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PATZ Attenuates the RNF4-mediated Enhancement of Androgen Receptor-dependent Transcription*

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PATZ is a transcriptional repressor affecting the basal activity of different promoters, whereas RNF4 is a transcriptional activator. The association of PATZ with RNF4 switches the activation to repression of selected basal promoters. Because RNF4 interacts also with the androgen receptor (AR) functioning as a coactivator and, in turn, RNF4 associates with PATZ, we investigated whether PATZ functions as an AR coregulator. We demonstrate that PATZ does not influence directly the AR response but acts as an AR corepressor in the presence of RNF4. Such repression is not dependent on histone deacetylases. A mutant RNF4 that does not bind PATZ but enhances AR-dependent transcription is not influenced by PATZ, demonstrating that the repression by PATZ occurs only upon binding to RNF4. We also demonstrate that RNF4, AR, and PATZ belong to the same complex *in vivo* also in the presence of androgen, suggesting that repression is not mediated by the displacement of RNF4 from AR. Finally, we show that the repression of endogenous PATZ expression by antisense expression plasmids in LNCaP cells results in a stronger androgen response. Our findings demonstrate that PATZ is a novel AR coregulator that acts by modulating the effect of a coactivator. This could represent a novel and more general mechanism to finely tune the androgen response.

Androgens are critical in development, maintenance of the male reproductive system, and for growth of normal prostate and prostate cancer (1, 2). The effects of androgens are mediated through the androgen receptor (AR),[1] a member of a large family of ligand-dependent transcriptional factors that belongs to the steroid receptor superfamily (3, 4).

AR can interact directly with basal transcription factors, such as TBP (5), TFIIB (6), and TFIIF (7). The activation of target gene expression by nuclear receptors is not just the result of ligand availability, but it is also tightly modulated by several coregulatory proteins, which function in many cases as signaling intermediates between receptors and general transcription or chromatin-modeling machineries. Coregulators interact with different regions of AR and can act as coactivators (*e.g.* SRC-1, CBP, ARA-70, ARA-54, SNURF/RNF4, ANPK, ARIP-3, ARIP-4, E6-AP) or corepressors (*e.g.* nuclear factor-κB, SMAD3, D-type cyclins) (8–13). However, the physiological significance of coregulators and their activating or repressing functions on AR-dependent transcription are still under investigation.

One of the most recently described AR coactivators is *RNF4*/*SNURF* (14). *RNF4* codes for a 21-kDa protein containing a RING finger motif (14, 15) and associates with the DNA binding domain of AR to enhance transcriptional response to androgens (14). RNF4 also functions as a coactivator of SP1-regulated promoters (16). RNF4, in turn, associates with a variety of transcription factors (HMGI-Y, PATZ, gscl, SPBP) (17–19) probably by virtue of the RING finger, which is a domain specialized in the formation of protein complexes. Thus, RNF4 could function as an adapter protein to form protein complexes associated to the androgen receptor.

We have recently shown that RNF4 colocalizes in nuclear speckles and can form a complex with PATZ, a novel POZ-AT hook-zinc finger protein (17). PATZ is a transcriptional repressor that acts in a selective manner on different promoters, and RNF4, a transcriptional activator, acts as a corepressor of basal transcription in association with PATZ (17). However, the effects of PATZ on AR-dependent transcription have not been investigated.

In this work, we considered the possibility that PATZ might function as an AR coregulator in a direct or RNF4-mediated manner. We show that PATZ has no direct effects on the androgen-dependent transcription, whereas in the presence of RNF4, PATZ can significantly repress the RNF4-mediated activation of AR-dependent transcription. We further demonstrate that the PATZ-repressing potential on RNF4 activation is not compromised by the specific histone deacetylases (HDACs) inhibitor trichostatin A (TSA), suggesting that the transcriptional repression by PATZ is achieved by mechanisms that are different than the recruitment of HDACs.

EXPERIMENTAL PROCEDURES

Materials—5α-Dihydrotestosterone (DHT) was obtained from Sigma, and TSA was obtained from Biomol.

Plasmids—pCMVβ was purchased from CLONTECH. The cDNA-encoding human AR was obtained from pSG5-AR (kindly provided by Dr. A. Migliaccio) and subcloned in pcDNA3.1 (Invitrogen) under the control of the CMV promoter (pCMV-hAR). The reporter gene construct, $pARE_2$-TATA-LUC, regulated by two AREs in front of E1b TATA sequence, was kindly provided by Dr. Palvimo (University of Helsinki, Helsinki, Finland). pcDNA-RNF4, pcDNA-PATZ, pcDNA-PATZ-ΔPOZ, and pHA-RNF4 expression vectors have been described previously (17).

