, polyacrylamide gel electrophoresis; Mes, 4-morpholineethanesulfonic acid.

of the signal transduction components is thought to have important consequences for cellular transformation. In lung and breast cancer cells, overexpression of caveolin-1 results in reduced transformation phenotypes (24, 25), suggesting a tumor suppressor role of caveolin-1. A reciprocal relationship between Her2/Neu tyrosine kinase activity and caveolin-1 expression has been documented in mammary adenocarcinoma. Ectopic overexpression of caveolin-1 inhibits Her2/Neu activity *in vivo*, a further suggestion of a tumor suppressor role of caveolin-1 (26). In cells derived from an mouse prostate reconstitution model, however, overexpression of caveolin-1 promotes resistance to apoptosis induced by androgen withdrawal, suggesting a promoter role of caveolin-1 in prostate tumor progression (12). Steroid hormone estrogen receptors have been shown to be potentiated by caveolin-1 in their transcriptional activities (27). Furthermore, nongenomic estradiol stimulation of nitric oxide release has been shown to be mediated by estrogen receptors localized in caveolae (28).

In light of these findings, we investigate the potential interactions between AR signaling and caveolin-1. We demonstrate that overexpression of caveolin-1 potentiates ligand-dependent AR activation and, conversely, that down-regulation of caveolin-1 expression by a caveolin-1 antisense expression construct can down-regulate ligand-dependent AR activation. We also demonstrate an association between these two molecules by finding co-localization of AR with caveolin-rich, low-density membrane fractions isolated by an equilibrium sucrose gradient centrifugation method. Co-immunoprecipitation and GST fusion protein pull-down experiments demonstrated that interaction between AR and caveolin-1 is an androgen-dependent process, offering further evidence for a physiological role of this interaction. Using a mammalian two-hybrid assay system, we determine that the caveolin-1 NH_2 terminus region is responsible for the interaction with both the NH_2-terminal domain and the ligand-binding domain (LBD) of AR.

EXPERIMENTAL PROCEDURES

Materials—Dihydrotesteosterone (DHT) was purchased from Sigma, polyclonal antibody to caveolin-1 from Transduction Laboratories (Lexington, KY), monoclonal antibody against AR from Santa Cruz Biotechnology (Santa Cruz, CA), and monoclonal antibody to hemagglutinin antigen (HA) from Berkeley Antibody (Berkeley, CA). A kit for selection of transfected cells, Capture-Tec System, was purchased from Invitrogen (Carlsbad, CA) and charcoal-dextran treated fetal bovine serum from HyClone (Denver, CO).

Expression Vector Constructs—Human AR pSV-hAR (29) was cloned into pCDNA3.0 (Invitrogen) downstream of a CMV immediate early promoter. Sense and antisense caveolin-1 constructs, a gift from H. Chapman (Harvard Medical School), were cloned into the pCEP4 and pMEP4 vectors, respectively (Invitrogen). An androgen-responsive luciferase reporter construct driven by a minimal promoter was constructed by inserting four synthetic tandem repeats of the androgen-responsive element (ARE) primers (5′-TGTACAGGATGTTCTGAATTCCATGTACAGGATGTTCT-3′ and 5′-AGAACATCCTGTACATGAATTCAAGAACATCCTGTACA-3′) in front of an E1b minimal TATA box sequence, followed by a firefly luciferase gene. A renilla luciferase reporter gene driven by a CMV promoter was used as a transient transfection internal control. Mammalian two-hybrid expression vectors expressing fusion protein of VP16-AR (full-length), AR-N-(1–500), DBD-(501–660), and LBD-(661–919) were constructed by cloning the corresponding fragments in-frame with partial herpes transactivating protein (VP16, residues 411–456) as a fusion gene into the pACT vector (Promega, Madison, WI). Caveolin-1 full-length and truncated mutants (Cav-(1–60), Cav-(58–178), Cav-(60–100), and Cav-(135–178)) were cloned in-frame fused with GAL4-DBD (residues 1–147) in a pBIND vector (Promega). Luciferase reporters pTet-off (encoding a tetracycline repressor protein driven by CMV promoter) and pTRE-luc (luciferase reporter driven by seven tandem repeats of tetracyclin-responsive element-fused minimal promoter) (CLONTECH, Palo Alto, CA), pGA-L-VP16 (CMV-driven expression vector of Gal4 DNA-binding domain fused with VP16 transactivating domain) (O. Gjoerup, Dana-Farber Cancer Institute), and pG5-Luc (luciferase driven by five tandem-repeats of Gal4 binding sequence-fused minimal promoter) (Promega) were used as nonandrogen responsive reporter control vector for monitoring general transcriptional activities.

Cell Culture and Transient Transfection—PC3 and LNCap cells were kept in RPMI 1640 supplemented with antibiotics (penicillin and streptomycin) and 10% fetal bovine serum. HEK293 cells were grown in Dulbecco's modified Eagle's medium (high glucose) supplemented with antibiotics and 10% fetal bovine serum. HEK293 cells overexpressing caveolin (293-Cav) or harboring an antisense caveolin driven by a metallothionine promoter (293-AS), gift of Dr. Chapman (30), were kept in the same medium as 293 cells supplemented with hygromycin. For androgen stimulation experiments, cells were grown in the same medium supplemented with 10% charcoal stripped fetal bovine serum (HyClone). Cells were transfected by electroporation with a total of 10 μg of plasmid DNA using a Bio-Rad Gene Pulser (Bio-Rad), and a luciferase assay was performed 48 h after transfection.

Establishing Stable LNCap Cells Expressing Caveolin-1—The pCDNA3.0 vector (Invitrogen) was used to subclone the full-length human caveolin-1 cDNA downstream of the CMV promoter. LNCap cells were transfected with pCDNA-Cav (LNCap-Cav), and an empty vector was used as a noncaveolin expressing LNCap parental control. Clones were selected in medium containing 300 μg/ml G418.

Preparation of Caveolae-enriched Membrane by Subcellular Fractionation—Low-density, caveolae-rich membrane fractions were isolated as described previously (31). Four 100-mm dishes of caveolin-1-expressing LNCap cells (LNCap-Cav) were grown to confluence (a total 2×10^7 cells), scraped into 1.5 ml of 0.5 M sodium carbonate buffer (pH 11.0), homogenized with a hand-held Polytron on ice (three 3-s bursts at medium speed), and sonicated (three 1-min bursts at 90% output with a Branson 450 sonicator). The resulting cell lysates were cleared by centrifugation on a bench-top centrifuge at 600 $\times$ *g* for 5 min. Supernatants were mixed with equal amounts of 90% sucrose in Mes-buffered saline (MBS; 50 mM Mes, pH 6.8, 150 mM NaCl) to make a concentration of 45% sucrose. Lysates containing the 45% sucrose were transferred to a centrifuge tube and overlaid with 4 ml each of 35 and 5% sucrose in MBS containing 0.25 M carbonate. The gradient was centrifuged at 39,000 rpm (200,000 $\times$ *g*) for 21 h with a Beckman SW41Ti rotor. The resulting gradient fractions were analyzed by collecting 12 1-ml fractions from the bottom of the gradient. The fractions were subjected to Western blot analysis with specific antibodies against either AR or caveolin-1.

