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Modulation of Androgen Receptor Transactivation by the SWI3-Related Gene Product (SRG3) in Multiple Ways

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The SWI3-related gene product (SRG3), a component of the mouse SWI/SNF complex, has been suggested to have an alternative function. Here, we demonstrate that in the prostate transactivation of the androgen receptor (AR) is modulated by SRG3 in multiple ways. The expression of SRG3, which is developmentally regulated in the prostate, is induced by androgen through AR. SRG3 in turn enhances the transactivation of AR, providing a positive feedback regulatory loop. The SRG3 coactivation of AR transactivation is achieved through the recruitment of coactivator SRC-1, the protein level of which is upregulated by SRG3, providing another pathway of positive regulation. Interestingly, SRG3 coactivation of AR transactivation is fully functional in BRG1/BRM-deficient C33A cells and the AR/SRG3/SRC-1 complex formed in vivo contains neither BRG1 nor BRM protein, suggesting the possibility of an SRG3 function independent of the SWI/SNF complex. Importantly, the AR/SRG3/SRC-1 complex occupies androgen response elements on the endogenous SRG3 and PSA promoter in an androgen-dependent manner in mouse prostate and LNCaP cells, respectively, inducing gene expression. These results suggest that the multiple positive regulatory mechanisms of AR transactivation by SRG3 may be important for the rapid proliferation of prostate cells during prostate development and regeneration.

Androgens are important in prostate development as well as in male sexual differentiation (13, 18). In the prostate, androgens promote mitosis and differentiation of ductal epithelium and furthermore appear to inhibit apoptosis of the differentiated cells (25). Within 2 days of androgen ablation, the prostate undergoes cell death, expressing apoptotic marker genes such as TRPM-2 (10). Androgen withdrawal thus causes the prostate to regress, while subsequent androgen injection allows the prostate to regenerate (8, 16). However, the mechanisms underlying the androgen-regulated regression and regeneration of the prostate as well as development of the normal prostate are not as yet understood.

Androgens act through the androgen receptor (AR), which is a member of the nuclear receptor superfamily. The structure of AR is similar to that of other nuclear receptors, having three separate functional domains, a variable N-terminal activation domain (AF-1), a central DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) (44). Upon ligand binding, the AR undergoes conformational changes that facilitate the formation of AR dimers in complex with specific DNA sequences, called androgen response elements (AREs), to enhance the transcription of target genes (33, 51). Transcriptional activation of nuclear receptors, including AR, appears to involve interactions with coactivator molecules and chromatin remodeling complexes so as to increase the transcription rate of their target genes (9, 22, 37, 52). Coactivators potentiate ligand-dependent transactivation of the receptor by diverse modes of action, including direct interactions with basal transcription factors and covalent modification of histones and other proteins (2, 24, 40, 43). Chromatin-remodeling complexes enhance the accessibility of nucleosomal DNA to transcription factors (11, 32, 45) by generating a "remodeled" state of chromatin.

SWI/SNF, a chromatin-remodeling complex, is required for the regulation of transcriptional processes associated with development, cellular differentiation, and proliferation (6, 26, 42). SWI/SNF is a 2-MDa multisubunit assembly that is highly conserved in eukaryotes. Mammalian SWI/SNF complexes contain one of two core ATPase subunits, BRM or BRG1, and further diverge in the composition of their subunits, containing another 7 to 14 BRG1- or BRM-associated factors (BAFs) (47). The direct recruitment of chromatin-remodeling enzymes by nuclear hormone receptors for attainment of full function has been described for AR, glucocorticoid receptor, estrogen receptor, and retinoid receptors (14, 15, 17, 36, 38, 46).

The SWI3-related gene product (SRG3), a mouse homologue of yeast SWI3 and human BAF155, was initially isolated as a gene highly expressed in the thymus, testis, and brain (26). It has been shown to be a core component of mouse SWI/SNF (26). In the thymus, SRG3 has been implicated in the glucocorticoid sensitivity of T cells, associating with the glucocorticoid receptor. Peripheral T cells of transgenic mice overexpressing

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SRG3 become more sensitive to glucocorticoid-induced apoptosis (20), and downregulation of SRG3 expression by Notch1 allows developing thymocytes to be resistant to glucocorticoid-induced apoptosis (7).

In this study, to understand the functional role of SRG3 in the androgen regulation of prostate development, we investigated its expression, gene regulation, and interaction with AR in the prostate. Here, we show that SRG3, the expression of which is upregulated by androgen through AR in the prostate, enhances the transcriptional activity of AR, providing a positive feedback regulatory loop. Furthermore, SRG3 overexpression elevates the protein level of SRC-1, which is recruited by SRG3 bound to AR, resulting in further enhancement of AR transactivation. Thus, SRG3 may play an important role in the rapid proliferation of cells during prostate development and regeneration by amplifying AR function in multiple different ways.

MATERIALS AND METHODS

Animals. Sprague Dawley (SD) rats were purchased from a commercial supplier (Daehan Laboratories, Korea). Transgenic mice overexpressing SRG3 were generated by microinjecting a DNA fragment of the chicken β-actin promoter/SRG3 open reading frame, which was cut out from the pCAGGSBS/mycSRG3 construct, into fertilized mouse eggs as previously described (20). Animals were kept in a cage with water and chow available and maintained under controlled conditions (12-h light and dark photoperiods, 50% humidity, 22°C). The ethical treatment of animals in this study was carried out according to National Institutes of Health standards.

Plasmids. The mammalian expression vectors of pcDNA3-SRC-1, -p300, -ARA70 and –mouse AR were previously described (34). The $pARE_2$-TATA-Luc and PSA-Luc reporter plasmids were previously described (27). Glutathione *S*-transferase (GST)-mycSRG3, pcDNA3-mycSRG3, and LexA-mycSRG3 were generated by cloning full-length mycSRG3 from the pCAGGSBS/mycSRG3 expression construct into pGEX4T-3, pcDNA3, and LexA202 vectors, respectively. The SRG3-Luc reporter plasmid was constructed by cloning an SRG promoter region (−1200 to +3, +1 being the A base in the translation initiation codon) into vector pGL3basic (Promega). SRG3 promoter deletion mutants were constructed by self-ligation after digesting SRG3-Luc with Acc65I and BclI (−691 to +3), KpnI and ApaI (−315 to +3), or Acc65I and XhoI (−134 to +3). Two other deletion mutants of SRG3 promoter were generated by PCR amplification using two primer sets: forward, 5′-GTTCCGAGAGACCAGAGG-3′, and reverse, 5′-GAGTTTTCACTGCATACGACG-3′ for the −277 to +3 region and forward, 5′-ATGCGTACGCCCGCTCTC-3′, and reverse, 5′-GAGTTTTC ACTGCATACGACG-3′, for the −210 to +3 region.

The human BRG1 and BRM expression vectors were kind gifts from J. W. Lee (Baylor College of Medicine) (29, 39). The GST-fused SRG3 deletion mutants were constructed by cloning EcoRI-ScaI, ScaI-NotI, MfeI-HincII, and HincII-XbaI fragments into either pGEX4T-3 or pGEX4T-2. pBluescript-mutSRG3 was generated by cloning a region spanning the Myb-like region and leucine zipper motif (26), which is amplified by PCR using primer set forward, 5′-TTG CAGAACTTTGGTCTTCG-3′, and reverse, 5′-CTGTCTCTGTTGTTCTAGA G-3′. Hemagglutinin (HA)-mutSRG3-1 was constructed by cloning the EcoRI-XbaI fragment of pBluescript-mutSRG3 into the pcDNA3HA vector. mutSRG3-2 was constructed by self-ligation of pcDNA3-mycSRG3 partially digested with XmnI. The B42-AR deletion mutants were generated by cloning the regions spanning Tau, DBD/hinge, and LBD of mouse AR into vector B42 digested with EcoRI and XhoI restriction enzymes.

The SRG3-siRNA construct was generated by cloning annealed short interfering DNA (siDNA) oligomers sense, 5′-CGTGACAGAACAGACCAATTTC AAGAGAATTGGTCTGTTCTGTCACGTTTTTT-3′, and antisense, 5′-AATT AAAAAACGTGACAGAACAGACCAATTCTCTTGAAATTGGTCTGTTC TGTCACGGGCC-3′, into the pSilencer 1.0-U6 vector (Ambion, Inc.) digested with ApaI and EcoRI enzymes. The BAF57 expression construct (pSG5-flag-BAF57,4) and BAF47 siRNA construct (pREP4-PUR/u6-BAF47,12) were kindly provided by M. G. Parker (Imperial College Faculty of Medicine, United Kingdom) and K. Zhao (National Institutes of Health, Bethesda, Md.), respectively. Two SRC-1 siRNAs were utilized, siRNA 1a (5′-AACACGACGAAAU AGCCAUAC-3′) and 1b (5′-AAGUGAUGACUCGUGGCACUG-3′) (35). A scrambled siRNA (5′-AACCCGAAAGAUAACCGAUAC-3′) was used as a control for nonspecific gene disruption.

