The androgen receptor (AR) belongs to the steroid hormone nuclear receptor superfamily. The AR remains in the cytoplasm until it is activated by ligand binding. Upon ligand binding, the receptor dissociates from its heat-shock protein chaperones, becomes dimerized, and is translocated into the nucleus where it binds to specific androgen response elements (AREs) to regulate, along with other transcription factors, the transcription of its target genes. The AR plays important roles in male sexual development, prostate cell proliferation, and the progression of prostate cancer.

The AR has been shown to be modified by small ubiquitin-like modifier 1, SUMO-1 (sumoylation), in vivo (2). Lysine residues at amino acid positions 386 and 520 of the human AR (hAR) are major sumoylation sites. Mutation of these residues blocks sumoylation and increases the transactivation ability of AR, suggesting that SUMO modification negatively regulates AR activity. However, the precise function of the sumoylation of AR remains unknown. Also SUMO-E3 ligase toward AR has not been identified.

SUMO-1 has been shown to conjugate to target proteins through an isopeptide linkage between a glycine residue in the terminus and the ε-amino group of a lysine residue of the target protein. Sumoylation has multiple functions that include involvement in protein targeting, stabilization, and transcriptional regulation. Sumoylation of RanGAP1 results in movement of the protein from the cytoplasm to the nuclear pore complex (3–5). In the case of PML (promyelocytic leukemia) proteins, sumoylation regulates their subnuclear localization to structures termed PML nuclear bodies (6–9). Sumoylation of inhibitor of NFκB acts antagonistically to ubiquitinylation and protects the sumoylated molecule itself from ubiquitin-mediated proteolysis (10). In addition, sumoylation of p53 has been proposed to regulate the transcriptional activity of p53 (11, 12).

Sumoylation of target proteins seems to occur in a manner analogous to the ubiquitylation reaction. Heterodimeric E1 enzyme (Sua1/Uba2) and E2 enzyme (Ubc9) have already been detected by us and by other groups (13–18), and recently we identified PIAS1 as a SUMO-E3 ligase toward p53 (19). Also, we and other groups have shown yeast Siz1/Uhl1 protein to have SUMO-1/Smt3-E3 activity (20, 21). PIAS1 and Siz1/Uhl1 share significant homology in their critical RING finger-like domain (SP-RING) (22). More recently, PIASy was shown to markedly stimulate the sumoylation of LEF1 and multiple other proteins in vitro and to function as a SUMO-E3 ligase toward LEF1 in vitro (23). PIAS1 and PIAS3 were originally discovered as transcriptional co-regulators of the Janus kinase-STAT pathway (24, 25). The human PIAS family consists of several homologous proteins, including PIAS1, PIAS3, PIASx, PIASxα, PIASxβ, and PIASy, and a hypothetical protein (GenBankTM accession no. CAB6507). All of them have a RING finger-like domain (SP-RING) (22). The-binding of a ligand, e.g. testosterone, to the AR was required for the sumoylation of AR in intact cells. Although AR-dependent transcription was enhanced by PIAS proteins without sumoylation of the receptor, PIAS1 and PIASxα repressed AR-dependent transcription in a manner dependent on the ectopic expression of SUMO-1 and their RING finger-like domain. Furthermore, the sumoylation sites of the AR were necessary for the full repressive effect on AR-dependent transactivation, indicating that the sumoylation of AR was crucial for the repression of transactivation of the AR. Thus, PIAS1 and PIASxα regulate the AR-dependent transactivation, which, at least in part, can be attributed to their SUMO-E3 activity toward AR.
was shown to interact with the AR (26). Rat ARIP3 (androgen receptor interacting protein 3), a protein found to bind to AR (27), is an ortholog of human PIASxα. These PIAS proteins influence AR-dependent transcription. Furthermore, PIAS1 and ARIP3/PIASxα are highly expressed in the testis (26, 27). These findings raise the possibility that PIAS1 and ARIP3/PIASxα may function as SUMO-E3 ligases toward the AR.

Here we present evidence that both PIAS1 and PIASxα act as specific SUMO-E3 ligases for AR in intact cells as well as in vitro and that sumoylation of the AR represses AR-dependent transcriptional activation.