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[1] The abbreviations used are: AR, androgen receptor; HDAC, histone deacetylases; TSA, trichostatin A; DHT, dihydrotestosterone; hAR, human androgen receptor; LUC, luciferase; HA, hemagglutinin; FBS, fetal bovine serum; PSA, prostate-specific antigen.

To construct the PATZ antisense expression vector, pcDNA-PATZ-AS, a PCR-generated full-length PATZ cDNA was inserted into the *Bam*HI-*Eco*RI sites of pCDNA3.1 expression vector (Invitrogen). The primers used were P3 BAM (5′-ACGTGGATCCGCCAA CAGGCCACTGGGT-3′) and A23 K (5′-ACGTGAATTGCGGCCATGGAGCGG-3′). pMyc-PATZ was made by cloning a PCR-generated full-length PATZ fragment into the *Eco*RI and *Hin*dIII sites of pcDNA3.1(−)/Myc-histone A (Invitrogen). The primers used were A23 K and A23 HINDIII (5′-ACGTAAGCTTGGC-CAACAGGCCACTGGGT-3′). To construct the pHA-RNF4-CS and pcDNA-RNF4-CS, the plasmid pEGFP-RNF4-CS (20) was digested with *Eco*RI, and the mutated RNF4 fragment was subcloned into the *Eco*RI sites of pCEFL-HA (17) and of pcDNA.3.1 (Invitrogen). pMyc-MBD2 was generated by cloning a PCR-generated full-length MBD2 fragment into the *Eco*RI and *Bam*HI sites of pcDNA3.1(−)/Myc-histone A (Invitrogen). The primers used were DM4 (5′-AGTCGAATTCAGCA-CAATGGATTGCCC GGCCCTCCCC-3′) and DM5 (5′-AGTCGGATCC-GGCTTCATCTCCACTGTCCAT-3′).

Cell Culture and Transfections—All mammalian cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). CV-1, HeLa, and 293T cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS), 50 μg/ml streptomycin, and 50 units/ml of penicillin. CV-1 and HeLa cells transfections were carried out by calcium phosphate precipitation using Calphos (CLONTECH). The cells (3.5×10^5) were plated on 60-mm dishes 24 h before the indicated amount of expression and reporter vectors was added. The β-galactosidase expression plasmid, pCMVβ (1 μg/60-mm plate), was used as an internal control for transfection efficiency. At 18 h after transfections, the medium was changed to one containing charcoal-stripped 2% (v/v) FBS (Sigma) in the presence or absence of DHT as depicted in the figure legends. Protein concentration was determined by using Bio-Rad protein assay reagents. Luciferase and β-galactosidase activities were assayed as described previously (17, 21). For the dose-response curves of TSA, the CV-1 and HeLa cells were treated for 24 h with 10 nM DHT with or without the pretreatment of cells with various amounts of TSA as indicated in the figure legends. Cells were then washed twice with PBS, resuspended in 0.1 M Tris-HCl, pH 7.8, and lysed by three freeze-thaw cycles, and the transcriptional activity was measured by luciferase assay.

The LNCaP androgen-sensitive human prostate adenocarcinoma cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS, penicillin (50 units/ml), and streptomycin (50 μg/ml). FBS was replaced with charcoal-stripped fetal bovine serum (Sigma) to remove any steroid contaminant. For transient transfection, LNCaP cells were grown in phenol red-free RPMI 1640 media with 5% charcoal-stripped fetal bovine serum 24 h before experiments. After depletion of steroids, cells were seeded in 100-mm dishes and transiently transfected with 5 μg of both the sense (pcDNA-PATZ) and antisense (pcDNA-PATZ-AS) PATZ expression plasmids. Cells were transfected by LipofectAMINE Plus reagents (Invitrogen) according to the instructions by the manufacturer. Control cultures were transfected with the same vector lacking the DNA-coding sequence for PATZ. LNCaP cells, grown at 70–75% confluence, were deprived of serum and incubated at 37 C°. At 4 h after transfections, charcoal-stripped 5% (v/v) FBS was added to the cultures, and cells were maintained for 48 h in fresh steroid-reduced medium in the presence or absence of 10 nM DHT. As internal control for transfection, efficiency cells were cotransfected with 2 μg of green fluorescent protein expression vector, and the number of green fluorescent protein expressing and nonexpressing cells was determined. Cells were harvested for the preparation of total RNA.