Transient transfection assays. The Cos-7, HeLa, L929, and C33A cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. The LNCaP cell line was purchased from the American Type Culture Collection (ATCC CRL-1740) and maintained in RPMI 1640 (Life Technologies, Inc.) supplemented with L-glutamine and 10% fetal bovine serum. Cells were plated in 24-well plates and transfected with the indicated amount of expression plasmids, a reporter plasmid, and the control *lacZ* expression plasmid pCMVβ (Clontech) by using Effectene reagent (QIAGEN, Germany) according to the manufacturer's instructions. Total amounts of expression vectors were kept constant by adding appropriate amounts of pcDNA3. Cells were treated with 10 nM dihydrotestosterone (DHT) for 24 h beginning 24 h following transfection. Luciferase and β-galactosidase activities were assayed as described previously (21). The levels of luciferase activity were normalized to *lacZ* expression.

Northern blot analysis. Adult male Sprague-Dawley rats (75 to 80 days old) were injected intraperitoneally with ethane dimethanesulfonate (EDS, 75 mg/kg body weight) in dimethyl sulfoxide-water (1:3, vol/vol). Four days later, the animals received an injection of 0.1 ml of 25 mg testosterone esters (Sustanon, Organon Korea Ltd., Seoul, Korea) for the indicated time periods. Ventral prostates were then collected and total RNAs were prepared using Tri reagent (Molecular Research Center Inc.). Twenty micrograms of total RNA was separated on a 1.2% denaturing agarose gel, transferred onto Zeta-probe nylon membrane (Bio-Rad), and immobilized by UV cross-linking. The membrane was hybridized with random-primed, ^{32}P-labeled mouse SRG3, human BRG1, and human BRM cDNA probes as described previously (21). The membrane was reprobed for glyceraldehyde-3-phosphate dehydrogenase as a loading control.

Real-time RT-PCR. Total RNAs were obtained from the prostates of 28-day-old nontransgenic and transgenic mice overexpressing SRG3 and LNCaP cells transfected with pcDNA3-mycSRG3 or pcDNA3 empty vector. Quantitative reverse transcription (RT)-PCR was performed with a real-time PCR machine (Corbett Research, Sydney, Australia) and QuantiTect SYBR Green RT-PCR (QIAGEN) according to the manufacturer's procedure. PCRs were carried out with the mouse SRC-1 primer set (forward primer, 5′-TGAATCCAATGATG GGCCAG-3′, and reverse primer, 5′-GATTCACTGTACACTGGACG-3′) or human PSA primer set (forward primer, 5′-GGCCAGGTATTTCAGGTCAG-3′, and reverse primer, 5′-CCACGATGGTGTCCTTGATC-3′) and the β-actin primer set (forward primer, 5′-GAGACCTTCAACACCCCAGCC-3′, and reverse primer, 5′-CCGTCAGGCAGCTCATAGCTC-3′) as a quantitative control. Signals were analyzed with the Rotor Gene 2000 (RG-2000) software (Corbett Research, Sydney, Australia).

Yeast two-hybrid assay. Plasmids encoding LexA fusions and B42 fusions were cotransformed into *Saccharomyces cerevisiae* EGY48 containing the *lacZ* reporter plasmid. The transformants were grown in inducing medium and processed for liquid β -galactosidase assays as described previously (21).

GST pull-down assay. GST, GST-AR domain mutant, and GST-SRG3 deletion mutant fusion proteins were expressed in *Escherichia coli* BL21 cells and isolated with glutathione-Sepharose 4B beads (Pharmacia Biotech AB, Sweden). Immobilized GST fusion proteins were then incubated with [^{35}S]methionine-labeled SRG3, AR, or SRC-1 protein produced by in vitro translation using the TNT coupled transcription-translation system (Promega). The binding reactions were carried out in 250 μl of GST binding buffer (20 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10% glycerol, 0.05% NP-40, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM dithiothreitol, and 1.5% bovine serum albumin) for 4 h at 4°C. The beads were washed three times with 1 ml of GST binding buffer. Bound proteins were eluted by adding 20 μl of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer and analyzed by SDS-PAGE and autoradiography.

Coimmunoprecipitation and Western blot assays. Coimmunoprecipitation assays were performed with untransfected L929 cells or Cos-7 cells which were transfected either with 500 ng of AR (or HA-AR-DBDh), 750 ng of mycSRG3, and 750 ng of SRC-1 expression plasmids or with 400 ng of AR, 400 ng of HAmutSRG3, 400 ng of SRC-1, 400 ng of BRG1, and 400 ng of BRM expression plasmids. Cells were treated with 10 nM DHT for 24 h following transfection and harvested in radioimmunoprecipitation assay cell lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2.5 mM EGTA, 1% Triton X-100, 50 mM NaF, 10 mM $Na_4P_2O_7$, 10 mM Na_3VO_4, 1 μg/ml aprotinin, 0.1 μg/ml leupeptin, 1 μg/ml pepstatin, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol). Whole-cell lysate (400 μg) was incubated with 2 μg of anti-AR or anti-HA antibody (Santa Cruz Biotechnology) for 4 h at 4°C and further incubated for another 4 h after adding 20 μl of protein A-agarose bead slurry (Invitrogen).

Agarose beads were washed three times with radioimmunoprecipitation assay buffer at 4°C, and bound proteins were separated by SDS-PAGE.

Total prostate proteins were prepared from 28-day-old nontransgenic and transgenic male mice overexpressing SRG3 in tissue lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.1 mM phenylmethylsulfonyl fluoride, 1% NP-40, 0.5% sodium deoxycholate, and 1 μg/ml aprotinin). HeLa cells were cotransfected with AR, SRC-1, and β-galactosidase expression plasmids (100 ng each) with or without 100 ng of SRG3 expression plasmid. Transfected cells were treated or not with 100 nM testosterone for 12 h after 24 h of transfection and harvested in radioimmunoprecipitation assay buffer. Tissue or whole-cell lysates were separated by SDS-PAGE. Proteins on the gels were transferred to a nitrocellulose membrane (Sigma), subjected to Western blot analysis with anti-SRG3 (26), anti-AR, anti-SRC-1, anti-p300, anti-ARA70, anti-HA, anti-BRG1, anti-BRM, anti-β-galactosidase, and anti-β-actin antibodies (Santa Cruz Biotechnology), and then detected with an ECL kit (Amersham Pharmacia).

Far-Western blot analysis. Whole L929 cell lysate prepared in RIPA buffer was separated by SDS-PAGE, and proteins on the gel were transferred to nitrocellulose membranes. The membrane was incubated with 1 μg/ml of purified GST, GST-SRG3, or GST-ARDBDh fusion protein in Hyb75 buffer (50 mM Tris-HCl, pH 8.0, 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 1 mM dithiothreitol, and 0.05% NP-40) at 4°C overnight after blocking with 5% nonfat milk. The membrane was then washed with Hyb75 buffer containing 1% nonfat milk three times at room temperature and subjected to Western blot analysis with anti-GST antibody.

Chromatin immunoprecipitation assay. The ventral prostates from 3-week-old male mice injected with flutamide (100 mg/kg body weight) for 12 h were dissected under the stereomicroscope, chopped, and cross-linked with 1% formaldehyde for 15 min at room temperature on a rotating platform. After 15 min, the cross-linking reaction was stopped by the addition of glycine to a final concentration of 0.125 M for 5 min at room temperature (50). LNCaP cells were transfected with pcDNA3-SRG3 expression plasmid, treated or not with 10 nM DHT for 6 h after 24 h of transfection, and cross-linked with 1% formaldehyde. After incubating the samples with TSE I [100 mM Tris-HCl (pH 9.4) and 10 mM dithiothreitol] for 20 min at 30°C, the cells were washed and processed for chromatin immunoprecipitation (ChIP) assays as previously described (21). Anti-AR (Santa Cruz Biotechnology), anti-SRG3 (26), or anti-SRC-1 (Santa Cruz Biotechnology) was used for immunoprecipitation.

