SRY Interacts with and Negatively Regulates Androgen Receptor Transcriptional Activity*

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This study investigated interactions between SRY, the Y chromosome encoded male sex determining factor, and the androgen receptor (AR). Coexpression of AR and SRY caused marked repression of AR transcriptional activity on a series of androgen-responsive reporter genes. Mammalian one- and two-hybrid experiments demonstrated an AR-SRY interaction mediated by the AR DNA binding domain. Precipitations with glutathione S-transferase fusion proteins indicated that AR-SRY interactions were direct and mediated by the AR DNA binding domain and the SRY high mobility group DNA binding domain. Transient expression of SRY in LNCaP prostate cancer cells repressed expression of an androgen-dependent prostate-specific antigen (PSA) reporter gene and stable SRY expression repressed the endogenous PSA gene. SRY protein expression was increased by proteosome inhibitors and by the androgen-ligated AR in transient and stable transfectants. AR transcriptional activity was also repressed by DAX1, and the effects of SRY and DAX1 on the AR were additive. These findings indicate that interactions between the AR, SRY, and DAX1 contribute to normal male development and function and suggest a general role for protein-protein interactions between high mobility group box proteins and steroid hormone receptors in regulating tissue-specific gene expression.

The androgen receptor (AR) is a steroid hormone receptor member of the larger nuclear receptor superfamily and is required for normal male development, with multiple AR mutations identified in patients with androgen insensitivity (1, 2). The AR also plays a critical role in the development and progression of prostate cancer, with most prostate cancers dependent upon androgens for their growth and responding to therapies that decrease androgen levels (3). The AR binds to specific DNA sequences (androgen-responsive elements (AREs)) as a homodimer through a central DNA binding domain (DBD) composed of two zinc fingers that are highly conserved among steroid hormone receptors. Ligand binding mediated by the conserved steroid hormone receptor C terminus ligand binding domain (LBD) induces conformational changes that result in dissociation from a heat shock protein 90 complex, dimerization, and movement of helix 12, which creates a binding site for Leu-X-X-Leu-Leu (LXXLL) motifs present in the steroid receptor coactivator (SRC-1, -2, and -3) family of transcriptional coactivator proteins (4–14). The N-terminal domain is least conserved among steroid hormone receptors, and in the case of the AR, it comprises about half of the protein and contains a strong autonomous transactivation function (15, 16). The AR N terminus can also interact directly with SRC proteins (11, 17, 18). In addition, LXXLL-like motifs identified in the N-terminal domain mediate ligand-dependent interactions between the AR N terminus and LBD (17, 19–22).

In addition to the SRC proteins, AR function may be further modulated by a growing list of other proteins shown to interact with the N terminus, DBD, or LBD of the AR or other nuclear receptors (23). This study demonstrates an interaction between the AR and human SRY, the testis-expressed protein encoded by the sex-determining region on the Y chromosome that mediates male sex determination (24–28). Human SRY is a 204-amino acid nuclear protein containing a central sequence specific high mobility group (HMG) box DNA binding domain (29, 30). Similar to other HMG box-containing proteins, SRY generates a sharp bend in DNA with distortion of local DNA structure (31). Significantly, human SRY lacks a C terminus transcriptional activation domain that is present in murine SRY and which is necessary for murine male sex determination (32, 33), suggesting that human SRY may function through interaction with additional transcriptional coactivator(s).

Initial studies suggested that SRY functioned in male sex determination by directly stimulating the expression of mullerian-inhibiting substance (MIS) (34, 35), but subsequent studies have indicated that SRY does not directly activate the MIS gene and may instead induce the expression of other transcription factors that regulate MIS (36–38). One such candidate direct regulator of the Mis gene is SOX9, an SRY related autosomal HMG protein (38–40). SRY function in male sex determination can also be antagonized by DAX1, a testis-expressed orphan nuclear receptor shown to function as a transcriptional corepressor (41). The major target of DAX1 on the Mis gene appears to be steroidogenic factor 1 (SF-1), an orphan nuclear receptor that positively regulates the Mis gene (37, 38, 42, 43).