Immunoprecipitation and Immunoblotting—CV-1 and 293T cell extracts were prepared in Nonidet P-40 lysis buffer (1% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 150 mM NaCl) with proteinase inhibitors. Immunoprecipitations were performed as described previously (17). The samples were immunoprecipitated with anti-AR or anti-Myc (Santa Cruz, CA) or with anti-HA (CA125, Roche Molecular Biochemicals) antibodies and resolved on 10% SDS-PAGE. The Western blot analysis was performed by using an anti-HA, anti-Myc, and AR antibody, 1:500 dilution in Tween-Tris-buffered saline (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20), and the antigenic proteins were detected by ECL kit (Amersham Biosciences, Inc.).

Northern Blot Analysis and Preparation of the Probes—Total RNA was extracted from LNCaP cells by using Trizol (Invitrogen) according to directions by the manufacturer. 15 μg each of total RNA sample was electrophoresed in a 1.2% agarose gel containing formaldehyde as a denaturing agent and then blotted to Hybond-N^+ nylon membrane (Amersham Biosciences, Inc.). cDNA probe for prostate-specific antigen (PSA) was labeled with [α-^{32}P]dCTP using the random primers DNA-labeling kit (Invitrogen). PSA cDNA probe (214 bp) was a RT-PCR product. The sequences of primers for a specific region of PSA cDNA were 5′-GAGGTCCACACACTGAAGTT-3′ and 5′-CCTCCTGAAGAA-TCGATTCCT-3′ (22). The hybridization was performed according to protocols CLONTECH with ExpressHyb hybridization solution. The film was autoradiographed at −70 °C.

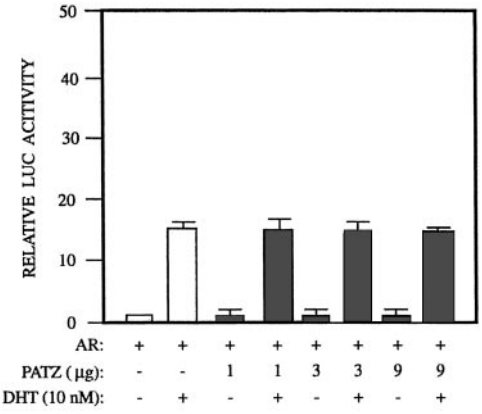

FIG. 1. **Influence of PATZ overexpression on AR-dependent transactivation.** CV-1 cells were transfected with 5 μg of pARE$_2$-TATA-LUC reporter plasmid along with 1 μg of pCMV-human AR or empty pCMV and the indicated amounts of PATZ expression vector (pcDNA-PATZ) or empty expression vector (pcDNA-3.1) in the presence or absence of 10 nM DHT as depicted. β-Galactosidase expression plasmid, pCMVβ (1 μg), was used as control for transfection efficiency. Reporter gene activities are expressed relative to that achieved with pCMV-hAR in the presence of DHT, and the means ± S.E. values of at least six experiments are given.

RESULTS

Effects of PATZ Expression on Androgen-dependent Transcription—Because RNF4 interacts with AR functioning as a coactivator and, in turn, RNF4 associates with PATZ, we tested whether PATZ had direct effects on AR-dependent transcription. For this purpose, we performed transient transfection experiments. CV-1 cells were transfected in different combinations with expression vectors for RNF4, AR, and PATZ along with the androgen-regulated pARE$_2$-TATA-LUC promoter cloned upstream to the luciferase reporter gene. In these cells in the absence of cotransfected AR, DHT did not influence reporter gene activity in the presence or absence of PATZ (data not shown). As shown in Fig. 1, AR activated the reporter gene approximately 10-fold in the presence of 10 nM DHT. Cotransfection of an increasing amount of PATZ expression plasmid did not alter either the basal activity of the pARE$_2$-TATA-LUC promoter or the transcriptional response to DHT. Western blot analysis of whole cell extracts showed that coexpressed PATZ did not alter AR concentration (data not shown). We conclude that PATZ has no influence on AR-dependent transcription. Next, because RNF4 associates with both AR and PATZ, we tested the possibility that the PATZ repressor protein could be recruited on an AR-responsive promoter when RNF4 is present. We transfected both expression vectors for PATZ and RNF4 along with AR and AR-responsive promoter. As shown in Fig. 2, coexpressed RNF4 further increased the AR-dependent transactivation. This result confirmed the data reported by Moilanen *et al.* (14) showing that the rat homologue of RNF4 (SNURF) had similar effects on AR-dependent transcription. Coexpression of increasing concentration of PATZ not only abolished the RNF4-mediated enhancement of AR-dependent transactivation but also led to a progressive reduction of AR transcriptional response to androgen. The above finding could not be explained by a decreased cellular concentration of either AR or RNF4, because immunoblotting analysis of cell extracts showed that coexpressed PATZ did not alter their concentration (Fig. 2). Similar results were obtained when another cell

