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SAP30, a Novel Protein Conserved between Human and Yeast, Is a Component of a Histone Deacetylase Complex

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Summary

Histone acetylation plays a key role in the regulation of eukaryotic gene expression. Recently, histone acetylation and deacetylation were found to be catalyzed by structurally distinct, multisubunit complexes that mediate, respectively, activation and repression of transcription. Here, we identify SAP30 as a novel component of the human histone deacetylase complex that includes Sin3, the histone deacetylases HDAC1 and HDAC2, histone binding proteins RbAp46 and RbAp48, as well as other polypeptides. Moreover, we describe a SAP30 homolog in yeast that is functionally related to Sin3 and the histone deacetylase Rpd3. The human SAP30 complex is active in deacetylating core histone octamers, but inactive in deacetylating nucleosomal histones due to the inability of the histone binding proteins RbAp46 and RbAp48 to gain access to nucleosomal histones. These results define SAP30 as a component of a histone deacetylase complex conserved among eukaryotic organisms.

Introduction

The eukaryotic genome is compacted with chromosomal proteins in the form of chromatin (van Holde, 1989). This allows for efficient storage of genetic information and also provides multiple levels for control of gene expression (Paranjape et al., 1994; Kadonaga, 1998). Recent studies have revealed two systems for alteration of chromatin structure. One system utilizes ATP-dependent multiprotein complexes (reviewed in Kingston et al., 1996; Wu, 1997, Cairns, 1998). The other system involves posttranslational modification, in particular, acetylation of lysine residues at the N-terminal tails of the core histones (reviewed in Brownell and Allis, 1996; Grunstein, 1997; Pazin and Kadonaga, 1997; Wade et al., 1997; Struhl, 1998).

Since the discovery of histone acetylation by Allfrey and colleagues more than three decades ago (Allfrey et al., 1964), a general correlation between core histone acetylation and transcription activity has been established. Hyperacetylation of histones correlates with gene activation, while hypoacetylation of histones correlates with gene repression (Hebbes et al., 1988; Braunstein et al., 1993). It is thought that acetylation of the lysine residues weakens histone interaction with DNA and thereby facilitates access of the transcription machinery to promoter DNA (Hong et al., 1993; Lee et al., 1993; Vettese-Dadey et al., 1996). Several lines of evidence are consistent with this model. First, several transcriptional coactivators have histone acetyltransferase (HAT) activity (reviewed by Brownell and Allis, 1996; Grunstein, 1997; Wade et al., 1997; Struhl 1998). Secondly, the global transcriptional repressor Sin3 associates with the histone deacetylase Rpd3 or its mammalian homologs HDAC1 and HDAC2 (Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Laherty et al., 1997; Kadosh and Struhl, 1997; Kasten et al., 1997; Nagy et al., 1997; Sommer et al., 1997; Zhang et al., 1997) and targets Sin3-HDAC/Rpd3 complexes to promoter elements resulting in transcriptional repression (Yang et al., 1996; Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Laherty et al., 1997; Nagy et al., 1997; Sommer et al., 1997; Zhang et al., 1997). Finally, recent studies using the HIV-1 promoter demonstrated that histone acetylation increases transcription by facilitating transcription reinitiation (Sheridan et al., 1997). Nevertheless, proof that histone acetylation controls gene activity requires direct analysis of chromatin structure of genes that have been targeted by HATs or HDACs.

Although the above model is generally correct, exceptions have been reported. For example, transcriptional silencing of the mating-type loci of budding yeast and centromeric heterochromatin of *D. melanogaster* requires lysine 12 of histone H4 to be preferentially acetylated (Turner et al., 1992; Braunstein et al., 1996), and the histone deacetylase Rpd3, which has a specificity for lysine 12, counteracts heterochromatic silencing (De Rubertis et al., 1996; Rundlett et al., 1996; Vannier et al., 1996). One explanation is that the acetylation state/residues of the histone polypeptides serve as signals for recruitment of different protein complexes. In this regard, the following observations are particularly relevant. First, the N-terminal tails of histones H3 and H4 interact with the silent information regulator proteins, SIR3 and SIR4 (Hecht et al., 1995). Secondly, the repression domain of the yeast global repressor Tup1 preferentially interacts with underacetylated histone H3 and H4 (Edmondson et al., 1996). Finally, hyperacetylation of fission yeast centromeric histones disrupts the localization of Swi6p, a component of centromeric heterochromatin (Ekwall et al., 1997). It seems that transcriptional regulation through histone acetylation or deacetylation is likely position dependent.

Identification of HATs and HDACs has allowed the relationship between histone acetylation and transcriptional regulation to be addressed at the molecular level. However, a mechanistic understanding of this relationship requires reconstitution of the enzymatic activities

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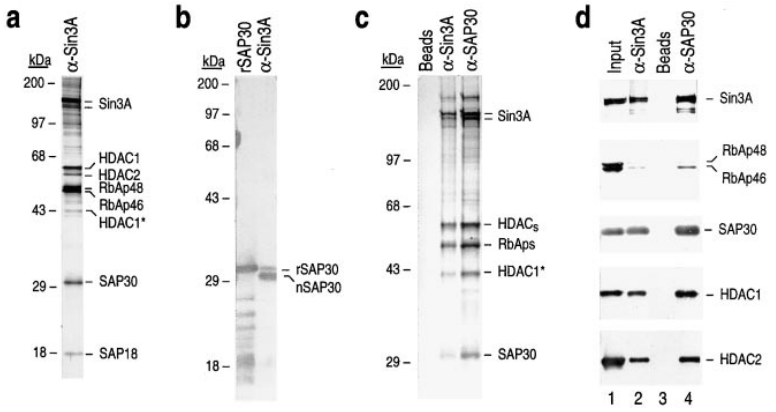

Figure 1. SAP30 Is a Component of the Human HDAC/Sin3 Complex

(a) Silver staining of an SDS–PAGE of the HDAC/Sin3 complex purified from HeLa cell extracts. The positions of protein size markers and the identity of the major polypeptides are indicated.

(b) Western blot analysis. Affinity-purified SAP30 antibodies react with recombinant SAP30 (left lane) and native SAP30 presented in the Sin3 affinity-purified complex (right lane). The positions of protein size markers are indicated.

(c) Silver staining of an SDS–PAGE of the complexes purified with anti-Sin3A and anti-SAP30 antibodies. Beads represent unrelated anti-GST antibodies. The identity of the polypeptides and protein size markers are indicated. HDACs and RbAps doublets did not resolve well due to the gel condition.