Immunoprecipitated DNA and input-sheared DNA were subjected to PCR using either mouse SRG3 primer pairs (sense, 5′-CGGAAGCGTCATTCGTCG-3′, and antisense, 5′-CATAGTCACCG TCCGTCG-3′), which amplify a 317-bp region (−314 to +3) spanning the putative androgen response element of the mouse SRG3 promoter, or mouse PSA primer pairs (sense, 5′-TGAGAAA CCTGAGATTAGGA-3′, and antisense, 5′-ATCTCTCTCAGATC CAGGCT-3′), which amplify a 229-bp region (−4271 to −4043) spanning the androgen response element of the PSA promoter. As a negative control, PCRs were performed using actin primer pairs (sense, 5′-GAGACCTTCAACACCCCAG CC-3′, and antisense, 5′-CCGTCAGGCAGCTCATAGCTC-3′), which amplify a 362-bp region spanning exon 4 of the β-actin gene.

RESULTS

Androgen regulates the expression of SRG3 in rat prostate. The proliferation rate of rat prostate increases from birth to 3 weeks of age, and thereafter it gradually decreases until it stops by 8 weeks (49). To examine whether SRG3 expression coincides with prostate proliferation, we performed Northern blot analysis with rat prostates at different developmental stages (Fig. 1A). The SRG3 expression was high in 2-week-old rat prostate and gradually decreased until adulthood, suggesting a role in prostate development. SRG3 expression in mouse prostate also showed a similar pattern to that in rat prostate (data not shown). On the other hand, the expression of BRG1 and BRM, also core components of the SWI/SNF complex, exhibited no significant changes during prostate development (Fig. 1A).

Because androgen is important for prostate proliferation, we investigated whether SRG3 expression is regulated by androgen in the prostate by treating rats with ethane dimethanesulfonate (EDS), a cytotoxic compound that specifically destroys testicular Leydig cells and eventually leads to androgen deprivation in adult male rats (3, 27, 28). The expression of SRG3 in the prostate of EDS-treated rats was lowered compared to that of vehicle-only-treated rats and reached a maximum approximately 6 h after androgen administration (Fig. 1B). Furthermore, administration of the AR antagonist flutamide decreased the expression of SRG3 in the prostates of adult male mice (Fig. 1C). These results suggest that SRG3 expression in the prostate is controlled by androgen.

The gene regulation of SRG3 by androgen was further investigated by transient transfection analyses with 1.2 kb of the SRG3 promoter. In LNCaP cells transfected with an SRG3 promoter-reporter construct, SRG3-Luc, luciferase activity was increased approximately fourfold by treatment with 10 nM DHT (Fig. 1D). Furthermore, in Cos-7 cells cotransfected with an AR expression plasmid and SRG3-Luc, SRG3 promoter activity was upregulated up to sixfold by AR in a dose-dependent manner when the cells were treated with DHT (Fig. 1E).

Transient transfection analyses with serial deletion mutants of the SRG3 promoter indicated that androgen response elements (AREs) might reside within the 67-bp region (−277 to −211, +1 being the A base in the translation initiation codon) of the SRG3 promoter (Fig. 1F), although sequence analysis of the region did not show any good match with either canonical or noncanonical AREs. The presence of putative AREs within the SRG3 promoter region was further confirmed by chromatin immunoprecipitation assay (ChIP) with the prostate of 3-week-old male mice. As shown in Fig. 1G, AR was recruited onto the SRG3 promoter region (−314 to +3) containing the putative AREs, and recruitment was abolished by injection of the AR antagonist flutamide. Together, these results suggest that androgen regulates SRG3 expression at the transcriptional level via AR.

SRG3 enhances the transactivation of AR. SWI/SNF chromatin remodeling complexes have been implicated in the modulation of transactivation of several nuclear receptors (14, 15, 17, 36, 38, 46). Because SRG3 is a core component of the mouse SWI/SNF complex, we examined whether SRG3 modulates the transactivation of AR. In HeLa cells, coexpression of SRG3 with AR increased the expression of a luciferase reporter gene in a dose-dependent manner when the cells were treated with DHT (Fig. 2A). SRG3 also enhanced the transactivation of endogenous AR on a natural AR target promoter, PSA, in LNCaP cells (Fig. 2B), and also on the SRG3 promoter (Fig. 2C), which had been shown to be an AR target promoter (Fig. 1D to G). We then checked whether SRG3 is necessary for the transactivation of AR in L929 cells, which endogenously express AR and SRG3 (Fig. 6A) by silencing SRG3 expression with siRNA. As shown in Fig. 2D, blocking SRG3 expression inhibited the transactivation of AR, suggesting the necessity of SRG3 for the full transactivation of AR in L929 cells.

To examine whether SRG3 acting as an AR coactivator depends on the SWI/SNF chromatin-remodeling complexes, we tested its function in C33A cells, which are doubly deficient in BRG1 and BRM (36, 38, 46). Unexpectedly, SRG3 enhanced AR transactivation in C33A cells as well as in other cells, such as HeLa and LNCaP, in a dose-dependent manner (Fig. 2E). Furthermore, the coexpression of either BRG1 or

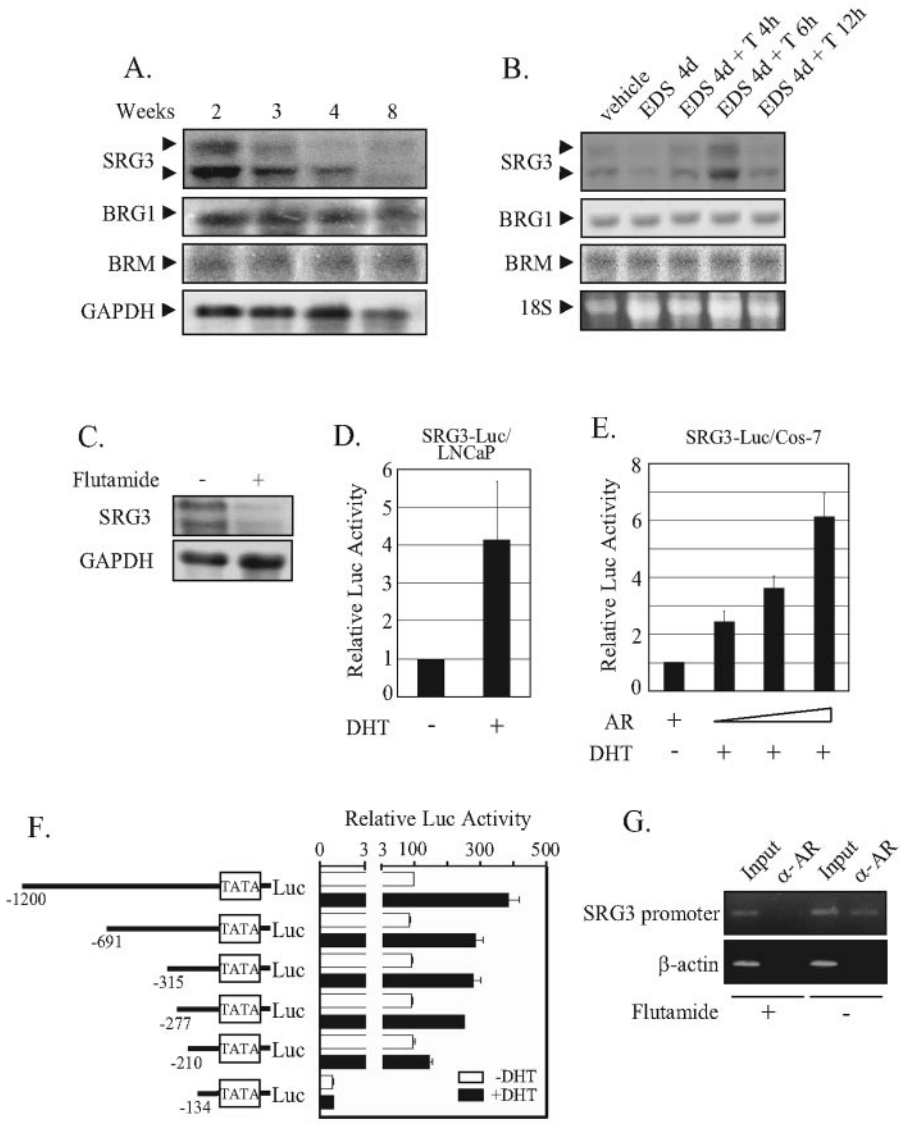

FIG. 1. Expression of SRG3 is induced by androgen. (A) SRG3 expression during rat prostate development. Northern blot analysis of total RNAs from prostate at different developmental stages was performed using ^{32}P-labeled SRG3, BRG1, or BRM cDNA as a probe. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. (B) The transcript level of SRG3 is upregulated by androgen in EDS-treated rat prostate. Rats were treated with EDS, and after 4 days the animals were supplemented with testosterone for the indicated time. The expression of 18S rRNA was used as an internal control in Northern blot analysis. (C) SRG3 expression is downregulated in mouse prostate by the administration of flutamide, an AR antagonist. Three-week-old male mice were injected or not with flutamide for 12 h. Total prostate RNAs were subjected to Northern blot analysis. (D) The SRG3 promoter is induced by DHT in LNCaP cells expressing the endogenous AR. The SRG3-Luc reporter (1,300 ng/well) containing the 1.2-kb fragment of the SRG3 promoter was transfected into LNCaP cells. (E) The SRG3 promoter is induced by androgen in an AR dose-dependent manner. Cos-7 cells were cotransfected with 30 ng of SRG3-Luc and AR expression plasmids (25, 50, and 150 ng). (F) Androgen target region within the SRG3 promoter was defined. Deletion mutants of the SRG3 promoter (30 ng) were cotransfected with 150 ng of AR expression plasmid into Cos-7 cells. Cells were treated or not with 10 nM DHT for 24 h. (G) AR was recruited onto the SRG3 promoter region containing putative AREs. ChIP assays were performed with prostates dissected from 3-week-old male mice which had been injected or not with flutamide. The immunoprecipitates were analyzed by PCR using a pair of specific primers spanning the SRG3 region containing putative AREs. A control PCR for nonspecific immunoprecipitation was performed using primers specific to the β-actin coding region.