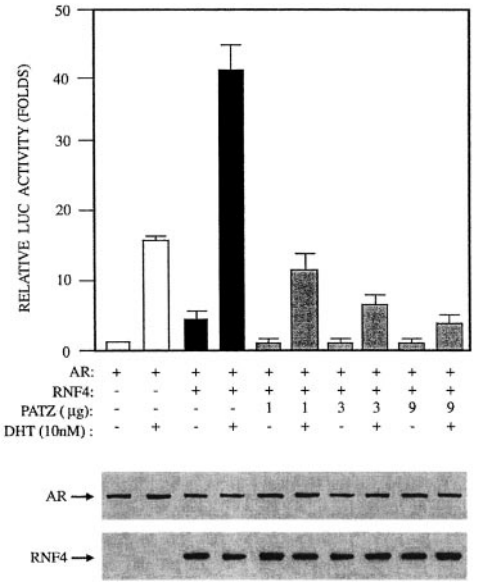

FIG. 2. **Simultaneous coexpression of PATZ and RNF4 represses the RNF4-mediated androgen activation.** CV-1 cells were cotransfected with 1 μg of pCMV-hAR, 5 μg of pARE$_2$-TATA-LUC reporter, 1 μg of pCMVβ, and 3 μg of pcDNA-RNF4 alone or together with the indicated amounts of pcDNA-PATZ in the absence or presence of DHT as depicted. LUC activity is expressed relative to the transactivation observed in the absence of DHT and without RNF4 and normalized by using β-galactosidase activity. The means $\pm$ S.E. values of least five experiments are shown. Immunoblot analysis of AR and RNF4 is shown at the *bottom*. 20 μg each of total cell extracts was resolved on 10% polyacrylamide gel under denaturing condition and subjected to immunoblotting with polyclonal AR and RNF4 (20) antibodies.

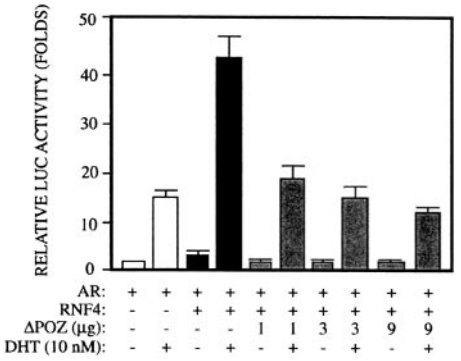

FIG. 3. **Effect of PATZ-Δ-POZ and RNF4 on the transcriptional activity of AR.** CV-1 cells were cotransfected with 1 μg of pCMV-hAR, 5 μg of pARE$_2$-TATA-LUC reporter, 1 μg of pCMVβ, and 3 μg of pcDNA-RNF4 alone or together with increasing amounts of pcDNA-PATZ-Δ-POZ in the absence or presence of DHT as depicted. LUC activity is expressed relative to the transactivation observed in the absence of DHT and without RNF4. The means $\pm$ S.E. values of at least five experiments are shown. Transcriptional activities are expressed as relative LUC activity normalized by using β-galactosidase activity.

line (HeLa) was employed (data not shown).

These findings suggest that PATZ can function as an AR corepressor only in the presence of RNF4 protein that possibly behaves as an adapter protein, allowing PATZ repressor protein to complex with AR.