Mammalian Two-hybrid Assay—A mammalian two-hybrid assay was performed with the vector system, pBIND and pACT, commercially available from Promega. Interaction between AR and caveolin-1 was determined with pACT-AR and its truncated mutants derived from full-length AR with pBIND-caveolin constructs. HeLa cells were transfected with the above expression constructs along with a luciferase reporter construct driven by a minimal promoter fused with GAL4-binding element. Activity was determined by a dual luciferase assay (Promega) in the presence and absence of 1 nM DHT. Fold induction relative to basal activity of cells transfected with straight pACT and pBIND control vectors was calculated and normalized to the renilla luciferase internal transfection control.

Immunoprecipitation—A standard protocol was used for immunoprecipitation of AR and caveolin-1. In brief, cells were lysed in immunoprecipitation RIPA buffer containing 50 mM Tris (pH 7.4), 135 mM NaCl, 1% (v/v) Triton X-100, and 60 mM octylglucoside and supplemented with protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 5 mM diisopropyl fluorophosphate, 5 μg/ml pepstatin, and 1 mM EDTA). Lysates were cleared by centrifugation at 12,000 $\times$ *g* for 30 min at 4 °C. Supernatants were incubated with individual antibodies (1 μg) and protein A-Sepharose beads (20 μl of packed beads) at 4 °C for 1 h. At the end of incubation, beads were washed 5 times with lysis buffer. The resulting immunoprecipitated immunocomplexes were solubilized in 40 μl of Laemmli sample buffer, resolved by SDS-PAGE, and transferred to a nitrocellulose membrane. The protein complex was detected by Western blot analysis and developed by ECL (Amersham Pharmacia Biotech, Piscataway, NJ).

GST Fusion Protein Pull-down Experiment—GST-caveolin fusion protein was created by fusing full-length caveolin-1 in-frame to the 3′ end of glutathione transferase using a PGEX-2T vector (Amersham Pharmacia Biotech) under the regulation of a tac promoter. *In vitro* translated AR labeled with [^{35}S]methionine was obtained with the TNT system for coupled transcription and translation *in vitro* (Promega). GST-Cav fusion proteins were immobilized on glutathione beads and resuspended in binding buffer (100 mM NaCl, 1 mM EDTA, 0.05% Nonidet P-40, 0.2% bovine serum albumin, 20 mM Tris, pH 8.0). GST-Cav packed beads (20 μl) were incubated with *in vitro* translated AR

labeled with [^{35}S]methionine in a total of 40 μl of binding buffer and incubated at 4 °C in the presence or absence of 10 nM DHT for 4 h. After incubation, the beads were washed 5 times with 1.5 ml of washing buffer (100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 20 mM Tris, pH 8.0). The resulting GST·Cav complexes were resolved by SDS-PAGE and visualized by autoradiography.

Enrichment of Positive Transfectants using Hapten-coated Magnetic Colloidal Beads—To enrich positive transfectants in a transient transfection experiment, we employed the commercially available kit Capture-Tec (Invitrogen). The procedure for isolation of positive transfectants was performed following the manufacturer's instructions. In brief, PC3 cells for AR reporter assay experiments were co-transfected with pHook-1 plasmid encoding a membrane-anchored single-chain antibody (sFv) directed against the hapten 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one. 16 h after transfection, cells were harvested with phosphate-buffered saline/EDTA and resuspended in phosphate-buffered saline; 10 μl of hapten-coated colloidal beads was added to 2×10^6 transfected cells and incubated at 37 °C with constant mixing for 30 min. Transfected cells expressing sFv were selected by immobilizing cells on a magnetic stand in an Eppendorf tube, while cells not expressing sFv were washed away. The resulting cells were subjected to biochemical analysis or replating for the AR reporter gene assay. To determine the recovery efficiency for positive transfectants, we used PC3 cells that were co-transfected with pHook-1 and green fluorescence protein and then counterstained with Hochst dye in a parallel experiment to monitor recovery rate by counting green fluorescent cells under a fluorescence microscope. We were constantly able to enrich cells from 90% to 95% with positive green fluorescence protein expression as compared with <10% positive transfectants in a preselected population.

Gel Electrophoresis and Immunoblotting—Proteins were separated by SDS-PAGE with a standard reducing condition protocol. After electrophoresis, proteins were electroblotted to a nitrocellulose membrane. The protein bands were visualized by Ponceau S staining. Blots were blocked by 5% nonfat dry milk, 0.05% Tween 20 in Tris-buffered saline (10 mM Tris, pH 8.0, 135 mM NaCl). Immunoblotting was performed with designated antibodies and visualized with an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech) following the manufacturer's protocol.

RESULTS

Overexpression of Caveolin-1 Enhances AR-mediated Transcriptional Activity and Increases the Sensitivity of AR to Ligand-dependent Activation—High levels of caveolin-1 expression have been observed to correlate with the sensitivity of murine metastatic prostate cancer cells to androgen withdrawal (12), a finding that suggests caveolin plays a role in AR signaling. To test this hypothesis, we used a cell culture model to determine whether changes in the levels of caveolin-1 expression alter ligand-dependent AR activation. The HEK293 cells expressed detectably lower levels of caveolin-1 compared with the PC3 cells, allowing for greater manipulation of caveolin-1 expression by transfection (data not shown). We used HEK293 lines stably transfected with either a wild-type caveolin-1 construct (293-Cav) or an antisense caveolin-1 construct (293-AS) driven by either CMV or an inducible metallothionine promoter, respectively. As shown in Fig. 1*A*, 293-Cav constitutively expresses levels of caveolin-1 ~10 times higher than those expressed by the parental vector control HEK293 line, as determined by video densitometry. Expression of caveolin-1 in the 293-AS cells was further reduced to 25% of the level of parental 293 control group when an antisense construct driven by a metallothionine promoter was induced by inclusion of 1 μM cadmium in the culture medium.