(d) Western blot analysis of the affinity-purified samples shown in Figure 1c. All antibodies used for Western were affinity-purified and are indicated to the right. HDAC1 and HDAC2 were hybridized separately with specific antibodies in two blots. Single bands indicate that the two antibodies do not cross-react.

with defined components. Mutational studies have unequivocally demonstrated that Rpd3 is the catalytic subunit of a yeast histone deacetylase (Kadosh and Struhl, 1998). However, the mammalian and yeast histone deacetylases exist in multiprotein complexes in the cell. It is likely that other polypeptides (subunits) are required for the regulation of the enzymatic activity, and more importantly, for the targeting of the histone deacetylase complexes to specific promoters. Interestingly, a polypeptide of 48 kDa, previously identified as a retinoblastoma (Rb) binding protein, copurifies with HDAC1 (Taunton et al., 1996). Moreover, the histone deacetylase complex HDA contains four polypeptides (Carmen et al., 1996). Along the same line, we also isolated a histone deacetylase complex containing multiple subunits (Zhang et al., 1997). This complex contains, in addition to the corepressor Sin3, the histone deacetylases HDAC1 and HDAC2 (Taunton et al., 1996; Yang et al., 1996), the Rb-associated proteins RbAp46 and RbAp48 (Qian et al., 1993; Qian and Lee, 1995), and two novel Sin3-associated polypeptides, SAP30 and SAP18. The exact functions of the RbAps and SAPs in this complex are not known. However, the RbAps have been found to interact with core histones H2A and H4 (Verreault et al., 1997) and to be present in different protein complexes involved in histone modification (Parthun et al., 1996; Taunton et al., 1996; Verreault et al., 1997; Zhang et al., 1997; Martinez-Balbas et al., 1998), nucleosome assembly (Tyler et al., 1996; Verreault et al., 1996; Kaufman et al., 1997), and nucleosome remodeling (Tsukiyama and Wu, 1995; Martinez-Balbas et al., 1998). It is likely that RbAps function as molecular bridges to bring histone/nucleosome modifying enzymes to their targets.

To extend our previous study, we characterized this complex further with the focus on the novel protein SAP30. We report identification and cloning of cDNAs encoding both human and yeast SAP30 and demonstrate that SAP30 is functionally conserved. We provide biochemical and genetic evidence that SAP30 is a true component of the histone deacetylase complex.

Results

SAP30 Is a Component of Human Histone Deacetylase Complexes

Using antibodies against the PAH2 domain of the mouse corepressor mSin3A, we previously isolated a Sin3A-containing complex (Zhang et al., 1997). The complex contains, in addition to mSin3A and other minor polypeptides, the histone deacetylases HDAC1 and HDAC2, the Rb-associated proteins RbAp46 and RbAp48, and two novel polypeptides designated SAP30 and SAP18. The cloning and characterization of SAP18 was reported previously (Zhang et al., 1997).

To further characterize this histone deacetylase–containing complex, we isolated a human cDNA encoding full-length SAP30 (see Experimental Procedures). A search of the GenBank databases using the human SAP30 sequence identified a potential yeast homolog, Sap30. Alignment of the human and yeast SAP30 sequences is shown in Figure 2a. Although limited sequence similarity was observed (38.9% similar, 18.7% identical), biochemical and genetic evidence demonstrate that these two proteins are functional counterparts (see below). The human and yeast *SAP30* ORFs were subcloned into bacterial expression vectors. The expressed proteins were purified and used to raise polyclonal antibodies. Antibodies directed against human SAP30 recognized a 30 kDa polypeptide present in the mSin3A affinity-purified complex (Figure 1b). The recombinant SAP30 polypeptide migrated slower than the native protein due to the presence of a histidine tag. The faster migrating species present in the recombinant SAP30 preparation most likely represent degradation products.

Having established that SAP30 is a component of the human Sin3A complex, we next asked whether a similar complex could be isolated using antibodies directed against human SAP30. An affinity column using immunopurified SAP30 antibodies and the same materials used

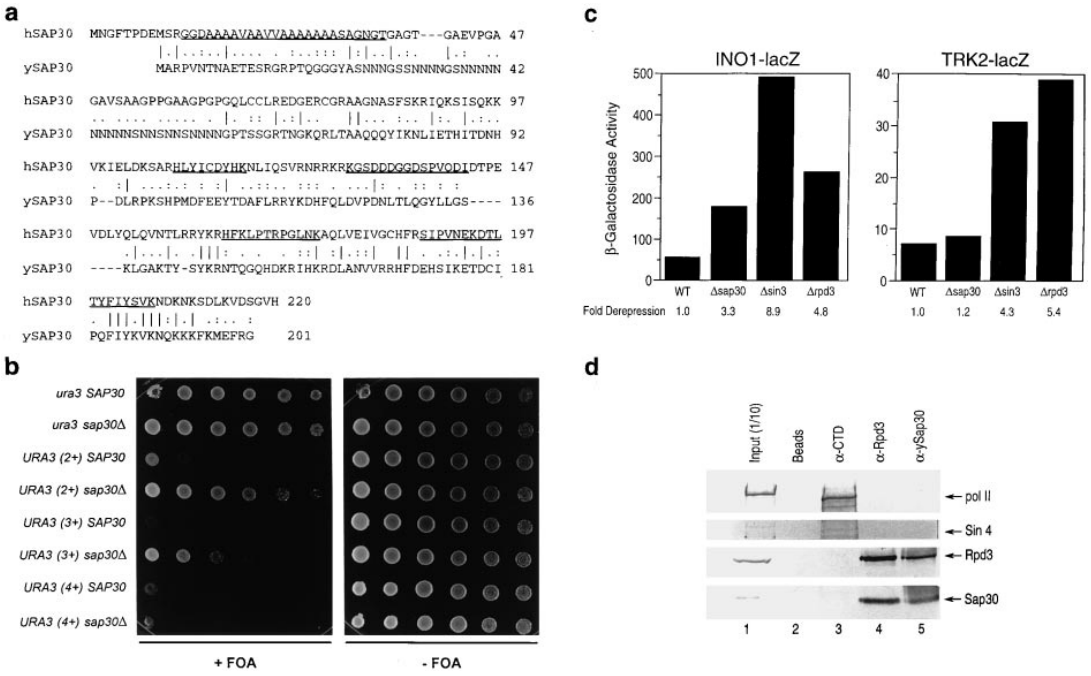

Figure 2. SAP30 Is Conserved between Human and Yeast

(a) Amino acid sequence alignment of human SAP30 (hSAP30) with its *S. cerevisiae* homolog (ySAP30). Sequence alignment was performed using the GCG Gap program (University of Wisconsin-Madison). Peptide sequences obtained from microsequencing of the band labeled SAP30 in Figure 1a are underlined. The yeast homolog was identified by BLAST analysis of Saccharomyces Genome Database (Stanford University, accession number P38429). The cDNA sequences that encode human SAP30 have been deposited into GenBank (AF055993). Vertical lines represent identical amino acids; dashes represent gaps; dots represent similar amino acids.

(b) Effect of *sap30*Δ on telomere position effect (TPE). The *SAP30* gene was disrupted (*sap30*Δ) in isogenic strains YPH250, UCC506 (*URA3-TEL-VR 2*+), UCC508 (*URA3-TEL-VR 3*+), or UCC510 (*URA3-TEL-VR 4*+); strain YPH250 lacks a functional *URA3* gene. Ten-fold serial dilutions of the indicated strains were spotted onto glucose-minimal medium that either contains (+FOA) or lacks (−FOA) 5-fluoro-orotic acid and were incubated at 30°C for 2 days. Growth of *URA3* strains on FOA medium is indicative of telomeric silencing.