BRM with SRG3 failed to cooperatively activate AR transactivation in C33A cells (Fig. 2F), while either BRG1 or BRM by itself was able to increase the transactivation of AR, as recently reported (36, 38, 46). Similarly, neither BAF57 nor BAF47, other components of the SWI/SNF complex, cooperated with SRG3 for the enhancement of AR transactivation, either when BAF57 was overexpressed (Fig. 2G) or when BAF47 gene expression was silenced with siRNA (Fig. 2H). Together, these results suggest that SRG3 enhances the transactivation of AR and probably does so in a manner independent of the SWI/SNF complex.

SRG3 specifically cooperates with SRC-1 to modulate AR transactivation. Because SRG3 itself has no histone acetyltransferase activity, we hypothesized that it cooperates with

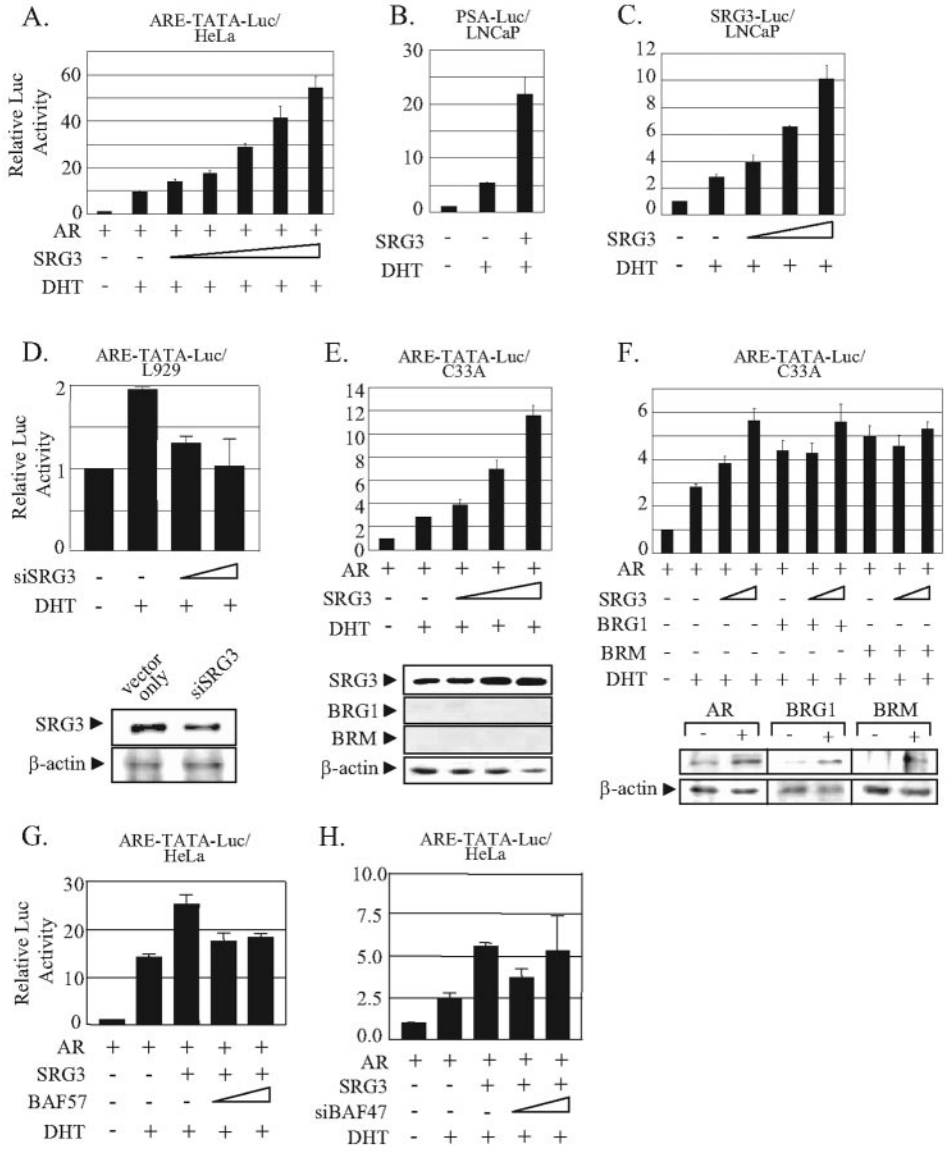

FIG. 2. SRG3 modulates AR transactivation. (A) SRG3 coexpression enhances AR transactivation. ARE2-TATA-Luc (50 ng) containing two copies of androgen response elements was cotransfected with 5 ng of AR and increasing amounts of SRG3 expression plasmids (5, 10, 25, 50, and 150 ng) into HeLa cells. (B) SRG3 increases the transactivation of endogenous AR with the native androgen response PSA promoter. LNCaP cells were cotransfected with 900 ng of PSA-Luc and 600 ng of SRG3 expression plasmids. (C) SRG3 enhances its own promoter activity by up-regulating the transactivation of the endogenous AR. LNCaP cells were cotransfected with 900 ng of SRG3-Luc and increasing amounts of SRG3 expression plasmids (200, 400, and 600 ng). (D) SRG3 is necessary for the transactivation of AR in L929 cells. L929 cells were cotransfected with 55 ng of ARE2-TATA-Luc together with 250 or 500 ng of SRG3 siRNA expression plasmid. Decreased protein expression of the endogenous SRG3 gene with transfection of 500 ng of SRG3 siRNA plasmid is shown at the bottom. (E) SRG3 activates AR transactivation in a dose-dependent manner in C33A cells. C33A cells were cotransfected with 55 ng of ARE2-TATA-Luc together with 20 ng of AR and increasing amounts of SRG3 expression plasmids (20, 40, and 100 ng). Increased protein expression of SRG3 and absence of induced expression of BRG1 and BRM are shown at the bottom. The SRG3 antibody used also recognizes the human BAF155 protein, so that a certain level of the signal appeared in C33A cells transfected without SRG3. Western blot analysis with anti-human BAF155 antibody (Santa Cruz Biotechnology) gave a similar result (data not shown). (F) SRG3 does not cooperate with either BRM or BRG-1 to enhance AR transactivation. ARE2-TATA-Luc (50 ng) was cotransfected with 20 ng of AR and a combination of the indicated expression plasmids (20 or 60 ng of SRG3 and 20 ng of BRG1 or BRM). Expression of AR, BRM, and BRG1 proteins from the expression plasmids in C33A cells is shown at the bottom. (G) BAF57 does not affect the SRG3-mediated enhancement of AR transactivation. HeLa cells were cotransfected with 55 ng of ARE2-TATA-Luc, 20 ng of AR, and 100 ng of SRG3 expression plasmids together with 25 or 50 ng of BAF57 expression plasmid. (H) BAF47 does not affect the SRG3-mediated enhancement of AR transactivation. HeLa cells transfected with 60 or 120 ng of the BAF47 siRNA construct or empty vector were selected in 1 μg/ml of puromycin for 2 days (12) and then cotransfected with 55 ng of ARE2-TATA-Luc, 20 ng of AR, and 100 ng of SRG3. Cells were treated or not with 10 nM DHT for 24 h.

