SHP Represses Transcriptional Activity via Recruitment of Histone Deacetylases†

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ABSTRACT: The orphan receptor short heterodimer partner (SHP) is a common partner for a great number of nuclear receptors, and it plays an important role in many diverse physiological events. In a previous study, we described SHP as a strong repressor of the androgen receptor (AR). Herein, we addressed the mechanism of action of its negative activity on transcription. We first investigated the intrinsic repressive potential of SHP and mapped two core repressive domains to the amino acids 170−210 and 210−240. From GST pull-down assays, we demonstrated a direct interaction between SHP and diverse histone deacetylases (HDACs) as well as a strong interaction between HDAC1 and SHP inhibitory domains. We further supported the evidence for an interaction between SHP and HDAC1 by showing their co-immunoprecipitation and provided evidence for the existence of a ternary complex comprising AR, SHP, and HDAC1. The use of trichostatin A (TSA), a specific inhibitor of HDAC activity, confirmed that HDACs significantly contribute to the intrinsic transrepressive activity of SHP. Finally, we showed that TSA reversed SHP-induced repression of AR, further emphasizing the relevance of the interaction between SHP and HDACs. This latter action affected in a very similar manner SHP-mediated repression of estrogen receptor α (ERα) transactivation. Altogether, our results indicate that SHP mediates most of its repressive effect through recruitment of HDACs and suggest that the physiological actions of SHP could be affected by HDAC inhibitors.

The short heterodimer partner (SHP)[1] is an atypical member of the nuclear receptor superfamily, with no conventional DNA-binding domain (DBD). *In vivo*, SHP was shown to inhibit the bile acid synthesis through a physiological loop where its expression is directly under the control of bile-acid-activated RXR/FXR heterodimers (*1*). A recent work further emphasized the role of SHP in bile acid production and detoxification of the liver through interactions with PXR (*2*). Interestingly, natural occurring mutations of SHP were reported to be associated with obesity and increased birth weight (*3*).

Since its isolation as a CAR-interacting protein (*4*), SHP was shown to interact with a wide variety of nuclear receptors, including estrogen receptor α (ERα) and ERβ (*5*), androgen receptor (AR) (*6*), HNF4α, as well as RXR (*7*), LRH-1 (*1*, *8*), and PXR (*2*) and to inhibit their transactivation. SHP was shown to interact with the AF-2 domain of ERs and therefore to compete with coactivators for interaction with the receptor-activating domain (*5*). Previously, SHP was evidenced to inhibit the constitutively active GAL4-VP16 when fused to this protein, further demonstrating that SHP alone was also capable of active repression (*9*).

The chromatin structure plays a crucial role in the regulation of gene expression. Histone acetylation and deacetylation are essential actors in modifying chromatin structure and regulating gene expression in eukaryotes. It was originally observed that hyperacetylated histones were found in transcriptionally active genes, whereas hypoacetylated histones were evidenced in transcriptionally silent regions such as heterochromatin (*10*). Acetylation is catalyzed by histone acetyltransferases, whereas the removal of the acetyl group is carried out by histone deacetylases (HDACs). To date, 18 different proteins with histone deacetylase activity have been isolated and grouped into three classes. The identification of the first HDAC revealed the existence of a family of proteins in higher eukaryotes related to the yeast protein, RPD3 (*11*). These proteins are 400−500 amino acid long, share a common domain organization, and define a class, known as class I HDAC, which includes HDAC1 (*12*), HDAC2 (*13*), HDAC3 (*14*), HDAC8 (*15*), and HDAC11 (*16*). A search for other HDACs resulted in the discovery of a second class of enzymes related to yeast HDA1 (*17*). The members of the class II are about 1000 amino acid long and include HDAC4, HDAC5, HDAC6 (*18*), HDAC7 (*19*), HDAC9 (*20*), and HDAC10 (*21*). Finally,

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[1] Abbreviations: AR, androgen receptor; ER, estrogen receptor; SHP, short heterodimer partner; HDAC, histone deacetylase; R1881, methyltrienolone; E2, 17-β-estradiol; ICI182780, (7,17-[9[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5-(10)-triene-3,17-diol); EBL$^+$, ERE-β-globin-luciferase; AF-2, activation function domain 2; GST, glutathione *S*-transferase; PCR, polymerase chain reaction; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; TSA, trichostatin A; RIP140, receptor interacting protein; DBD, DNA-binding domain.

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another yeast protein, SIR2, was shown to have HDAC activity, and its mammalian homologues have also been identified as HDACs. This diversity is thought to reflect the involvement of such proteins in different functions. HDACs are the catalytic component of large multiproteic complexes, which do not bind directly to DNA but are recruited to specific promoters through interactions with transcription factors. For example, CtBP interacts with HDAC4 and HDAC5 to act as a transcriptional repressor (*22*). SMRT and N-CoR, two extensively studied corepressors interact with both class I and class II HDACs through different domains (*23*−*25*).

Histone methylation also regulates eukaryotic gene expression. Many studies evidenced that methylation of H3 lysine 9 (H3-K9) is associated with transcriptionally inactive chromatin (*26*, *27*). Recently, Boulias et al. demonstrated that SHP could interact not only with the G9a H3-K9 methyltransferase but also with lysine 9-methylated histone 3, pointing out the role of SHP in negative regulation of gene expression (*28*). In an attempt to elucidate SHP-mediated repression, Kemper et al. showed that SHP could recruit the mSin3A-Swi/Snf complex to the CYP7A1 promoter to exert chromatin remodeling (*29*).

