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Modulation of YY1 activity by SAP30☆

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Abstract

Yin Yang 1 (YY1) is a highly conserved and multifunctional transcription factor. The diverse activities of YY1 are regulated and sometimes modified by interaction with various other proteins. By using a yeast two-hybrid screening system, SAP30 was identified as a protein that associates with YY1 and it is able to enhance YY1-mediated repression in a dose-dependent manner. SAP30 is a 30 kDa nuclear protein and is a component of the human histone deacetylase complex. In this study, the interaction of SAP30 and YY1 was confirmed both by in vitro and in vivo assays. The interaction domains between YY1 and SAP30 were mapped to the C-terminal segment of YY1 (295–414) and the C-terminal 91 amino acid region of SAP30. The observation that YY1, SAP30, and HDAC1 form a complex in vivo provides evidence that YY1 also recruits HDAC1 indirectly via its binding to SAP30. These results describe a novel mechanism for YY1-mediated repression.

Keywords: YY1; SAP30; HDAC1; HDAC complex; Transcription repression

Yin Yang 1 (YY1, also known as δ, NF-E1 and UCRBP) is a multifunctional transcription factor and is highly conserved across species ranging from *Xenopus* to human. It has been shown that YY1 is able to function as a repressor, an activator or even an initiator of transcription. The C-terminal domain of YY1 contains four C_2H_2 zinc fingers that can bind the specific DNA sequence CGCCATNTT, which can be found in many different promoters and such binding alters transcriptional activity. Many cellular and viral gene promoters have been reported to be regulated by YY1. Most of these gene products have important functions in cell growth and differentiation (reviewed in [1,2]). There is ubiquitous expression of YY1 in actively growing, differentiated, and growth-arrested cells [3] and this suggests that YY1 may participate in a wide range of cellular activities as cell conditions vary. Its function as an activator or a repressor of gene expression has been linked to the specific association of YY1 with other proteins. For example, YY1 represses transcription of the adeno-associated virus (AAV) P5 promoter in the absence of adenovirus E1A, but activates transcription in the presence of E1A [4]. It is generally believed that how YY1 function is manifested depends on the promoter context [5], interactions with cell-type-specific factors [6–10], and interactions with other regulatory proteins. Indeed, it has been shown that YY1 is able to interact with the TATA-binding protein [3], TFIIB [11], Sp1 [12,13], c-Myc [6], ATF/CREB [14], C/EBP [10], E1A [15], and the retinoblastoma protein (Rb) [16]. These interactions modify the transcription activity of specific promoters.

Recent studies suggest that YY1 might also be involved in chromatin remodeling functions via interaction with histone acetyltransferases (HAT) and histone deacetylases (HDAC). The p300 and the CREB-binding protein (CBP), two closely related transcription coactivators associated with HAT activity, were found to interact with YY1 and thereby modulate YY1's functions [3,9,17]. YY1 can interact with 3 HDACs (HDAC1, HDAC2, and HDAC3) in vitro and potentially with

☆ *Abbreviations:* RPD3, reduced potassium dependency 3; HDAC, histone deacetylase; SAP30, Sin3-associated polypeptide p30; PAH, paired amphipathic helix.

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HDAC2 in vivo [18,19]. HDAC1, HDAC2, and HDAC3 are class I HDACs that have a high degree of homology to the *Saccharomyces cerevisiae* global transcriptional regulator Rpd3p (reviewed in [20]). These two groups of enzymes, the histone deacetylases (HDAC) and histone acetyltransferases (HAT), can modify histones, and this modification is proposed to alter chromatin structure [21]. Typically, acetylated histones are localized in active chromatin, whereas deacetylated histones are co-localized with transcriptional inactive chromatin [22]. When these enzymes are directed to a promoter through a DNA-binding factor, the promoter can be activated or repressed [23–25].

Sin3-associated polypeptide p30 (SAP30) is a 30 kDa nuclear protein associated with Sin3 and a component of the human histone deacetylase complex that includes mSin3, the histone deacetylases HDAC1 and HDAC2, the histone-binding proteins RbAP46 and RbAP48, as well as other polypeptides [26]. Sin3, or its mammalian orthologs mSin3A and mSin3B, is a large multidomain protein and is believed to function as a scaffold upon which the rest of the complex assembles. The mSin3 proteins have been identified as corepressors associated with the Mad family of DNA-binding transcriptional repressors [27,28]. Repression by Sin3 is mediated through its interaction with transcription factors and accessory proteins, which in turn facilitate activity of the Sin3 complex [29]. The Sin3 corepressor complexes are conserved from yeast to mammals and SAP30 is also evolutionarily conserved; it can be detected in all cell lines and most human adult tissues. SAP30 can bind to the PAH3 (paired amphipathic helix) domain of mSin3. SAP30 is associated with histone deacetylase activity and is capable of repressing transcription when tethered to DNA [30]. In yeast, strains with a disruption of the SAP30 locus have a similar phenotype to those with a disruption of either Rpd3 or Sin3. It has been suggested that SAP30 is required for the normal functioning of the Rpd3 complex. All the biochemical and genetic evidences support the conclusion that SAP30 exists in a complex with the histone deacetylases Rpd3/HDAC in both yeast and mammalian cells [26].