Repression of AR Transactivation by PATZ Does Not Depend on POZ Domain or on Histone Deacetylation—To explore the mechanisms underlying the PATZ repression potential on AR-dependent transcription, we tested whether the POZ domain of PATZ is essential for such activity. In fact, it has been reported that the POZ domain can bind and recruit the HDACs on target promoters mediating the repression potential of several POZ containing proteins (23, 24). We used an expression vector (pcDNA-PATZ-ΔPOZ) (17) containing the PATZ-coding sequence lacking the first 145 amino acids constituting a region of high homology with other POZ domains. pcDNA-PATZ-ΔPOZ was cotransfected in place of the complete PATZ in CV-1 cells in a series of transcription assays. As expected, pcDNA-PATZ-ΔPOZ did not influence AR-dependent transcription in the absence of RNF4 (data not shown). Conversely, although at a lesser extent, pcDNA-PATZ-ΔPOZ was still able to repress AR-dependent transcription in the presence of RNF4 (Fig. 3).

These data indicated that the POZ domain is not essential for the repression of androgen-dependent transcription. However, a repression mechanism involving histone deacetylation could not be ruled out from these experiments. We then used a specific inhibitor of HDACs, TSA, in a series of transfection assays. Fig. 4 shows that an increasing amount of TSA (5–900 ng/ml) did not influence the transcriptional response to androgen in the presence of PATZ and RNF4. Our data seem to exclude the involvement of HDACs in the PATZ repression of androgen-mediated transactivation. Very interestingly, we observed that the PATZ repression potential on basal transcription was strongly affected by both the deletion of the POZ domain (17) and by TSA.[2] Thus, it is very likely that the repressive activity of PATZ on basal or on androgen-dependent transcription occurs through different mechanisms.

Effects of Mutations Affecting the PATZ/RNF4 Association on PATZ Repression Potential—To confirm that the association of PATZ with RNF4 was critical for the repression of AR transactivation activity, we needed to find a mutant RNF4 that retained the ability to enhance transcriptional response to androgens but was not able to interact with PATZ. The integrity of the RING finger domain of RNF4 was probably essential for PATZ association (17), whereas mutations destroying the RING structure were described to affect only partially the ability of RNF4 to enhance AR-dependent transactivation (14). Thus, an expression vector for RNF4 bearing two-point mutations converting two cysteines of the RING finger domain in serines (RNF4/CS) was transfected along with PATZ and AR in 293T cells for immunoprecipitation and in CV-1 cells for transcription assays. First, we found that RNF4/CS failed to coimmunoprecipitate with PATZ (Fig. 5*A*), confirming that the integrity of the RING structure was essential for such association. We then found that RNF4/CS retained the ability to enhance AR-dependent transcription, confirming the observations by Moilanen *et al*. (14), but we also found that such activity was not affected by transfection of increasing concentrations of PATZ protein (Fig. 5*B*). Western blot analysis confirmed that in our assays, the stability and the levels of expression of the respective proteins were comparable (Fig. 5, and data not shown).

These findings demonstrate that PATZ repression potential on AR-dependent transcription is strictly dependent on the association with RNF4.

PATZ, RNF4 and AR Belong to the Same Protein Complex—Two main mechanisms for AR repression by PATZ could be invoked. PATZ could either destroy AR/RNF4 association or associate with AR/RNF4, rendering the complex not functional. To answer this question, we performed a series of coimmunoprecipitation experiments. After transfection of PATZ, RNF4, and AR expression vectors, each protein was independently immunoprecipitated, and Western blot analysis was performed with antibodies against the other two. In all cases, we found that PATZ, RNF4, and AR belong to the same complex *in vivo* both in the presence and in the absence of androgen (Fig. 6). When PATZ was substituted with another Myc-tagged nuclear

[2] R. Pero, unpublished results.