(c) Effect of *sap30*Δ, *rpd3*Δ, and *sin3*Δ deletions on *lacZ* expression from *INO1-lacZ* and *TRK2-lacZ* reporter plasmids. Isogenic strains YMH171 (WT), YMH277 (*sap30*Δ), YMH265 (*sin3*Δ), and YMH270 (*rpd3*Δ) were transformed with plasmid pJS325 (*INO1-lacZ*) or pAB137 (*TRK2-lacZ*), and β-galactosidase activities were assayed as described. The indicated activities were obtained by duplicate assays of three independent transformants; standard deviations were <25% in each case.

(d) Western blot analysis of immunoprecipitates from partially purified yeast whole cell extracts. The antibodies used for immunoprecipitation are indicated at the top. Antibodies used for Western blots are indicated to the right. Rpd3 and Sap30 can immunoprecipitate each other, but neither can immunoprecipitate RNA pol II holoenzyme components (lanes 4 and 5).

for purification of the mSin3A complex were used. Polypeptides retained on the anti-SAP30 column were compared to those present in the Sin3A affinity-purified complex. Silver staining of an SDS–PAGE gel demonstrated that both antibodies immunoprecipitated almost identical polypeptides (Figure 1c). Western blot analysis confirmed the presence of Sin3A, RbAp48 and RbAp46, and the histone deacetylases HDAC1 and HDAC2 in both complexes (Figure 1d). In contrast, none of these polypeptides were present in the control column. These biochemical studies collectively establish that human SAP30 is present in the HDAC1 and HDAC2 histone deacetylase complexes.

Yeast Sap30 Is Functionally Related to Rpd3 and Sin3

The limited sequence similarity between mammalian and yeast SAP30 sequences (Figure 2a) raises the question of whether these two proteins are functionally related. We addressed this concern by determining the phenotypes associated with the loss of Sap30 function in yeast. We found that the *SAP30* gene, like the *RPD3* and *SIN3* genes, is important for normal cell growth, but is not essential for cell viability (data not shown).

Disruption of the *RPD3* and *SIN3* genes confers a similar set of phenotypes, including cycloheximide sensitivity at 37°C (Cyh^s), mating and sporulation defects, and enhanced silencing of genes ectopically positioned near telomeres (Vidal and Gaber, 1991; Vidal et al., 1991; De Rubertis et al., 1996; Rundlett et al., 1996; Vannier et al., 1996). Strains disrupted at the *SAP30* locus were scored for each of these phenotypes. Meiotic analysis of a *SAP30/sap30::LEU2* diploid strain resulted in 2:2 segregation of Cyh^s:Cyh^r and Leu^+:Leu^-; moreover, Cyh^s/Leu^+ cosegregated among all progeny (data not shown). Association of Cyh^s with *sap30*Δ was confirmed using isogenic *SAP30* and *sap30::LEU2* strains and compared to isogenic *rpd3::LEU2* and *sin3::LEU2* strains. Whereas the *SAP30* strain was Cyh^r, the *sap30::LEU2* strain was Cyh^s, and this phenotype was fully rescued

Table 1. Summary of Phenotypes Associated with Isogenic *sap30*Δ, *rpd3*Δ, and *sin3*Δ Mutants

Strain[a]	Growth on Cycloheximide[b]	Mating Efficiency[c]	Sporulation Efficiency[d]	Telomere Position Effect[e]
WT	+	+	+	−
*sap30*Δ	−	−	−	+
*rpd3*Δ	−	−	−	+
*sin3*Δ	−	−	−	+

[a] Isogenic yeast strains YMH171 (WT), YMH277 (*sap30*Δ), YMH270 (*rpd3*Δ), and YMH265 (*sin3*Δ) were used in this study.
[b] Cell growth was scored on synthetic complete medium containing 0.04 μg/ml cycloheximide at 37°C. The symbol (−) denotes distinctly impaired growth relative to growth of the WT strain (+).
[c] Mating efficiency was scored by a halo assay that measures growth arrest in response to pheromone secretion. A YPD plate was seeded with ~10^4 cells of strain 29-6 (*MAT***a** *sst2*Δ). Approximately 2.5 × 10^6 cells of the indicated strains were then spotted onto the surface of this plate and incubated at 30°C for 2 days. The symbol (+) denotes a halo of growth inhibition that correlates with efficient mating, whereas (−) denotes a distinctly smaller halo of growth inhibition.
[d] Sporulation efficiency was determined by crossing isogenic *SAP30* (YMH171) and *sap30*Δ (YMH277) strains with a *sap30*Δ mutant (YMH281) of opposite mating type and scoring the resulting diploid strains for tetrad formation following 3 days growth at 30°C on sporulation medium. Sporulation deficiencies of homozygous *rpd3*Δ and *sin3*Δ mutants were not determined in this study, but were reported previously (Vidal and Gaber, 1991; Vidal et al., 1991).
[e] Results are summarized from Figure 2b. The symbol (+) denotes enhanced silencing of the *URA3* gene ectopically positioned near the right telomere of chromosome V, scored as enhanced growth in the presence of 5-FOA.

by plasmid-borne *SAP30* (Table 1). Furthermore, the isogenic *rpd3::LEU2*, *sin3::LEU2*, and *sap30::LEU2* strains displayed nearly identical Cyh^s phenotypes.

Mating and sporulation phenotypes were also scored using the isogenic *SAP30* and *sap30*Δ strain pair. Mating competence was scored by a halo assay that detects mating pheromone secretion. As expected, the *SAP30* strain produced a distinct zone of growth inhibition, whereas the *sap30*Δ mutant resulted in a noticeably smaller halo, comparable to the mating defects of the isogenic *rpd3*Δ and *sin3*Δ mutants (Table 1). To determine whether *sap30*Δ affected sporulation, diploid strains constructed by crossing isogenic *SAP30* and *sap30*Δ strains with a *sap30*Δ strain of opposite mating type were induced to sporulate. In this case, the heterozygous *SAP30/sap30::LEU2* strain sporulated efficiently, whereas the homozygous *sap30*Δ/*sap30*Δ diploid failed to sporulate, even upon prolonged incubation under inducing conditions (Table 1). Thus, loss of Sap30 function causes mating and sporulation defects comparable to those associated with loss of Rpd3 and Sin3 function.