In the present study, we used the yeast two-hybrid assay to identify proteins that may interact with YY1. We found that SAP30 is a protein that can bind to YY1 and alter its function. Functional interaction between YY1, SAP30, and other factors is demonstrated in this study and may help to define how the wide range of functions reported for YY1 in the literature occur.

Materials and methods

Plasmid constructions. Plasmids pSV2CAT [31], p5 + 1SVECAT [4], Flag-SAP30 [26], and GST–SAP30 [30] have been described previously. pIE2, which express a fused protein made up of the Gal4–DNA-binding domain and HCMV IE2 protein, and pG5TKCAT were generous gifts from Dr. Lin Young-Sun [32]. The pAS2-1YY1 was constructed by inserting a DNA fragment encoding the full length of YY1 into the *Eco*RI sites of pAS2-1 (Clontech). The plasmids for in vitro transcription and translation: pCITE4a (+)-YY1 (1–414), pCITE4a (+)-YY1 (1–295), pCITE4a (+)-YY1 (1–207), pCITE4a (+)-YY1 (1–154), pCITE4a (+)-YY1 (1–85), pCITE4a (+)-YY1 (81–414), pCITE4a (+)-YY1 (146–414), pCITE4a (+)-YY1 (198–414), and pCITE4a (+)-YY1 (295–414) were cloned by inserting DNA fragments encoding the corresponding length of amino acids from the YY1 protein, between the *Eco*RI and the *Xho*I sites of pCITE4a (+) (Invitrogen). Plasmids pCITE4c (+)-SAP30 (1–220), pCITE4c (+)-SAP30 (65–220), pCITE4c (+)-SAP30 (129–220), pCITE4c (+)-SAP30 (1–129), and pCITE4c (+)-SAP30 (1–65) were cloned by inserting the DNA fragments encoding the corresponding peptides from SAP30, namely residues 1–220, 65–220, 129–220, 1–129, and 1–65, respectively, between the *Eco*RI and the *Xho*I sites of pCITE4a (+) (Invitrogen). pGal-YY1, which expresses Gal4–YY1, was constructed by inserting a full-length YY1 cDNA between the *Eco*RI and *Bam*HI sites of pSG424 [33]. pGal-YY1 1–295 and pGal-YY1 295–414 were constructed by inserting the corresponding YY1 fragments between the *Eco*RI and *Bam*HI sites of pSG424. pGEX-YY1, which expresses GST–YY1, was cloned by inserting YY1 cDNA into *Bam*HI and *Eco*RI sites of pGEX-5x (Amersham Pharmacia Biotech). pFlagSAP30 (1–129) was cloned by inserting a DNA fragment encoding residues 1–129 of SAP30 between the *Hin*dIII and *Xba*I sites of original Flag-SAP30 vector.

Yeast two-hybrid screen. A full-length cDNA of YY1 was cloned into pAS2-1 as a Gal4–DNA-binding domain fusion. This construct was used to screen a human HeLa S3 activation domain cDNA library (MATCHMAKER two-hybrid system; Clontech). Approximately 8.8×10^6 transformants were screened according to the manufacturer's protocol. cDNAs from the activation domain library that encoded proteins that interacted with YY1 were isolated and sequenced.

Cell culture, transfection, and CAT assay. H1299 was maintained in RPMI1640 supplemented with 10% fetal bovine serum. The human cervical carcinoma cell line HeLa was maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Approximately 5×10^5 cells were seeded in each well of 6-well plate 20–24 h before transfection. LipofectAMINE-mediated transfections were performed according to the manufacturer's methodology (Life Technologies). CAT activity was measured 48 h after transfection and quantified according to the method described by Carey et al. [34].

In vitro transcription and translation. In vitro transcription and translation were performed by the Single Tube Protein System 3, T7 (Novagen) according to the manufacturer's protocol. [^{35}S]Methionine (Amersham Pharmacia Biotech) was included in the reaction so that the synthesized proteins were labeled. The templates were pCITE4a (+)-YY1 (1–414), pCITE4a (+)-YY1 (1–295), pCITE4a (+)-YY1 (1–207), pCITE4a (+)-YY1 (1–154), pCITE4a (+)-YY1 (1–85), pCITE4a (+)-YY1 (81–414), pCITE4a (+)-YY1 (146–414), pCITE4a (+)-YY1 (198–414), pCITE4a (+)-YY1 (295–414), pCITE4a (+)-SAP30 (1–220), pCITE4a (+)-SAP30 (65–220), pCITE4a (+)-SAP30 (129–220), pCITE4a (+)-SAP30 (1–129), and pCITE4a (+)-SAP30 (1–65).