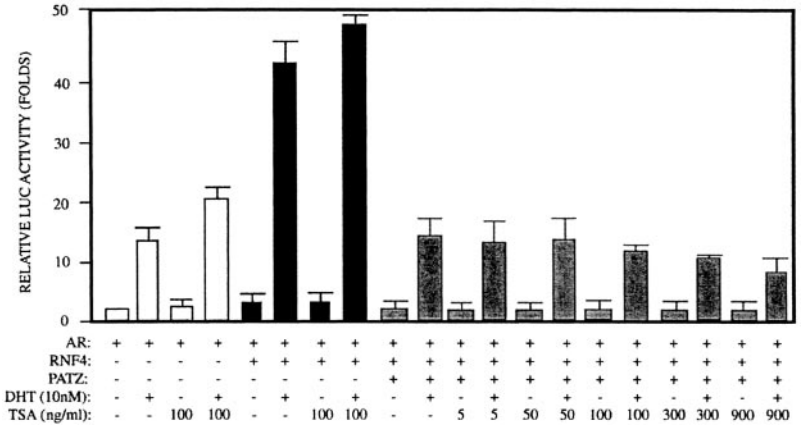

FIG. 4. **Deacetylase activity is not required for repression by PATZ.** CV-1 cells were cotransfected with 1 μg of pCMV-hAR, 5 μg of pARE$_2$-TATA-LUC reporter, 1 μg of pCMVβ, and 3 μg of pcDNA-RNF4 alone or together with increasing amounts of pcDNA-PATZ in the absence or presence of DHT as depicted. In particular, the CV-1 cells were treated for 24 h with 10 nM DHT with or without pretreatment for 18 h with various concentrations of TSA. LUC activity is expressed relative to the transactivation observed in the absence of DHT and without RNF4 and normalized by using β-galactosidase activity. The means ± S.E. values of least five experiments are shown.

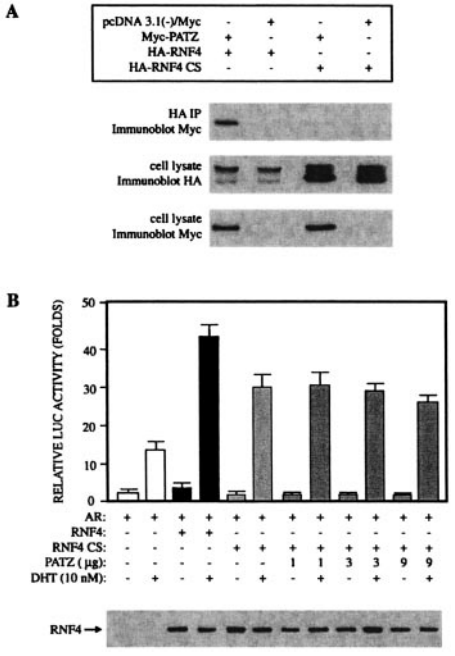

FIG. 5. **Comparison of RNF4 and RNF4-CS ability to associate with PATZ and the influence of PATZ/RNF4-CS on the AR-dependent transactivation.** *A,* whole cell extracts from 293T cells cotransfected with vectors encoding Myc-PATZ or empty plasmid together with vectors encoding HA-RNF4 or HA-RNF4-CS were immunoprecipitated by anti-HA antibody. The protein complexes were resolved on 10% polyacrylamide gel under denaturing condition and transferred to ECL nitrocellulose filters, and the coimmunoprecipitated PATZ protein was detected by using anti-Myc antibody. The same extracts were subjected to immunoblotting with anti-HA antibody and with anti-Myc antibody. *B,* CV-1 cells were cotransfected with 1 μg of pCMV-hAR, 5 μg of pARE$_2$-TATA-LUC reporter, 1 μg of pCMVβ, and 3 μg of pcDNA-RNF4 alone or 3 μg pcDNA-RNF4-CS alone or together with the indicated amounts of pcDNA-PATZ in the absence or presence of DHT as depicted. LUC activity is expressed relative to the transactivation observed in the absence of DHT and without RNF4 and normalized by using β-galactosidase activity. The means ± S.E. values of least five experiments are shown. Immunoblot analysis of RNF4 is shown at the *bottom*. 20 μg each of total cell extracts was resolved on 10% polyacrylamide gel under denaturing condition and subjected to immunoblotting with polyclonal RNF4 antibody (20).