We previously described the interaction between AR and SHP as well as its repressive effect on androgen-induced transcription (*6*). Herein, we went further into the mechanism of action of SHP by evidencing the role of HDACs. We first mapped two intrinsic repressive domains of SHP and demonstrated that they interact with HDAC1. We showed that SHP could be found in intact cells in a complex with AR and HDAC1, and finally, we observed that SHP-induced repression of AR as well as ERα was reversed by trichostatin A (TSA), a specific inhibitor of HDAC proteins. Altogether, this work demonstrates that SHP exerts most of its intrinsic inhibitory action through recruitment of HDACs.

EXPERIMENTAL PROCEDURES

Expression Plasmids. pCMV5-AR (*30*), pSG5-SHP (*31*), pSG5-ERα, and ERE-β-globin-luciferase (EBL^+) (*32*) were generous gifts of Terry Brown, Lotta Johansson, Pierre Chambon, and Patrick Balaguer, respectively.

cDNAs encoding full-length SHP and SHP(1−170) were amplified by PCR and then inserted into the p3XFLAG-myc-CMV-24 (Sigma) previously digested with *Eco*RI to create p3XFLAG-SHP and p3XFLAG-SHP(1−170), respectively. The sequence of the inserts were checked to make sure that they were correctly amplified.

The vector pGAL4/VP16 was constructed as follows: the cDNA encoding the fusion of the GAL4DBD to the VP16AD was PCR-amplified using pM3-VP16 (Clontech) as a template and then inserted into pVP16 previously digested with *Bgl*II and *Eco*RI.

The cDNA-encoding SHP was obtained by digesting pSG5-SHP with *Eco*RI and then inserted in pGAL4/VP16 at the same site to create pGAL4/VP16-SHP. The fragments corresponding to SHP(1−170), SHP(1−210), SHP(1−240), SHP(170−240), SHP(170−210), and SHP(210−240) were obtained by PCR amplification using pSG5-SHP as a template and then inserted into pGAL4/VP16 previously digested with *Eco*RI to create pGAL4/VP16-SHP(1−170), pGAL4/VP16-SHP(1−210), pGAL4/VP16-SHP(1−240), pGAL4/VP16-SHP(170−240), pGAL4/VP16-SHP(170−210), and pGAL4/VP16-SHP(210−240), respectively. All the inserts were sequenced and found to be correctly amplified.

The fragments corresponding to SHP(1−170), SHP(170−240), SHP(170−210), and SHP(210−240) were prepared as described above and then inserted into pGEX-4T-1 (Pharmacia) previouly digested with *Eco*RI to create pGEX-SHP-(1−170), pGEX-SHP(170−240), pGEX-SHP(170−210), and pGEX-SHP(210−240), respectively. pGEX-SHP was previously described (*6*).

Plasmids for *in vitro* translation: pcDNA-HDAC3 came from Dr. Emiliani, and both pcDNA-HDAC5 and pcDNA-HDAC6 came from Dr. Khochbin; pcDNA-HDAC1-c-myc, which was also used for immunoprecipitations, came from Dr. Seiser.

Cell Lines, Transfection, and Luciferase Assay. CV-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. They were transiently transfected, using the calcium phosphate method, in 12-well dishes with 0.1 μg of pCMV-hAR, 1 μg of *p*-mouse-mammary-tumor-virus-luciferase (MMTV-luc) used as an androgen-regulated gene, and 0.5 μg of pCMV-β-galactosidase for androgen-dependent transactivation or 0.1 μg of HEGO (pSG5-ERα), 0.5 μg of EBL^+, and 0.5 μg of pCMV-β-galactosidase for estrogen-dependent transactivation. In addition, 0.8 μg of pSG5-SHP was also transfected. When used, 0.4 μg of either GALVP16 alone or fused to a SHP deletion mutant was transfected together with 0.5 μg of 17M5X luciferase reporter plasmid. The amount of plasmid vector was kept constant in all experiments.

12 h after transfection, the cells were incubated with 1 nM R1881 to activate AR. When working with ER, 12 h after transfection, the cells were incubated with a phenol red-free medium supplemented with 5% charcoal-stripped fetal bovine serum and either 10 nM E2 or 100 nM ICI. When indicated, TSA was added to a final concentration of 50 nM, 16 h before lysis. After 24 h at 37 °C, the cells were harvested in a lysis buffer: 25 mM Tris-H_3PO_4 (pH 7.8), 2 mM DTT, 2 mM EDTA, 1% TritonX-100, and 10% glycerol. Aliquots were used for β-galactosidase and luciferase activity assays. To measure β-galactosidase activity, the lysate was mixed with a β-galactosidase buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgCl_2$, and 50 mM β-mercaptoethanol) to which was added ONPG to a final concentration of 2 mM. The activity was given by absorption at the optical density of 415 nm. The luciferase activity was measured by the reaction of lysate with the luciferin solution: 270 μM coenzyme A, 470 μM luciferin, 530 μM ATP, 20 mM Tris-H_3PO_4, 1.05 mM $MgCl_2$, 2.7 mM $MgSO_4$, 0.1 mM EDTA, and 33 mM DTT. Luciferase activity was measured as relative light units (RLU) on a luminometer (LKB Instruments, Rockville, MD). All values represent the mean RLU/β-galactosidase (±SD) from triplicates and were reproduced in at least three independent experiments.