GST fusion proteins and pull-down assay. GST, GST–YY1, and GST–SAP30 were expressed in *Escherichia coli* DH5α and purified according to standard protocols [35–37]. The ligand concentrations, using bovine serum albumin as a standard, were 2, 0.1, and 0.4 mg/ml of resin for GST, GST–YY1, and GST–SAP30, respectively. Aliquots (50 μl) of the GST and the GST fusion proteins were incubated overnight at 4 °C with in vitro translated [^{35}S]methionine-labeled proteins. After washing with buffer D (20 mM Hepes, pH 8.0, 20% glycerol, 100 mM KCl, and 0.2 mM EDTA; immediately prior to use phenylmethylsulfonyl fluoride (PMSF) and dithiothreitol (DTT) were added to 0.5 mM) [38], the bound proteins were eluted from beads with buffer D containing 0.1 M glutathione and the proteins thus released were analyzed by electrophoresis on a 10% SDS–polyacrylamide gel.

Co-immunoprecipitation. HeLa Cell extracts were prepared in lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 0.5% Na deoxycholate, and 0.1% SDS) containing 1× protease inhibitor mixture (Complete, Roche Molecular Biochemicals). Equal amounts of protein were incubated with the indicated antibodies overnight at 4 °C, followed by 2 h precipitation with protein agarose beads (Upstate Biotechnology). All immunoprecipitates were washed three times with washing buffer (20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 0.5% Nonidet P-40, and 1 mM EDTA). Bound proteins were collected by boiling in SDS sample buffer and analyzed by SDS–polyacrylamide gel electrophoresis. Proteins were transferred to an Immobilon membrane (Millipore) and this was followed by Western blotting analysis. Primary antibodies for the Western blotting were anti-YY1 (H-10, Santa Cruz) at 1:700 dilution and anti-SAP30 (Upstate Biotechnology) at 1 μg/ml dilution. Signals were detected by the ECL system (Pierce) according to the manufacturer's instructions.

Results

Identification of SAP30 as an YY1-interacting protein

To isolate proteins that can interact with YY1, a yeast two-hybrid screening was performed using a fusion protein that contained the YY1 fused to Gal4–DNA-binding domain. This bait was used to screen a human HeLa S3 activation domain cDNA library (MATCHMAKER two-hybrid system; Clontech). Approximately 8.8×10^6 colonies were screened. Several positive clones were identified and sequenced. One of them contained a cDNA encoding amino acids 65–220 of SAP30. The introduction of the plasmid pGal4DBD–YY1 alone into yeast gave no colonies on the selective medium. Similarly, no colonies were observed when the cells were co-transformed with pGal4DBD–LaminC and pGal4AD–SAP30 aa 65–220. This suggests that YY1 specifically interacts with SAP30 in the two-hybrid system (Fig. 1).

SAP30 binds the Zn-finger domain of YY1

To map the regions required for the interaction between SAP30 and YY1, a series of YY1 deletion mutants was generated and a GST–SAP30 affinity matrix was employed to study the interaction between SAP30 and the YY1 derivatives. The YY1 derivatives were translated in vitro in the presence of ^{35}S and added to GST–SAP30 resin. As shown in Fig. 2, full-length YY1 was retained by the GST–SAP30 fusion protein but not by GST alone (compare lanes 2 and 3). YY1 derivatives lacking the C-terminus failed to interact with SAP30. Data obtained from these YY1 derivatives in SAP30-binding assay indicated that the interaction domain of YY1 with SAP30 was located between amino acid residues 295 and 414 (lanes 16–27).

The YY1 interaction domain of SAP30

The interaction domain of SAP30 with YY1 was first mapped by GST pull-down assay. As shown in Fig. 3, full-length SAP30 was retained by the GST–YY1 fusion protein but not by GST alone (compare lanes 2 and 3). In addition to full-length SAP30, the C-terminal domain of SAP30 also interacts with YY1 (Fig. 3, lanes 3, 6, and 9). SAP30 derivatives lacking the C-terminus 91 amino acids failed to interact with YY1 (lanes 12 and 15). This result is consistent with data obtained from the yeast study. Interestingly, the C-terminal 91 amino acids of SAP30 are also required for and are sufficient for binding to mSin3A [30].

In vivo association of SAP30 and YY1

To demonstrate that YY1 associates with SAP30 in human cells, cell extracts were prepared from the HeLa