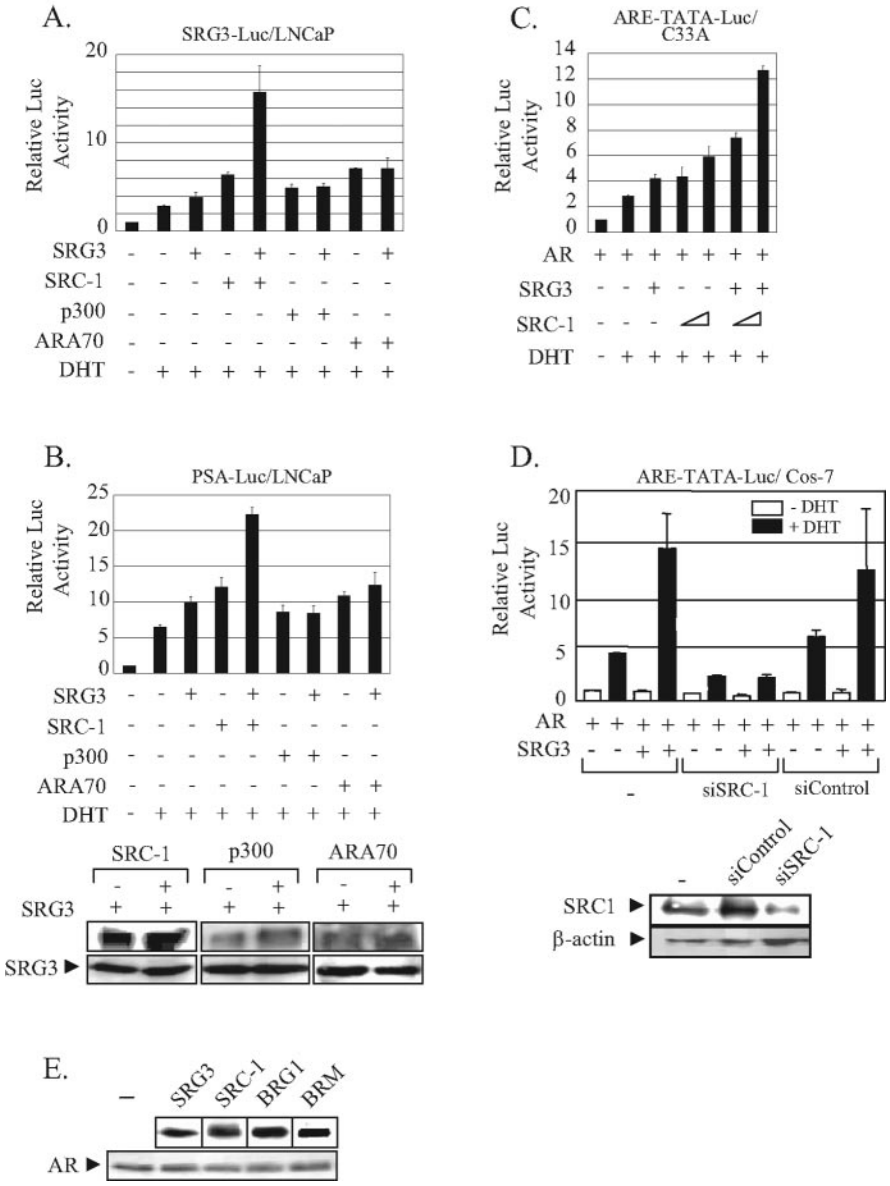

FIG. 3. SRG3 cooperates specifically with SRC-1 to enhance AR transactivation. (A) SRC-1 but neither p300 nor ARA70 enhances the ability of SRG3 to transactivate AR on its own promoter. LNCaP cells were cotransfected with 900 ng of SRG3-Luc and a combination of the indicated expression plasmids (200 ng of SRG3 expression plasmid and 400 ng of SRC-1, p300, or ARA70 expression plasmid). (B) SRC-1 enhances the ability of SRG3 to transactivate AR on the PSA promoter. LNCaP cells were cotransfected with 900 ng of PSA-Luc and a combination of the indicated expression plasmids (200 ng of SRG3 expression plasmid and 400 ng of SRC-1, p300, or ARA70 expression plasmid). Expression levels of SRC-1, p300, and ARA70 as well as SRG3 protein are shown at the bottom. (C) SRC-1 enhances the ability of SRG3 to transactivate AR in C33A cells. ARE2-TATA-Luc (55 ng) was cotransfected with 20 ng of AR and a combination of the indicated expression plasmids (20 ng of SRG3 plus either 20 or 60 ng of SRC-1). (D) SRC-1 recruitment is necessary for SRG3 coactivator function. Cos-7 cells were transfected with 40 ng of ARE2-TATA-Luc, 20 ng of AR, and 80 ng of SRG3 expression plasmids along with no siRNA, 100 nM each of both SRC-1 siRNA 1a and 1b, or 200 nM of scrambled siRNA as a control. The cells were then treated or not with 10 nM DHT for 24 h. Decreased SRC-1 protein expression in Cos-7 cells with transfection of both SRC-1 siRNA 1a and 1b is shown at the bottom. (E) AR receptor levels were little affected by coexpression of SRG3, SRC-1, BRG1, or BRM. Cos-7 cells were transfected with 100 ng of AR and 100 ng of the indicated cofactor expression plasmid and treated with 10 nM DHT for 24 h. Western blot analyses were performed with each of the corresponding anti-cofactor antibodies.

histone acetyltransferase to activate AR transactivation. We first tested SRC-1 and p300, which are known to act as AR coactivators, along with ARA70. As shown in Fig. 3A and 3B, the coexpression of SRG3 with SRC-1 cooperatively increased the expression of the reporter directed by the SRG3 and PSA promoters in LNCaP cells compared with controls expressing only SRG3 or SRC-1. However, SRG3 coexpression affected neither the p300 nor ARA70 coactivation of AR transactivation on either the SRG3 or PSA promoter. We observed the same cooperative coactivation of AR transactivation by SRG3 and SRC-1 in C33A cells (Fig. 3C).

We then tested whether the recruitment of SRC-1 is neces-

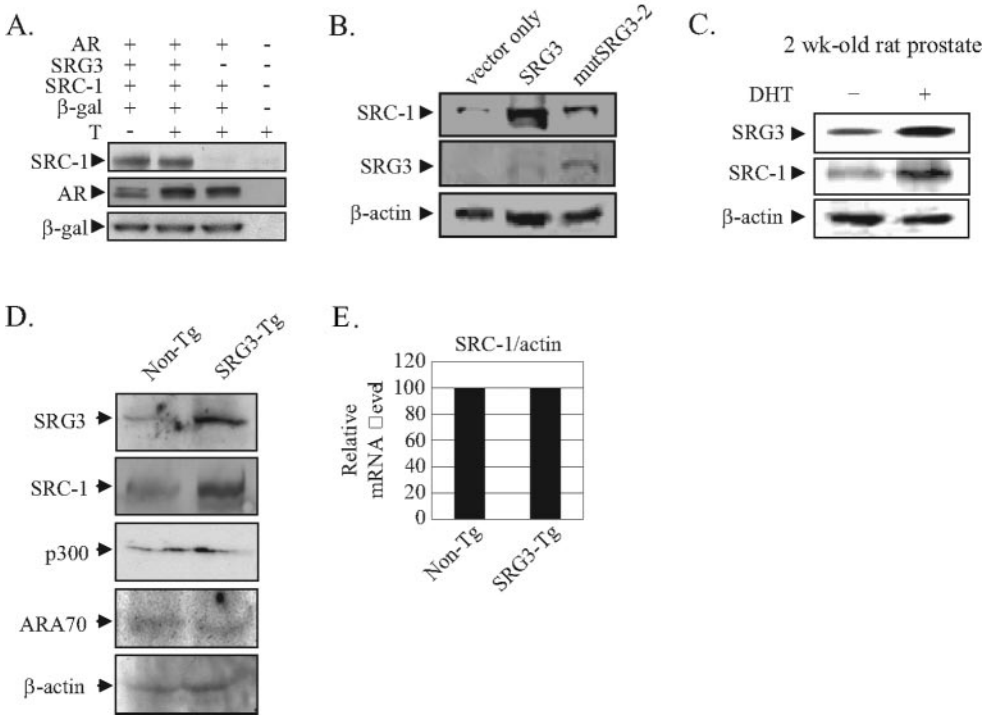

FIG. 4. SRG3 upregulates the level of SRC-1 protein. (A) Coexpression of SRG3 upregulates the level of SRC-1 protein. AR, SRC-1, and the control β-galactosidase expression plasmids (100 ng each) were cotransfected with or without SRG3 expression vector into HeLa cells. The cells were treated or not with 100 nM of testosterone (T). Western blot analyses were carried out with anti-SRC-1, anti-AR, and anti-β-galactosidase antibodies. (B) SRG3 overexpression upregulates the level of endogenous SRC-1 protein. L929 cells were transfected with empty vector, myc-SRG3, or myc-mutSRG3-2 expression plasmid in the presence of 10 nM DHT. Western blot analyses were carried out with anti-SRC-1, anti-myc (SRG3), and anti-β-actin antibodies. (C) Up-regulation of SRG3 expression by DHT in rat prostate causes the elevation of SRC-1 protein level. Two-week-old rats were injected with DHT at 3 mg/kg body weight for 24 h. (D) SRG3 overexpression causes the up-regulation of SRC-1 protein level. Prostate extracts were prepared from 4-week-old SRG3-transgenic and nontransgenic mice (four animals for each sample). Western blot analyses were performed with anti-SRG3, anti-SRC-1, anti-p300, anti-ARA70, and anti-β-actin antibodies. (E) The mRNA level of SRC-1 was not affected by SRG3 overexpression. Total RNAs were prepared from the prostates of 4-week-old SRG3-transgenic and nontransgenic mice. Mouse SRC-1 transcripts were analyzed by real-time RT-PCR. β-Actin was used as a quantitative control.