GST Pull-Down Assays. GST, GST-SHP, GST-SHP(1−170), GST-SHP(170−210), GST-SHP(170−240), and GST-SHP(210−240) were produced and purified as previously described (*6*). [^{35}S]-Labeled proteins were cell-free-synthesized using the TNT lysate system according to the instructions of the manufacturer (Promega) and incubated with purified GST fusion proteins in PDB buffer (PBS containing

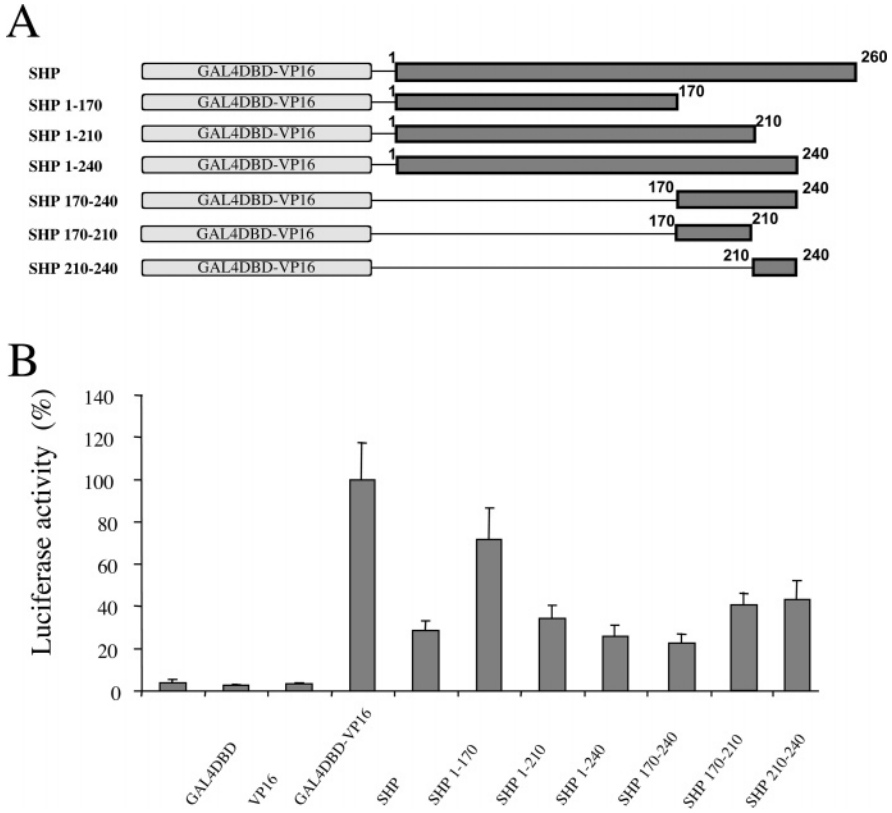

FIGURE 1: SHP presents two repressive domains. (A) Schematic representation of the chimera used throughout experiments. All deletion mutants of SHP were fused to the constitutively active transcription factor GAL4DBD-VP16. (B) Mapping of the inhibitory domains of SHP. The different plasmids described above were transfected in COS7 cells using the phosphate calcium method (see the Experimental Procedures). The results are expressed relative to GAL4DBD-VP16 alone. The mean ± SD values from at least three independent experiments are shown.

20 mM Hepes-KOH at pH 7.9, 10% glycerol, 100 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, and 0.2 mM PMSF), overnight at 4 °C. After washing 4 times with PDB buffer, SDS buffer (30 μL) was added to the beads and boiled for 5 min. Proteins were separated on a 12% SDS−PAGE. Gels were colored with Coomassie blue and dried, and autoradiographies were performed with KODAK biomax films. The figures are representative of at least three independent experiments.

Co-immunoprecipitation. COS7 cells were transfected as described above, except that 100 mm dishes were used and 10 μg of the corresponding plasmid were transfected. The cells were resuspended in 1 mL of lysis buffer [50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 1% NP40, and 1% Triton] in the presence of protease inhibitors, first incubated with either anti-Flag M2 (Sigma), anti-c-myc (Clontech), or anti-AR (AR-441, Santa Cruz) monoclonal antibodies for 2 h at 4 °C and then with Protein G-Sepharose for an additional 16 h at 4 °C. Protein G-Sepharose containing the immune complex was then washed 3 times with the washing buffer [50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, and 0.1% NP40] and resuspended in SDS-containing sample buffer. The proteins were resolved through a 10% SDS−PAGE and immunoblotted with either the anti-Flag M2 monoclonal antibody, the anti-c-myc monoclonal antibody, the anti-HDAC1 polyclonal antibody (H-51, Santa Cruz), or the anti-AR polyclonal antibody (N20, Santa Cruz).

