Differential Regulation of c-Jun-dependent Transcription by SUMO-specific Proteases*

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c-Jun is a transcription factor that plays an important role in regulating cell growth, apoptosis, differentiation, and transformation. The transcriptional activity of c-Jun can be regulated by both phosphorylation and sumoylation. It has also been shown that c-Jun transcription can be regulated by SuPr1, an alternatively spliced form of SUMO-specific protease 2 (SENP2). However, the ability of SuPr1 to enhance c-Jun transcription is dependent on promyelocytic leukemia but is independent of the de-sumoylation activity of SuPr1. Here, we show that SUMO-specific protease 1 (SENP1) also markedly enhances the transcription activity of c-Jun. The action of SENP1 on c-Jun transcription is independent of the sumoylation and phosphorylation status of c-Jun but is critically dependent on the desumoylation activity of SENP1. We further show that p300 is essential for SENP1 to enhance c-Jun-dependent transcription because SENP1 can desumoylate the CRD1 domain of p300, thereby releasing the cis-repression of CRD1 on p300. Thus, two SUMO-specific proteases regulate c-Jun-dependent transcription through entirely different mechanisms.

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SENP1 Induces c-Jun Activity

SENP1. Furthermore, SENP1 can desumoylate p300 and release the repression of SUMO-CRD1, leading to an increase in p300 transactivation. Our findings demonstrate a difference between SENP1 and SENP2 in the regulation of c-Jun-dependent transcription and provide a novel mechanism for regulating c-Jun-dependent transcription.

EXPERIMENTAL PROCEDURES

Plasmids—Gal4-p300, Gal4-p300ΔCRD1, Gal4-p300N, Gal4-p300NΔCRD1, Gal4-p300NΔCRDKR, SUMO-1, FLAG-SENP1, FLAG-SENP1 mutant (R630L, K631M), and FLAG-SENP2 have been described previously (22, 39). Gal4-c-Jun full-length, Gal4-c-Jun full-length mutant (K226R), His-p300 (1–1004), His-p300 (1–1045), GST-p300 (1–1045), HA-p300ΔCRD1, and FLAG-SENP2 mutant (R577L, K578M) were prepared by standard cloning and PCR-based mutagenesis. Details of the construction are available upon request. Plasmids Gal4-luciferase, Gal4-DBD, Gal4-c-Jun, Gal4-c-Jun S63A,S73A, and Jun (−79/+170)-luc were gifts from Dr. Bing Su (The University of Texas M. D. Anderson Cancer Center). The Jun (−79/ +170)-luc mutant was prepared by PCR-based mutagenesis with the oligonucleotide CGGGGATCCACAGTTGCGCT. Expression plasmids HA-p300, E1A, and E1AΔ38–38 were gifts from Dr. Yongzhong Wu (Virginia Commonwealth University). We used antibodies against FLAG (M2, Sigma), HA (HA-7, Sigma), His (HIS1, Sigma), Gal4-DBD (BD Biosciences), c-Jun, phospho-Jun (Ser73) (Upstate Biotechnology), and E1a (sc-430, Santa Cruz Biotechnology).

Cell Transfection and Luciferase Assays—PC-3 cells were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum. COS-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. After 24 h of cultivation, these cells were transiently transfected with expression plasmids by Lipofectamine (Invitrogen) according to the manufacturer’s instructions. These cells were starved for 18 h before luciferase was assayed as described previously (50). β-Galactosidase activity was used as an internal control.

TALON Resin Precipitation—TALON resin (Clontech) precipitation of His-300 was carried out as described in a previous publication (51). Briefly, total cell lysates were prepared in lysis buffer (6 M guanidine hydrochloride, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). DNA in the sample was sheared with a 25-gauge needle, and the lysate was centrifuged at 100,000 × g at 15 °C for 30 min. The supernatant was incubated with TALON resin beads for 1 h at room temperature. The beads were washed twice with washing buffer (8 mM urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8) and then twice more with another washing buffer (8 mM urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 6.0). Subsequently, the beads were washed with phosphate-buffered saline twice and treated in 2% sodium dodecyl sulfate treating solution for SDS-polyacrylamide gel electrophoretic analysis.

Western Blotting—Western blotting was carried out as described in our previous publication (52).