sary for SRG3 to enhance AR transactivation by silencing the endogenous SRC-1 expression with siRNA in Cos-7 cells. In cells treated with SRC-1 siRNA but not with scrambled siRNA as a control, the AR transactivation itself was significantly impaired and SRG3 no longer enhanced the AR transactivation in the presence of androgen (Fig. 3D). Meanwhile, AR receptor levels were little affected by coexpression of SRG3, SRC-1, BRG1, or BRM in transient transfection experiments (Fig. 3E). Together, these results suggest that SRG3 enhances AR transactivation by specifically recruiting SRC-1.

SRG3 upregulates the SRC-1 protein level. Interactions between two coactivators or between a nuclear receptor and its coactivator have been reported to increase the stability of one of the participant proteins, resulting in an upregulation of the protein level (1, 19, 31). Because SRG3 cooperated with SRC-1 in the enhancement of AR transactivation, most likely via physical association, we examined the effect of SRG3 on the SRC-1 protein level in HeLa cells cotransfected with AR, SRC-1, and the control β-galactosidase expression plasmids with or without the SRG3 expression vector.

As shown in Fig. 4A, the SRC-1 protein level was significantly higher in cells with SRG3 coexpression than in cells without it. Treatment with testosterone did not affect the level of SRC-1 protein. In addition, SRG3 overexpression in L929 cells upregulated the level of endogenous SRC-1 protein, while overexpression of an SRG3 mutant form (mutSRG3-2, Fig. 6D) that has its Myb-like region (MLR) deleted and is unable to enhance the AR transactivation, did not (Fig. 4B). Furthermore, when SRG3 expression in the prostate of 2-week-old male rats was upregulated by injecting DHT because SRG3 expression is androgen inducible (Fig. 1), the SRC-1 protein level was elevated too (Fig. 4C).

We also investigated the SRG3 effect on the SRC-1 protein level using transgenic mice overexpressing SRG3. As shown in Fig. 4D, the overexpressed SRG3 upregulated the protein level of SRC-1 but neither p300 nor ARA70 in the prostate. This could be due to the increased stability of the SRC-1 protein or the increased translational efficiency of its mRNA, because the level of the SRC-1 mRNA exhibited no difference in the SRG3-transgenic and nontransgenic mouse prostates (Fig. 4E).

SRG3 physically associates with AR and SRC-1 in vitro. To verify that the functional interaction between SRG3 and AR involves a physical association, we performed yeast two-hybrid analyses using SRG3 fused to the LexA DNA binding domain (LexA-SRG3) and AR domain mutants fused to the B42 acti-

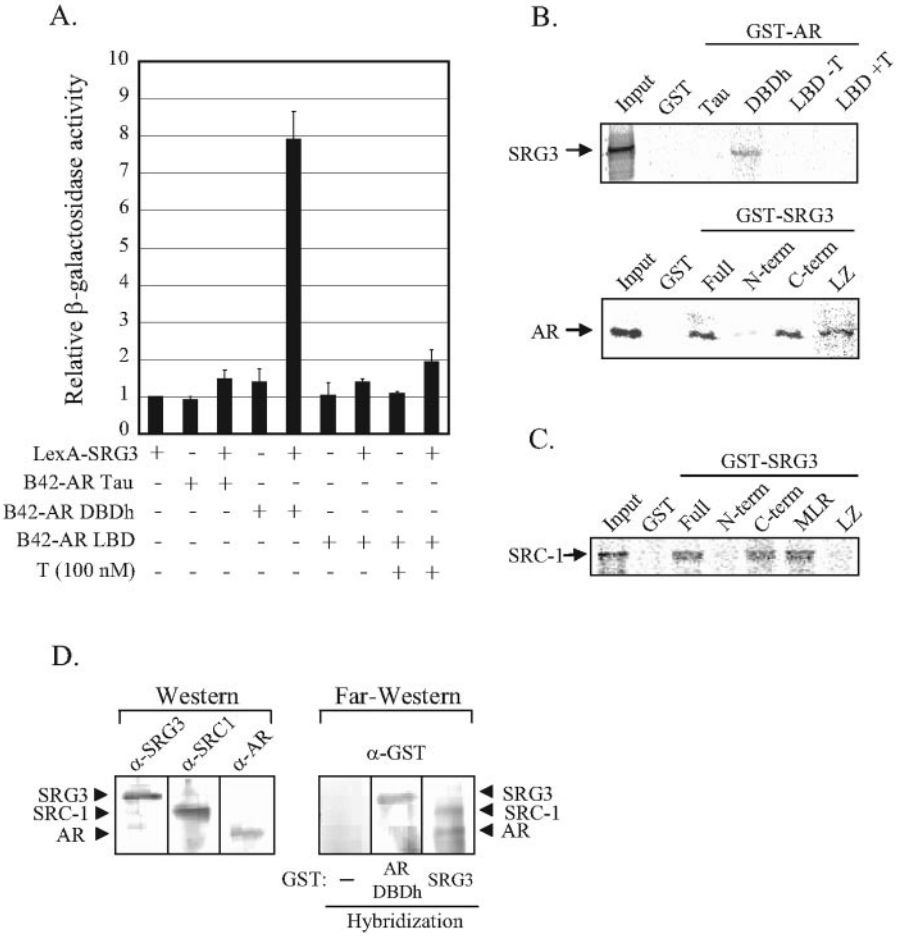

FIG. 5. SRG3 physically associates with AR and SRC-1 in vitro. (A) SRG3 interacts with AR through the DBDh region of AR. The interaction between AR and SRG3 was scored by activation of the β-galactosidase reporter in the yeast two-hybrid system. All values represent the mean ± standard error of the mean of at least three independent colonies. (B) The DBDh region of AR interacts directly with SRG3 through a region spanning a leucine zipper motif (LZ) in the SRG3 C terminus. [^{35}S]methionine-labeled SRG3 and AR were allowed to bind the GST fusion proteins of AR domain mutants and SRG3 deletion mutants, respectively. Reactions were carried out with an equivalent amount of each protein as determined by Coomassie blue staining (data not shown). Ten percent of the labeled protein used in the binding reaction was loaded as the input. (C) SRG3 interacts directly with SRC-1 through its Myb-like region (MLR). [^{35}S]methionine-labeled SRC-1 was allowed to bind the GST-fused SRG3 and its deletion mutants. (D) SRG3 interacts directly with both AR and SRC-1, while AR interacts with SRG3. Far-Western blot analyses were performed with L929 cell extracts. Nitrocellulose membranes containing proteins of the L929 cell extract were incubated with purified GST, GST-AR-DBDh, or GST-SRG3 fusion protein following Western blot analysis with anti-GST antibody.

vation domain (B42-AR domain mutants). As shown in Fig. 5A, LexA-SRG3 itself showed a low basal level of β-galactosidase activity, and so did B42-AR-Tau, B42-AR-DBDh, and B42-AR-LBD alone. However, the presence of LexA-SRG3 with B42-AR-DBDh strongly induced β-galactosidase activity, while the presence of LexA-SRG3 with B42-AR-LBD very weakly induced the expression of the reporter in the presence of testosterone. These results indicate that SRG3 interacts with AR and the DBDh region of AR is the major determinant for this interaction.

Direct physical interaction between SRG3 and AR was then assessed by glutathione *S*-transferase (GST) pull-down analyses (Fig. 5B). [^{35}S]methionine-labeled SRG3 produced by in vitro translation was incubated with the GST fusion protein of AR domain mutants, while [^{35}S]methionine-labeled AR was incubated with the GST fusion protein of SRG3 (full-length) and its deletion mutants. SRG3 interacted with GST-AR-DBDh but not with GST-AR-Tau or GST–AR-LBD. On the other hand, AR interacted with the GST-SRG3 full-length and C-terminal regions but not with the N-terminal region. Interestingly, AR interacted with the region spanning a leucine zipper motif of the C-terminal region (amino acid residues 722 to 942). These results suggest that SRG3 directly interacts with AR and the DBDh of AR and the leucine zipper-containing region of SRG3 are responsible for the interaction.