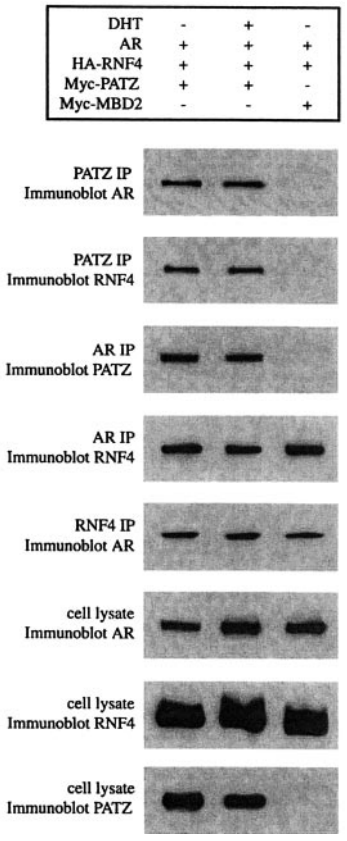

FIG. 6. **PATZ, RNF4, and AR belong to the same protein complex.** pMyc-PATZ or pMyc-MBD2 were cotransfected with pHA-RNF4 and pCMV-hAR in CV-1cells as indicated. After 30 h in culture in the absence or presence of 10 nM DHT, whole cell extracts were prepared and subjected to immunoprecipitation with mouse monoclonal anti-Myc antibody, rabbit polyclonal anti-AR antibody, and mouse monoclonal anti-HA antibody. Immunoprecipitated (*IP*) proteins were analyzed by immunoblotting with a rabbit anti-AR antibody, a mouse monoclonal anti-HA antibody, and anti-Myc antibodies and with a rabbit polyclonal anti-RNF4. On the *bottom* as indicated are represented portions of the cell lysates (5%) that were subjected to immunoblotting prior to immunoprecipitation.

protein (MBD2) expression plasmid as a negative control, we found that only the AR/RNF4 complex was formed. These data show that PATZ forms a ternary complex together with RNF4 and AR both in the presence and in the absence of androgen, thus ruling out interference on the physical formation of the complex and supporting the hypothesis that PATZ renders the complex not functional in the presence of hormone as shown by the transfection assays (Fig. 2).

Physiological Role of PATZ in the Androgen Response—We next tested whether PATZ had any effect on transcription of endogenous androgen-responsive genes. For this purpose, we used prostate cancer-derived cells (LNCaP), which are androgen-responsive (25) and express both RNF4 and PATZ at similar levels (data not shown). The transcription of the PSA, which is up-regulated by androgens (22), was evaluated after transfection of the sense and antisense PATZ expression plasmids. Cells were transfected with pcDNA-PATZ,

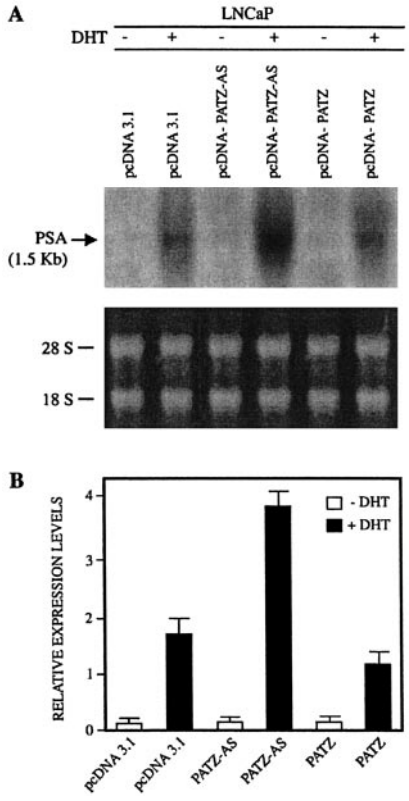

FIG. 7. **Effect of sense (pcDNA-PATZ) and antisense (pcDNA-PATZ-AS) PATZ expression vectors on PSA mRNA content in LNCaP Cells.** *A,* LNCaP cells were transfected with empty pcDNA3.1 vector or with pcDNA-PATZ or with pcDNA-PATZ-AS as indicated. After transfection, cells were treated for 48 h with 10 nM DHT or were grown in the absence of hormone. Transfection efficiency ranged between 40 and 50% as measured by cotransfection of 2 μg of green fluorescent protein expression vector. Total RNA was prepared from cells and used for the experiments. A specific radioactive probe for PSA was used for hybridization. The specific PSA mRNA (1.5 kb) is indicated. Ethidium bromide staining of the same RNA membrane shows equal loading of total RNA. *B,* relative PSA expression levels. Expression ratios are determined by normalizing all samples for 28 S and 18 S intensity relative to the LNCaP at steady state. Data are expressed as the mean ± S.E. values of three separate experiments.

pcDNA-PATZ-AS, or pcDNA empty vector and then treated with 10 nM DHT. Northern blot analysis showed that the expression of PSA was higher after DHT treatment but was down-regulated when PATZ was transfected (Fig. 7). This effect could not be attributed to the down-regulation of AR expression, because Western blot analysis showed that the amount of AR was comparable in all the samples. Conversely, when PATZ antisense expression plasmid was transfected, a slight but significant increase of PSA mRNA expression upon DHT treatment was observed (Fig. 7). Western blot analysis demonstrated that the levels of endogenous PATZ were very low in the PATZ antisense-transfected cells compared with the nontransfected cells (data not shown). These data indicate that PATZ could play a physiological role in the attenuation of androgen stimuli and suggest that changes in its expression may condition the physiological response to androgens.