Responses of AR signaling in cells expressing different levels of caveolin-1 expression were determined by transient co-transfection of pCDNA-hAR and p(ARE)$_4$-Luc into the above mentioned cell lines, followed by exposure to DHT (1 nM). As shown in Fig. 1*B*, in the absence of DHT stimulation, overexpression of caveolin-1 resulted in a 4-fold elevation of basal ARE-luciferase reporter activity in 293-Cav cells as compared with that of parental 293 vector control cells. These results suggest that caveolin-1 overexpression sensitizes cells to AR mediated signaling which induces a moderate ligand-independent activation of AR. This observation is consistent with the previous finding that caveolin-1 overexpression promotes estrogen receptor α ligand-independent signaling (27). In the DHT-stimulated groups (293 *versus* 293-Cav), overexpressing caveolin-1 dramatically up-regulated the expression of ARE-luciferase reporter which may be the result of cell sensitization. Down-regulation of caveolin-1 expression by a caveolin antisense construct limited the induction of ligand-dependent AR tran-

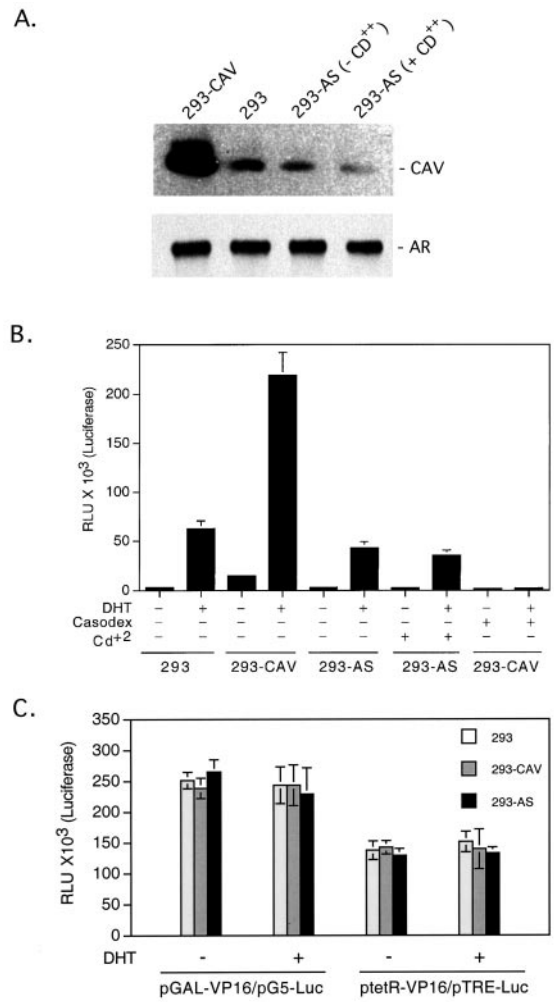

FIG. 1. **Caveolin-1 overexpression potentiates ligand-dependent AR transcriptional activity.** *A,* levels of expression of caveolin-1 protein were determined by Western blots in the 293 parental vector control, 293 cells overexpressing caveolin-1 (293-Cav), or 293 cells stably expressing antisense caveolin-1 (293-AS) under a cadmium (Cd^{2+})-inducible metallothionine promoter. In the *lower panel*, the levels of transient expression of AR from each transfection group were equal, as determined by anti-AR Western blot analysis. *B,* androgen-dependent AR transcriptional activities in 293 cells expressing different levels of caveolin-1 were determined by transient transfection with 0.5 μg of pCDNA-AR and 9.5 μg of p(ARE)$_4$-Luc. DHT (1 nM), vehicle or bicalutamide Casodex (100 nM) was added to the culture 24 h after cells were transfected with AR and luciferase reporter genes. The luciferase reporter assay was performed 24 h later. The luciferase activity was normalized by the internal control and the non-DHT-treated control groups. All experiments were repeated three times, with consistent results. Relative light unit (*RLU*) data represent the mean. *C,* non-AR-responsive control promoter/reporter systems, pGAL-VP16/pG5-Luc and ptetR-VP16/pTRE-Luc, are included to show that general transcription was not affected by changes in the levels of caveolin expression. The same 293 cell groups were transiently transfected with 0.5 μg of pGAL-VP16 expression vector with 9.5 μg of pG5-Luc reporter; or 0.5 μg of ptetR-VP16 expression vector with 9.5 μg of pTRE-Luc reporter. The luciferase reporter assay was carried out 48 h after transfection. Data represent the mean ± S.D. (n = 3).

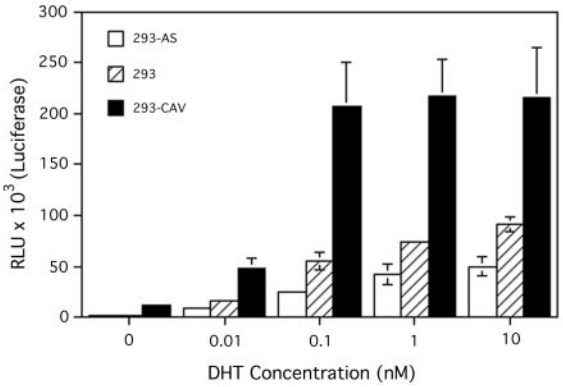

FIG. 2. **Caveolin-1 overexpression increases the sensitivity of AR to ligand-dependent transactivation.** This figure shows the dose-dependent (DHT: 0.01–10 nM) AR transcriptional response of 293 cells expressing various levels of caveolin-1. The IC_{50} for the ligand-dependent activation of 293 is approximated at 0.5 nM. To achieve the same fold stimulation, 293-Cav cells required 0.02 nM DHT, as determined by interpolation, which is 25-fold lower than that required by the parental 293 cells. The left-shift of the dose-response curve indicates an increase in the fold stimulation of AR in response to DHT in 293-Cav cells compared with parental 293 cells and 293-AS cells. Data represent the mean ± S.D. (n = 3)

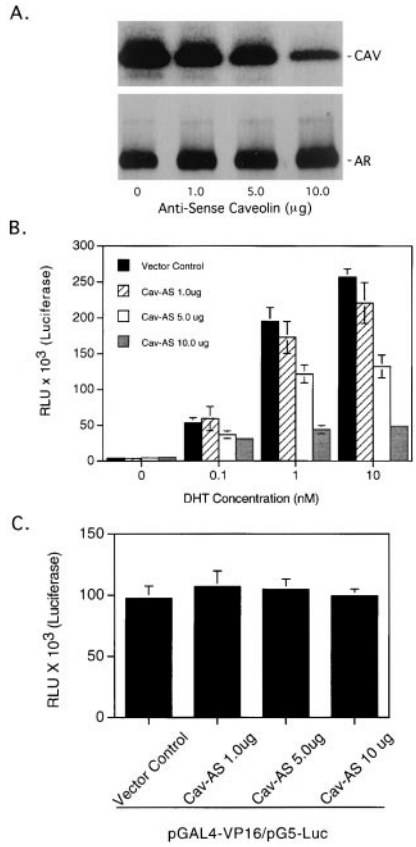

FIG. 3. **Antisense caveolin-1 down-regulates AR transactivation in PC3 cells.** *A,* antisense caveolin-1 reduces caveolin-1 protein expression in PC3 cells as determined by Western blot analysis. Various quantities (0, 1.0, 5.0, and 10.0 μg) of antisense caveolin-1 expression vector (pCEP-Cav-AS) were transiently transfected into PC3 cells together with 1 μg of pHook-1 expression vector for enrichment of the transfected cells (see "Experimental Procedures"). Cell lysates were collected 48 h afterwards. Caveolin expression was determined by immunoblot. Level of AR in the co-transfectants remained the same in the different transfection groups, as shown in the *lower panel*. *B,* transient AR transactivation experiments were performed in parallel to establish a dose-response curve for AR transactivation by co-transfecting pCDNA-AR and p(ARE)$_4$-Luc. Data represent the mean ± S.D. of firefly luciferase activity normalized to renilla luciferase activity by a dual luciferase assay (n = 3). *C,* non-AR-responsive control promoter/reporter system, pGAL-VP16 and pG5-Luc, are included to show that general transcription was not affected by changing levels of caveolin expression. The luciferase reporter assay was carried out 48 h post-transfection. Data represent the mean ± S.D. (n = 3).