Because transient transfection analyses showed that SRG3 specifically cooperates with SRC-1 to enhance AR transactivation (Fig. 3), the physical interaction between SRG3 and SRC-1 was investigated by GST pull-down analysis (Fig. 5C). SRC-1 interacted with GST-SRG3 full-length and C-terminal regions but not with the N-terminal region. Furthermore, SRC-1 interacted with the Myb-like region in its C-terminal (amino acid residues 611 to 721), but not with the leucine zipper-containing region. These results suggest that SRG3 di-

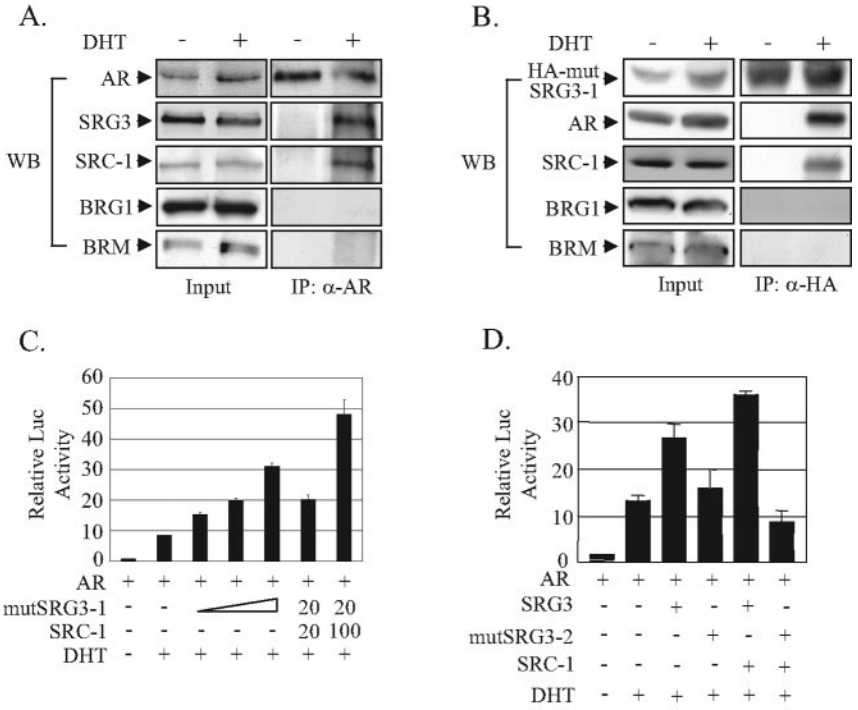

FIG. 6. AR and SRG3 form a complex with SRC-1 in vivo and may function as an AR coactivator in a manner independent of the SWI/SNF complex. (A) AR endogenously forms a complex with SRG3 and SRC-1 in the presence of androgen, and the complex does not contain BRG1 or BRM. L929 cells were treated or not with 10 nM DHT for 24 h. Coimmunoprecipitations were carried out with anti-AR antibody. (B) mutSRG3-1 containing only the Myb-like region and leucine zipper domain is able to associate with AR and SRC-1 but not with BRG1 or BRM in vivo. Cos-7 cells were cotransfected with HA-mutSRG3, AR, SRC-1, BRG1, and BRM expression plasmids. Coimmunoprecipitation experiments were carried out with anti-HA (mutSRG3-1) antibody. Western blot analyses of immunoprecipitated materials were performed with anti-AR or anti-HA, anti-SRG3, anti-SRC-1, anti-BRG1, and anti-BRM antibodies. Input Western blots are shown for the expression level of each protein. (C) mutSRG3-1 is able to enhance AR transactivation. Fifty ng of ARE2-TATA-Luc reporter plasmid was cotransfected along with 5 ng of AR and increasing amounts of mutSRG3-1 (20, 50, and 100 ng) into Cos-7 cells. Either 20 ng or 100 ng of SRC-1 expression plasmid was also cotransfected as indicated. (D) mutSRG3-2, a mutant that is no longer able to bind SRC-1, is unable to enhance AR transactivation. Fifty ng of ARE2-TATA-Luc reporter plasmid was cotransfected along with 5 ng of AR, 50 ng of either SRG3 or mutSRG3-2, and 50 ng of SRC-1 into Cos-7 cells. Cells were treated or not with 10 nM DHT for 24 h.

rectly interacts with SRC-1 and has a region for SRC-1 binding distinct from the region for AR binding.

Direct physical interactions between SRG3, AR, and SRC-1 were further confirmed by Far-Western blot analyses using purified GST fusion proteins. Proteins in the extract of L929 cells which endogenously express SRG3, AR and SRC-1 (Fig. 6A) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was then incubated with purified GST, GST-AR-DBDh, or GST-SRG3 fusion protein, and the L929 cell proteins which interacted with each GST fusion protein were detected with an anti-GST antibody. GST-SRG3 protein bound SRC-1 and AR, while GST-AR-DBDh protein bound SRG3 (Fig. 5D). These results indicate that SRG3 interacts directly with both AR and SRC-1, while AR interacts with SRG3.

SRG3 forms a complex with AR and SRC-1 in vivo and may function as an AR coactivator in an SWI/SNF complex-independent way. To determine whether AR protein forms a complex with SRG3 and SRC-1 in vivo, coimmunoprecipitation experiments were carried out using L929 cells which were treated or not with DHT (Fig. 6A). L929 is a mouse fibroblast cell line and endogenously expresses AR, SRG3, SRC-1, BRG1, and BRM. Immunoprecipitation of whole-cell extracts was performed using an anti-AR antibody. Western blot analyses of the immunoprecipitated material revealed that SRG3 and SRC-1 endogenously associated with AR, but only when the cells were treated with DHT. SRC-1 itself has been reported to interact directly with AR through both the AF-1 and LBD of AR (5). The recruitment of SRC-1 to AR through SRG3 was confirmed by coimmunoprecipitation experiments using an AR mutant containing only its DBDh region, which can interact with SRG3 but not with SRC-1. SRC-1 was associated with AR (DBDh) only in the presence of SRG3 (data not shown).

Because the earlier results revealed that SRG3 functions as an AR coactivator in the absence of BRG1 and BRM in C33A cells (Fig. 2E and F), we investigated whether BRG1 or BRM is associated with the endogenous AR/SRG3/SRC-1 complex in L929 cells. Western blot analyses of the AR-immunoprecipitated material revealed that AR/SRG3/SRC-1 complex did not contain either BRG1 or BRM (Fig. 6A), again suggesting the possibility of SRG3 coactivator function in a manner that is SWI/SNF complex independent.

To further address the findings that SRG3 coactivator function is independent of the chromatin-remodeling complex, we generated an SRG3 deletion mutant (amino acid residues 611

to 942) containing only the Myb-like region for the interaction with SRC-1 and the leucine zipper domain for the interaction with AR (Fig. 5B and C). Coimmunoprecipitation experiments were carried out using Cos-7 cells transfected with HA-tagged SRG3 mutant, AR, SRC-1, BRG1, and BRM expression vectors. Immunoprecipitation of whole-cell extracts was performed with an anti-HA (mutSRG3-1) antibody. As expected, the SRG3 mutant was able to associate with SRC-1 and AR but not with BRG1 and BRM in vivo (Fig. 6B). Transient transfection analyses with the SRG3 mutant revealed that the mutant was able to enhance the transactivation of AR in a dose-dependent manner (Fig. 6C), as did wild-type SRG3. Furthermore, coexpression of SRC-1 with the SRG3 mutant cooperatively increased the expression of the reporter. Meanwhile, a SRG3 mutant (deletion of amino acid residues 613 to 723, mutSRG3-2), which has its MLR region deleted and thus is no longer able to bind SRC-1, was significantly unable to enhance AR transactivation in the presence or absence of SRC-1 overexpression (Fig. 6D). Together, these results further support the view that the SRG3 coactivator function is independent of the SWI/SNF complex.