The telomere position-effect (TPE) in yeast causes a mosaic inactivation of telomere-proximal genes (Gottschling et al., 1990; Aparicio et al., 1991). This form of transcriptional silencing is counteracted by the *RPD3* and *SIN3* genes (De Rubertis et al., 1996; Rundlett et al., 1996; Vannier et al., 1996). We therefore asked if *SAP30* exerts a similar effect on telomeric silencing, using the TPE assay described previously (Gottschling et al., 1990). Accordingly, enhanced silencing associated with TPE correlates with an increased population of cells resistant to 5-fluoro-orotic acid (5-FOA) due to diminished expression of the *URA3* gene ectopically positioned 2.0, 3.5, and 6.5 kb from the right-end telomere of chromosome V. The *sap30*Δ strains with *URA3* positioned 2.0 kb (2+) and 3.5 kb (3+) from the telomere grew significantly better than the isogenic *SAP30* strains in the presence of 5-FOA (Figure 2b). Thus, Sap30 counteracts genomic silencing in a manner similar to Rpd3 and Sin3.

Since disruption of yeast *RPD3* and *SIN3* causes transcriptional derepression (Vannier et al., 1996), we asked if disruption of *SAP30* confers a similar effect. In these experiments, transcription from *INO1-lacZ* and *TRK2-lacZ* reporter plasmids was monitored in isogenic wild-type, *sap30*Δ, *rpd3*Δ, and *sin3*Δ strains. In strains harboring the *INO1-lacZ* reporter, β-galactosidase levels were enhanced 3.3-fold in the *sap30*Δ mutant, compared to 8.9- and 4.8-fold in the *sin3*Δ and *rpd3*Δ mutants, respectively (Figure 2c). A similar derepression of *IME2-lacZ* expression was observed for *sap30*Δ, *sin3*Δ, and *rpd3*Δ mutants (data not shown). β-galactosidase activities were also derepressed in the *sin3*Δ and *rpd3*Δ mutants harboring the *TRK2-lacZ* reporter; however, no *TRK2-lacZ* derepression was observed in the *sap30*Δ mutant. This result offers an explanation for why *sap30* did not turn up in the initial selection that uncovered *rpd3* and *sin3* as transcriptional repressors (Vidal and Gaber, 1991; Vidal et al., 1991) and demonstrates that Sap30 can exert differential effects on gene expression in a promoter-dependent manner.

The above data establish a genetic link between Sap30 and the Rpd3–Sin3 complex and suggest that Sap30 functions within the same pathway. Consistent with this premise, *sap30*Δ *rpd3*Δ and *sap30*Δ *sin3*Δ double mutants, like *rpd3*Δ *sin3*Δ double mutants, are not only viable, but exhibit phenotypes indistinguishable from the single mutants (data not shown). Moreover, the finding that SAP30 is an integral component of the human HDAC/Sin3 complex suggests that yeast Sap30 is a component of the yeast Rpd3–Sin3 complex. We investigated this possibility using polyclonal antibodies generated against yeast Sap30 and Rpd3. Western blot analysis demonstrated that yeast Sap30 copurifies with Rpd3 through several fractionation steps (data not shown). Furthermore, Sap30 and Rpd3 were coimmunoprecipitated using antibodies directed against Rpd3 (Figure 2d, lanes 4) and Sap30 (lane 5). This complex appears to be distinct from the RNA pol II holoenzyme complex since antibodies directed against the CTD domain of Rpb1 failed to immunoprecipitate either Rpd3 or Sap30, but did immunoprecipitate RNA pol II and the Sin4 component of the RNA pol II holoenzyme (lane 3; Li et al., 1995). This result is consistent with previous results demonstrating that human (Zhang et al., 1997)

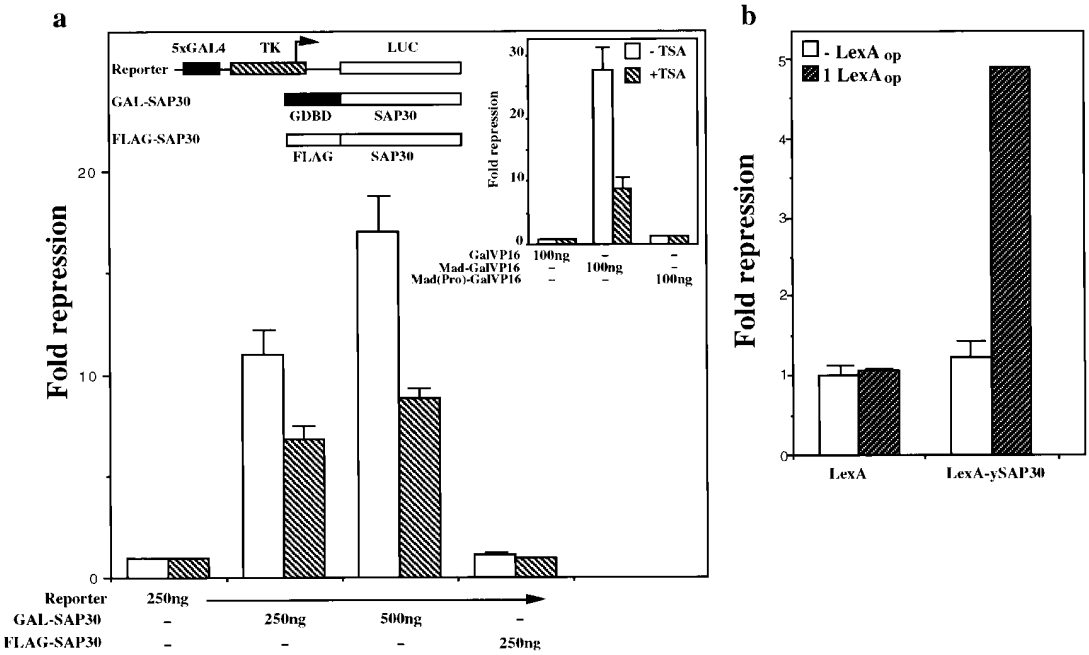

Figure 3. Human and Yeast Sap30 Repress Transcription In Vivo When Tethered to Promoter

(a) Human SAP30 represses transcription and this repression involves histone deacetylase. The top left insertion shows a schematic representation of the reporter and expression vectors used in the transfection experiments. 293T cells were transfected with Gal4-Tk-luc reporter in the presence or absence (−) of effector plasmids as indicated. The amount of plasmids used are also indicated. Transfected cells were either not treated (open bars) or treated (shadow bars) with 100 ng/ml Trichostatin A 8 hr before harvesting. The top right insertion shows that transcription repression conferred by histone deacetylase recruitment through Mad–Sin3 interaction is partially dependent on histone deacetylase activity as reported (Hassig et al., 1997). Transcription activity was determined by measuring luciferase activity. Transfection efficiencies were normalized using β-galactosidase assay. Transfections were repeated at least three times in duplicate.

(b) Yeast Sap30 represses transcription when tethered to promoter DNA. Yeast strain YMH171 (wt) was transformed with plasmid pM1175 (LexA-SAP30) or pBTM116 (LexA vector control), and either of two reporter plasmids, pLGΔ312S (*CYC1-lacZ*) or CK30 (*CYC1-lexAop-lacZ*). pLGΔ312S differs from CK30 by the presence of a single lexA operator site located upstream of the *CYC1* TATA element (Keleher et al., 1992). Three independent transformants of each strain were assayed for β-galactosidase activities. Data are presented as fold-repression relative to the activity of the control strain YMH171/pBTM116/pLGΔ312S and were determined for each strain by duplicate assays of three independent transformants. Absolute activities are 218 ± 34 (−LexAop/LexA), 176 ± 15 (1 LexAop/LexA), 235 ± 48 (−LexAop/LexA-SAP30), and 48 ± 1 (1 LexAop/LexA-SAP30) units of β-galactosidase activity.

and yeast (Kadosh and Struhl, 1997) Rpd3–Sin3 complexes are distinct from RNA polymerase II holoenzyme complexes.