scription activation to one-half of level of the control parental cells. Thus, caveolin-1 overexpression is sufficient to induce a moderate ligand-independent activation of AR, and caveolin-1 potentiates AR transcriptional activity in the presence of ligand. On the other hand, the anti-androgen bicalutamide (Casodex) completely blocked the AR-mediated transactivation of luciferase gene expression regardless of caveolin-1 overexpression. Overexpression of caveolin-1 does not alter AR ligand specificity since estrogen does not stimulate the AR response (data not shown). We determined that this modulated AR transactivation response was not the result of different levels of AR expression in various transfection groups. As demonstrated in Fig. 1*A* (*lower panel*), the expression level of AR is maintained at similar levels in each group. Two AR unresponsive negative control promoter/reporter systems, pGAL-VP16/pG5-luc and ptetR-VP16/pTRE-luc, were tested to demonstrate that changes in the level of caveolin-1 expression do not affect the general transcription activities (Fig. 1*C*).

We hypothesize that cultured cell lines overexpressing caveolin-1 sensitize AR (making it "hyperactive") by lowering the critical concentration of androgen required for AR activation. Using the same transient transfection assay, we tested this hypothesis by establishing an androgen dose-response curve for ligand-dependent AR activation in HEK293 cells expressing various levels of caveolin-1. As shown in Fig. 2, the IC_{50} for the ligand-dependent activation of vector control parental 293 cells was approximated at 0.5 nM DHT. In contrast, the 293-Cav cells required only 0.02 nM DHT to achieve the same fold stimulation (determined by interpolation), 25 times lower than that required by the parental 293 cells. The left-shift of the dose-response curve indicated an increase in fold stimulation of AR in response to DHT in 293-Cav cells compared with parental 293 cells and 293-AS cells. This experiment demonstrated that caveolin-1 overexpression in 293-Cav cells dramatically increased androgen receptor-mediated transcriptional activation as 293-CAV cells required a much lower concentration of androgen to achieve the same androgenic responses as that achieved by the 293 parental vector control cells.

To further confirm these results, we tested the prostate cancer PC3 cell line. Since PC3 expresses very high levels of caveolin-1, efforts to increase levels of caveolin expression appreciably by heterologous overexpression proved futile (data not shown). We used a transient system with an antisense caveolin-1 construct to down-regulate the expression. By co-transfecting a membrane-anchored mouse sFv receptor (see "Experimental Procedures") with antisense caveolin-1 into PC3 cells, we were able to enrich the positive transfectants harboring the caveolin antisense expression vector in a transient transfection experiment with the aid of colloidal magnets coated with sFv-binding hapten. As shown in Fig. 3*A*, an antisense vector dose-dependent down-regulation of caveolin-1 expression in PC3 cells was observed, whereas the levels of AR protein remained equal in various groups. This system was used in parallel to test our hypothesis concerning caveolin-1 modulation of AR transcription. As shown in Fig. 3*B*, down-regulation of caveolin-1 expression in PC3 cells by antisense caveolin-1 reduced the androgen sensitivity of AR. This reduction in AR transactivation activity, as shown in Fig. 3*A*, was not due to reduced levels of AR expression. Down-regulation of caveolin-1 expression by antisense did not affect AR-unresponsive general transcriptional activity of the pGAL-VP16/pG5-

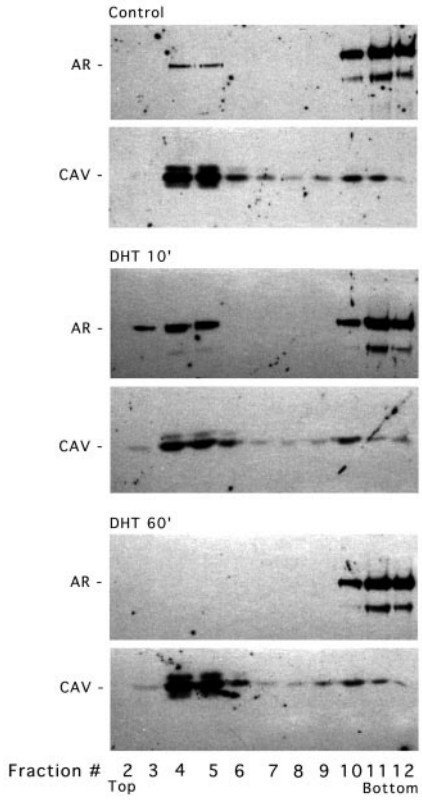

FIG. 4. **Co-sedimentation of AR with caveolin-rich caveolae membrane domain fractions.** LNCap cells stably expressing caveolin-1 (LNCap-Cav) were treated without (vehicle) or with DHT (1 nM) for 10 and 60 min. Cells were lysed in alkaline lysis buffer as described under "Experimental Procedures." The resulting cell lysates were subjected to equilibrium sucrose density gradient centrifugation. Twelve 1-ml fractions were collected from the gradients and resolved by SDS-PAGE and then by transblotting to a nitrocellulose membrane. Immunoblot analysis was performed with anti-Cav and anti-AR, respectively. As shown, the association of AR with the caveolin-rich caveolae membrane fraction in response to DHT treatment was transiently increased, whereas the distribution of caveolin-1 remained unchanged.

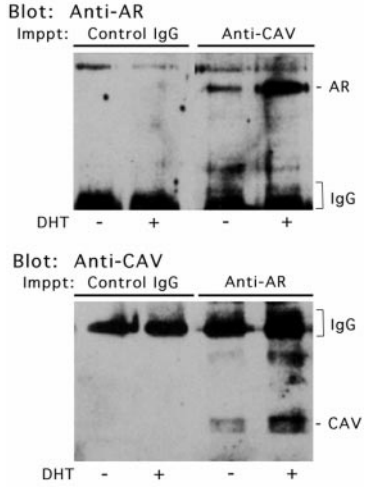

FIG. 5. **Co-immunoprecipitation of AR and caveolin-1 from intact cells.** Cell lysates from LNCap-Cav cells stimulated for 10 min with 1 nM DHT or vehicle were subjected to immunoprecipitation by antibodies against caveolin-1 (polyclonal, anti-CAV) or AR (monoclonal, anti-AR). Immunoprecipitates were resolved by SDS-PAGE and transblotted to nitrocellulose membranes. Membranes were subjected to immunoblot analysis, reciprocally probed with anti-AR or anti-CAV, respectively, and then developed with enhanced ECL. Rabbit IgG and mouse IgG were used, respectively, as the control IgG in these experiments. As indicated in the blot, an increased association of AR and caveolin-1 was detected in response to DHT treatment.

Luc reporter system (Fig. 3*C*). These results strongly suggest that cross-talk occurs between the AR activation pathway and the caveolin-1-associated signal complex and further indicate that the level of caveolin expression correlates positively with AR-mediated transcriptional activity.