Murine SRY appears to be expressed only briefly in the gonadal ridge at the time of testis determination (27, 36),...
SRY and AR Interaction

whereas human SRY is expressed in adult as well as fetal Sertoli cells and in adult germ cells (44), although its functions in the adult testis are not known. SRY expression has also been reported in human tissues outside of the testis, including prostate and prostate cancer (44–48), and loss of SRY and other Y-chromosome-encoded genes is a frequent finding in prostate cancer (47). These findings suggest possible roles for human SRY in maintaining as well as in establishing the male phenotype.

It was previously shown that human SRY can interact through its unique C terminus with a novel PDZ domain-containing nuclear protein of uncertain function, SIP-1 (49). SRY was also reported to interact with calmodulin through its HMG domain (50). The interaction between the AR and SRY described in this report was mediated by the respective highly conserved zinc finger and HMG box DBDs and resulted in repression of AR transcripational activity. An inhibitory interaction was also demonstrated between the AR and DAX1. These results indicate that AR, SRY, and DAX1 interactions may contribute to male sexual development and function. Significantly, previous studies have shown that the ubiquitously expressed HMG-1 and -2 proteins, which contain non-sequence-specific HMG box DBDs, augment DNA binding and transcriptional activity of steroid hormone receptors including the AR (51–56). In conjunction with these findings, the present study suggests a more general role for interactions between HMG box proteins and steroid hormone receptors in controlling the expression of tissue-specific and developmentally regulated genes.

EXPERIMENTAL PROCEDURES

Plasmids—A human AR expression vector, pSVARo, was from A. Brinkmann (57), pBl-CMV, pG5-Luc, pBind, and pACT vectors were from Promega (Madison, WI). GST-AR fusion protein expression vectors and the MMTVpA3-Luc reporter regulated by the MMTV-LTR (from R. Pestell) were described previously (58). VP16 activation domain-AR expression vectors were generated in pACT containing the full-length AR (pACT-hAR), the N-terminal (amino acids 1–500, pACT-AR-N), the DBD (amino acids 501–680, pACT-ARDBD), and the LBD (amino acids 681–926, pACT-AR-LBD) (59). The ARE–Luc reporter contained four tandem AREs cloned into pGL3 (5). The PsA7kb-Luc reporter containing a 7-kb genomic fragment upstream from the Prostate-specific antigen (PSA) gene was cloned into pGL3 from T. Libermann (60).

An expression vector encoding full-length human SRY with a C terminus Myc epitope tag, pcDNA-hSRY, was generated by polymerase chain reaction amplification of the single exon SRY-coding region from human genomic DNA using the primers SRY5’ (5′-gaggaattccatctacatcttcgccttccgacg-3′) and SRY3′ (5′-aagggatccctgccgaagaattgcagt-3′). The polymerase chain reaction product was cloned into EcoRI and BamHI sites in the pcDNA3.1Myc-His(+) expression vector (Invitrogen, Carlsbad, CA). The GST-SRY fusion protein expression vectors were generated in pGEX-2T by inserting polymerase chain reaction amplification products for full-length SRY or the N terminus, HMG domain, or C terminus. Primers for the N-terminal vector, GST-SRY1–57), were 5′-N-SRY (5′-aaggaattccatctacatggtctgtc-3′) and 3′-SRY (5′-gggatccctgccgaagaattgcagt-3′). Primers for the HMG domain vector, GST-SRY58–137, were 5′-HMG-SRY (5′-aaggaattccatctacatggtctgtc-3′) and 3′-HMG-SRY (5′-gggatccctgccgaagaattgcagt-3′). Primers for the C terminus vector, GST-SRY138–204, were 5′-C-SRY (5′-aaggaattccatctacatggtctgtc-3′) and 3′-C-SRY (5′-gggatccctgccgaagaattgcagt-3′).

VP16-1 SRY expression vector was generated by inserting full-length human SRY into the EcoRI site of the VP16 activation domain vector AASVPVP16 (61) using the SRY primers 5′-SRYVPIVP16EcoRI (5′-gtgaggaattccatctacatggtctgtc-3′) and 3′-SRYVPIVP16EcoRI (5′-gtgaggaattccatctacatggtctgtc-3′). The SRY expression vector was transfected into 293FT cells, and the mouse DAX1 expression vector was from Y. Sadovsky (62).