RESULTS

SHP Exhibits Two Intrinsic Repressive Domains. In a previous work, we evidenced that SHP could target AR and inhibit its ligand-dependent activation (*6*). To further characterize its mechanism of action, we first addressed the question of the intrinsic repressive potential of SHP. The SHP cDNA was cloned into a pGAL4/VP16 vector to express a protein fused to the constitutively active GAL4DBD-VP16 chimera (Figure 1A). We also constructed a series of SHP mutants for which part of the cDNA encoding the protein was deleted. As indicated in Figure 1B, SHP induced a 70% decrease of the GAL4DBD-VP16 transactivation, confirming that SHP *per se* had a transrepressive activity. To rule out the possibility of a steric interference for the repressive effect on VP16 activation domain, we measured the ability of GAL4DBD-SHP to transrepress the activity of a LexA-VP16 fusion protein on a L8G5-Luc reporter plasmid and found SHP to be as inhibitory as in our system (*33*). The inhibitory effect of the mutant SHP(1−170) was weak, whereas both SHP(1−210) and SHP(1−240) displayed a strong repressive potential identical to that of full-length SHP. These results indicated that a silencing domain could be assigned to the carboxy-terminal region comprised between the amino acids 170 and 240. When isolated, this region supported a strong inhibitory activity comparable to that of the full-length protein. We then generated two shorter constructs spanning that region, i.e., SHP(170−210) and SHP(210−240). Remarkably, the two mutants SHP(170−210) and SHP(210−240) separately still induced a 62 and

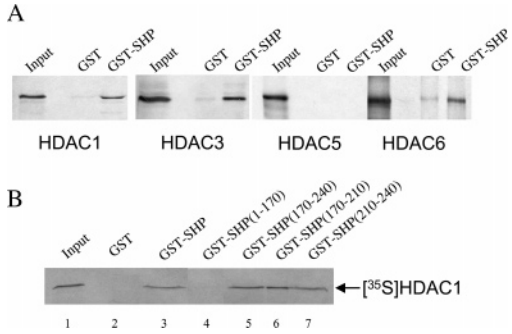

FIGURE 2: SHP interacts *in vitro* with HDACs. (A) Pull-down assays were undertaken as described in the Experimental Procedures using [^{35}S]-labeled HDACs and purified GST or GST-SHP. The inputs represent 10% of the *in vitro* translated HDAC used in each assay. (B) HDAC1 interacts with the core repressive domains of SHP. Pull-down assays were carried out as described in the Experimental Procedures using [^{35}S]-labeled HDAC1 and purified GST-fused proteins. The input represents 10% of the *in vitro* translated HDAC1 used in each assay.

60% decrease of the GAL4DBD-VP16 transactivation. From this series of experiments, we conclude that two domains comprised between amino acids 170−210 and 210−240 can function independently as SHP inhibitory domains.

SHP Interacts with HDACs. The intrinsic repressive potential of SHP suggests that the protein could recruit inhibitory proteins. Moreover, histone deacetylation appears to be the best characterized mechanism underlying transcription repression. Therefore, to shed light on results obtained above, we first investigated the interaction between SHP and HDACs by *in vitro* GST pull-down assays (Figure 2A). We used four different HDACs representative of the main two classes (class I and class II). As shown in Figure 2A, we did not detect any interaction between GST alone and *in vitro* translated HDAC1, HDAC3, or HDAC5, whereas a faint band was observed for the interaction between GST and HDAC6. However, the figure clearly shows that GST-SHP interacted with HDAC1, HDAC3, and HDAC6, whereas no binding could be detected with HDAC5, thus indicating a specificity of interaction.

We then asked whether the repressive domains evidenced in Figure 1 would recruit HDACs. Because HDAC1 emerges as the best studied member of the HDAC family, we therefore elected it to further investigate the interaction between SHP and HDACs. To check whether HDAC1 could associate with some of the SHP mutants described above, we performed *in vitro* GST pull-down assays between GST-fused SHP mutants and *in vitro* translated [^{35}S]-HDAC1. Coomassie staining of the gel indicated that the amount of GST-SHP mutants was kept constant in all experiments (data not shown). As evidenced in Figure 2B, no band could be detected for the interaction between GST and HDAC1 (lane 2), whereas GST-SHP displayed a strong binding to HDAC1 (lane 3). GST-SHP(170−240) (lane 5), which spanned the repressive domain of SHP evidenced above, as well as the two core inhibitory domains, displayed an interaction with HDAC1 comparable to that of wild-type SHP (lanes 6 and 7). In sharp contrast, the mutant GST-SHP(1−170) retained no interaction with HDAC1 (lane 4). These results further support the data obtained in Figure 1 and link the transrepressive effects of SHP to an interaction with HDAC1.