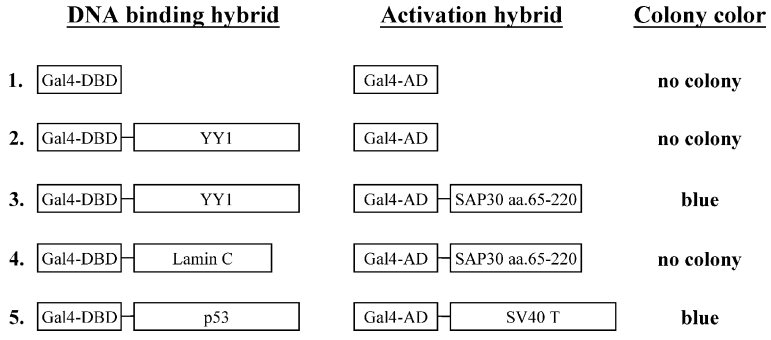

Fig. 1. Interaction of YY1 with SAP30. YY1 specifically interacts with SAP30 in the yeast two-hybrid system. DNA-binding hybrid and activation hybrid were cotransformed into yeast strain Y190. Diploids were assayed by selecting for growth in the absence of histidine and for expression of the LacZ promoter by β-gal activity according to the manufacturer's protocol (Clontech). The following plasmids were cotransformed: Gal4DBD and Gal4AD were used as negative control (row 1); Gal4DBD–YY1 and Gal4AD were used to rule out that YY1 can turn on LacZ promoter by itself (row 2); Gal4AD–SAP30 (aa. 65–220) and Gal4DBD–YY1 (row 3) were used to indicate YY1 association with SAP30; Gal4DBD–Lamin C and Gal4AD–SAP30 (aa. 65–220) (row 4) were also used as controls; and Gal4DBD–p53 and Gal4AD–SV40T (row 5) are positive controls. Yeast colony color was observed after incubation for a standard period of time.

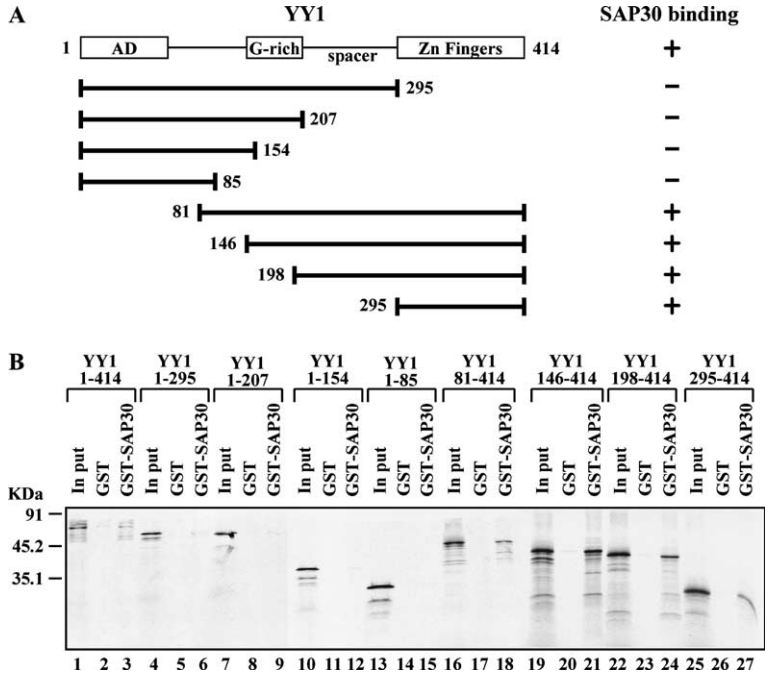

Fig. 2. Mapping the SAP30-binding domain of YY1. (A) A schematic drawing of YY1 and the various YY1 deletion mutants. The location of the activation domain (AD), the glycine-rich region (G-rich), and the zinc-finger domain (Zn fingers) are indicated based on [2]. The ability of each YY1 deletion mutant to bind GST–SAP30 is indicated (+ or −). (B) Identification of the SAP30-binding domain of YY1. In vitro translated and ^{35}S-labeled YY1 or YY1 fragment proteins were incubated with equal amounts of GST or GST–SAP30 fusion proteins and then immobilized on glutathione–Sepharose 4B beads. Bound proteins were eluted from the beads and separated on a 10% SDS–PAGE and analyzed by autoradiography.

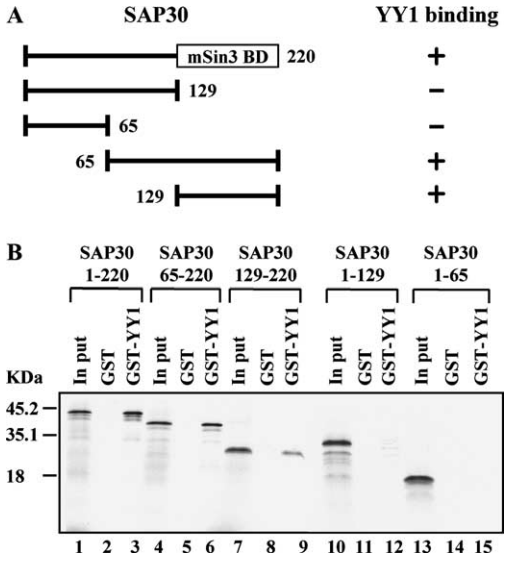

Fig. 3. Interaction of SAP30 with YY1 through a C-terminal domain. (A) Schematic drawing of SAP30 and its deletion fragments. The ability of each SAP30 deletion fragment to bind GST–YY1 is indicated (+ or −). (B) Identification of the YY1 interaction domain of SAP30. In vitro translated ^{35}S-labeled SAP30 and SAP30 mutant proteins were incubated with equal amounts of GST or GST–YY1 fusion proteins and then immobilized on glutathione–Sepharose 4B beads. Bound proteins were eluted from the beads and separated on a 10% SDS–PAGE and analyzed by autoradiography.