Transient Transfections and Reporter Gene Assays—CV1 cells were cultured in 24-well plates in DMEM with 5% FCS (Hyclone, Logan, UT) to ∼80% confluence. Cells were transfected by mixing the indicated amounts of plasmid DNA with 2 µl of LipofectAMINE 2000 (Invitrogen) in a final volume of 100 µl of Opti-MEM for 45 min at room temperature. Each transfection mix was then mixed with 0.5 ml of DMEM with 10% charcoal-dextran-tripped fetal bovine serum (CS-FCS) (Hyclone) and added to the cell culture well. After 24 h the culture medium was replaced with 0.5 ml of DMEM, 10% CS-FCS with or without 10–50 nM dihydrotestosterone (DHT) as indicated. After another 24 h, the cells were rinsed once with PBS and subsequently lysed with 100 µl of PBS/marine buffer and measured for luciferase activity using a dual-luciferase reporter assay system (Promega). The firefly luciferase results were divided by the control Renilla luciferase to give relative light units, and the results reflect the mean ± S.D. from triplicate or quadruplicate samples. LNCaP cells were transfected similarly, except the cells were initially in RPMI 1640 and grown in 5% CO2 instead of 10%, as described for the CV1 cells. GST Pull-down Assays—GST-AR and GST-SRY fusion proteins were purified on glutathione-agarose beads as described previously (58).

S3S-Labeled AR and SRY were generated by in vitro transcription/translation (TNT T7 quick coupled transcription/translation system, Promega). The pcDNA-AR or pcDNA-SRY vectors (2 µg) in 50 µl of reticulocyte lysate with 20 µCi of [35S]methionine were incubated for 1.5 h at 30 °C. GST fusion proteins on glutathione-agarose beads (5 µg/sample) were mixed with 10 µl of the programmed lysates in 0.5 ml of phosphate-buffered saline, pH 7.4, 1 mM dithiothreitol, and protease inhibitors. After binding for 4 h at 4 °C, the beads were washed in the same buffer except with 0.05% Nonidet P-40. Proteins were eluted with SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer and run on 10% SDS-PAGE gels under reducing conditions.

Stable Transfections—pcDNA-AR or pcDNA vectors were transfected into LNCaP cells in conjunction with a pBABE-puro vector using LipofectAMINE 2000. Stable cell lines were selected with puromycin (3 µg/ml).

Protein Analysis—AR protein was detected by immunoblotting with rabbit polyclonal Abs directed against an N-terminal or C terminus peptide (Santa Cruz Biotechnology, Santa Cruz, CA), using 1:2,000 of the primary and 1:5,000 of a secondary HRP conjugated anti-rabbit antibody (Promega). SRY protein was detected with an anti-c-Myc antisemur (Santa Cruz Biotechnology) at 1:1,000 and a secondary horseradish peroxidase-conjugated anti-mouse antibody at 1:2,000. PSA levels were measured using a clinical enzyme-linked immunosorbent assay kit. Proteosome inhibitors LLNL and MG132 were dissolved in Me2SO.

RESULTS

AR Transcriptional Activity Is Repressed by SRY—A strong androgen-regulated transcripational enhancer for the PSA gene that contains a consensus ARE and multiple weak non-consensus AREs has been mapped to a 441-bp fragment 4.3 kb upstream from the PSA transcriptional initiation site (63–65). A search for additional consensus cis-elements in this enhancer revealed multiple potential DNA binding sites for SRY (not shown). In addition to being expressed in the embryonic urogenital sinus, human SRY is expressed in adult tissues including adult testis and prostate as well as prostate cancer cell lines (44–46, 48). Therefore, potential interactions between the AR and SRY in the regulation of the PSA gene were initially assessed.

Cotransfection experiments with AR and SRY expression vectors and a luciferase reporter gene regulated by the PSA promoter/enhancer (pSACTkb-Luc) were used to test the possible role for SRY in regulating this gene. The PSA reporter was strongly stimulated by the AR in the presence of DHT, and this activation was markedly repressed by cotransfection with SRY (Fig. 1A). This repression did not appear to be due to transcriptional or toxic effects, as SRY did not affect the basal activity of this reporter gene in the absence of DHT (Fig. 1B), and similar repression was not observed with control CMV- or SV40-regulated Renilla reporter genes (data not shown). To determine whether the repression might be because of decreased AR expression, lysates from transfected cells were immunoblotted for AR. AR protein expression was greater in the presence of DHT, but cotransfection with SRY did not substantially alter the level of AR expression in the presence or absence of DHT (Fig. 1C). To determine whether the SRY repression was dependent upon DNA binding to an SRY cis-element in the 7-kb PSA

