Sds3 (Suppressor of Defective Silencing 3) Is an Integral Component of the Yeast Sin3-Rpd3 Histone Deacetylase Complex and Is Required for Histone Deacetylase Activity*

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SDS3 (suppressor of defective silencing 3) was originally identified in a screen for mutations that cause increased silencing of a crippled HMR silencer in a rap1 mutant background. In addition, sds3 mutants have phenotypes very similar to those seen in sin3 and rpd3 mutants, suggesting that it functions in the same genetic pathway. In this manuscript we demonstrate that Sds3p is an integral subunit of a previously identified high molecular weight Rpd3p-Sin3p containing yeast histone deacetylase complex. By analyzing an sds3Δ strain we show that, in the absence of Sds3p, Sin3p can be chromatographically separated from Rpd3p, indicating that Sds3p promotes the integrity of the complex. Moreover, the remaining Rpd3p complex in the sds3Δ strain had little or no histone deacetylase activity. Thus, Sds3p plays important roles in the integrity and catalytic activity of the Rpd3p-Sin3p complex.

Numerous studies in the past have linked acetylation of core histones to transcriptional regulation (1). The identification of co-activator proteins as histone acetyltransferases (HATs)¹ has strengthened the connection between histone acetylation and transcription (2–5). In yeast several distinct HAT complexes have been identified that modify nucleosomal histones (5–9). Acetylation of nucleosomal histones by these HAT complexes stimulates transcription from preassembled chromatin templates (10, 11), and these complexes are targeted by direct interactions with transcriptional activators (12, 13).

To counteract the effect of HAT complexes, it is necessary to reverse acetylation by efficiently deacetylating nucleosomal histones. Histone deacetylase complexes (HDACs) perform this reaction. HDACs have been isolated and characterized in several organisms as multiprotein complexes that are associated with DNA binding repressors and co-repressors (14–22). The majority of these complexes contain members of the Rpd3p-HDAC-related protein family as catalytic subunits (23). In addition to these Rpd3p-related HDAC complexes, two non-Rpd3p-related deacetylase complexes have been identified in Zea mays (24, 25). Moreover, yeast HDAC complexes containing Hda1p and Hso3p as catalytic subunits have been identified (26, 27).

Two yeast multicomponent HDAC complexes have been found to contain Rpd3p (26, 28). The larger of these complexes was also found to contain the Sin3p co-repressor (28). Sin3p is thought to mediate interactions of the Sin3-Rpd3 complex with sequence-specific DNA binding repressors such as Ume6p, which leads to a localized deacetylation of histones H3 and H4 and repression of transcription in vivo (19, 29). RPD3 and SIN3 were both originally identified in genetic screens as regulators of gene expression (30, 31). Mutations in RPD3 and SIN3 affect transcription of the same set of genes (32). SIN3 and RPD3 were identified among at least 19 other genes in a screen for extragenic suppressors of a silencing defective rap1 allele (rap 1-12) (33, 34). Another gene identified in this screen is SDS3 (suppressor of defective silencing 3). Although mutations in SDS3 were shown to cause several phenotypes in common with sin3 and rpd3 mutants, they did not appear to derepress a plasmid-borne TRK2 gene, raising the possibility that SDS3 might function independently of SIN3 and RPD3 (33). However, a recent study found that a mutation in SDS3 reduced Sin3p-mediated repression and that Sds3p and Sin3p could be co-immunoprecipitated from cell extracts (35). These results illustrated that SDS3 functions in the same genetic pathway as SIN3 and that Sds3p and Sin3p can interact in some way.

In this manuscript we demonstrate that Sds3p is an integral subunit of a Rpd3p-Sin3p-containing yeast HDAC complex with an apparent molecular mass of 1.2 MDa. By analyzing a sds3Δ strain we show that, in the absence of Sds3p, Sin3p could be chromatographically separated from Rpd3p, indicating that Sds3p promotes the integrity of this complex. In addition, the remaining Rpd3p complex in the sds3Δ strain had little or no histone deacetylase activity. Thus, Sds3p plays important roles in the integrity and catalytic activity of the Rpd3p-Sin3p complex.

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⁴ The abbreviations used are: HATs, histone acetyltransferases; HDAC, histone deacetylase complex; HA, hemagglutinin; bp, base pair(s); PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.
EXPERIMENTAL PROCEDURES

Yeast Strains—The sds3A strain in the W303 background (36) was generated as described previously (37). The Sds3-HAp construct was made by inserting three copies of the HA epitope in the 3′-end of the SDS3 open reading frame. A unique NotI site was engineered into +978 of the 984-bp SDSL3 open reading frame. A NotI cassette containing three copies of the HA epitope (YPYDDDDY) was inserted and cloned into 2-μm plasmid pRS423. The resulting clone, DV-246, complements the sds3A mutation in a manner identical to the untagged SDSL3 gene.