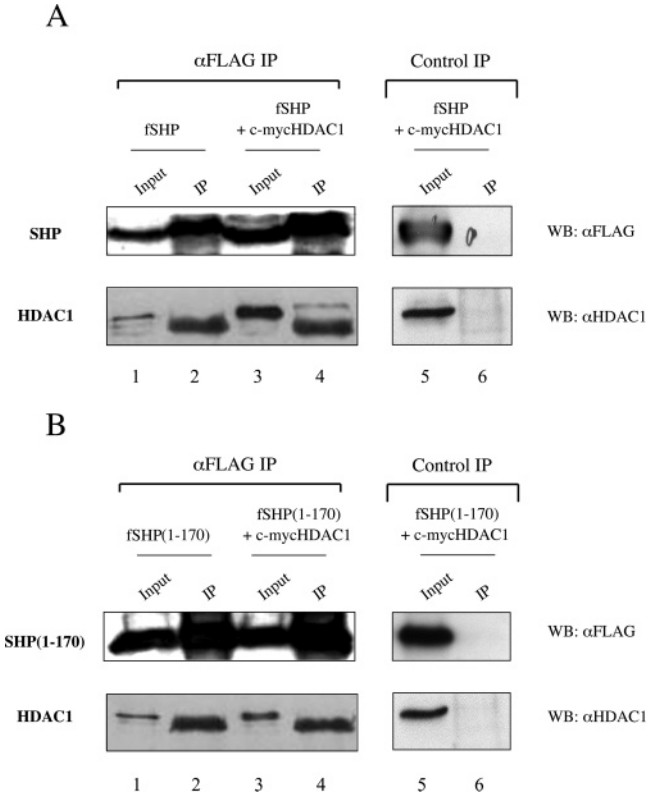

FIGURE 3: HDAC1 co-immunoprecipitates with SHP. (A) COS7 cells were transfected with either fSHP alone (lanes 1 and 2) or fSHP and c-mycHDAC1 (lanes 3−6). The immunoprecipitations followed by Western blots were undertaken with the indicated antibodies (see the Experimental Procedures). The control IP was performed with the monoclonal anti-AR antibody. The inputs, lanes 1, 3, and 5, represent 10% of the cell extract used in the assays. (B) SHP(1−170) does not interact with HDAC1. COS7 cells were transfected with either fSHP(1−170) alone (lanes 1 and 2) or fSHP-(1−170) and c-mycHDAC1 (lanes 3−6). Immunoprecipitations, Western blots, and inputs were done as described in A.

HDAC1 Co-immunoprecipitates with SHP. To answer the question whether the interaction between HDAC1 and SHP could occur in a cellular context, we performed co-immunoprecipitation experiments (Figure 3). COS7 cells were cotransfected with either FLAG-SHP (fSHP) alone (lanes 1 and 2 of Figure 3A) or fSHP and c-mycHDAC1 (lanes 3−6 of Figure 3A). As evidenced on Figure 3A, the use of an anti-FLAG antibody not only immunoprecipitated full-length fSHP (lanes 2 and 4, upper panel) but also c-mycHDAC1 (lane 4, lower panel) when coexpressed. As a control immunoprecipitation, when fSHP and c-mycHDAC1 are coexpressed, the use of an irrelevant anti-AR antibody could pull down neither SHP nor HDAC1 (lane 6, upper and lower panels). We did similar immunoprecipitations using COS7 cells transfected with either fSHP(1−170) alone (lanes 1 and 2 of Figure 3B) or fSHP(1−170) and c-mycHDAC1 (lanes 3−6 of Figure 3B) to try to corroborate *in vitro* data obtained in Figure 2B. fSHP(1−170) appeared to be expressed in larger quantities as compared to fSHP (lanes 1, 3, and 5), and the use of the anti-FLAG antibody enriched fSHP(1−170). In that context, it is remarkable that the anti-FLAG antibody could not co-immunoprecipitate c-mycHDAC1, which not only strengthens the specificity of the interaction but also the data evidencing the interaction domains. When fSHP(1−170) and c-mycHDAC1 are coexpressed, an irrelevant anti-AR antibody could pull down neither fSHP-

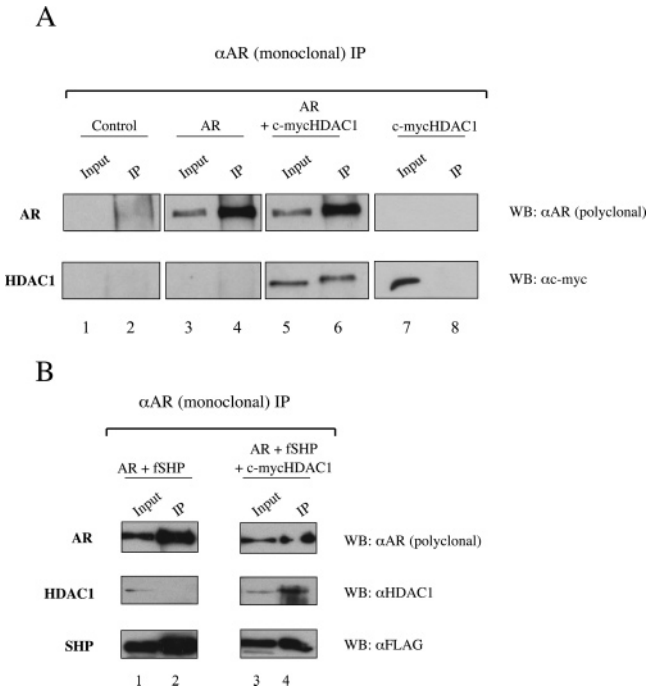

FIGURE 4: Evidence for an AR/HDAC1/SHP complex. (A) HDAC1 co-immunoprecipitates with AR. COS7 cells were either nontransfected (lanes 1 and 2) or transfected with AR alone (lanes 3 and 4), AR and c-mycHDAC1 (lanes 5 and 6), or c-mycHDAC1 alone (lanes 7 and 8) and treated with R1881 (10^{-9} M). The immunoprecipitations were undertaken using an anti-AR monoclonal antibody, and the Western blots were done with either an anti-AR polyclonal antibody (upper panel) or an anti-c-myc monoclonal antibody (lower panel). (B) SHP and HDAC1 co-immunoprecipitate with AR. COS7 cells were transfected with either FLAG-SHP and AR (lanes 1 and 2) or FLAG-SHP, AR, and c-mycHDAC (lanes 3 and 4) and treated with R1881 (10^{-9} M). The immunoprecipitations were done with an anti-AR monoclonal antibody, and the Western blots were undertaken with the indicated antibodies (see the Experimental Procedures).