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promoter/enhancer, additional AR-regulated reporter genes were assessed. The mouse mammary tumor virus (MMTV) long terminal repeat contains two androgen-responsive elements and was strongly stimulated by the AR in the presence of DHT (Fig. 2A). Similar to the result with the PSA7kb-Luc reporter, SRY markedly repressed DHT-stimulated transcription from the MMTV long terminal repeat. A third AR-regulated reporter containing a multimerized synthetic androgen-responsive element, ARE4-Luc, was also examined. SRY-mediated repression of ligand-dependent AR transactivation was also observed with this ARE4-Luc reporter (Fig. 2B). Taken together, these findings indicated that SRY repressed AR transactivation and that this repression did not require SRY binding to consensus cis-elements.

**AR Interacts with SRY in Mammalian One- and Two-hybrid Assays**—To determine whether the repression reflected a direct interaction between SRY and AR, mammalian one-hybrid experiments were carried out using full-length SRY fused to the VP16 transactivation domain. It should be noted that murine SRY has a strong C terminus transactivation domain that is not present in human SRY (32). The VP16-SRY fusion protein alone in the absence of AR had no substantial effect on an ARE4-luciferase reporter gene, supporting the conclusion that SRY does not directly bind to this reporter (Fig. 3A). However, in contrast to the result with the native SRY, ligand-dependent AR transactivation activity was augmented ~2-fold by the VP16-SRY fusion protein. As a further control, the VP16 transactivation domain alone did not stimulate AR transactivation activity. These results indicated that there was a direct AR-SRY protein interaction.

The converse mammalian two-hybrid experiment was also carried out using SRY fused to the Gal4 DNA binding domain and AR fused to VP16 transaction domain. As shown previously, the SRY-Gal4 fusion protein did not show any transcriptional activity when transfected with a Gal4-luciferase reporter (pG5-Luc) (Fig. 3B and data not shown) (32). However, transcription was stimulated by cotransfection of a VP16-AR fusion protein (pACT-hAR), and this activity was further stimulated by DHT, supporting a direct AR-SRY interaction. To determine whether the interaction was mediated by a discrete region of the AR, Gal4-SRY-mediated transcriptional activation was assessed with cotransfection of VP16 fusions to the AR N-terminal (pACT-N), the central DNA binding domain (pACT-DBD), or the C terminus ligand binding domain (pACT-LBD). Transcription was stimulated only by the pACT-DBD, indicating that the AR-SRY interaction was mediated by the AR DBD (Fig. 3B). This activity was DHT-independent, consistent with the absence of the LBD that sequesters unliganded AR. The greater enhancement by the full-length AR construct, pACT-hAR, may reflect dimerization or the additional intrinsic transcriptional activity of the AR N terminus.

**AR and SRY Interact Directly in GST Pull-down Assays**—GST fusion protein pull-down experiments were next carried out to determine whether there was a direct AR-SRY interaction. SRY was 35S-labeled by coupled in vitro transcription/translation, and binding to a series of GST-AR fusion proteins was assessed. SRY bound specifically to a GST-AR-(505–919) fusion protein, encoding the 3’ end of exon 1, the DBD and LBD of the AR (Fig. 4A). SRY also bound to the GST-AR-(553–635)
AR–SRY interactions.

A

Ang), ARE4–Luc reporter (250 ng), pRL–CMV (0.1 ng), and AASVVP16-VP16 units. and normalized luciferase activity was determined. RLU

Cells were cultured in DMEM, 5% CS–FCS, Gal4–SRY (100 ng), and the pACT–AR fusions (500 ng) as indicated. transfected with pG5–Luc reporter (250 ng), pRL–CMV control (0.1 ng), and normalized luciferase activity was determined.