RNA Interference—The 21-nucleotide SENP1 small interfering RNA (siRNA) (GGTAAACCAACTCCGTATTC) was synthesized by Dharmacon (39). The same sequence in the inverted orientation was used as a nonspecific siRNA control. The SENP1 and nonspecific siRNA oligonucleotides were inserted into the pShuttleNeo vector (IMAGEGen Corporation) according to the manufacturer’s instructions. PC-3 cells were grown in 6-well plates and transfected with the siRNA plasmid (1 μg) three times at 12-h intervals using Lipofectamine 2000 (Invitrogen). The cells were harvested 72 h after transfection. Expression of SENP1 and c-Jun was detected by using real-time PCR (for SENP1) and Western blot (for c-Jun).

RESULTS

SENP1 Is a Stronger Activator of c-Jun than SENP2—Best et al. (32) reported that SuPr-1, an alternatively spliced form of SENP2, could induce c-Jun-dependent transcription. Because both SENP1 and SENP2 belong to the SUMO-specific protease family with broad substrate specificity (41), we speculated that SENP1 might also be an activator of c-Jun. To test this hypothesis, we performed a luciferase reporter gene assay by using GAL4 fused to the transactivation domain (1–223) of c-Jun (G4-c-Jun) and the GAL4-luciferase reporter plasmid. When expressed in PC-3 cells, SENP1 markedly induced G4-c-Jun-dependent transcription (Fig. 1A). SENP1 exhibited stronger activation of c-Jun-dependent transcription than SENP2. Treatment of SENP1 showed a dose-dependent effect of SENP1 on c-Jun-dependent transcription (Fig. 1B). The effects of SENP1 in different cell lines, such as 293, MCF-7, HeLa, and U-2OS cells, were tested, and cell type specificity was not observed (data not shown). These results suggest that SENP1 can function as a strong activator of c-Jun-dependent transcription.

A previous study indicated that desumoylation activity was not required for SuPr-1, a splice variant of SENP2, to induce c-Jun activity (32). We similarly observed that the catalytic mutant of SENP2 actually induces more c-Jun activity than wild-type SENP2 (Fig. 1A). However, these two proteases SENP1 and SENP2 diverged in their effects on c-Jun-dependent transcription. In contrast to SENP2, the action of SENP1 on c-Jun is dependent on its catalytic activity, as the catalytic inactive mutation markedly reduced the effect of SENP1 on c-Jun-dependent transcription (Fig. 1A). We confirmed this result by increasing the amount of SENP1 mutant transfected, which did not prompt any significant change in c-Jun-dependent transcription (Fig. 1B). Western blot analysis insured that the wild-type and mutant SENP1 were expressed at similar levels and did not alter G4-c-Jun expression (Fig. 1C). Collectively, these data suggest that the action of SENP1 on c-Jun-dependent transcription, unlike that of SENP2, is mediated through a desumoylation mechanism.

To determine whether SENP1 could affect transcription of an endogenous promoter, we examined the effect of SENP1 on the c-Jun promoter (−79/+170), which contains AP-1 binding sites in the −72 position (53, 54). As shown in Fig. 1D, SENP1 induced c-Jun promoter activity in a dose-dependent manner. The catalytic activity of SENP1 is also required for this effect. We also examined whether the effect of SENP1 on the c-Jun promoter is through the AP-1 binding site. The mutation of the AP-1 binding site markedly abolished the activity of the c-Jun promoter by SENP1 (Fig. 1D). To further confirm the effect of SENP1 on c-Jun-dependent transcription, we used siRNA to silence endogenous SENP1 and then examined whether the expression of endogenous c-Jun, a target dependent on c-Jun transactivation, was affected. The transfection of the SENP1-specific siRNA plasmid into PC-3 cells decreased endogenous SENP1 expression by 53% (real-time PCR analysis, data not shown), whereas expression of SENP1-siRNA reduced endogenous c-Jun expression by 60% (Fig. 1E). Collectively, these data indicate that SENP1 can strongly activate c-Jun-dependent transcription through its desumoylation activity.