(1−170) nor HDAC1 (lane 6, upper and lower panels). Furthermore, both fSHP(170−210) and fSHP(210−240) co-immunoprecipitated c-mycHDAC1 (data not shown). This first series of immunoprecipitations showed that SHP could interact with HDAC1 in a cellular context.

Evidence for a Ternary Complex between AR, SHP, and HDAC1. As we previously showed that SHP was an AR partner (*6*), we designed experiments to investigate the formation of a ternary complex between SHP, HDAC1, and AR in COS7 cells. The initial experiment was to demonstrate that AR and HDAC1 could be associated in our experimental conditions. As shown in Figure 4A, the use of an anti-AR antibody immunoprecipitated the receptor from AR-transfected COS7 cells (lane 4). When coexpressed with AR, c-mycHDAC1 was co-immunoprecipitated with the receptor (lane 6). As a control experiment, when expressed alone c-mycHDAC1 could not be immunoprecipitated by an anti-AR antibody (lane 8, lower panel). We concluded from this experiment that AR was able to recruit HDAC1.

We then investigated the ability of SHP to recruit HDAC1 when it interacts with AR. To address that question, we performed immunoprecipitations with an anti-AR antibody in COS7 transfected with either AR and fSHP (lanes 1 and 2 of Figure 4B) or AR, fSHP, and c-mycHDAC1 (lanes 3 and 4 of Figure 4B). As shown in lane 2 of Figure 4B, the use of the anti-AR antibody not only enriched AR but also co-immunoprecipitated SHP. When HDAC1 was coexpressed together with AR and fSHP, the AR not only co-immunoprecipitated fSHP but also HDAC1 (lane 4, middle panel, Figure 4B). It must be stated that the anti-AR antibody immunoprecipitated neither fSHP nor HDAC1 in the absence of the coexpression of AR (data not shown). When the three proteins were coexpressed, it is noteworthy that the amount of AR pulled down was not as important as obtained when AR was expressed with only fSHP (compare right and left panels of Figure 4B). It can be hypothesized that the conformation of the receptor or accessibility to the receptor in the context of the interaction with SHP and HDAC1 is somehow modified, which in turn, hampers the interaction between the antibody and AR. This series of immunoprecipitations allowed us to propose the existence of a complex composed of AR, SHP, and HDAC1.

TSA Reverses SHP-Mediated Transactivation. The enzymatic activity of HDACs can be antagonized by drugs such as TSA (*34*). In line with the interaction data obtained above, we investigated whether TSA could affect the transrepressive activity of SHP. Using SHP mutants described in Figure 1, we first demonstrated that the transcriptional activity of GAL4DBD-VP16 fused to full-length SHP, in COS7 cells, was augmented by TSA, although we did not recover the full transcriptional activity of GAL4DBD-VP16 (Figure 5A). This may be due to the fact that the full-length protein may not only recruit HDAC proteins but also other inhibitory proteins insensitive to TSA. In contrast, on the three mutants SHP(170−240), SHP(170−210), and SHP(210−240), TSA completely restored the activation potential of GAL4DBD-VP16, indicating that the core repressive domains directly involve histone deacetylase activity. As described in Figure 1, SHP(1−170) displayed a weak inhibition, which was not significantly modified by the addition of TSA. This latter result confirmed that the amino-terminal part of SHP is not associated with any HDAC activity.

The effect of TSA can vary according to the partners with which the corepressor interacts. Indeed, it was already shown for the co-repressor LcoR that TSA affected very differently its activity depending on the nuclear receptor with which it interacted (*35*). Therefore, to correlate the existence of a complex between AR/SHP/HDAC to a transcriptional effect, we investigated whether TSA would affect SHP-dependent repression of AR. On the left panel of Figure 5B, we first confirmed that SHP induced an 80% reduction of AR-dependent transactivation in CV1 cells. We then assayed the effect of 50 nM TSA on that repression (right panel of Figure 5B) and observed that TSA relieved almost completely AR transcriptional inhibition.

SHP was also shown to target other nuclear receptors, and its inhibitory effects on ER were already evidenced (*5*). We therefore tested whether the effect of TSA could be specific for AR or whether it could be extended to another steroid receptor such as ERα. As illustrated in Figure 5C, when SHP was transfected in CV1 cells, we observed a total inhibition of E2-induced ERα transactivation (left panel). The use of TSA increased the basal level of transactivation and thus drastically affected the E2 induction of ERα activation. Nevertheless, TSA completely reversed the repressive effects of SHP (right panel). Altogether, these results indicate that histone deacetylase activity is implicated in SHP-mediated repression of AR and ERα transcriptional activity.