cells and then immunoprecipitated with an antibody against SAP30. YY1 was detected in immunoprecipitates by Western blotting using an antibody against YY1. This result showed that YY1 could only be co-immunoprecipitated with anti-SAP30 antibody (Fig. 4A, lane 3) and pre-immune serum did not bring down any detectable amount of YY1 (Fig. 4A, lane 2). Thus, it is clear that endogenous YY1 and SAP30 form a complex in vivo.

To confirm the interaction between SAP30 and YY1 in vivo, plasmids expressing Flag-SAP30 and Flag-SAP30 (1–129) were used for transient transfection study. Because of the low transfection efficiency of HeLa cells, H1299 cells were used for this experiment. Cell lysates were prepared and subjected to immunoprecipitation by YY1 antibody. The immunoprecipitates were then resolved on SDS–PAGE, followed by Western blotting using an anti-Flag antibody. As shown in Fig. 4B, the Western blotting result for cell lysates before immunoprecipitated with YY1 antibody demonstrated that the expression levels of transfected Flag-SAP30 and Flag-SAP30 (1–129) were equivalent (lanes 2 and 3). Only the full-length Flag-SAP30 was co-immunoprecipitated with the YY1 (lane 5) in contrast to Flag-SAP30 (1–129), which failed to be precipitated (lane 6). This also indicates that the C-terminal region of SAP30 is required for binding to YY1. As a negative control, H1299 cells were also transfected with a Flag-tagged vector alone and no Flag signal was detected in the co-immunoprecipitates with YY1 (lane 4). This result demonstrates that YY1 is associated with SAP30 in vivo and the C-terminal region of SAP30 is critical to YY1 binding.

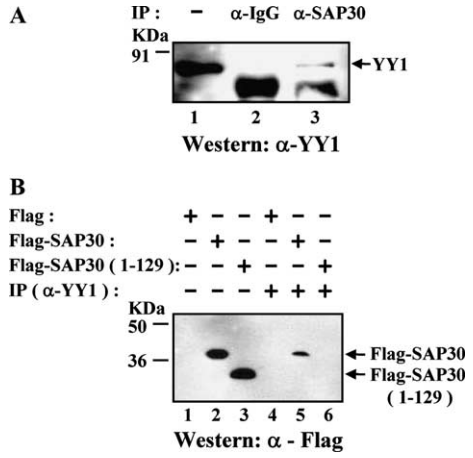

Fig. 4. Association of SAP30 with YY1 in vivo. (A) Immunoprecipitations (IP) were performed with cell extracts of 6×10^6 HeLa cells by using either an antibody against SAP30 (lane 3) or normal mouse IgG as a negative control (lane 2). Precipitates were subjected to SDS–PAGE and the association was detected by Western blotting using anti-YY1 antibody. To demonstrate the recognition of YY1, a Western blotting of HeLa cell nuclear extract is shown in lane 1. (B) The C-terminus of SAP30 is required for SAP30–YY1 interaction in vivo. Flag-tagged SAP30 (Flag-SAP30), truncated Flag-tagged SAP30 (1–129), or Flag vector alone, were transfected into H1299 cells. After 48 h of transfection, cell lysates were collected and subjected to immunoprecipitation with an anti-YY1 antibody (lanes 4–6). The immunocomplex was then separated by a 10% SDS–PAGE and detected by Western blotting using an anti-Flag antibody. YY1 was co-precipitated with full-length Flag-SAP30 (lane 5), but not with Flag-SAP30 (1–129) (lane 6) or Flag vector alone (lane 4).

SAP30 augments the transcriptional repression activity of YY1

Modulation of YY1 transcriptional activity by SAP30 was investigated by a transient transfection assay. Initially, HeLa cells were co-transfected with a SAP30 expression plasmid and a reporter plasmid containing natural YY1-binding sites (p5 + 1) or a control reporter (pSV2CAT) without the YY1-binding site. As shown in Fig. 5A, the p5 + 1 promoter was repressed by SAP30 in a dose-dependent manner. A threefold increase in YY1-mediated repression activity was observed as the amount of SAP30 was increased from 0.3 to 1.8 μg. In contrast, SAP30 had little effect on pSV2CAT. To rule out the possibility that SAP30 may repress transcription via interaction with factors other than YY1, a reporter containing five Gal4–DNA-binding sites upstream of the thymidine kinase promoter (pG5TKCAT) was co-transfected into HeLa cells with a mammalian expression vector encoding YY1 fused to Gal4–DNA-binding domain (Gal4–YY1) and an increasing dose of Flag-tagged SAP30. Fig. 5B demonstrated that overexpression of Gal4–YY1 repressed the pG5TKCAT promoter slightly, whereas the presence of SAP30 decreased the promoter activity dramatically. In this study, Gal4–IE2, another chimeric transcription factor that had been shown to repress transcription of the pG5TKCAT promoter [32], was also incorporated to check the specificity of SAP30. As shown in the right panel of Fig. 5B, SAP30 did not produce a dose-dependent transcriptional repression modification of Gal4–IE2. Together, these results