Cosedimentation of AR with Caveolin-1-enriched Caveolae Membrane Domain Fractions—The results of AR activity modulated by caveolin-1 expression suggest potential physical interactions between these two molecules. To evaluate the association of AR with caveolin-rich caveolae membrane complex, we performed equilibrium sucrose density gradient centrifugation (31) to determine whether these two molecules co-localize in the caveolin-enriched membrane fractions. Equilibrium sucrose density gradient centrifugation is widely used for isolation of caveolae-enriched membrane fractions because these membrane subdomains contain high levels of cholesterol and sphingolipids with characteristic low buoyant density (16). Since LNCap cells express AR but do not express detectable amounts of caveolin-1 (9, 32),[2] we established a stable LNCap cell line expressing caveolin-1 constitutively (LNCap-Cav). LNCap-Cav cells were stimulated with vehicle or 1 nM DHT for 10 and 60 min. Caveolin-containing low-density membrane fractions were collected by equilibrium sucrose gradient as described under "Experimental Procedures." As shown in Fig. 4, only a small amount of AR was associated with the caveolin-rich density membrane fractions in nonandrogen-stimulated LNCap-Cav cells (*upper panel*, control), as detected by Western blot. Association of AR with the low-density, caveolin-rich membrane fractions increased with androgen stimulation (*middle panel*, 10 min). By 60 min, this association became undetectable (*lower panel*) as most of the AR became nucleus bound. Distribution of caveolin-1 in the caveolae membrane fractions remained constant throughout the time course. These results indicate that AR redistributes to the caveolin-rich membrane fractions in response to androgen stimulation and that such redistribution is a dynamic transient process since AR is no longer detected in the caveolin-rich membrane fractions once it becomes nucleus bound.

Co-immunoprecipitation of AR with Caveolin-1 in Response to Androgen Stimulation—The association of AR with caveolin-1-rich, low-density membrane fractions provides further evidence that this receptor is somehow interacting with caveolin-1 in response to androgen stimulation. However, the co-localized subcellular distribution does not indicate a direct interaction between these two molecules. Two approaches, *in vivo* co-immunoprecipitation and *in vitro* GST fusion protein pull-down, were undertaken to further address this issue. Co-immunoprecipitation studies in intact cells were performed with LNCap cells stably expressing caveolin-1 (LNCap-Cav). These cells were maintained in hormone-free medium for 72 h prior to the experiment. They were stimulated with 1 nM DHT or vehicle for 10 min at 37 °C. Cell lysates were collected for immunoprecipitation with antibodies against caveolin-1 (rabbit polyclonal), against AR (mouse monoclonal), or with mouse or rabbit IgG and then Western blotted. The membranes were probed reciprocally with antibodies against either AR or caveolin-1 and developed with horseradish peroxidase-coupled specific second antibodies in an enhanced ECL system. As shown in Fig. 5 (*upper panel*), association of AR with caveolin-1 was detected in anti-caveolin-1 immunoprecipitates. Consistent with the sucrose gradient AR partition assay described above, AR associated with caveolin-1 was detected at a low level in the nonandrogen-treated group, and the association increased after cells

[2] M. L. Lu and X. Zhang, unpublished observations

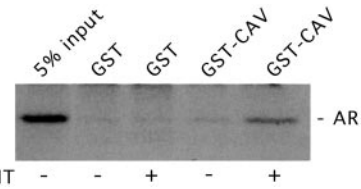

FIG. 6. ***In vitro* GST fusion protein binding studies of AR interactions with caveolin-1.** Androgen-dependent interaction of AR and caveolin-1 was tested by incubating *in vitro* translated [^{35}S]methionine AR with glutathione-agarose-immobilized GST-caveolin fusion protein or with GST alone in the presence or absence of 10 nM DHT. The incubation and washing were performed as described under "Experimental Procedures." The input lane represents 5% of the [^{35}S]methionine AR used per reaction.

were treated with 1 nM DHT for 10 min. Reciprocally (Fig. 5, *lower panel*), caveolin-1 also was detected in the anti-AR immunoprecipitation complex. In the control IgG groups, neither AR nor caveolin-1 was detected by Western blot analysis.

Direct Interaction between AR and Caveolin-1 as Determined by GST-caveolin Fusion Protein Pull-down Experiment—The co-immunoprecipitation of AR and caveolin-1 was further substantiated by a GST fusion protein pull-down experiment. Bacteria-expressed GST-caveolin fusion protein was immobilized on glutathione-Sepharose beads, which were incubated with *in vitro* translated AR labeled with [^{35}S]methionine in the presence or absence of androgen. As shown in Fig. 6, *in vitro* association of AR with immobilized caveolin is also an androgen-dependent event. Together, these results demonstrate that association between AR and caveolin-1 is a ligand-dependent process.

Determination of Interaction between AR Submolecular Domain and Caveolin-1 by Mammalian Two-hybrid Assay—The results described above indicate a direct interaction between AR and caveolin-1 during AR ligand-dependent activation. To further characterize this interaction at the submolecular level, we performed a mammalian two-hybrid assay. This approach was previously described in mapping the interaction between caveolin-1 and endothelial nitric-oxide synthase (33). As depicted in Fig. 7*A*, we divided AR into several submolecular domains: NH_2-terminal (AR-N, residues 1–500), NH_2-terminal with DNA-binding domain (AR-N/DBD, residues 1–660), DNA-binding domain alone (DBD, residues 500–660), and ligand-binding domain alone (LBD, residues 660–919). The full-length AR and submolecular domains were cloned in-frame with a herpes VP16 transactivation protein epitope tagged with HA using a pACT vector (see "Experimental Procedures"). Full-length caveolin-1 was cloned in-frame with Gal4 DNA-binding protein tagged with HA in a pBIND vector. Expression of the fusion proteins was confirmed by Western blot (Fig. 7*B*) using 12CA5 monoclonal antibody against HA tag. Consistent with the biochemical results, the two-hybrid results show that interaction between full-length AR and caveolin-1 is androgen-dependent, *i.e.* 46.6 ± 6.5-fold of induction ($p < 0.001$; two-tailed Student's t test for samples with unequal variances) as compared with vector control (Fig. 7*C*). Inclusion of bicalutamide (Casodex), an anti-androgen, also promoted the interaction between AR and caveolin (64.5 ± 6.5-fold of induction, $p < 0.005$), similar to an agonist effect, a finding consistent with the ligand dependence of this interaction observed above. The higher luciferase induction in the Casodex group may reflect a higher concentration of Casodex used for the treatment. Both AR-N and LBD domains showed an interaction with caveolin-1, with induction of 73.6 ± 10.4-fold and 14.2 ± 0.7-fold, respectively ($p < 0.005$), whereas AR-DBD showed no detectable interaction with caveolin-1. Constructs with overlapping DBD domains, AR-N/DB, exhibited the same levels of interaction as