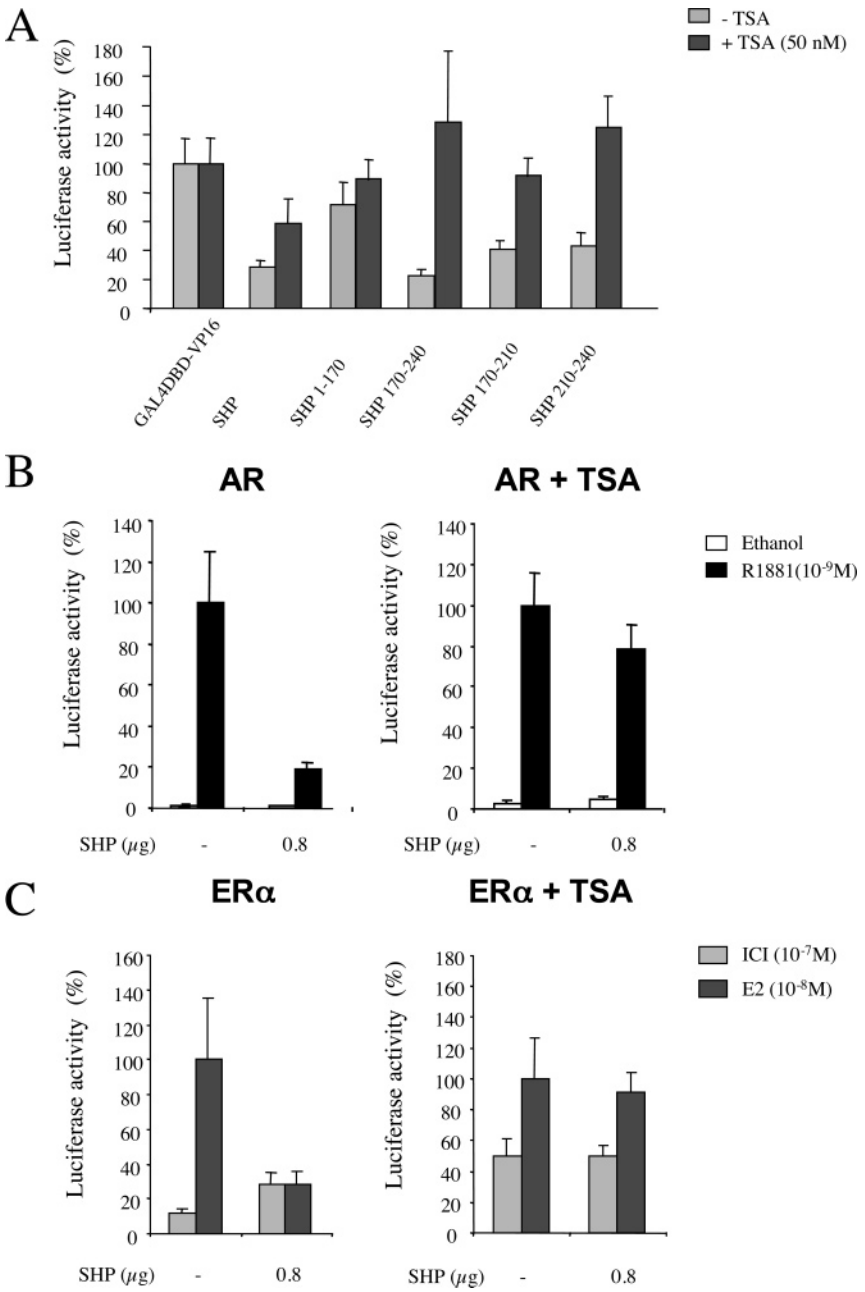

FIGURE 5: TSA reverses SHP-mediated repression. (A) Transrepression mediated by SHP inhibitory domains is sensitive to TSA. GAL4DBD-VP16 constructs used in Figure 1 were transfected in COS7 cells using the phosphate calcium method. The experiments were performed with or without TSA (50 nM). The mean ± SD values from at least three independent experiments are shown. (B) TSA reverses the inhibitory effect of SHP on AR-dependent transactivation. CV1 cells were transiently transfected with AR and SHP (0.8 μg) when indicated, using the phosphate calcium method as described in the Experimental Procedures (left panel). On the right panel, the same experiment was done in the presence of TSA (50 nM). The luciferase activity was normalized with the β-galactosidase value and expressed as the percentage of the activity given by the AR in the presence of R1881 (without SHP). (C) TSA reverses the inhibitory effect of SHP on ERα-dependent transactivation. The experimental conditions are the same as described above, except that ERα was transfected instead of AR, in CV1 cells. The luciferase activity was normalized with the β-galactosidase value and expressed as the percentage of the activity given by ERα in the presence of E2 (without SHP). In B and C, the mean ± SD values from at least three independent experiments are shown.