Human SAP30 Is Functionally Associated with Histone Deacetylase

The above biochemical data establish that SAP30 is a component of a histone deacetylase complex in both human and yeast. Furthermore, the genetic data establish that Sap30 is required for the function of the Rpd3 complex because deletions of SAP30, RPD3, and SIN3 confer similar pleiotropic phenotypes. Since the HDAC/Rpd3 complex is involved in histone deacetylation and transcriptional repression, we asked whether the SAP30 complex functions in a similar manner. Previously, it was demonstrated that targeting histone deacetylase to a promoter, directly or through components of the histone deacetylase complex, resulted in transcriptional repression (Yang et al., 1996; Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Laherty et al., 1997; Nagy et al., 1997; Sommer et al., 1997; Zhang et al., 1997). Since SAP30 is a component of the histone deacetylase complex, we speculated that targeting SAP30 to a promoter would result in transcriptional repression. Accordingly, a reporter containing five Gal4-DNA binding sites upstream of the thymidine kinase (TK) promoter was cotransfected into 293T cells with a mammalian expression vector encoding SAP30 fused to the Gal4-DNA binding domain [Gal4(BD)-SAP30] or a Flag-tagged SAP30 (Figure 3a). The results demonstrated that targeting SAP30 to a promoter resulted in repression of transcription. Importantly, repression was dependent on histone deacetylase activity since repression was partially relieved in the presence of the histone deacetylase inhibitor trichostatin A (TSA, Figure 3a). The effect of TSA on overcoming SAP30-mediated repression was similar to that observed when Sin3 was tethered to the promoter via a *Mad-Gal4-VP16* DNA construct (Hassig et al., 1997; see top right insert in Figure 3a).

A similar effect on transcription was observed when Sap30 was tethered to promoter DNA in yeast (Figure 3b). For this experiment, we used *CYC1-lacZ* (pLGΔ312S) or *CYC1-lexAop-lacZ* (CK30) reporter plasmids that were introduced into strain YMH171 (wt) harboring plasmid DNA that expresses either LexA alone (pBTM316) or

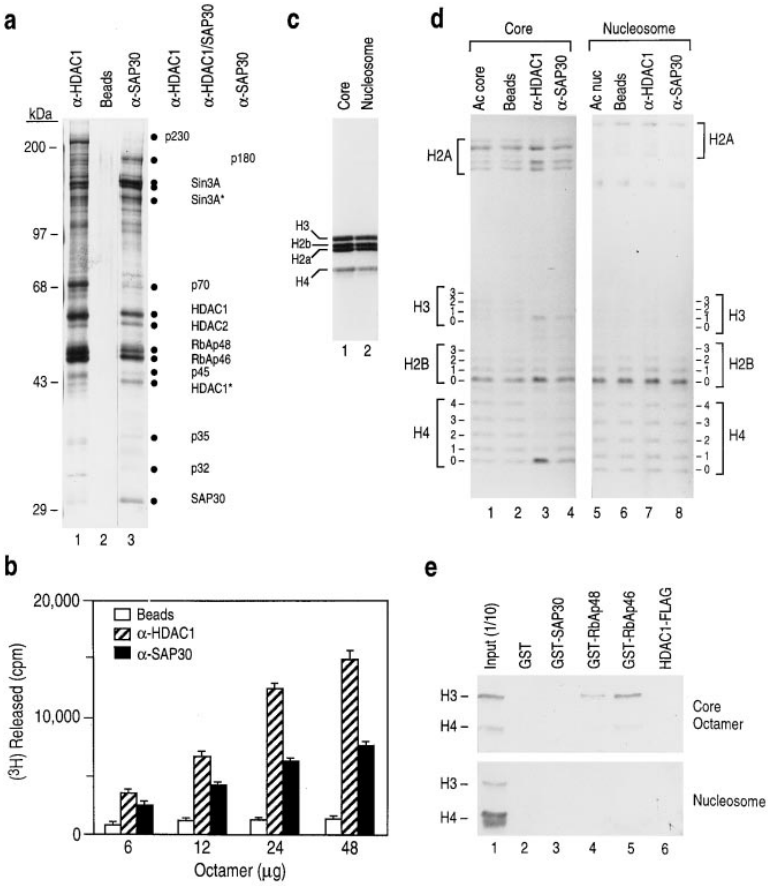

Figure 4. Both HDAC1 and SAP30 Complexes Have Histone Deacetylase Activity

(a) Silver staining of an SDS–PAGE of histone deacetylase complexes purified with anti-HDAC1 and anti-SAP30 antibodies. The previously characterized proteins and newly identified polypeptides are indicated. The newly identified polypeptides (p followed by a number indicating their apparent molecular weight) have been sequenced. Protein size markers are indicated.

(b) Histone deacetylase assays with ^{3}H-labeled HeLa core histone octamers. Histone deacetylase complexes used are parallel immunoprecipitates with that shown in (a). The amounts of substrates used are indicated. The reaction and quantitation are described in Experimental Procedures. Data shown represent the average of two independent assays with standard deviations. Beads represent immunoprecipitation with a control antibody.

(c) Coomassie staining of 2 μg of core histone octamers and nucleosomes resolved in an 18% SDS–PAGE. These histones are used as substrates for deacetylation assays shown in (d).

(d) Substrate specificity of the HDAC1 and SAP30 complexes assayed in TAU gels. 6 μg of highly acetylated HeLa core histone octamers (lanes 1–4) and nucleosomes (lanes 5–8) was deacetylated without (lanes 1 and 5) or with (lanes 2–4, lanes 6–8) immunoprecipitates using antibodies as indicated. The composition of the enzymes is shown in (a). Deacetylation reactions were performed as described in Experimental Procedures. The whole reactions were loaded onto and resolved in TAU gels and visualized with Coomassie staining. The different acetylated forms of histones H4, H3, and H2B are indicated.

(e) Western blots of GST pull-down assays. 2 μg of GST or GST fusion or Flag-tagged HDAC1 proteins was bound to 10 μl of glutathione-agarose beads or anti-FLAG M2 affinity gel and incubated with 5 μg of core histone octamers (upper panel) or nucleosomes (lower panel). The proteins remained bound after washing (see Experimental Procedures), were eluted, and were resolved in an 18% SDS–PAGE followed by Western blot analysis with antibodies specific for histones H3 and H4.

a LexA-Sap30 fusion protein (pM1175). LexA-Sap30 is functional since it complements the Cyh^{s} phenotype associated with *sap30*Δ (data not shown). CK30 differs from pLGΔ312S by the presence of a single LexA binding site located between the UAS and TATA elements of the *CYC1* promoter (Keleher et al., 1992). The LexA–Sap30 fusion resulted in 5-fold repression, and this effect was dependent upon the LexAop (Figure 3b). A similar tethering experiment demonstrated that a lexA–Rpd3 fusion caused repression of reporter gene expression (Kadosh and Struhl, 1997). Thus, targeting of either human or yeast Sap30 to promoter DNA confers transcriptional repression in a manner similar to targeted Rpd3 repression.