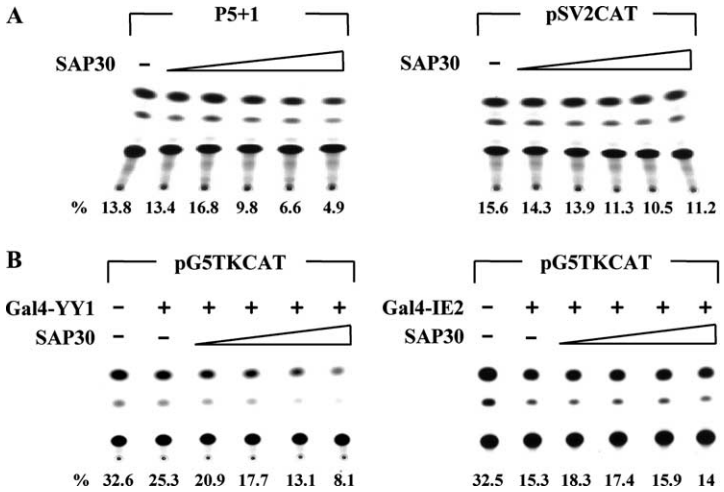

Fig. 5. Augmentation by SAP30 of the repression activity of YY1. (A) YY1 responsive promoter (P5 + 1) is repressed by SAP30. Transient transfections were performed in HeLa cells, using p5 + 1 or pSV2CAT as a reporter. p5 + 1 was derived from AAV and contains natural YY1-binding sites; pSV2CAT was driven by the SV40 promoter/enhancer. The amount of reporter construct used for transfection was 1 μg and the amount of SAP30 expression plasmids used in the transfections were 0.15, 0.3, 0.6, 1.2, and 1.8 μg. Acetylation rate is indicated as a percentage (%). (B) SAP30 enhances transcriptional repression of Gal4–YY1, but not Gal4–IE2. pG5TKCAT contains 5× Gal4–DNA-binding sites upstream of the thymidine kinase promoter. The reporter construct pG5TKCAT (1 μg) and either the Gal4–YY1 (0.1 μg) or the Gal4–IE2 (0.1 μg) expression plasmid were transfected into HeLa cells, in the absence (–) or presence of an increasing dose of the SAP30 expression plasmid. The amounts of SAP30 expression plasmid used for transfection were 0.15, 0.3, 0.6, and 1.2 μg. At least three experiments were performed and similar results were obtained for each.

indicate that SAP30 specifically enhances YY1-mediated repression in a dose-dependent manner.

SAP30 enhances YY1-mediated repression by interaction with the Zn-finger domain of YY1

Since amino acids 295–414 of YY1 are required for the YY1-SAP30 interaction, the next question is whether this sequence is sufficient for the repression. A set of experiments similar to those in Fig. 5B were conducted with increasing amounts of SAP30. As shown in Fig. 6A, a YY1 derivative lacking amino acids 295–414 and fused to the Gal4–DNA-binding domain was incapable of repression (compare lanes 1 and 2), whereas a Gal4 fusion protein containing amino acids 295–414 conferred transcriptional repression (Fig. 6B, lanes 1 and 2). This result is consistent with a previous report that the repression domain of YY1 lies near the zinc-finger region and that deletion of C-terminal sequences from YY1 leads to high-level constitutive transactivation [39]. Inclusion of the SAP30 protein further increases the repression activity of Gal4–YY1 (295–414) (Fig. 6B, lanes 3–6), but has no effect on the Gal4–YY1 derivative (1–295) for the pG5TKCAT promoter (Fig. 6A, lanes 3–6). This strongly suggests that the zinc-finger domain of YY1 located at the C-terminal is not only required for binding to SAP30 in vitro, but is also sufficient for transcriptional repression in vivo. This result also demonstrated that the ability of SAP30 to enhance YY1-mediated repression depends on an association between SAP30 and YY1. SAP30 could not repress transcription by itself and requires other factors to tether it to promoter.