DISCUSSION

To date, a consistent number of studies on SHP revealed that it interacts with a great number of different nuclear receptors. In a previous work, we already evidenced a repressive effect of SHP on AR-dependent transactivation (*6*). Although it was shown that SHP can compete away a coactivator peptide for the interaction with ERα (*5*), the mechanism of action of the orphan receptor is still poorly characterized. In this study, we provided the first evidence for the role of HDAC proteins in its repressive activity. First, we pinpointed the core repressive domains of SHP and showed that they interacted strongly with HDAC1. Additionally, we demonstrated that, when AR, SHP, and HDAC1 were cotransfected, the three proteins could be partners of a ternary complex pulled down by an anti-AR antibody. Furthermore, the HDAC inhibitor TSA reversed the intrinsic repressive effects of SHP, emphasizing the role of HDACs. Last, when studied on either AR or ERα-dependent transactivation, the repressive effect of SHP could also be reversed by TSA addition. Altogether, the results obtained with TSA

together with protein−protein interaction data prompt us to propose that AR could form a ternary complex with SHP and HDAC1, which would be responsible for SHP-mediated repression.

It must be stated that AR can be immunoprecipitated together with HDAC1 when coexpressed (Figure 4A), which is in agreement with a previous report (*36*). Thus far, no data clearly ascertained a direct interaction between a HDAC protein and AR. In contrast, indirect interactions were recently investigated: DJBP and ARR19 were found in interaction with AR and were shown to modulate AR-dependent transcription through recruitment of HDACs (*37*, *38*). Whether the recruitment of proteins with histone deacetylase activities affect repression directly through regulation of the acetylated status of AR (*36*) or through modification of the chromatin structure remains to be addressed.

In this study, the repressive domains of SHP were mapped to a region comprised between amino acids 170 and 240. When we used the HDAC inhibitor, TSA, on the GAL4DBD-VP16-SHP chimera, it must be observed that we did not recover the totality of the transactivation resulting from GAL4DBD-VP16 alone. In sharp contrast, full activity was always recovered with the shorter parts of SHP that included the core repressive domains. These results let us think that SHP would exert its repressive effects not only through histone deacetylase activities but also through other non-TSA-sensitive proteins. Along with this hypothesis, Båvner et al. described the interaction between SHP and a non-HDAC protein, EID1 (*39*). The mechanism of action of EID1 was not hypothesized to be HDAC-mediated but was rather thought to antagonize the CBP-dependent coactivator functions. Recently, a study reported the role of helices H6 and H7 in transcription repression (*40*). Interestingly, deletion of this region, which covers the region spanning amino acids 128−139, results in the loss of EID1 interaction. Because TSA did not modify SHP(1−170)-dependent repression, it is tempting to hypothesize that EID1 would be responsible for the non-TSA-sensitive repression of SHP.

Other transcription repressors such as RIP140 were shown to exert their repressive effects through binding to HDAC1 and 3 (*41*) and through interactions with non-HDAC proteins such as the coinhibitor CtBP (*33*, *42*). The mechanism of action of RIP140 can be even closer to that of SHP when considering that RIP140 was shown to compete with coactivators to bind to nuclear receptors (*43*). More recently, another coinhibitor, LCoR, was also shown to repress ERα through recruitment of HDACs and CtBP (*35*). In the same study, the authors pointed out that LCoR-dependent repression can be very differently affected by TSA according to the nuclear receptor with which it interacts. Finally, CtBP itself was also shown to exert its effects through binding not only HDACs but also proteins such as PcG (for a review, see Chinnadurai 2002).

Two recent studies dealt with the role of SHP in chromatin silencing. The first one by Kemper et al. showed that a bile acid treatment resulted in SHP-mediated recruitment of mSin3A and Swi/Snf complex to the CYP7A1 promoter, which is associated to gene repression (*29*). In that study, the authors also evidenced an interaction between HDAC1 and GST-SHP but did not address the role of HDACs in mediating SHP-dependent silencing. The second one by Boulias et al. showed that SHP was able to recruit the G9a H3-K9 methyltransferase and therefore mediate SHP transcriptional silencing (*28*).

According to our findings and data from the literature, we can hypothesize that, from an activated status where coactivators interact with nuclear receptors such as AR or ER, SHP would compete away the coactivators and then complete the repressive effect by recruiting HDACs and methyltransferases. The mechanism by which SHP represses the activity of the receptors that it interacts with could be a general mechanism of action, and according to the availability of intracellular repressive proteins, SHP would recruit HDACs and/or non-HDAC proteins.

Both the almost ubiquitous nature of SHP and the fact that it was shown to interact with a great number of nuclear receptors lead us to speculate that what we evidenced herein could also be true for the well-documented role of SHP on LXR, FXR, or LRH (*1*, *8*, *44*−*46*). The physiological loop where SHP is under the control of RXR/FXR heterodimers compares to that implying ER and estrogens. Indeed, a recent work illustrated the stimulatory role of ER on the expression of SHP (*47*). From results obtained both in ERαKO mice (*48*) and inactivating mutations in the SHP gene associated with mild obesity in the Japanese population (*3*), the authors speculate that the regulation of SHP levels would be important for the mechanism by which estrogens regulate adipose tissue. Recently, a work by Bektic et al. (*49*) reported the role of an ERβ ligand, the isoflavonoid genistein, on the repression of AR activity. ERβ stimulation by specific ligands could inhibit AR via the upregulation of SHP expression.

SHP was shown to play a major role in pathological dysfunctions such as mild obesity. It was also demonstrated to be important for bile acid production or physiology of both estrogens and androgens as shown herein. Altogether all of these events could well be under the regulation of HDAC proteins, and our study suggests that some of these physiological actions of SHP could be significantly affected by the diverse anti-HDAC drugs under use in clinical studies.

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