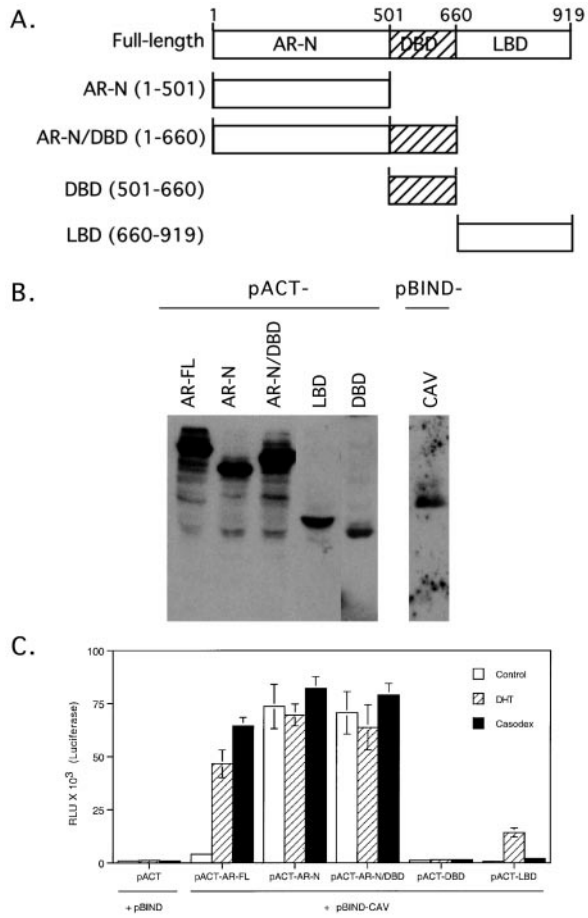

FIG. 7. **Mapping of AR submolecular domain responsible for caveolin-1 interaction by mammalian two-hybrid assay.** *A,* full-length AR and truncated mutants of AR encompassing various functional domains were cloned in-frame downstream of VP16 transactivation domain with a HA tag using a pACT vector. Caveolin-1 was cloned in-frame downstream of GAL4 DBD with a HA tag using a pBIND vector. *B,* immunoblot analysis of correspondent fusion proteins transiently expressed in HeLa cells, Gal4-caveolin (pBIND-Cav), VP-AR full-length (pACT-AR-FL), and truncated domains (AR-N, AR-N/DBD, LBD, DBD), by the anti-HA antibody 12CA5. *C,* a mammalian two-hybrid assay was performed by co-transfecting HeLa cells with 5 μg of pACT-AR or AR truncated mutants, as indicated, with 5 μg pBIND-caveolin and 5 μg of pG5-Luc vector. Cells were treated with vehicle control, DHT (10 nM), or bicalutamide Casodex (5.0 μM) for 20 h before the dual luciferase assay. A renilla luciferase reporter activity was used as a transfection internal control. The luciferase activity was normalized by the internal control and the vector basal control groups. All experiments were repeated three times, with consistent results. Data represent the mean ± S.D. ($n = 3$).

their nonoverlapping counterpart AR-N, an indication that DBD does not participate in the interaction. The interaction between caveolin-1 and LBD, although weaker, appeared to be androgen-dependent. It is interesting that the anti-androgen bicalutamide (Casodex) did not promote caveolin/LBD interaction. The interaction between the NH_2-terminal of AR (AR-N or AR-N/DBD) and caveolin-1 was not affected by treatment with the antagonist.

Mapping the Submolecular Regions of Caveolin-1 Supports the Interaction with AR—To determine the submolecular region required for the interaction of caveolin-1 with AR, we cloned caveolin-1 truncated mutants, Cav-(1–60), Cav-(58–100), and Cav-(135–178) (Fig. 8*A*), which encompass both the amino and carboxyl termini of cytoplasmic domains of caveolin-1 in-frame with the GAL4DBD in a pBIND vector with an HA tag. The expression of the corresponding constructs was confirmed by a Western blot analysis with use of anti-HA

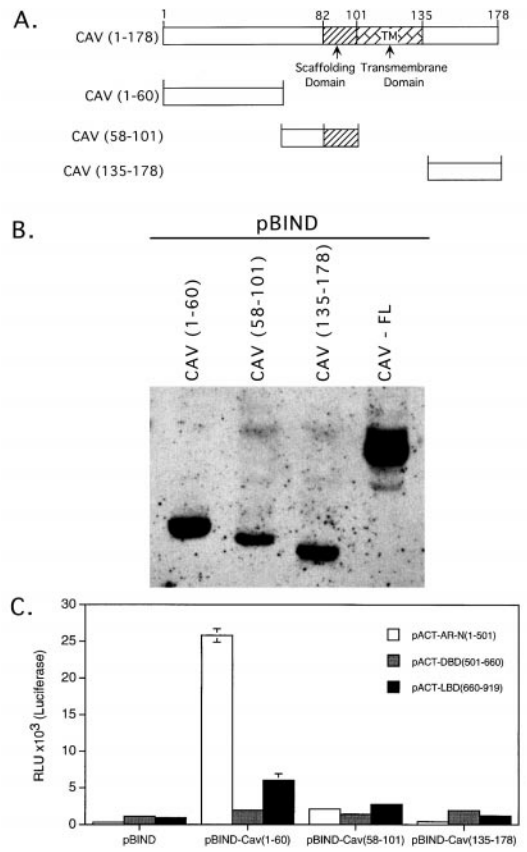

FIG. 8. **Mapping of caveolin-1 submolecular domain responsible for AR interaction by mammalian two-hybrid assay.** *A,* three truncated fragments of caveolin-1 were cloned in-frame with a GAL-4 DNA-binding domain using a pBIND vector with an HA tag toward NH_2 terminus. These domains encompass cytoplasmic portions of the caveolin-1. *B,* Western blot analysis of the expressed GAL-caveolin truncated fragment fusion protein with anti-HA (clone 12CA5). *C,* a mammalian two-hybrid assay was performed by co-transfecting HeLa cells with VP fusion of various AR domains (using a pACT vector), GAL4-caveolin truncated domain fusion (using a pBIND vector), and pG5-Luc vector. Cells were treated with vehicle control or DHT (10 nM) for 20 h before the dual luciferase assay. A renilla luciferase reporter was used as a transfection internal control. The luciferase activity was normalized by the internal control and the vector basal control groups. All experiments were repeated three times, with consistent results. Data represent the mean ± S.D. ($n = 3$).