AR, SRG3, and SRC-1 are recruited to AR target promoters in response to androgen in vivo. To determine whether AR, SRG3, and SRC-1 form a complex on AR target promoters in vivo, we performed ChIP assays with prostates dissected from male mice which were injected with flutamide or not (Fig. 7A). In the absence of flutamide injection, AR, SRG3, and SRC-1 associated with the SRG3 promoter in the prostate, while flutamide injection resulted in the failure of recruitment of these factors to the SRG3 promoter. We also performed ChIP assays with LNCaP cells transfected with an SRG3 expression plasmid and then treated or not with DHT (Fig. 7B). Androgen treatment induced AR, SRG3, and SRC-1 to associate with the PSA promoter, while none of them was recruited to the promoter in the absence of DHT treatment. Furthermore, SRG3 overexpression in LNCaP cells upregulated androgen-dependent transcription of the endogenous PSA gene (Fig. 7C). Together, these results suggest that in vivo the AR/SRG3/SRC-1 complex occupies AREs on the SRG3 and PSA promoters in an androgen-dependent manner, inducing gene expression.

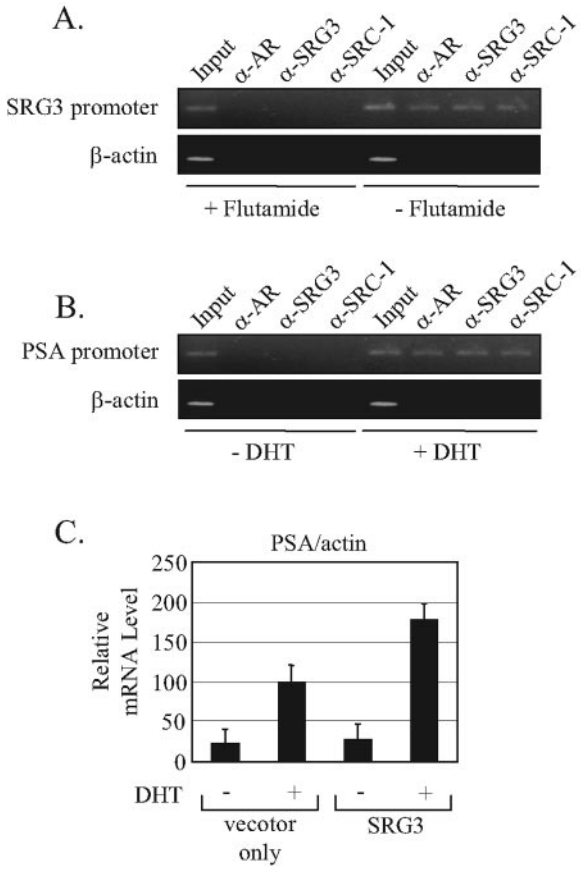

FIG. 7. AR, SRG3 and SRC-1 are recruited to AR target promoters in response to androgen in vivo. (A) ChIP assays were performed with prostates dissected from 3-week-old male mice that had been injected or not with flutamide at 100 mg/kg body weight for 12 h. (B) ChIP assays were performed with LNCaP cells transfected with SRG3 expression plasmid and then treated or not with 10 nM DHT for 6 h. Anti-AR, anti-SRG3, and anti-SRC-1 antibodies were used for immunoprecipitations. The immunoprecipitates were analyzed by PCR using a pair of specific primers spanning the region containing the AREs of the SRG3 promoter in mouse prostate and the PSA promoter in LNCaP cells. A control PCR for nonspecific immunoprecipitation was performed using primers specific to the β-actin coding region. (C) SRG3 overexpression elevates androgen-dependent transcription of the endogenous PSA gene. LNCaP cells were transfected with SRG3 expression plasmid or empty vector and then treated or not with 10 nM DHT for 24 h. PSA transcripts were analyzed by real-time RT-PCR of total RNAs. β-Actin was used for a quantitative control.

DISCUSSION

In this study, it is demonstrated that SRG3 functions as an AR coactivator, recruiting SRC-1 to AR target promoters such as SRG3 and PSA. This SRG3 function is probably independent of the BRG1/BRM-containing SWI/SNF complex because SRG3 works in BRG1/BRM-deficient C33A cells as well as in other normal cells, the AR/SRG3/SRC-1 complex contains neither the BRG1 nor BRM protein in vivo, and an SRG3 mutant (mutSRG3-1) which is unable to associate with BRG1 or BRM is able to enhance AR transactivation, as does wild-type SRG3.

SRG3, known to be a mouse homologue of yeast SWI3, *Drosophila* MOIRA, and human BAF155, is also a core component of the SWI/SNF chromatin-remodeling complex (6, 26, 42). However, the expression pattern of SRG3 has been shown to be basically similar to but somewhat different from that of both BRG1 and BRM during mouse embryogenesis, which suggests that some SRG3 proteins may not be components of either the BRG1- or BRM-containing SWI/SNF complexes and may have an alternative novel function(s) (30). In addition, the expression of the SRG3 homologue BAF155 is different from that of the major components of the SWI/SNF complex in certain human cell lines (48). Furthermore, in this study, SRG3 was different in expression pattern from BRG1 and BRM during rat prostate development and in the expression response to androgen in rat prostate. These findings further support the suggestion that SRG3 has an alternative function in addition to being a subunit of the SWI/SNF complex.

Recently, SRG3 has been reported to associate with glucocorticoid receptor to regulate glucocorticoid receptor transactivation in a thymoma cell line (20). However, it has not been

made clear whether SRG3 interacts directly with glucocorticoid receptor or whether the modulation of glucocorticoid receptor transactivation by SRG3 is BRM/BRG1 independent. Meanwhile, the SWI/SNF chromatin-remodeling complex has been reported to be needed for glucocorticoid receptor transactivation (17, 39, 41, 53), in which BAF60a and BAF57 but not BRG1, BAF155, or BAF170 turn out to be direct links between glucocorticoid receptor and the chromatin-remodeling complex (23). BAF60a also interacts with progesterone receptor b, estrogen receptor alpha, and farnesoid receptor. In addition, BAF57 has been reported to be a direct link between estrogen receptor and the SWI/SNF complex (4). Thus, this report of SRG3 interaction with AR is the first evidence of a direct interaction between SRG3 (BAF155) and the steroid nuclear receptor. This may be further evidence for the SWI/SNF complex-independent function of SRG3, although the possibility that SRG3 mediates the interaction between AR and the chromatin-remodeling complex cannot be ruled out.

A previous study has shown that Notch1, a transcription factor, downregulates the expression of SRG3 in macrophages (7). Deletion analysis of the SRG3 promoter in the present study revealed that the 76-bp region (−210 to −135) contains a sequence element(s) that is essential for the high level of SRG3 basal expression, activating expression over 100-fold (Fig. 1F). The downregulation of SRG3 expression during prostate development may be related to the transcription factor that interacts with this sequence element. Developmental downregulation of the expression of this transcription factor can maintain low levels of SGR3 expression during the course of prostate differentiation. The identification and characterization of the main factor or factors that regulate SRG3 expression are important subjects and under active investigation.

The present study also demonstrates that androgen upregulates the expression of SRG3 and that SRG3 is a direct target gene of AR. To the best of our knowledge, this is the first report that the expression of core components of the SWI/SNF complex is regulated by signals such as steroid hormones. Besides having a role in the positive feedback regulation of AR action, the biological significance of SRG3 induction by androgen remains to be elucidated in relation to the SWI/SNF chromatin-remodeling complex.

Upon ligand binding, hormone receptors become activated and recruit coactivators which adopt diverse modes of action, including direct interaction with basal transcription factors and covalent modification of histones and other proteins to induce the expression of target genes (2, 24, 40, 43). Interestingly, SRG3 increases the protein level of SRC-1 (Fig. 4A to C) as well as recruiting SRC-1 to enhance AR transactivation. Increased protein stability following an interaction between a transcription factor and its coactivator has been reported. For example, Jab1 (Jun activation domain-binding protein 1) interacts with HIF-1α (hypoxia-inducible factor 1α) and enhances HIF-1α stability, resulting in an increase in the HIF-1α protein level (1). In a similar way, hepatitis B virus X protein and p300 increase the half-life of activating signal cointegrator 2 and sterol regulatory element binding proteins (SREBPs), respectively (19, 31). Thus, the increase of protein stability by protein-protein interaction is likely another action mode of transcription factor/coactivator to enhance gene expression.

Numerous studies have demonstrated the modulation of AR function by its coregulators. However, the molecular regulatory mechanism of prostate development or regeneration by androgen is as yet unclear. In this study, we demonstrate that androgen induces the expression of SRG3 and SRG3 in turn enhances the transcriptional activity of AR, which provides a positive feedback regulatory mechanism for AR transactivation. Furthermore, SRG3 upregulates the protein level of SRC-1, with which SRG3 cooperates to enhance AR transactivation, providing another regulatory mechanism for AR transactivation by SRG3. These multiple positive regulatory mechanisms of AR transactivation by SRG3 may be important for the rapid proliferation of cells during prostate development and regeneration, during which the SRG3 expression level is high.

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