A complex containing YY1, SAP30, and HDAC1 exists in vivo

SAP30 is a component of the human Sin3A complex and directly interacts with other components, such as mSin3A, HDAC1, RbAP46, and RbAP48 [26]. Both HDAC1 and HDAC2 have been reported to bind to YY1 [19]. The fact that SAP30, the other component of the mSin3 complex, also binds to YY1, prompts us to ask whether SAP30, YY1, and other components coexist as a complex. To address the question, plasmids expressing Flag-tagged HDAC1, HDAC2, RbAP46, RbAP48, and SAP30 were generated and then transfected into cells separately. As shown in Fig. 7A, upper panel, all the proteins were expressed to similar levels. The immunoprecipitates brought down by anti-YY1 antibody were examined by Western blotting using an antibody against FLAG. Only HDAC1, HDAC2, or SAP30 were co-immunoprecipitated by the YY1 antibody, but not RbAP46 and RbAP48 (Fig. 7A, lower panel). It was necessary to further investigate whether a complex of SAP30, YY1, and HDAC1 or HDAC2 exists in vivo. Flag-tagged HDAC1 and YY1 or HDAC2 and YY1 were co-transfected into cells and the immunoprecipitates collected by anti-SAP30 antibody were then examined by Western blotting using an antibody against FLAG. Only HDAC1 was co-immunoprecipitated with YY1 by anti-SAP30 antibody (Fig. 7B). In contrast, an association between HDAC2, YY1, and SAP30 was not detected. However, we cannot rule out an association of HDAC2, YY1, and SAP30, because the expression level of co-transfected HDAC2 was much lower. However, these results do demonstrate that HDAC1 forms a complex with YY1 and SAP30 in vivo.

Discussion

We have isolated SAP30 as an YY1-interacting protein by a yeast two-hybrid system. The specific association of SAP30 and YY1 is further supported by in vitro and cell extract studies. SAP30 enhanced the repression activity of YY1 in a dose-dependent manner. Association between YY1 and SAP30 requires the zinc-finger

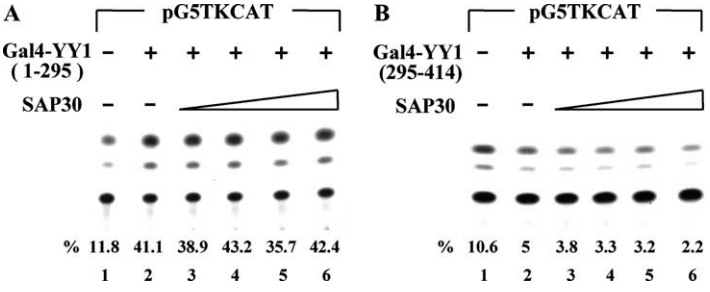

Fig. 6. Enhancement by SAP30 of the YY1-mediated repression through its interaction with the Zn-finger domain of YY1. (A) C-terminal deleted YY1 abrogates the ability of SAP30 to enhance repression of YY1. pG5TKCAT contains five Gal4–DNA-binding sites located upstream of the thymidine kinase promoter. pG5TKCAT reporter construct (1 μg) and 0.5 μg of expression plasmid Gal4–YY1 (1–295) were transiently transfected into HeLa cells in the absence (−) (lanes 1 and 2) or presence of 0.15 (lane 3), 0.3 (lane 4), 0.6 (lane 5), and 0.12 μg (lane 6) of the SAP30 expression plasmids. Acetylation rate is indicated as percentage (%). (B) The zinc-finger domain of YY1 is required for SAP30-mediated repression. The experimental conditions were the same as described in (A) except that Gal4–YY1 (1–295) was replaced by Gal4–YY1 (295–414), which contains the zinc-finger domain of YY1. At least three experiments were performed and similar results were obtained for each.

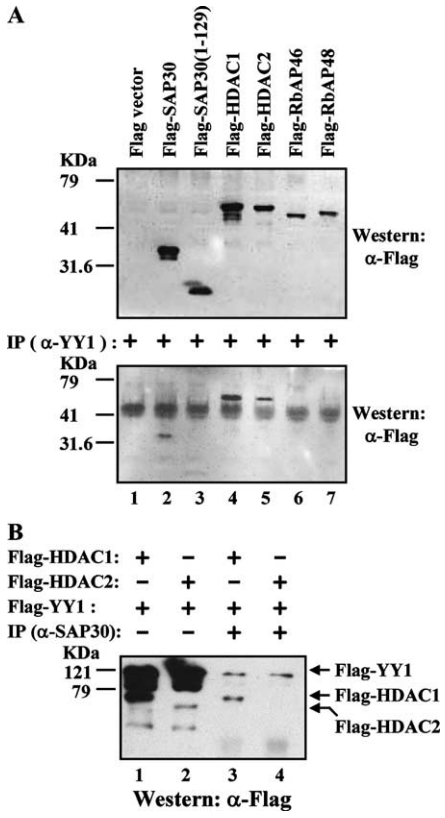

Fig. 7. Co-existence of SAP30, YY1, and HDAC1 in a complex in vivo. (A) Identification of the binding components in the SAP30–YY1 complex. Plasmids of Flag, Flag-SAP30, Flag-SAP30 (1–129), Flag-HDAC1, Flag-HDAC2, Flag-RbAP46, or Flag-RbAP48 were constructed for transfection. H1299 cells were transfected with plasmids as indicated. After 48 h of transfection, cell lysate was collected and proteins were separated on a 10% SDS–PAGE and analyzed by Western blotting using an anti-Flag antibody to detect the expression product (upper panel). Immunoprecipitations (IP) were also performed with cell lysates using anti-YY1 antibody. Proteins were separated by a 10% SDS–PAGE and analyzed by Western blotting using an anti-Flag antibody (lower panel) to detect the associated proteins. (B) A complex of SAP30, YY1, and HDAC1 co-exists in vivo. The experimental method was the same as described in (A). Flag-YY1 was co-transfected with Flag-HDAC1 or Flag-HDAC2 into H1299 cells. Immunoprecipitation was performed using anti-SAP30 antibody. Proteins were separated by a 10% SDS–PAGE and analyzed by Western blotting using anti-Flag antibody.