PSA gene. In the initial experiments, LNCaP cells were modulated the transcriptional activity of an endogenous AR on Therefore, LNCaP cells were used to determine whether SRY production are both androgen-stimulated. LNCaP cells have also been reported to express SRY transcripts, which can be transcribed/translated AR bound specifically to full-vitro binding of full-length AR to SRY GST fusion proteins. The results were consistent with the above mammalian two-hybrid results interaction being mediated through the AR DNA binding do-

B

SRY (VP16-SRY) or empty AASVVP16 (VP16) as indicated. Cells were cultured for 24 h in DMEM, 5% CS–FCS, SRY (VP16) (Fig. 4B). Binding was not affected by the inclusion of DHT, consistent with the above experiments. AR and SRY have been shown to bind to the same DNA recognition sequence. Taken together, these results indicated that SRY-AR binding was modulated by the AR DBD. The converse experiments were carried out to directly assess binding of full-length AR to SRY GST fusion proteins. The in vitro transcribed/translated AR bound specifically to full-length SRY fusion protein, GST-SRY-(1–204) (Fig. 4B). Binding was not affected by the inclusion of DHT, consistent with the interaction being mediated through the AR DNA binding domain (not shown). To determine whether binding was mediated by a discrete region of SRY, GST-SRY-(1–57), -(58–137), and -(138–204) fusion proteins containing the SRY N terminus, the central HMG domain, and the C terminus, respectively, were generated. The AR bound specifically to the GST-SRY-(58–137) fusion protein, encoding only the DBD, but not to fragments of the AR N-terminal or C terminus to this domain. These results were consistent with the above mammalian two-hybrid results and indicated that there was a direct SRY binding interaction mediated by the AR DBD.

Fig. 3. Mammalian one- and two-hybrid protein analysis of AR-SRY interactions. A, CV1 cells were transfected with pSVARo (50 ng), ARE4–Luc reporter (250 ng), pRL–CMV (0.1 ng), and AASVVP16-SRY (VP16-SRY) or empty AASVVP16 (VP16) as indicated. Cells were cultured for 24 h in DMEM, 5% CS–FCS, +/– DHT (10 nm) as indicated, and normalized luciferase activity was determined. B, CV1 cells were transfected with pG5–Luc reporter (250 ng), pRL–CMV control (0.1 ng), Gal4–SRY (100 ng), and the pACT–AR fusions (500 ng) as indicated. Cells were cultured in DMEM, 5% CS–FCS, +/– DHT (10 nm) for 24 h, and normalized luciferase activity was determined. RLU, relative light units.

cotransfected with SRY and the PSA7kb-Luc reporter. Transcription of the PSA7kb-Luc reporter gene was stimulated by DHT, and this was strongly repressed by cotransfection with SRY (Fig. 5A). This indicated that transfected SRY could repress the activity of the endogenous LNCaP AR.

SRY function was then assessed by generating a LNCaP cell line stably transfected with an SRY expression vector, LNCaP-SRY. Expression of the transfected SRY was confirmed by immunoblotting (see below). Consistent with the results from transient SRY transfections, expression from the transfected PSA7kb-Luc reporter gene was markedly diminished in the SRY-expressing line (Fig. 5B). To assess SRY effects on expression of the endogenous PSA gene, PSA protein production by the LNCaP-SRY cells was determined by enzyme-linked immunosornt assay on culture supernatants. PSA production in nontransfected LNCaP cells grown in steroid hormone-depleted medium was stimulated by DHT (Fig. 5C), although it should be noted that production of endogenous PSA was less androgen-dependent than expression of the PSA7kb-Luc reporter gene in the same cells. It is not yet known whether this androgen-independent PSA production in LNCaP cells is AR-dependent or -independent. PSA production from a control LNCaP cell line stably transfected with the pcDNA3 expression vector (LNCaP-REF#1) was similar to the nontransfected cells. In contrast, androgen-dependent and -independent PSA production were markedly repressed in the SRY-expressing LNCaP cells (Fig. 5C).

Immunoblotting was used to determine whether repression in the LNCaP-SRY cells was because of decreased AR protein expression. AR expression in the LNCaP-SRY cells was comparable with nontransfected LNCaP or control-transfected LNCaP cells (Fig. 5D). AR levels in the absence of DHT were moderately decreased in each of the cells, reflecting a previously demonstrated increase in turnover of the unliganded AR protein (67). Taken together, these results showed that stably expressed SRY could strongly repress expression of an endogenous AR-regulated protein.