To analyze the function of SAP30 further, we purified the Sap30 and HDAC1 complexes using antibodies specific for each polypeptide. The polypeptide compositions of the two complexes are presented in Figure 4a. The identities of the polypeptides are assigned based on data obtained from protein microsequencing as well as from Western blot analysis (data not shown). Both complexes contain Sin3A, the Rb-associated polypeptides RbAp46 and RbAp48, the histone deacetylases HDAC1 and HDAC2, SAP30, and several other novel polypeptides. The relative abundance of these polypeptides varied between the two complexes. Moreover, the HDAC1 complex contains a polypeptide of 230 kDa that was apparently absent in the SAP30 affinity-purified complex. Similarly, the SAP30 affinity-purified complex includes a polypeptide of 180 kDa that was apparently absent, or drastically reduced, in the HDAC1 affinity-purified complex.

To analyze whether the above complexes have histone deacetylase activity, ^{3}H-labeled core histone octamers or oligonucleosomes were purified from HeLa cells and used as substrates. Equal amounts of enzyme complexes were incubated with different amounts of labeled substrate. Results shown in Figure 4b demonstrate that both complexes, when compared to the mock immunoprecipitates, are active in deacetylating core histones. The amount of label released is proportional to the amount of substrates used. The HDAC1-immunopurified complex was more active than the SAP30-immunopurified complex. Interestingly, neither complex was active when labeled nucleosomes were used as substrates (data not shown; see below).

To analyze the substrate specificity of the SAP30 and HDAC1 complexes, highly purified acetylated core histones as well as oligonucleosomes with an average size of 20 nucleosome array (Figures 4c and 4d, lanes 1 and 5) were used as substrates. Changes to the acetylation state of histone polypeptides were visualized using Tri-

ton acetic acid urea (TAU) gels. This analysis revealed that both complexes have similar activity in deacetylating histone H3. However, the HDAC1 complex is more efficient in deacetylating histone H4 compared to the SAP30 complex (Figure 4d, lanes 3 and 4). In agreement with the ^{3}H-acetate release assay presented above, neither complex was able to deacetylate nucleosomal histones (Figure 4d, lanes 7 and 8).

To understand why the native complexes were not able to deacetylate nucleosomal histones, we asked whether components of the complexes were able to recognize nucleosomal histones. Previous studies suggested that the Rb-associated polypeptides, which are present in different complexes involved in nucleosome remodeling and histone metabolism (Parthun et al., 1996; Taunton et al., 1996; Tyler et al., 1996; Verreault et al., 1996, 1997; Kaufman et al., 1997; Zhang et al., 1997; Martinez-Balbas et al., 1998), are likely to tether different complexes to histones. Therefore, we assayed the ability of RbAp46 and RbAp48, as well as SAP30 and HDAC1, to interact with core histone octamers or nucleosomal histones. A GST pull-down assay followed by Western blot analysis using antibodies specific for histones H3 and H4 demonstrated that RbAp46 and RbAp48 were capable of interacting with the histone octamer (Figure 4e, upper panel). However, these interactions were precluded when the histones were in association with DNA, i.e., in the form of nucleosomes (Figure 4e, lower panel). Identical results were observed when acetylated core octamers or acetylated oligonucleosomes were used in this assay (data not shown). These results support the idea that deacetylation of nucleosomal histones requires recruitment of the histone deacetylase complexes to DNA.

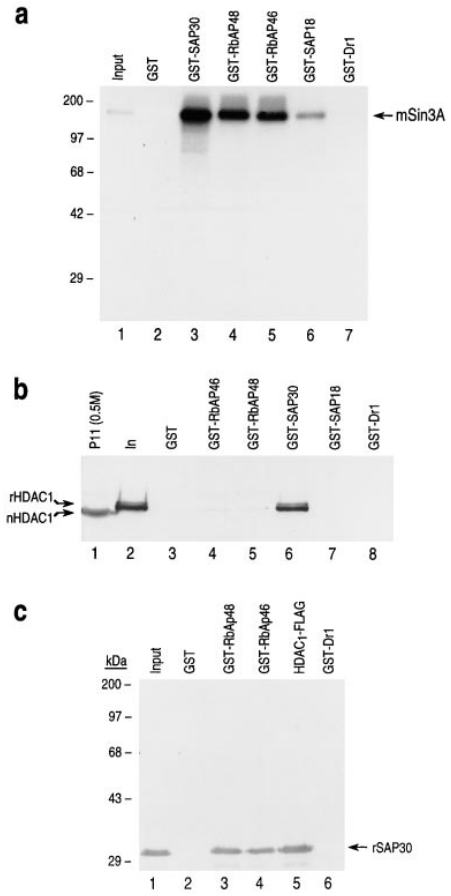

Figure 5. SAP30 Directly Interacts with HDAC1

(a) GST pull-down assays show that Sin3A strongly interacts with SAP30 in vitro. Equal amounts (3 μl) of in vitro translated and labeled Sin3A were incubated with 10 μl of glutathione-agarose beads covered with 2 μg of GST or GST fusion proteins as indicated. After extensive wash, bound proteins were eluted, resolved on SDS–PAGE, and visualized by autoradiography. About 1/10 μl of labeled Sin3A was loaded as Input.

(b) SAP30 directly interacts with HDAC1 in vitro. Western blot analysis of GST pull-down assays using antibodies against HDAC1. The GST pull-down was performed as described in (a). 1 μg of Flag-tagged HDAC1 purified from baculovirus-infected Sf9 cell extracts was used as input for the pull-down. Lane 2 represents 1/10 of the amount used in the pull-down.

(c) SAP30 directly interacts with HDAC1, RbAP46, and RbAp48 in vitro. GST pull-down assays were performed as in (b). Bound proteins were revealed by Western blot analysis using anti-SAP30 antibodies. Lane 1 represents 1/10 of the amount used in the pull-down.

SAP30 Interacts Directly with HDAC1

To gain insight into the function of SAP30 within the histone deacetylase complexes, we analyzed protein–protein interactions among the characterized components. Because Sap30 was originally identified as a component of Sin3A complex, we first analyzed whether Sap30 interacts with Sin3A. Since our efforts to express full-length Sin3A in different expression systems failed, we translated Sin3A in vitro using the reticulocyte lysate system and performed GST pull-down assays. Sin3A strongly interacted with Sap30, as well as with the RbAp polypeptides in this assay (Figure 5a). Consistent with our previous finding, Sap18 also interacted with Sin3A, although to a lesser extent than Sap30 (Figure 5a). All interactions were specific, since neither GST alone nor GST fused to the repressor Dr1 interacted with Sin3A (Figure 5a).