EXPERIMENTAL PROCEDURES

Plasmid Constructions and Expression of Recombinant Proteins—pEGFP-hAR (either the wild-type or K386R/K520R), pEGFP-PIAS1 (either wild-type or C350A), pEGFP-PIAS3, pEGFP-PIASxα (either wild-type or C362A), and pEGFP-PIASxβ were constructed by in-frame insertion of the cDNAs of these enzymes into the pEGFP-C1 vector (Clontech) with fusion to the green fluorescent protein (GFP) at the amino terminus of the enzyme. pFLAG-hARs (either wild-type or K386R/K520R) were constructed by inserting the cDNA into the pFLAG-CMV-2 vector (Sigma). Preparation of pFLAG-SUMO-1(IGG) and pFLAGx2-SUMO-1(IGG) was described previously (19). pFLAG-SUMO-1(ΔGG) was constructed by inserting SUMO-1 cDNA, which encodes from the first methionine to threonine 95, into pFLAG-CMV-2, hAR (either the wild-type one or K386R/K520R), PIAS1, and PIASxα cDNAs were amplified and cloned into the pBacGST vector, which was modified pFastBac vector (Invitrogen) having a glutathione S-transferase (GST) gene; and the recombinant proteins were expressed in SF9 cells by use of a baculovirus protein expression system and were purified with glutathione-Sepharose 4B (Amersham Biosciences). The substitution mutant K386R/K520R was amplified by PCR with specific mutation primers. pGV-ARE2-TATA-LUC was constructed by insertion of two copies of the AREs of the C3 (1) gene, generated by annealing 2 mutation primers. pGV-ARE2-TATA-LUC was constructed by insertion the two oligonucleotides 5′-GGGCTGATATGCACTGATTTGTTCGCTTAGCTAGTTTCA-3′ (sense) and 5′-GATCTGAGAACATCGTACAGTACGTCGAAGTCATGAGGTTCA-3′ (antisense) at the MluI-BglII site of the pGFP2 vector (Wako) and then by replacing the SV40 promoter sequence with the E1b TATA sequence generated by annealing the two oligonucleotides 5′-GATCCGGGGCTTAAAGGGTA-GATCTGAGAAC-3′ (antisense) and sense 5′-AGCCTCATATATTACCTTTCGTTGTGAAAGTGGGCCTGCTAGCCATGATACCGAGATCTGAGAACATCGTACAGTACGTCG-3′ (antisense) at the BglII-HindIII site.

Cell Culture and DNA Transfections—U2OS human osteosarcoma cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cells were transfected by using FuGENE 6 reagent (Roche) according to the manufacturer’s instructions. For immunoblotting, cells were plated in 35-mm diameter culture plates (~1 × 106 cells/plate) 24 h before transfection, and 4 h before transfection the cells received fresh medium with 10% charcoal-stripped FBS. At 8 h after transfection, the medium was changed to DMEM containing 2% charcoal-stripped FBS in the presence or absence of 100 nM testosterone. The cells were harvested 24 h after transfection. For reporter activity assays, cells were plated in 12-well plates (~3 × 105 cells/well) 24 h before transfection, and 4 h before transfection the cells received fresh medium with 10% charcoal-stripped FBS. Transfections were performed by using FuGENE 6 reagent. At 24 h after transfection, the medium was changed to DMEM containing charcoal-stripped FBS with or without 100 nM testosterone. The cells were harvested an additional 24 h later.

Immunoblotting and Immunoprecipitation—Cells were washed with ice-cold phosphate-buffered saline and directly lysed with SDS-sample buffer. For immunoprecipitation, the cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 2 mM dithiothreitol, 3 mM ATP, 100 ng of purified GST-hAR (either the wild-type one or K386R/K520R), 0.36 µg of purified E1 (GST-Sua1/His-Uba2), 20 ng or 500 ng of Ubc9, 500 ng of SUMO-1, and 100 ng of GST-PiASxα proteins. Ubc9 and SUMO-1 were expressed in Escherichia coli (BL21, LysS) by using a pET vector. E1, hAR, and PIAS proteins were expressed in SF9 cells by use of the baculovirus protein expression system (Invitrogen). Purification of enzymes was performed as described previously (14). After incubation for 30 min in the presence or absence of 1 µM testosterone, the reaction mixture was subjected to SDS-PAGE and immunoblotting with a monoclonal anti-hAR antibody (Santa Cruz Biotechnology, Inc.).