SENP1 Activation of c-Jun Is Independent of Phosphorylation—c-Jun is a transcription factor involved in the JNK signaling pathway (2, 3, 7, 8, 55–57). JNK modulates c-Jun-dependent transcription through phosphorylation of c-Jun at Ser-63 and Ser-73 (8). To investigate the mechanism underlying c-Jun activation by SENP1, we first examined whether SENP1 could indirectly induce phosphorylation of c-Jun. Anti-phosphorylated c-Jun antibody was used to identify G4-c-Jun phosphorylation. As shown in Fig. 2A, phorbol 12-myristate 13-acetate (PMA), a stimulator of the JNK pathway, strongly induced c-Jun phosphorylation; however, co-expression of SENP1 and the SENP1 mutant could not induce the phosphorylation of G4-c-Jun (Fig. 2A). We also used the G4-c-Jun S63A,S73A construct in which Ser-63 and Ser-73 are mutated to Ala to perform the luciferase reporter assay. Mutation of both Ser-63 and Ser-73 to Ala did not affect the ability of SENP1 to induce G4-c-Jun activity (Fig. 2B), whereas PMA induction of c-Jun was abolished by the mutation (Fig. 2C). These results suggest that the action of SENP1 on c-Jun-dependent transcription is not mediated through a phosphorylation mechanism.

SENP1-Inducing c-Jun Transcriptional Activity Occurs Independently of c-Jun Desumoylation—c-Jun could be conjugated by SENP1.
**FIG. 1.** SENP1 induces c-Jun-dependent transcription. A, SENP1 is a stronger activator than SENP2 in G4-c-Jun-dependent transcription. PC-3 cells were transfected with Gal4-luciferase (G4-Luc) (100 ng) and Gal4-DBD or G4-c-Jun plasmid (50 ng) in the absence or presence of SENP1 or SENP2 wild-type and mutant plasmids (100 ng). Luciferase activity was measured. Transfection efficiency was normalized by using a β-galactosidase expression construct. B, dose response of SENP1 action. PC-3 cells were transfected with Gal4-luciferase (100 ng) and Gal4-DBD or G4-c-Jun plasmid (50 ng) in the absence or presence of increasing amounts of SENP1 wild-type or mutant plasmids (10, 50, 200 ng). Luciferase activity was measured as described in A. C, Western blots of cell extracts from B. D, SENP1 induces c-Jun promoter (-79/+170) activity. PC-3 cells were transfected with Jun (-79/+170)-luciferase or Jun (-79/+170)-luciferase mutant reporter plasmid (100 ng) in the absence or presence of increasing amounts of SENP1 wild-type or mutant plasmids (10, 50, 200 ng). Luciferase activity was measured as described in A. E, silencing endogenous SENP1 reduces endogenous c-Jun expression. PC-3 cells were transfected with c-Jun luciferase or c-Jun luciferase mutant reporter plasmid (100 ng) in the absence or presence of increasing amounts of SENP1 wild-type or mutant plasmids (10, 50, 200 ng). Luciferase activity was measured as described in A.

**FIG. 2.** The effect of SENP1 is independent of c-Jun phosphorylation and sumoylation status. A, Western blotting analysis of Gal4-c-Jun phosphorylation. Lysates of PC-3 cells transfected with G4-c-Jun and empty vector, SENP1, or the SENP1 mutant or treated with PMA (100 nm) for 30 min were analyzed with an anti-phospho-Jun antibody. B, SENP1 action is independent of c-Jun phosphorylation. PC-3 cells were transfected with Gal4-luciferase and G4-c-Jun or G4-c-Jun S63A,S73A and increasing amounts of SENP1 or SENP1 mutant plasmids, and luciferase activity was measured. C, PMA activates G4-c-Jun, which is dependent on phosphorylation. PC-3 cells were transfected with G4-c-Jun or G4-c-Jun S63A,S73A. Cells were treated with PMA (100 nm) or vehicle for 12 h before luciferase activity was measured. The expression levels of G4-c-Jun wild type (WT) and mutant were analyzed by Western blotting with the anti-Gal4DBD antibody. D, SENP1 action is independent of c-Jun desumoylation status. PC-3 cells were transfected with Gal4-luciferase (G4-Luc) (100 ng) and G4-c-Jun full-length or G4-c-Jun full-length mutant (K229R) plasmid (50 ng) in the presence of SENP1 or SENP1 mutant plasmids (100 ng). Luciferase activity was measured as described in Fig. 1A. The c-Jun expression level was measured by using Western blotting with anti-Gal4DBD antibody. mut, mutant; FL, full-length.
SENP1 Induces c-Jun Activity