domain of YY1, and this region is also sufficient for SAP30 to enhance YY1-mediated repression. We suggest that YY1 is able to recruit SAP30 to a promoter and augments YY1 transcriptional repression activity.

Multiple models of YY1-mediated repression have been previously reported [2]. In the first model, the repression is achieved by displacement by YY1 of an activator. The α-actin promoter, where the YY1-binding site occludes the serum response factor binding element (SRE), is a prototype of this model [40]. In the second model, YY1 can prevent activator function by interfering with its association with other activation factors. One such case is YY1 repression of Sp1- and CREB-mediated transcription through its blocking of communication between these activators and their targets within the general transcription machinery [41]. The third proposed model is that YY1 recruits a corepressor that either interferes with activators or modifies the chromatin structure. This model is exemplified by YY1-mediated-transcriptional repression by interaction with HDAC2 [18]. Structural analysis of the repression domains of YY1 reveals how complex these interactions are. Numerous studies have demonstrated that the repression domain is located within the C-terminal zinc fingers of YY1 [2]. In addition to the C-terminal repression domain, a region encompassing residues 170–200 of YY1 is also reported to bind HDAC2 and thus is also a possible repression domain. Mutations in this region abolish its ability to bind with HDAC2 and thus eliminate YY1's transcriptional repression function [18]. Recently, Yao et al. reported that two regions of YY1, residues 170–200 and residues 201–333, can interact with HDACs in vivo. However, co-immunoprecipitates with the YY1 region from amino acids 261–333 alone exhibit histone deacetylase activity. Residues 170–200 of YY1 bind HDAC in vitro and in vivo, but residues 261–333 of YY1 alone interact with HDAC in vivo [42]. These results suggest that a bridge factor might exist in the cell that links HDAC to YY1 through the C-terminal region of YY1. The association of YY1 with SAP30 through the same region as identified in this study leads us to propose that the bridge factor linking HDAC to YY1 is SAP30. It has been reported that SAP30's repression activity when tethered to a promoter, is through a modification of histone deacetylase activity [26]. The association of YY1 with SAP30 and HDAC1, as demonstrated in this study, sheds light on a possible repression mechanism whereby YY1 recruits HDACs indirectly via SAP30.

In this study, the C-terminal region of SAP30 (amino acids 129–220) was found to be necessary for binding to YY1 protein. These C-terminal 91 amino acids of SAP30 have been shown to be required and to be sufficient for binding to the mSin3A transcriptional repression system [30]. We have tried, but have not been able to successfully detect the presence of mSin3A in the co-immunoprecipitated complex of YY1 and SAP30 (data not show). Since YY1 and mSin3A associate with the same region of SAP30, it is reasonable to suggest that the interactions of SAP30 with mSin3A and YY1 may be mutually exclusive. Consistent with this argument, Zhang et al. [43] have found that YY1 was absent in the mSin3-containing complex, even though the mSin3 component HDAC2 has been found to interact with YY1 [18]. Zhang et al. [26] also demonstrated that SAP30 interacts directly with purified recombinant HDAC1. The interaction of SAP30 with HDAC1 suggests that histone deacetylase might be recruited to promoter DNA in a Sin3-independent manner.

SAP30, like Sin3 and Rpd3, can modulate the silencing effect at the cryptic *HM* mating loci, the telomeric loci, and the rDNA locus in *S. cerevisiae* [44]. Pho, a *Drosophila* homolog of YY1, encoded by *pleiohomeoti*, is a member of the Polycomb group (PcG) genes [45]. Polycomb group proteins (PcG) repress homeotic genes in cells where these genes must remain inactive during *Drosophila* and vertebrate development. This repression depends on *cis*-acting silencer sequences, called the Polycomb group response elements (PREs). It is conceivable that the *Drosophila* YY1 homologs, Pho, and Pho-like, bind to PREs and this is required for the silencing of homeotic genes [46]. Both Pho and Pho-like sequence contain four zinc fingers that share 80–96% sequence homology to the four zinc fingers of YY1. Since both SAP30 and YY1 are involved in gene silencing, the association of the YY1 zinc fingers with SAP30 offers a possible explanation for this mechanism of gene silencing.

Although we have identified a nuclear protein SAP30 as a protein that interacts with YY1 and have also examined its possible functional relevance, however, the exact implications of this for transcriptional regulation needs to be explored further in future studies.

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