RESULTS

PIAS1 and PIASxα Specifically Enhanced the Sumoylation of AR in Intact Cells—The hAR has been reported to interact with PIAS1 and PIASxα and to be modified by SUMO-1. Recently, we and other groups showed that PIAS family proteins have SUMO-E3-like activity. Thus, the interaction between the AR and PIAS proteins raises the possibility that PIAS proteins might function as SUMO-E3 ligases for this receptor. Although PIAS proteins were shown to act as a co-regulator (26–29), direct regulation of the sumoylation of the AR by PIAS proteins has not been reported. Therefore, we investigated whether the sumoylation of AR could be enhanced by PIAS proteins in intact cells. U2OS cells were made to co-express GFP-hAR, FLAGx2-tagged SUMO-1, and GFP-PIAS proteins in the absence or presence of testosterone. Then the sumoylation of AR was checked by immunoblotting or by immunoprecipitation of the cell lysate with an anti-AR antibody and incubating the blotted proteins with anti-AR or anti-FLAG antibody. Using cell extracts for immunoblotting with the anti-hAR, we detected GFP-hAR protein bands at 130 kDa, whereas the endogenous hAR was hardly detected in U2OS cells (data not shown). In the case of no expression of PIAS proteins, no bands representing modified AR were detected in the presence or absence of testosterone (Fig. 1A, lanes 1 and 2). In contrast, when PIAS1 or PIASxα was co-expressed, some additional slow-migrating bands representing the sumoylated AR appeared in the presence of testosterone. Only one faint slow-migrating
neither PIAS3 nor PIASx
absence of 100 nM testosterone. Twenty-four hours post-transfection the encoding GFP-hAR (wild-type or K386R/K520R mutant), FLAGx2-intact cells.

U2OS cells were co-transfected with expression plasmids in a manner (Fig. 1A, lanes 4 and 8), whereas the expression of neither PIAS3 nor PIASxβ enhanced the sumoylation of the receptor (Fig. 1A, lanes 5, 6, 9, and 10). Consistent with previously published results (2), the hAR mainly was sumoylated at two sites. However, we also noted other less intense bands. Next, to confirm that these AR antibody-reactive, slower-migrating bands were indeed sumoylated hARs, we transfected U2OS cells with the same combination of plasmids as in the previous experiment. Immunoprecipitation of the lysates prepared from the transfected cells was performed with anti-AR antibody, and then immunoblotting was conducted with anti-FLAG antibody or anti-AR antibody (Fig. 1, B and C, respectively). When anti-FLAG antibody was used for the detection, FLAGx2-SUMO-1-conjugated GFP-hAR bands were detected at the same place on the gel as in Fig. 1A. Therefore, we considered these bands to represent sumoylated hARs. In this experiment, additional bands of hAR that had shifted to a higher molecular weight than the former band, representing hAR sumoylated at two sites, were also detected. Overexpressed PIAS1 or PIASxα may induce sumoylation at other sites or polysumoylation, analogous to polyubiquitination. When cells expressed K386R/K520R, the hAR with 2 mutated sumoylation sites, no sumoylated bands were detected in the absence or presence of testosterone (Fig. 1C, lanes 7–10). These data thus indicate that the slower-migrating bands were sumoylated hARs. Under the condition of no ectopical expression of SUMO-1, the sumoylation of the hAR was also enhanced in the presence of testosterone, although the enhancement was weaker than that in the presence of FLAGx2-SUMO-1 (Fig. 1B, lanes 8 and 10). Furthermore, when cells expressed the PIAS1 mutant (C350A) or PIASxα mutant (C362A), the sumoylation of hAR was only a little enhanced in the presence of C350A or C362A compared with that in the absence of PIAS proteins. The enhancement was very much smaller than that in the presence of wild-type PIAS1 or PIASxα (Fig. 2A). This result is much the same as that found previously with p53 used as the substrate for sumoylation. Thus, mutations that interfere with Ubc9 prevent the enhancement of the sumoylation (described below). Taken together, the above data obtained from the transfection assay system clearly indicate that PIAS1 and PIASxα enhanced the sumoylation of hAR in intact cells in a ligand-dependent and RING finger-like domain-dependent manner. PIASxα and PIASxβ are thought to be alternatively spliced forms from the same gene because their amino acids 1–550 are identical but their C termini are different from each other. The C-terminal deletion mutant PIASxα (1–550) could not enhance the sumoylation of hAR (Fig. 2B), indicating that the C-terminal domain (amino acids 551–572) of PIASxα is essential for its SUMO-E3 ligase activity toward hAR.