A. HDAC does not involve G4-c-Jun activity by SENP1. PC-3 cells were transfected with Gal4-luciferase (100 ng) and G4-c-Jun (50 ng) in the absence or presence of SENP1 (100 ng) plasmids. Cells were treated with TSA (0, 10, 100, 200 nM) for 18 h before luciferase activity was measured. B. E1A markedly reduces the effect of SENP1 on c-Jun. PC-3 cells were transfected with Gal4-luciferase (100 ng) and G4-c-Jun (50 ng) plus SENP1 (100 ng), E1A (10, 50, 100 ng), and E1AΔ2–36 (10, 50, 100 ng) as indicated, and luciferase activity was measured. The expression level of E1A and E1AΔ2–36 was measured by using Western blotting with anti-E1A antibody. C. SENP1 enhances the transactivation of p300 on c-Jun-dependent transcription. PC-3 cells were transfected with Gal4-luciferase (100 ng) and G4-c-Jun (50 ng) plus empty vector, SENP1 (100 ng), or the SENP1 mutant (100 ng) in the absence or presence of p300 (50 ng) plasmids. Luciferase activity was measured.

Figure 3. p300 is essential for SENP1 action on G4-c-Jun. A. HDAC activity induced by SENP1 is dependent on its sumoylation status in vivo. To test this possibility, we generated G4-c-Jun full-length and G4-c-Jun full-length sumoylation mutant (K229R) plasmids. The mutant was then compared with the wild-type protein in the Gal4-luciferase reporter system. Consistent with the previous study (40), the mutant exhibited higher transcriptional activity than wild-type c-Jun (2-fold) (Fig. 2D, lane 2 versus lane 5). However, co-expression of SENP1 still markedly enhanced the c-Jun mutant’s transcriptional activity by 28-fold when compared with vector control (Fig. 2D, lane 5 versus lane 6). The effect of SENP1 on the c-Jun mutant was similar to that of the c-Jun wild type and was also dependent on its catalytic activity (Fig. 2D). These data suggest that most of the enhancement of c-Jun-dependent transcription by SENP1 is independent of the sumoylation status of c-Jun.

p300 Is Essential for Induction of c-Jun Activity by SENP1—Because most of the c-Jun-mediated transcription regulated by SENP1 is not through desumoylation of c-Jun itself, the co-regulator might be the mediator in the effect of SENP1 on c-Jun transcriptional activity.

HDACs act as repressors to down-regulate transcription (58). Among the HDAC family, HDAC1 and HDAC4 can be sumoylated (33, 39, 59, 60). HDAC1 has been shown to be desumoylated by SENP1 in regulating androgen receptor-dependent transcription (39). It is possible that desumoylation of HDAC by SENP1 plays a role in inducing c-Jun activity. We first examined whether HDACs are required for SENP1 action on G4-c-Jun activity. Cells were treated with the HDAC inhibitor trichostatin A (TSA) after co-transfection with the G4-c-Jun reporter system and SENP1 or empty vector. TSA could not augment G4-c-Jun activity (Fig. 3A), suggesting that HDACs were not involved in the regulation of G4-c-Jun in our reporter system. Because HDACs had no effect on G4-c-Jun activity, TSA could not alter the effect of SENP1 on G4-c-Jun either (Fig. 3A).

Another important co-regulator for c-Jun activity is p300, which can interact with c-Jun and enhance c-Jun-dependent transcription (16). Importantly, p300 could be modified by SUMO, and the sumoylation of p300 is essential for the cise-repression function of CRD1 on p300 transactivation (22). Therefore, we tested whether p300 is involved in the activation of c-Jun by SENP1. Because adenovirus E1A specifically interacts with p300 and inhibits p300 activity (16, 61–63), we tested whether E1A could inhibit SENP1 activity on G4-c-Jun. As shown in Fig. 3B, overexpression of E1A strongly repressed c-Jun-dependent transcription (lane 2 versus lane 1), whereas E1AΔ2–36, a p300 binding-defective mutant (64), did not. This suggests that p300 is a crucial cofactor for G4-c-Jun transcriptional activity. When SENP1 was co-expressed with E1A, the enhancement of G4-c-Jun transcription by SENP1 was almost completely inhibited (Fig. 3B). We further confirmed that the effect of E1A is dependent on its ability to bind to p300 by using E1AΔ2–36. The effect of E1A on SENP1 was severely impaired by mutation of the p300 binding region of E1A (Fig. 3B). These results suggest that SENP1 action on G4-c-Jun activity is mediated by p300.