Inhibition of SRY Protein Degradation by the AR—Expression of the SRY protein in the stably transfected LNCaP-SRY cells was assessed by immunoblotting. Significantly, SRY protein expression in these cells was strongly stimulated by DHT (Fig. 6A). An unidentified protein migrating more slowly than SRY was also detected by the anti-Myc antibody in all of the LNCaP lysates and was not changed by DHT. Because SRY...
transcription in these cells was driven by a CMV promoter, which is not known to be androgen-dependent, it appeared unlikely that the decreased SRY level was because of reduced transcription. Therefore, it was next determined whether the decrease in SRY in the absence of DHT reflected increased SRY degradation. Consistent with this hypothesis, treatment of the cells with proteosome inhibitors (LLNL or MG132) markedly augmented the level of SRY protein in the presence or absence of added DHT (Fig. 6B). This was not because of an increase in AR protein levels, because AR levels were not markedly altered by the proteosome inhibitors (Fig. 6B).

These data suggested that SRY protein was stabilized by the DHT-ligated AR, but they were also consistent with the hypothesis that both the liganded and unliganded AR stimulated SRY degradation, with the unliganded AR more efficient. Therefore, the effect of the AR on SRY protein levels in transiently transfected AR negative cells was assessed by immunoblotting. SRY protein level was not increased by DHT in the absence of SRY (Fig. 6C). In contrast, a substantial DHT-dependent increase in SRY expression was observed when cells were cotransfected with AR. Taken together, these results indicated that SRY protein was degraded by the proteosome and that this degradation was inhibited by the DHT-bound AR.

**SRY Repression of the AR Is Not Antagonized by DAX1**—SRY stimulation of male sexual determination can be antagonized by increased expression of DAX1 (41), a protein related to the nuclear receptor superfamily that can function as a transcriptional corepressor (62–67). This dosage-sensitive sex reversal by DAX1 may reflect its ability to inhibit SF-1, although it only occurs in conjunction with a weak Sry allele, suggesting additional interactions. In any case, it was of interest to determine whether DAX1 could modulate the SRY-mediated AR transcriptional repression.

CV1 cells were cotransfected with AR and SRY expression vectors alone or with a DAX1 expression vector. As shown above, SRY repressed DHT-stimulated AR transcriptional activity (Fig. 7). DAX1 did not antagonize this repression and instead resulted in further inhibition. Significantly, DAX1 alone also functioned as a potent repressor of AR transcriptional activity, and the effects of SRY and DAX1 together were additive (Fig. 7). These results indicated that DAX1 did not antagonize SRY effects on the AR and that DAX1 was also a repressor of AR transcriptional activity.

**DISCUSSION**

Although SRY has been clearly established as the male sex-determining gene on the Y chromosome, its mechanism of action in sex determination and its subsequent functions in human adult testis and other tissues remain unclear. This study demonstrated an interaction between SRY and the AR that was mediated by their respective DNA binding domains and that resulted in repression of ligand-stimulated AR transcriptional activity on a series of AR-regulated genes. The AR is expressed early during male sex determination by the Wolffian duct, and development of this structure is testosterone-dependent. As a result, patients with inactivating AR mutations and, hence, complete androgen insensitivity lack Wolffian duct-derived structures (73). However, these patients with complete androgen insensitivity nonetheless also lack the Mullerian duct-derived fallopian tubes, uterus, and cervix, indicating normal SRY-dependent production of MIS in the fetus. Therefore, a critical early role for direct AR-SRY interactions in male sex determination appears unlikely. Moreover, there is no evidence for coexpression of AR and SRY, respectively, in fetal Sertoli cells or in Wolffian duct.

In contrast to the case in the developing testis, SRY and AR are coexpressed in Sertoli cells and germ cells in adult testes (44). SRY appears to play a role in germ cell development since the testes appear only as a gonadal streak without germ cells in the absence of SRY (74), suggesting a role for AR-SRY interactions in germ cell development. AR and SRY are also expressed...
in adult prostate epithelium, and frequent loss of the Sry gene has been reported in prostate cancer (44–48). This study found that SRY could repress the transcriptional activity of the endogenous AR in LNCaP prostate cancer cells. Conversely, the AR increased the stability of the SRY protein in LNCaP cells, further supporting a functional interaction between these proteins. The majority of prostate cancers are androgen-dependent and regress in response to androgen ablation therapies, demonstrating a critical role for the AR in stimulating prostate cancer growth (3). Therefore, SRY function as a negative regulator of AR transcriptional activity suggests that its loss in prostate cancer may augment AR activity.