DISCUSSION

For several years the mechanism of steroid receptor action has been considered very simple consisting in hormone binding followed by nuclear import, recognition of specific DNA motifs, and direct transcriptional activation (or repression) (3, 4). Extensive research has demonstrated the existence of an increasing array of coregulator functioning, in most cases, as bridging proteins between the receptor and component of transcription machinery (9, 10, 12). Each of these proteins potentially interacts with multiple signaling systems including most steroid receptors. Thus, the steroid response is strongly dependent on the activity of coregulators. In this paper, we describe that PATZ may function as a novel androgen receptor coregulator. In contrast to most AR coregulators, PATZ has a unique feature in that it does not influence directly the androgen response but acts by attenuating the coactivation activity of RNF4/SNURF. Our finding demonstrates that PATZ repression potential on AR-dependent transcription is strictly dependent on the association with RNF4. It will be very interesting to assess whether PATZ could associate with other AR coregulators, or whether it is specific for RNF4. However, it could be hypothesized that a mechanism of attenuation of AR coactivators by proteins that have no direct influence on AR activity could be a more general phenomenon and could represent a mechanism for a finer control of the nuclear receptor activity. In fact, in other instances, AR-dependent repression of transcription occurs through the attenuation of the function of other transcription factors, such as proteins interacting with AP-1 and nuclear factor-κB sites (12).

A particular feature of several coregulators is their ability to influence the histone acetylation status through the interaction with specific histone acetyltransferases or deacetylases, resulting in transcriptional activation or repression, respectively (9). Because PATZ contains a POZ domain, which has been described to bind HDACs, we tested the possibility that PATZ repress RNF4-mediated AR activation through chromatin modifications. Our results using POZ deletion mutants and a pharmacological inhibitor of histone deacetylases (TSA) indicate that histone deacetylation is not involved in PATZ repression. Because PATZ repression potential on basal transcription is strongly affected by both the deletion of the POZ domain (17) and by TSA,[3] we suggest that the repressive activity of PATZ may occur through different mechanisms.

Our data can exclude that the observed repression of AR-dependent transcription by PATZ is a simple consequence of PATZ overexpression. In fact, (i) RNF4, AR, and PATZ belong to the same complex, (ii) a mutant RNF4 that is not able to bind PATZ enhances AR-dependent transcription, and it is not influenced by PATZ demonstrating that our observation may not be attributed to "squelching" mechanisms, and (iii) repression of endogenous PATZ expression by antisense expression plasmid in androgen responsive cells, expressing similar amounts of endogenous RNF4 and PATZ, results in a stronger androgen response as measured by an increase in PSA expression. Our results also show that transfected PATZ is able to repress androgen-induced PSA expression in LNCaP cells although at a low level. We believe that the excess of PATZ can only slightly alter the equilibrium of the endogenous complex formed by AR, RNF4, and PATZ, which are expressed at similar levels in these cells.

The mechanisms by which RNF4 modulates androgen-dependent transcription have been investigated (12, 14, 16, 26). RNF4 can interact with both DNA and proteins (transcription factors) and may promote an assembly of nucleoprotein structures (12). It has been shown that RNF4 modulates the transcriptional activities of androgen receptor and SP1 via different domains, and it may act as a functional link between steroid- and SP1-regulated transcription (16). Moreover, it has been shown that RNF4 facilitates AR import to nuclei and retards its export on hormone withdrawal, suggesting that RNF4-me-

[3] R. Pero, F. Lembo, E. A. Palmieri, C. Vitiello, M. Fedele, A. Fusco, C. B. Bruni, and L. Chiariotti, unpublished results.

diated tethering of AR in nuclei represents a novel mechanism for activating steroid receptor functions (27). Thus, upon binding to RNF4, PATZ might interfere with one of the above mechanisms. Further experiments will be necessary to establish the precise mechanisms by which PATZ represses RNF4-mediated enhancement of AR-dependent transcription. However, our data seem to exclude that PATZ interferes with the binding of RNF4 to AR, because all three proteins belong to the same complex *in vivo* both in the presence and in the absence of androgen. Finally, both PATZ and RNF4 have been involved in the regulation of cell growth and in human tumors (20, 28). It will be very interesting to assess the specific role of these molecules and their association in the androgen-dependent growth of normal and tumor cells.

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PATZ Attenuates the RNF4-mediated Enhancement of Androgen Receptor-dependent Transcription

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