In addition, we overexpressed p300 to evaluate its contribution to SENP1-mediated activation of c-Jun. As shown in Fig. 3C, p300 synergized with SENP1 in enhancing G4-c-Jun-mediated transcription. Overexpression of p300 alone could induce G4-c-Jun activity by 3.5-fold. In the presence of SENP1, however, the transactivation activity of p300 was enhanced
SENP1 Increases p300 Transactivation by Desumoylating the CRD1 Domain—Although previous reports suggest that the CRD1 domain of p300 is sumoylated, it was unknown whether SENP1 could remove SUMO from the conjugated CRD1 domain. We first determined whether p300 could associate with SENP1 in vivo. SENP1 was co-precipitated with p300 (1–1045) in cell extracts (Fig. 5A). We then performed a co-transfection experiment to examine whether the CRD1 domain is the target of the desumoylation activity of SENP1. SENP1 was co-expressed with His-p300 (1–1045), a fragment containing the CRD1 domain, and HA-SUMO-1 in COS-7 cells. As shown in Fig. 5B, p300 (1–1045) was conjugated by SUMO-1 (Fig. 5B, lane 4), SENP1 removed all SUMO-conjugated bands (Fig. 5B, lane 5), but the SENP1 mutant did not (Fig. 5B, lane 6). Consistent with another study (22), a CRD1 domain-deleted form of p300 (1–1004) cannot be conjugated by SUMO (Fig. 5B, lane 2). These results clearly show that SENP1 desumoylates p300.

To determine a direct effect of SENP1 on p300 activity, we used the Gal4-DBD reporter system in which p300 is fused to the Gal4 DNA-binding domain (Fig. 5C). When expressed in PC-3 cells, SENP1 induced the G4-p300 activity by 30.8-fold, but SENP1 mutant did not (Fig. 5D). Deletion of the CRD1 domain increased p300 transactivation by 8-fold (Fig. 5D, lane 4 versus lane 7), whereas it severely impaired the effect of SENP1 on p300 transactivation (Fig. 5D). These data suggest that the CRD1 domain is required for the action of SENP1 on p300 transactivation.

To further determine whether SENP1 action is mediated through desumoylating the CRD1 domain of p300, we used a Gal4-p300N+minimal CRD1 domain (mCRD1) fusion construct. The mCRD1 is required for CRD1-mediated repression and contains two sites for SUMO modification (22) (Fig. 5C). The p300N+mCRD1 fusion protein exhibited only 4% of the activity of p300N (Fig. 5E, lane 1 versus lane 4). However, expression of SENP1, but not the SENP1 mutant, completely reversed mCRD1 repression up to the level of p300N (Fig. 5E). Substitution of both lysines of mCRD1 by Arg (Fig. 5C) completely relieved its repression and reduced the effect of SENP1 on p300 transactivation (Fig. 5E). These data suggest that the action of SENP1 on p300 transactivation is mediated through desumoylation of the CRD1 domain.

**DISCUSSION**

c-Jun, a major component of AP-1, plays an important role in cell growth, apoptosis, differentiation, and transformation (1–5, 10, 57, 65–67). Numerous reports from biochemical and functional studies have established that activation of JNK leads to increased c-Jun Ser-63 and Ser-73 phosphorylation and transcriptional activity (1, 6–8, 17, 68). Results from a knock-in mouse model showed, however, that some biological functions of c-Jun did not require its N-terminal phosphorylation (12–14), indicating that JNK-c-Jun signaling transduction is not the only mechanism for regulating c-Jun activity. SuPr-1, a spliced form of SENP2, was recently identified as an activator of c-Jun independent of c-Jun phosphorylation (32). The mechanism underlying SuPr-1 action on c-Jun activity is through SuPr-1 binding of SUMO-modified PML, altering the distribution of promyelocytic leukemia and promyelocytic leukemia oncogenic domain-associated proteins (32). The effect of SuPr-1 on c-Jun activity is independent of its desumoylation activity (32).