Toward reconstituting histone deacetylase activity in vitro, we asked which polypeptides directly interact with HDAC1, the catalytic subunit of the histone deacetylase complex. GST pull-down assays demonstrated that recombinant HDAC1 purified from baculovirus-infected Sf9 cells directly interacts with bacterially produced GST-Sap30 (Figure 5b). The interaction was specific because GST alone or GST fused to the Rb-associated polypeptides or GST-Dr1 failed to pull-down HDAC1 (Figure 5b). Importantly, a similar interaction between Sap30 and HDAC1 was demonstrated when HDAC1 was bound to anti-FLAG coated beads (Figure 5c, lane 5). The inability of the Rb-associated polypeptides to interact with HDAC1 was not the result of inactive polypeptides, as these polypeptides directly interacted with Sap30 (Figure 5c). Therefore, Sap30 interacts directly with HDAC1, as well as with the Rb-associated polypeptides.

The inability to demonstrate a direct interaction between HDAC1 and RbAp48 was surprising since initial purification of the histone deacetylase HDAC1, using trapoxin affinity matrix, identified HDAC1 as well as an RbAp48 (Taunton et al., 1996). Therefore, we analyzed the interaction further using in vitro translated HDAC1

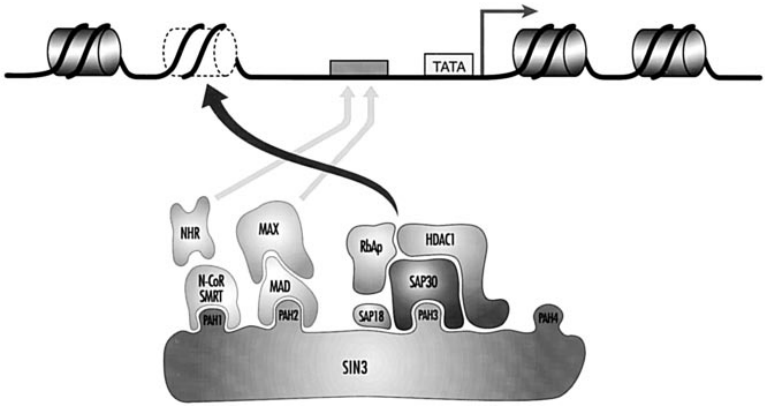

Figure 6. Model for Nucleosomal Histone Deacetylation and Transcriptional Repression

Histone deacetylase complexes are recruited to promoter elements through DNA-binding proteins that interact with Sin3 or other components of the complex. This in turn enables RbAp to interact directly with core histones of the remodeled nucleosome, allowing deacetylation of the core histones. Deacetylation of core histones results in the formation of more compact nucleosomes leading to transcriptional repression. The polypeptides reported to be in Sin3-containing complexes are shown. The polypeptides are not shown according to their mass, but only denoting their interactions among components of the complex.

and found that both GST-RbAp46 and GST-RbAp48 interacted under these conditions (data not shown). Moreover, an interaction between RbAp48 and HDAC1 could be demonstrated from Sf9 cell extracts derived from a coinfection (data not shown). However, to our surprise, RbAp48 failed to coimmunoprecipitate with HDAC1 if separately infected cell extracts were mixed (data not shown). This result suggests that HDAC1 and RbAp48 may need to fold together to form a stable complex. Alternatively, a molecular chaperon may be required to form the RbAp48–HDAC1 complex.

Discussion

In the present study, we present biochemical as well as genetic evidence demonstrating that the novel protein Sap30 exists in a complex with the histone deacetylases Rpd3/HDAC in yeast and mammalian cells. We also show that this association is functional since antibodies directed against Sap30 immunoprecipitated a protein complex that is active in deacetylating histone octamers, in particular H3. Consistent with our finding that Sap30 is a component of histone deacetylase complexes and the notion that targeted histone deacetylation results in transcriptional repression, Sap30 represses transcription when tethered to a promoter. Histone deacetylase activity is at least partially responsible for this repression. The fact that *sap30* and *rpd3* deletions confer similar phenotypes indicates that Sap30 plays a critical role in the normal function of the Rpd3 complex in vivo.

SAP30 Is Functionally Associated with Histone Deacetylase HDAC/Rpd3

Sap30 was isolated as a component of the Sin3/HDAC complex. Protein–protein interaction studies demonstrated that Sap30 interacts directly with multiple components of the Sin3/HDAC complex. Of particular interest is the ability of Sap30 to interact directly with the histone deacetylase HDAC1 (Figures 5b and 5c). Several lines of evidence indicate that this interaction is functionally important. First, disruption of *SAP30* in yeast confers a set of phenotypes similar to those associated with disruption of *RPD3* and *SIN3*, including cycloheximide sensitivity, mating and sporulation defects, and enhanced telomeric silencing (Table 1 and Figure 2). In light of the fact that human and yeast Sap30 exist in the same complex with HDAC/Rpd3 (Figures 1 and 2), the similar phenotypes strongly suggest that Sap30 is required for the normal function of the Rpd3 complex. Secondly, targeting of human and yeast Sap30 to promoter DNA resulted in transcriptional repression, and mammalian Sap30-mediated repression is at least partially dependent upon histone deacetylase activity (Figure 3).

The observation that HDAC1 complexes isolated by different laboratories have different compositions suggests that multiple HDAC1 complexes may exist in vivo (Taunton et al., 1996; Zhang et al., 1997; and Figure 4a). It appears that Sap30 might be present in a subset of these complexes. This is supported by biochemical experiments demonstrating that Sap30 eluted in a sharp peak corresponding to the beginning of a broad elution profile of HDAC/Sin3 in a gel filtration column (data not shown). It is also supported by the fact that SAP30 can be depleted from nuclear extracts that still contain HDACs (data not shown). Consistent with the premise that Sap30 is not present in all HDAC/Rpd3 complexes is the observation that *TRK2* gene expression is not affected by disruption of *SAP30*, but is derepressed by disruption of either *RPD3* or *SIN3* (Figure 2c). The notion that Sap30 is present only in a subset of HDAC complexes raises the possibility that Sap30 may regulate HDAC/Rpd3 activity. Direct interaction of Sap30 with HDAC1 suggests that histone deacetylase might be recruited to promoter DNA in a Sin3-independent manner. In support of this possibility, yeast Sap30 can repress transcription in a *sin3*Δ mutant (data not shown). In addition, we consistently found that Sap30 immunoprecipitates have histone H3 deacetylase activity similar to HDAC1 immunoprecipitates, yet have much weaker activity directed toward histone H4 (Figure 4d, lanes 3 and 4). The ability of HDAC1 immunoprecipitates to deacetylate both H3 and H4 in vitro is consistent with the substrate specificity of yeast Rpd3 observed in vivo (Rundlett et al., 1996).