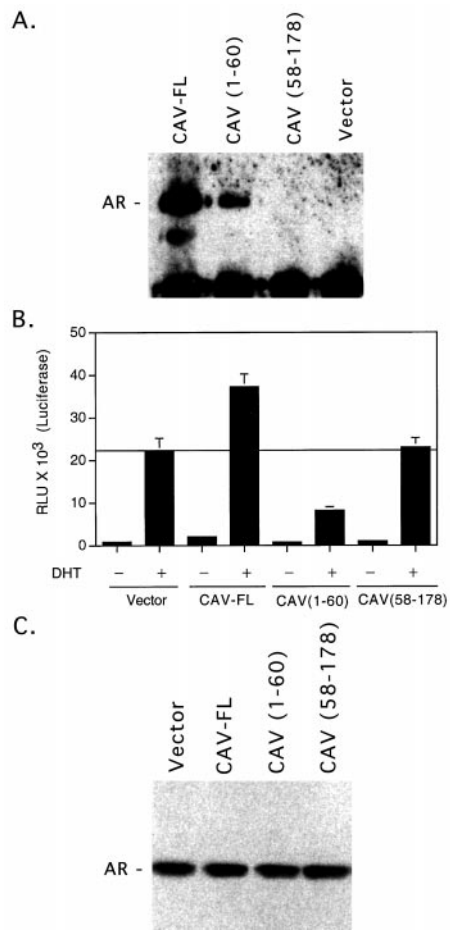

FIG. 9. **Caveolin-1 AR-binding domain deletion mutant does not potentiate AR transactivation activity.** *A,* co-immunoprecipitation of AR with caveolin and Cav-(1–60) but not with Cav-(58–178). 293 cells were co-transfected with 5 μg of pCDNA-AR and 5 μg of pCDNA harboring HA-tagged caveolin-1, truncated fragments, or an empty pCDNA3 vector. 24 h later, cell lysates from each group were subjected to immunoprecipitation by antibodies against HA (clone 12CA5). Immunoprecipitates were resolved by SDS-PAGE and transblotted to nitrocellulose membranes. Membranes were then subjected to immunoblot analysis probed with anti-AR and developed with enhanced ECL. *B,* androgen-dependent AR transcriptional activity in 293 cells transiently expressing caveolin-1 deletion mutants. 293 cells were co-transfected with 0.5 μg of pCDNA-AR and 4.5 μg of pCDNACav(FL), pCDNA-CAV-(1–60), or pCDNA-CAV-(58–178) along with 5 μg of $p(ARE)_4$-Luc. DHT (1 nM) was added to the culture 24 h after transfection. The luciferase assay was performed 48 h post-transfection. The luciferase activity was normalized by the internal control and the non-DHT-treated control groups. All experiments were repeated three times with consistent results. Data represent the mean ± S.D. ($n = 3$). *C,* the expression level of AR remained the same in each transfection group, as determined by anti-AR Western blot analysis.

12CA5 monoclonal antibody (Fig. 8*B*). A mammalian two-hybrid assay was carried out to detect the interactions between these various fragments with various AR domains, amino terminus (AR-N), DBD, or carboxyl domains (LBD), by transient co-transfection. As shown in Fig. 8*C*, a strong interaction was detected between caveolin NH_2-terminal amino acid residues 1–60 and the AR-N (25.8 ± 2.5-fold of induction; $p < 0.005$), while a weaker interaction was detected between the LDB domain of AR and the same caveolin-1 fragment (6.0 ± 0.3-fold of induction; $p < 0.05$). Since no functional domain was previously designated in the NH_2-terminal region of caveolin-1 (14), our result defines for the first time a potentially new interacting domain for caveolin-1. It was previously reported that deletion of the NH_2-terminal corresponding region in caveolin-3, a muscle-specific isoform of caveolin-1, results in no functional defect in the mediation of Ha-Ras signals (34). In this context, our results suggest that this domain may function in special signal events. Cav-(58–101) and Cav-(135–178) exhibit no detectable interactions with any of the submolecular domains of AR since only basal luciferase activities were detected in these groups.

Caveolin-1 Mutants with Deleted AR-binding Domain Do Not Interact with AR—To validate the physiologic relevance of the caveolin AR-binding domain identified from the two-hybrid assay, we used two of our deletion mutants of caveolin-1, Cav-(1–60) (the AR-binding domain), and Cav-(58–178) (deletion of AR-binding domain), to test their ability to interact with AR *in vivo* in co-immunoprecipitation and AR response transcriptional reporter assays. AR- and HA-tagged full-length caveolin (Cav-FL), Cav-(1–60), or Cav-(58–178) were co-transfected into 293 cells. HA-tagged full-length caveolin and deletion mutants were immunoprecipitated by anti-HA (clone 12CA5). The resulting immunoprecipitates were probed with polyclonal antibody to AR in a Western blot analysis. As expected, AR could be co-immunoprecipitated by HA-tagged Cav-(1–60), although at a lower level than the full-length caveolin (Fig. 9*A*). Conse-

quently, the AR-binding domain deletion mutant Cav-(58–178) exhibits no physical interaction with AR, as evidenced by the absence of AR in the immunoprecipitation complex. Next, using a transient co-transfection reporter assay, we characterized these deletion mutants of caveolin for their ability to modulate AR transactivation activity. As shown in Fig. 9*B*, co-transfection of caveolin wild type up-regulates AR transactivation activity as compared with activity in the vector control (1.73 ± 0.13-fold; $p < 0.005$). Co-transfection of the newly identified AR-binding domain deletion mutant Cav-(58–178) had no modulatory effect on AR transactivation. However, co-transfection of the caveolin AR-binding domain (Cav-(1–60)) with AR down-regulated AR transcriptional activity to 40% of the vector control. Since 293 cells expressed moderate levels of caveolin-1, down-regulation of AR transactivation by overexpression of the cytosolic soluble Cav-(1–60) fragment may have resulted from the interference of the interaction between AR and endogenous caveolin-1. The expression of AR in different transfection groups remained at similar levels as determined by Western blot (Fig. 9*C*).

DISCUSSION

In the current study, we demonstrate the interaction between AR and caveolin-1 and show that overexpression of caveolin-1 potentiates AR-mediated transcription. Our results support the notion that overexpression of caveolin-1 sensitizes AR signaling by lowering the critical concentration of androgen required for AR activation. It is therefore plausible that, in some prostate cancers, overexpression of caveolin-1 is one way by which the tumors become unresponsive to androgen deprivation and survive in the milieu characterized by a castration-like androgen concentration. The correlation of the level of caveolin-1 expression with disease progression demonstrates the changing physiology of cancer cells during cancer progression. The "neo-expression" of caveolin-1 in cancerous prostate cells appears to be a gain-of-function step during tumor progression. Immunohistochemical analysis of a prostate specimen (9, 13) demonstrated that normal human prostate and BPH epithelial cells do not express detectable levels of caveolin-1. Furthermore, detection of caveolin-1 expression in an organ-confined prostate cancer specimen predicts a shorter time to disease progression (13). These observations suggest that, in normal prostate epithelium, AR activation pathways interact with signal components associated with a caveolae functional equivalent, the cholesterol-rich raft microdomain, for signal transduction (35).