SRY is the founding member of a large family of proteins with homologous HMG box DBDs, termed the SRY-type HMG box (SOX) proteins, which are widely expressed and appear to contribute to the expression of developmentally regulated and tissue-specific genes (75, 76). SRY and the SOX proteins all bind similar DNA motifs, and their specificity appears to be regulated by tissue-specific expression and cooperative binding with other transcription factors. Indeed, recent data demonstrate that SOX9 binds to a cis-element in the Mis gene and cooperates with SF-1 to directly regulate MIS protein expression during male sex determination (38–40). SOX9 was further found to bind directly to SF-1 through the HMG domain, although in contrast to the SRY-AR interaction, binding was mapped to the LBD of SF-1 (38). Nonetheless, the interaction between the SRY HMG box and the AR DBD, both of which are highly conserved among SOX proteins and steroid hormone receptors, respectively, suggests that additional SOX proteins may similarly interact with the AR and other steroid hormone receptors. If this is the case, then these interactions could make major contributions to developmentally regulated and tissue-specific gene expression.

Further support for a more general interaction between HMG box proteins and steroid hormone receptors comes from previous studies showing that the HMG-1 and -2 proteins can enhance DNA binding and transcriptional activity of steroid hormone receptors, including the AR (51–56). HMG-1 and -2 are ubiquitously expressed HMG box proteins distantly related to SRY that similarly bind to and bend DNA, but without sequence specificity. HMG-1 and -2 appear to enhance steroid hormone receptor-DNA binding by stabilizing the conformation of DNA in the steroid hormone receptor-DNA complexes, but weak protein-protein interactions have been shown between HMG-1 and the progesterone receptor (38). Therefore, the AR-SRY interaction reported here in conjunction with these previous HMG-1 and -2 studies suggest that interactions between HMG domains and steroid hormone receptor DBDs may be highly conserved.

Human SRY has not been shown to encode autonomous repressor or transactivation domains, so the mechanism by which SRY overexpression represses AR transcriptional activity is not yet clear (32). It is possible that SRY destabilizes AR-DNA complexes by binding to cryptic SRY-responsive elements, although such elements were not detected with the VP16-SRY fusion protein. Moreover, as noted above, HMG-1 appears to stabilize rather than disrupt steroid hormone receptor-DNA complexes. An alternative hypothesis is that overexpressed SRY competes with one or more other proteins, including HMG proteins, for AR binding. If this is the case, then a physiological function for SRY may be to enhance AR binding and transcriptional activity on promoters with adjacent androgen- and SRY-responsive elements. Finally, the transcriptional activation of a Gal4-SRY fusion protein by VP16-AR fusion proteins suggests that the AR may also function as a coactivator for SRY.

A further finding in this study was that DAX1, similar to SRY, strongly repressed AR transcriptional activity. DAX1 is an atypical orphan nuclear receptor with a C terminus that is homologous to nuclear receptor LBDs, but it lacks the conserved nuclear receptor DBD (68–70). DAX1 is encoded on the X chromosome (68), and its loss causes congenital adrenal
hypoplasia and hypogonadotrophic hypogonadism (69, 77), whereas DAX1 overexpression due to gene amplification results in a sex reversal phenotype similar to SRY loss (41). This sex reversal appears due in part to increased DAX1 repression of SF-1 activity on the Mis promoter (42, 43), but there may be additional mechanisms as sex reversal in mice is only observed in conjunction with a weak Sry allele (41).

DAX1 functions as a transcriptional repressor through recruitment of the corepressors NCoR and Alien (62, 71). A recent study showed that DAX1 could also bind to and repress the estrogen receptor α and β through LXXLL motifs in the DAX1 N terminus (72), which is presumably the mechanism by which it represses the AR. DAX1 is highly expressed in testes, where it may function as a physiological negative regulator of AR, as it represses the AR. DAX1 is highly expressed in testes, where DAX1 overexpression due to gene amplification results in a sex reversal phenotype similar to SRY loss (41). This thus may function as a physiological negative regulator of AR, with increased AR repression possibly contributing to the gonadal dysgenesis seen with duplication of the DAX1 gene (41). In addition to expression in adrenal, testes, and ovary, DAX1 is also expressed in the hypothalamus and pituitary (78). Therefore, AR (and/or estrogen receptor) hyperstimulation due to loss of repression by DAX1 may contribute to the hypogonadotrophic hypogonadism associated with DAX1 loss.

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