PIAS1 and PIASxα Act as SUMO-E3 Ligases Toward AR in Vitro—Next, we conducted in vitro sumoylation by using purified recombinant hAR proteins as the substrate (Fig. 3). To confirm that PIAS1 and PIASxα indeed have SUMO-E3 ligase activity toward the hAR, we prepared and purified the recombinant proteins and assayed their activities. GST-tagged PIAS1, PIASxα, and hAR (either the wild-type or the K386R/K520R mutant) were expressed in SF-9 cells, purified by use of glutathione-Sepharose 4B, and then used for the sumoylation assay in the presence of purified recombinant E1 (SuA1 and hUba2 heterodimer) and E2 (hUbc9). In the absence of PIAS proteins, no sumoylated hAR bands were detected. When a 25× greater amount of hUbc9 than normal was used, only one sumoylated band was detected slightly in this assay system (Fig. 3, lane 2). In contrast, the addition of PIAS1 or PIASxα to the assay system enhanced the sumoylation of the hAR. Interestingly, these reactions were independent of the addition of testosterone, indicating that the reaction does not require a conformational change in the receptor by ligand binding in vitro. When reactions were performed in the absence of hUbc9, no enhancement of the sumoylation was observed (Fig. 3, lanes 5 and 8). The K386R/K520R mutant AR was not sumoylated in the presence of PIAS1 or PIASxα. Thus, PIAS1 and PIASxα
seemed to enhance the sumoylation at the same sites both in vivo and in vitro.

These data described above strongly suggest that PIAS1 and PIASxα function as SUMO-E3 ligases toward the hAR.

Wild-type PIAS1 and PIASxα, but Not Their RING Finger-like Domain Mutants, Interact with Ubc9 in the Yeast Two-hybrid Assay—By use of the yeast two-hybrid assay, we previously showed that PIAS1 bound to hUbc9 but that when a cysteine residue in the RING finger-like domain was mutated to alanine, the mutant could not bind to hUbc9. Therefore, we also examined the interaction between the wild-type or RING finger-like domain mutant of PIASxα with hUbc9 by using the same assay. As shown in Fig. 4, wild-type PIASxα interacted with hUbc9. In contrast, the C362A mutant could not bind to hUbc9.

These data suggest that the interaction between PIAS proteins and Ubc9 through the RING finger-like domain of these proteins was necessary for the SUMO-E3 ligase activity of PIAS.

PIAS1 and PIASxα Specifically Repress the AR-dependent Transactivation in the Presence of Ectopically Expressed SUMO-1—PIAS family proteins have been shown to influence AR-dependent transcription. It has been suggested that the sumoylation negatively regulates AR activity because the mutation of sumoylation sites increases the transactivation ability of AR. To investigate the influence of the sumoylation mediated by PIAS1 or PIASxα on AR-dependent transactivation, we performed transient transfection assays using 293T cells transfected with a luciferase reporter having AREs in the promoter. As shown in Fig. 5A, in the absence of PIAS proteins the wild-type hAR increased the reporter gene expression level by 10-fold upon addition of testosterone. When PIAS1 was co-expressed with the wild-type hAR, this ligand-dependent activation was slightly increased. Co-expression of PIASxα with wild-type hAR further enhanced the luciferase activity. In contrast, co-expression of the wild-type hAR with PIAS1 or PIASxα and SUMO-1(GG) strongly reduced the ligand-dependent activation. However, co-expression of SUMO-1(GG), which could not be conjugated to target proteins, had no effect on the ligand-dependent hAR activation in the presence of PIAS1 or PIASxα. PIAS3 or PIASxβ, which had shown no SUMO-E3 ligase activity toward hAR, strongly enhanced the transactivation activity in a dose-dependent manner. Interestingly, co-expression of SUMO-1(GG) did not reduce the activation by PIAS3 or PIASxβ (Fig. 5A, right panel). Ectopic expression of SUMO-1 enhanced the sumoylation of hAR by PIAS1 and PIASxα in U2OS cells (Fig. 1B). These data suggest that the sumoylation of hAR may repress the ligand-dependent hAR activity.