In the present study, we have found that SENP1, another member of the SENP family, strongly induces c-Jun-dependent transcription. We observed similar results in the regulation of c-Jun activity by SENP2 as observed for SuPr-1 (32); neither protease requires its catalytic activity to regulate c-Jun. In contrast to SENP2, the desumoylation activity of SENP1 is required for enhancement of c-Jun activity. In our study, the modulation of c-Jun by SENP1 was not via desumoylation of c-Jun. Instead we show that the action of SENP1 on c-Jun transcription is mediated through desumoylation of p300.
co-activator for c-Jun-dependent transcription (7, 16–18, 69). Expression of p300 enhances c-Jun activity (16). A previous study identified a transcriptional repression domain, CRD1, within p300 (27), and Girdwood et al. (22) showed that this domain represses p300 transactivation in a sumoylation-dependent manner. We hypothesized that SENP1 induces c-Jun-dependent transcription via desumoylation of the CRD1 domain of p300. This hypothesis was based on the function of the CRD1 domain of p300 on the c-Jun-dependent transcription and on the finding that the sumoylation sites are located exclusively in the repressive domain (32). The hypothesis was confirmed by the following results. First, p300 is essential for SENP1 action on c-Jun-dependent transcription (Fig. 4A and B). Second, the CRD1 domain represses p300 transactivation on c-Jun-dependent transcription (Fig. 4A) and is required for the action of SENP1 on c-Jun (Fig. 4B). Third, the CRD1 domain is a direct target of SENP1, and SENP1 could induce p300 transactivation through the desumoylation of this domain (Fig. 5). We proposed a SENP1 action model (Fig. 6). In this model, SENP1 deconjugates SUMO from CRD1, relieving cis-repression of SUMO-CRD1 on p300 transactivation and thereby increasing c-Jun-dependent transcription.

SUMO modification could repress the activity of several transcription factors (22, 31). HDAC has been shown to be recruited to sumoylated Elk1 and p300, repressing the transactivation activity of these proteins (22, 31). In the present study, we did not observe any effect of TSA on G4-c-Jun on SENP1 action on G4-c-Jun (Fig. 3A). This suggests that at least the members of class I and II histone deacetylase families were not involved in either the regulation of G4-c-Jun activity or the enhancement of G4-c-Jun activity by SENP1. This is in contrast to the profound TSA effect on the SENP1 effect on androgen receptor-dependent transcription that we reported earlier (39). The reason for the lack of TSA effect is not precisely known at present. Recently Pestell and co-workers (70) reported that in SirT1, a member of the class III histone deacetylase family, repression of p300 required sumoylation on the CRD1 domain of p300, indicating that sumoylated p300 could also recruit SirT1 to repress p300 transactivation independent of TSA.

A growing list of transcription factors and co-regulators have been shown to be modified by SUMO (32–38), indicating that SUMO modification plays an important role in the regulation of transcription. Like ubiquitination, sumoylation requires three enzymatic reactions (E1, E2, E3) to form an isopeptide bond between the C-terminal Gly of SUMO and specific Lys residues of the sumoylated protein (34, 41). The enzymatic reaction mediated by SUMO-specific proteases is a key step in

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**FIG. 5.** SENP1 enhances p300 transactivation through desumoylation. A, SENP1 physically interacts with p300 in vivo. Precipitates with glutathione-Sepharose (GST, top panel) or whole-cell lysates (bottom panel) from COS-7 cells transfected with the indicated expression plasmids were analyzed by Western blot (WB) with anti-FLAG M2. B, SENP1 de-sumoylates p300. Whole-cell lysates from COS-7 cells transfected with the indicated expression plasmids were precipitated with TALON resin and analyzed by Western blot with anti-HA antibody (top panel) or anti-His antibody (bottom panel). C, schematic diagram of p300 and p300-truncated constructs used in these experiments. D, effect of SENP1 on p300 transactivation requires CRD1. PC-3 cells were transfected with Gal4-luciferase (100 ng) and Gal4-DBD (G4-DBD) (50 ng), G4-p300 (50 ng), or G4-p300ΔCRD1 (50 ng) plus SENP1 or the SENP1 mutant (100 ng) as indicated. The luciferase activity was measured. E, effect of SENP1 on p300 transactivation is dependent on the sumoylation of CRD1. PC-3 cells were transfected with Gal4-luciferase (100 ng) and G4-p300N (50 ng), G4-p300N+mCRD1 (50 ng), or G4-p300N+mCRD1KR (50 ng) plus SENP1 or the SENP1 mutant (100 ng) as indicated. The luciferase activity was measured. mut, mutant.
regulating sumoylation and therefore could profoundly alter the activity of sumoylated proteins (37, 41). It has been reported that SENP1 could enhance androgen receptor-dependent transcription through desumoylation of HDAC1 (39). In this study, we demonstrated that p300 is another target of SENP1. SUMO modification of CRD1 within p300 could cis-repress transactivation of p300, and SENP1 could reverse the repressive function of CRD1 through its desumoylation activity.

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\[ \text{SUMO-p300} \]

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