Nucleosomal Histone Deacetylation Requires Targeting

The discovery that histone deacetylase complexes contain polypeptides that can directly interact with core histones raises the possibility that histone deacetylases

may work on a genome-wide basis through RbAp–histone interaction. If this is the case, loss of histone deacetylase activity might be expected to cause a general increase in histone acetylation, resulting in a global increase in gene expression. However, disruption of *RPD3* in yeast, or treatment of human cells with histone deacetylase inhibitors, did not cause global gene activation. On the contrary, only a limited number of genes are affected, and some genes are repressed rather than activated (Vidal and Gaber, 1991; Vidal et al., 1991; Van Lint et al., 1996). One possible explanation is that histone deacetylase is recruited to only a set of genes through the interaction between DNA-binding proteins and components of the histone deacetylase complex. This hypothesis gained experimental support when Sin3, a corepressor known to interact with DNA-binding protein, was found in the same complex as HDAC/Rpd3 (Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Kasten et al., 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997).

To reconstitute transcriptional repression through histone deacetylation, first we investigated whether nucleosomal histones could be deacetylated. Using a histone deacetylase complex isolated from HeLa cell extracts, we showed that this histone deacetylase complex is able to deacetylate core histone octamers but unable to deacetylate nucleosomal histones (Figure 4d). This result is surprising since the histone deacetylase complex contains the RbAp46 and RbAp48 polypeptides that are capable of interacting directly with core histones (Verreault et al., 1997; Y. Z. and D. R., unpublished data). One possible explanation for this result comes from a detailed mapping of the histone domains involved in histone–RbAp interaction. Helix 1 of histone H4 is involved in the histone–RbAp interaction (Verreault et al., 1997; Y. Z. and D. R., unpublished data), yet helix 1, which contains multiple DNA contact sites, is sequestered when complexed with DNA (Luger et al., 1997). In fact, our GST pull-down assays (Figure 4e) demonstrated that RbAps were unable to interact with nucleosomal histones. Inability to access nucleosomal histones may also explain why the Hat1 holoenzyme complex, which contains RbAp46 or its yeast homolog, was unable to acetylate nucleosomal histones (Parthun et al., 1996; Verreault et al., 1997).

The inability of the histone deacetylase complex to interact with nucleosomal histones provides evidence for targeted histone deacetylation. As shown in Figure 6, histone deacetylase complexes can be recruited to a promoter by sequence-specific DNA-binding proteins such as Mad-Max (Hassig et al., 1997; Laherty et al., 1997; Zhang et al., 1997), nuclear hormone receptor (Alland et al., 1997; Heinzel et al., 1997; Nagy et al., 1997), DP1-E2F (Brehm et al., 1998; Luo et al., 1998; Magnaghi-Jaulin et al., 1998), or other DNA-binding proteins. After recruitment, the histone deacetylase could be further targeted to the opened nucleosomal histones through RbAp–histone interactions. Deacetylation of the core histone tails resulted in the formation of a more compacted nucleosomal structure, which prevents preinitiation complex formation and reinitiation (Sheridan et al., 1997). In light of the observations that nonhistone proteins can also be acetylated (Gu and Roeder, 1997; Imhof et al., 1997), recruited histone deacetylase may repress transcription by deacetylating these factors. The availability of a histone deacetylase complex in conjunction with a nucleosome assembly system (Orphanides et al., 1998) will allow us to test these possibilities.

Experimental Procedures

Purification of mSin3, HDAC1, and SAP30 Complexes and SAP30 Cloning

Purification of mSin3, HDAC1, and SAP30 complexes was based on previously published procedures (Zhang et al., 1997) using the DEAE-52 fraction as input. Affinity-purified antibodies against the PAH2 domain of mSin3, or against the C-terminal domain of HDAC1, or against the full-length of SAP30 were coupled to 1 ml of protein A–agarose beads (Repligen) as described (Harlow and Lane, 1988). The resin was incubated overnight at 4°C with the DEAE-52 pool (4 ml, 1.8 mg/ml). Immunoadsorbed complexes were then washed extensively with buffer C containing 700 mM KCl. The bound proteins were either used directly for enzymatic assays or eluted with 0.1 M glycine (pH 2.6). Eluates derived from the immunoaffinity column steps were concentrated on a micro Mono S column using the SMART system. The Ponceau S–stained SAP30 band was excised, in situ tryptic-digested, and fractionated, and selected fractions were sequenced as described (Zhang et al., 1997). The obtained peptide sequences match several EST cDNA clones. One of the EST clones (AA128147) was obtained from Research Genetics (Huntsville, AL) and used to make probes for screening a HeLa λZAP cDNA library (Stratagene). The predicted human SAP30 amino acid sequences were used to identify a yeast homology (P38429). The *SAP30* ORF of yeast was cloned by PCR.

Yeast Strains

Strains YMH265 (*sin3::LEU2*), YMH270 (*rpd3::LEU2*), and YMH277 (*sap30::LEU2*) were derived from strain YMH171 by one-step gene disruption of the indicated locus. Strains UCC506 (URA3-TEL-VR 2+), UCC508 (URA3-TEL-VR 3+), or UCC510 (URA3-TEL-VR 4+) were derived from strain YPH250 by integration of the *URA3* gene 2.0 (2+), 3.5 (3+), or 6.5 (4+) kb from the right end of chromosome V and were described previously (Gottschling et al., 1990).

Purification of HeLa Nucleosomes and Core Histones

Nucleosomes and core histones were purified from HeLa cells using a modification of a published procedure (Ausio and van Holde, 1986). For the purification of acetylated nucleosomes and core histones, the same procedure was followed except sodium butyrate (final of 10 mM) was added to the cell culture 24 hr before harvesting. The same concentration of sodium butyrate was present in all buffers during purification and was eliminated at the last step by dialyzing into storage buffer (10 mM HEPES [pH 7.5], 1 mM EDTA, 10 mM KCl, 0.2 mM PMSF, 10% glycerol). A published procedure (Carmen et al., 1996) was followed for the labeling of histones in HeLa cells. After labeling at 37°C for 2 hr, the labeled nucleosomes and core histones were purified in the presence of 10 mM of sodium butyrate using the above procedure. The purified histone octamer has a specific activity of 3×10^6 cpm/mg.

Histone Deacetylase Assays and TAU Gel

Histone deacetylase assays were performed using a modification of a published procedure (Hendzel et al., 1991). Immunoprecipitated complexes using 10 μl of antibody-coated protein A–agarose beads were washed extensively as described above. The complexes were equilibrated with histone deacetylation buffer (75 mM Tris [pH 7.0], 150 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 1 mM PMSF) before incubating with nucleosomes or core histone octamers at 30°C for 1 hr. The released [^{3}H]acetic acid was extracted with ethyl acetate and quantified by liquid scintillation counting as described (Hendzel et al., 1991) (Figure 4b). Alternatively, deacetylation was monitored by resolving histones in TAU gel followed by Coomassie staining using a modification of a published procedure (Lennox and Cohen, 1989).

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