Sumoylation Sites of hAR Are Required for the Repressive Effect on Ligand-dependent Activation—To further investigate the role of sumoylation of the AR, we used the K386R/K520R mutant, lacking the two sumoylation sites of hAR, in the same reporter assay. As shown in Fig. 5B, in the absence of PIAS proteins, the K386R/K520R mutant hAR increased the reporter gene expression level 2-fold over that of the wild-type hAR. This result is consistent with a previously published finding (2). At the amounts indicated in Fig. 5, PIAS1 or PIASxα alone did not influence this ligand-dependent transactivation. In contrast to the results obtained with the wild-type hAR, however, co-expression of PIAS1 or PIASxα with SUMO-1(GG) still repressed the transactivation of the K386R/K520R mutant hAR. The repression level of the transactivation of K386R/K520R, however, was weaker than that of the wild-type hAR. These results suggest that sumoylation is required for the full repression of the AR-mediated transactivation.

RING Finger-like Domain Is Required for the Repression of the AR-dependent Transactivation—Next, we examined whether the RING finger-like domain mutant of PIAS1 or PIASxα, which did not enhance the sumoylation of hAR in intact cells, could repress the ligand-dependent hAR activation. As shown in Fig. 5C, co-expression of the wild-type hAR with the PIAS1 C350A or PIASxα C362A mutant could also enhance the ligand-dependent transactivation. Interestingly, PIAS1 C350A decreased this enhancement in a dose-dependent manner. Co-expression of the wild-type hAR with the PIAS1 C350A or PIASxα C362A mutant and FLAG-SUMO-1(GG) had no effect on the ligand-dependent activation. These data suggest that PIAS1 and PIASxα repress AR-dependent transactivation in a manner dependent on their RING finger-like domains, which are required for their SUMO-E3 ligase activity. When we performed the same reporter gene assays as above by using COS-7 cells, essentially similar results were obtained (data not shown).

Taken together, these results strongly suggest that PIAS1 and PIASxα repressed the AR-dependent transactivation.
through the sumoylation of AR by their SUMO-E3 ligase activity.

**DISCUSSION**

Recently, we and other groups identified PIAS family proteins as SUMO-E3 ligases toward p53 or LEF1. These findings suggested that PIAS family proteins might act as SUMO-E3 enzymes for other substrates as well. The AR has been shown to be sumoylated and to interact with PIAS1 and ARIP3/PIASxα. Therefore, in the present study we sought to obtain evidence that PIAS1 and PIASxα have SUMO-E3 ligase activity toward the hAR. In this study PIAS1 and PIASxα, but not PIAS3 and PIASxβ, could enhance the sumoylation of hAR. PIAS3 and PIASxβ were shown to interact with the AR (28, 29), but they could not enhance the sumoylation of hAR. The protein interaction with PIAS proteins seems to be necessary for, but not sufficient to enhance, the sumoylation by PIAS proteins. A recent report showed that PIAS1 and PIASxα also acted as SUMO-E3 ligases for p53 and c-Jun (30) but not for other substrates such as Sp100. Taken together, these observations suggest that PIAS family proteins have substrate specificity. Because the PIAS family consists of a small number of family members, one of them may catalyze different substrates, but also multiple PIAS proteins may react with the same substrate. However, we cannot rule out the possible existence of yet undiscovered types of SUMO-E3 ligase.

In the case of the addition of Ubc9, 20 ng of protein was used except for lane 2, which contained 500 ng of the protein. The reaction mixture was subjected to SDS-PAGE and immunoblotted with monoclonal anti-hAR antibody.

**Fig. 3.** PIAS1 and PIASxα enhance the sumoylation of hAR in vitro. GST-hAR (either the wild-type or K386R/K520R mutant) and GST-PIAS proteins expressed in Sf-9 cells and purified by use of glutathione-Sepharose 4B resin were subjected to the in vitro sumoylation assay described under “Experimental Procedures.” In the case of the addition of Ubc9, 20 ng of protein was used except for lane 2, which contained 500 ng of the protein.

**Fig. 4.** Interaction of PIAS1 or PIASxα with Ubc9 in yeast two-hybrid assay. Wild-type PIAS1 or PIASxα and their RING finger-like domain mutants (C350A and C362A, respectively) were cloned into pGAD424 as GAL4 activation domain fusion constructs. The two-hybrid assay was performed with Y190 cells transformed with a pGAD424-PIAS1 (wild-type or C350A) or pGAD424-PIASxα (wild-type or C362A) and a bait plasmid expressing the GAL4 DNA-binding domain fused to the indicated protein. All the transformants were plated on selective medium lacking tryptophan (Trp+), leucine (Leu+), and histidine (His+), but containing 25 mM 3-amino-1,2,4-triazole (3AT+), to test cell growth.