Caveolin-1 is a versatile protein that has a very complex functional domain organization. Several recent studies have elegantly defined domains involved in targeting of Golgi (residues 60–80 and 135–178), homologous oligomerization (residues 61–101), heterologous oligomerization (residues 168–178), scaffolding domain (residues 80–101), and transmembrane domain (residues 102–134) (36–38). Caveolin has been well recognized as a primary scaffolding protein in the membrane invagination caveolae. It has also been implicated in membrane trafficking of nonclathrin-dependent endocytosis and intracellular cholesterol transport. As a signal complex scaffold protein, caveolin-1 has been postulated to organize and modulate the signal outputs. The conventional "caveolae signaling hypothesis" proposed by Lisanti and colleagues (22, 26) suggests that caveolar localization of various signaling molecules provides a compartmental basis for their regulation and serves as a convergence point for cross-talk between different signaling pathways. Interaction between AR and caveolin may represent one such regulatory mechanism. Our data on the transient interaction between AR and caveolin upon ligand stimulation suggest a functional interplay between the caveolin-scaffolding signal complex and liganded AR. As demonstrated in the present study, AR does not associate with caveolin until it is activated by ligand binding. Interaction between AR and the "preassembled scaffold signal complex" may function by providing an organizational mechanism for molecular interaction and by maximizing the signal output. This hypothesis is consistent with our finding of a positive correlation between the level of caveolin expression and AR transactivation activity.

The association of various signal molecules with caveolin is mediated by a conserved caveolin scaffolding domain located in the membrane-proximal region as determined by domain mapping (39, 40). This domain recognizes a well defined caveolin-binding motif ($\phi X \phi XXXX \phi$ or $\phi XXXX \phi XX \phi$, where ϕ is a hydrophobic residue and X is any amino acid residue). Many signal components, including small GTPase, protein kinase-α, protein kinase-β, phospholipase Cγ, Src family kinases, mitogen-activated protein kinase, and receptor tyrosine kinases, have been proven to interact with caveolin-1 via this domain (14). Therefore, it is conceivable that AR is brought to the proximity of a preformed activation complex for further biochemical interaction via caveolin-1 association. Because AR is a phosphoprotein with dynamic regulation of its phosphorylation status (41, 42), many signal pathways regulated through the caveolin complex are also implicated in AR transactivation process, including mitogen-activated protein kinase (43) and epidermal growth factor receptor (44). It is reasonable to predict, although it remains to be proven, that AR-caveolin interaction may facilitate at least part of the AR phosphorylation and/or dephosphorylation process. Consequently, a recent report by Migliaccio *et al.* (45) demonstrates an agonist-dependent interaction and activation of tyrosine kinase Src by the androgen receptor and the estrogen receptor in prostate cancer cells. Although the occurrence of this interaction has not yet been clearly defined, it will be interesting to determine whether AR is brought to the proximity of Src by association with caveolin-1 upon ligand-dependent activation. Moreover, these interactions may be part of a general mechanism of steroid receptor regulation since caveolin has been shown to potentiate the transactivation process of estrogen receptor α (27). Furthermore, caveolin-1 also co-localizes with estrogen receptor α in an estradiol-dependent manner in a co-immunoprecipitation study. In agreement with our data, 4-hydroxytamoxifen, an antiestrogen, remains effective in inhibiting estrogen receptor α transactivation in the presence of caveolin-1 overexpression (27).

Alternatively, multiple lines of evidence demonstrate that caveolin-1 is also involved in the intracellular membrane trafficking. Caveolin has been shown to directly bind to cholesterol (46, 47). Treating cells with cholesterol oxidase causes a "retrograde" movement of caveolin from membrane caveolae to endoplasmic reticulum. The returning of caveolin-1 from endoplasmic reticulum to membrane caveolae has been determined to be microtubule-dependent (48). Furthermore, it has been shown that, while transporting cholesterol intracellularly, caveolin forms a complex with chaperone consisting of hsp56, cyclophylin 40, and cyclophilin A (49). The same subset of chaperones is also involved in AR transformation and nuclear translocation (50, 51), suggesting a potential overlap in the intracellular trafficking machinery. It is estimated that up to 10 to 15% of caveolin resides in the cytosol rather than being membrane-bound in 3T3 cells (49). It raises the possibility that the interaction between AR and caveolin may be targeted at AR nuclear translocation as part of the steroid-receptor transformation process. Moreover, a recent study by Ozanne *et al.* (52) shows that filamin, an actin cross-linking protein, is an AR-interacting protein. The authors show that ligand-dependent

AR nuclear translocation is facilitated by filamin interaction since AR remains in the cytoplasm even after prolonged incubation with androgen in filamin-deficient M2 cells. They propose a potential role of filamin in the organization of an active chaperone complex for AR nuclear translocation. Filamin has previously been identified as interacting with caveolin through the caveolin NH_2-terminal half (residues 1–101) (53). Interrelations between these observations and our results further underscore the possibility that the functional role of the AR-caveolin interaction may be targeted at AR nuclear translocation. Indeed, in the study by Schlegel *et al.* (27), overexpression of caveolin appears to potentiate estrogen receptor α nuclear translocation in a ligand-independent manner.

Our finding that the NH_2-terminal 60 amino acids of caveolin interact with AR is somewhat unexpected since the NH_2 terminus of caveolin (residues 1–60) has not previously been designated to be a functional domain. Schlegel *et al.* (27) has previously demonstrated that Cav-(1–60) expresses as a soluble polypeptide in cytoplasm. The ability of this peptide fragment to down-regulate AR transactivation in 293 cells, which express moderate level of caveolin-1, may be a result of competitive interference of AR and endogenous caveolin interaction. This notion is further mirrored by the ineffectiveness of Cav-(58–178) to modulate AR transcriptional activity in the transient transfection reporter assay. Unlike that of NH_2-terminal of AR with caveolin, interaction between LBD and caveolin depends on androgen but not on Casodex (Fig. 8*C*), suggesting that this interaction is conformation-dependent. A potential caveolin binding motif composite ($\phi X\phi XXXX\phi$ + $\phi XXXX\phi XX\phi$ = $\phi X\phi XXXX\phi XXXX\phi XX\phi$) from residue ^{739}YSWMGLMVFAMGWRSF^{754} of AR is spotted in helix 5 of the ligand-binding domain (54, 55). This composite binding motif is similar to a previously identified composite binding motif in the platelet-derived growth factor receptor and the endothelin receptor (20). In two-hybrid studies, we were unable to demonstrate the interaction between LBD and submolecular domains of caveolin. We did, however, detect a moderate interaction between LBD and the full-length caveolin in an agonist-dependent fashion. It remains to be determined whether this putative motif actually participates in the interaction between AR and caveolin. It may suggest that a ligand-induced conformational change is involved in the interaction. A weak, ligand-dependent interaction, less than 1% of AR input, was detected in a GST pull-down experiment. On the one hand, this weak interaction is consistent with the nature of a transient association of these two molecules. On the other hand, it suggests that other auxiliary interactions or molecules, which may not be present in the *in vitro* translation lysate, are required for the interaction.

In summary, we have demonstrated the potential cross-talk between the caveolin-1-associated signal pathway and AR-mediated transcriptional activity in a cell culture model. Several lines of evidence, biological as well as biochemical, support the notion of an androgen-dependent physiological interaction between these two pathways. Overall, our results favor the notion that a transient and dynamic association between AR and caveolin-1 may play a role in promoting AR ligand-dependent transcriptional activation. Although the functional significance of this interaction remains to be determined, it suggests that caveolin-1 plays a role as a convergent point for AR cross-talk with other cellular signal transduction pathways. These findings pave the way to further define the underlying signal cross-talk in AR-mediated transactivation.

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