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2 T. Nishida and H. Yasuda, unpublished data.
was required to repress the hAR-dependent transactivation because a RING finger-like domain mutant of PIAS1 or PIASx could not repress the ligand-dependent hAR activation in the presence of ectopically expressed SUMO-1. This domain in other proteins of the PIAS family also seems to be important for their biological activities. PIASy targets LEF1 to nuclear bodies and represses the transcriptional activation of LEF1, and this repression requires the RING finger-like domain of PIASy. Because the RING finger-like domain mutant failed to target both itself and LEF1 to nuclear bodies, this subnuclear sequestration by PIASy correlated with the repression of LEF1 (23). Similarly, RING finger-like domain mutant PIASxβ slightly induced the transactivation of p53, although wild-type PIASxβ repressed the transcriptional activity of p53 (30). In contrast, another group reported that PIAS1 stimulated p53-dependent transcriptional activation and that a PIAS1 mutant lacking the RING finger-like domain also activated p53-mediated gene expression as efficiently as did the wild-type (34). These findings suggest that the RING finger-like domain of PIAS proteins may not only play a role in the sumoylation of substrates but also in another function, such as protein-protein or protein-DNA interaction. For instance, Ubc9 interacts with the AR and activates AR-dependent transcription independently of its SUMO-E2 enzyme activity (35). The RING finger-like domain of PIAS proteins may recruit Ubc9 to the AR and/or stimulate the co-regulatory activity of Ubc9 toward the receptor.

The effects of PIAS proteins on AR-dependent transcription activity are yet obscure because they seem to depend on the cell type, the reporter gene promoter context, and expression levels of PIAS protein in a cell. For instance, when the minimal ARE2 TATA promoter or probasin promoter were used as the reporter, PIAS3 and ARIP3/PIASx decreased AR-dependent transcription activity in a dose-dependent manner in HeLa, CV-1, and COS-1 cells; whereas in HepG2 cells, all of the PIASs increased AR-dependent transcription in a dose-dependent manner (28, 36). It has been proposed that PIAS1 and PIASxβ possess an inherent transcription-activating function but that ARIP3/PIASxα and PIAS3 are devoid of this activity (28). In prostate cancer cells, however, PIAS1 and PIAS3 enhanced the transcriptional activity of AR, but PIASy acted as a potent inhibitor of AR (37). These different effects of PIAS proteins may be caused by different expression levels of other cellular factors binding to the AR and/or PIAS proteins involved in AR-dependent transcriptional regulation. For example, PIAS1 interacts with other members of the PIAS family in a cell, for co-expression of PIAS1 with other members of the PIAS proteins influenced the effect of PIAS1 on AR-dependent transcription (38).
Our present data provide evidence that PIAS1 and PIASxα act as SUMO-E3 ligases for the AR and repress AR-dependent transactivation, at least in part, through the sumoylation of the AR by their SUMO-E3 ligase activity. However, the mechanisms by which several PIAS proteins regulate AR-dependent transactivation are not yet clear. Some PIAS proteins may sequester ARs from the transcriptional complex and target them to the nuclear matrix, as in the case of LEF1 by PIASy. In our preliminary immunofluorescence microscopy experiments, although hARs were not co-localized to the nuclear matrix with them to the nuclear matrix, as in the case of LEF1 by PIASy. In sequester ARs from the transcriptional complex and target transactivation, at least in part, through the sumoylation of the act as SUMO-E3 ligases for the AR and repress AR-dependent regulators, its DNA binding ability, and its stability. Further important to know what degree of sumoylation is needed to reflect the true physiological subcellular localization. It is also the results from overexpression studies sometimes do not reflect the true physiological subcellular localization. It is also the true physiological subcellular localization. It is also important to know what degree of sumoylation is needed to alter the properties of the AR. Sumoylation of the AR may alter the association of this receptor with other transcriptional co-regulators, its DNA binding ability, and its stability. Further studies will be needed to address these issues.

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Note Added in Proof—During the completion of this study, Kotaja et al. also reported similar findings (39).

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