Preparation of Whole Cell Extracts and Purification of the Rpd3p Complex—Strains were grown to an optical density of 1.5 as described previously (37), and cells of a 12-liter culture were harvested by centrifugation at 3000 × g for 10 min. The cell pellets were resuspended, and lysed using a glass bead-beater (Biospec), and the resulting extracts were loaded onto Ni²⁺-agarose as described (37). After Ni²⁺-agarose chromatography, the imidazole eluate was loaded directly onto a Mono-Q HR5/5 column (Amersham Pharmacia Biotech) to separate HDAC complexes. Bound proteins were eluted with a linear gradient of 0 to 500 mM NaCl in 40 mM Hepes, pH 7.8, 10% glycerol, 0.1% Tween 20, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 1 mM PMSF, 0.5 mM DTT). Fractions of 0.5 ml were collected and assayed for HDAC activity as described previously (38). In general, 10 μl of each fraction was incubated with 3 μg of trichium-labeled chicken reticulocyte core histones (38). Samples were collected for 30 min at 30 °C, and the released radioactivity was measured as described (38).

Fractions containing HDAC activity were pooled separately, concentrated to 500 μl, and processed over a Mono-Q column and a Superose 6 column (Amersham Pharmacia Biotech) to determine the native molecular weight. The column was run in 350 mM NaCl in 40 mM Hepes, pH 7.8, 10% glycerol, 0.1% Tween 20, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 1 mM PMSF, 0.5 mM DTT at a flow rate of 0.2 ml/min. Fractions of 0.5-ml volume were collected and assayed for HDAC activity. Aliquots of fractions containing HDAC activity were applied to SDS-PAGE and subject to Western blot analysis as described (39). The flow-through fraction of 0.6- and 1.2-MDa Rpd3p complexes were collected and assayed for HDAC activity. Fractions containing HDAC activity were applied to SDS-PAGE and subject to Western blot analysis as described (39).

Immunoprecipitation, Modified HDAC Assay for Immunoprecipitation—Antibodies for HDAC (Covance) and Rpd3p (Upstate Biotechnology) were coupled to protein A-Sepharose beads. 40 μl of 50% bead slurry was washed twice with 200 μl of binding buffer (150 mM NaCl, 40 mM Hepes, pH 7.5, 10% glycerol, 0.1% Tween 20, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 2 μg/ml PMSF, 0.2 mM DTT, and 1 mM PMSF). Beads were recovered by centrifugation at 110 × g for 1 min in a table-top centrifuge after each wash step. 20 μl of antibody solution (20 μg) was added to the beads, and the antibodies were bound to the beads by rotation on a wheel for 1 h at room temperature. Antibodies were cross-linked to beads as described previously (39). Superose 6 chromatography peak fractions were diluted to the appropriate salt concentration of 150 mM NaCl with binding buffer. Beads were concentrated with Microcon 10 concentrators (Amicon) to the original volume, and 20 μl of sample was added to 20 μl of antibody beads. The binding reaction was performed for 4 h or overnight with a rotation wheel at 4 °C. After the binding reaction, beads were recollected by centrifugation, the supernatant was removed and saved, and beads were washed twice with binding buffer. HDAC assays were performed with beads and supernatant. 20 μl of 1 μg/ml radiolabeled chicken erythrocyte core histones were added to samples and incubated for 3 h at 30 °C with rotation. HDAC activity was measured as described previously (38).

Whole cell extract for immunoprecipitation was prepared from 100 ml of the Sds3-HAp expression strain by glass bead disruption into extraction buffer (40 m1 Hepes, pH 7.5, 350 mM NaCl, 10% glycerol, 0.1% Tween 20, 1 mM PMSF, 1 mM DTT, 2 μg/ml pepstatin A, 2 μg/ml leupeptin). For immunoprecipitation, 200 μg of whole cell extract total protein, as determined by Bradford assay, was diluted to 150 mM NaCl in extraction buffer lacking NaCl. The diluted extract was mixed for several hours at 4 °C in the presence or absence of 4 μg of antibody directed against Rpd3 or 1 μl of antibody directed against Sin3. Immune complexes were collected by mixing antibody- or mock-treated extracts with protein A-Sepharose for several hours at 4 °C. After several washes in binding buffer (see above), input extract, unbound supernatants, and bound bead material were subjected to Western blot analysis using antibody against the HA epitope tag.

For Western blot analysis, beads and supernatant were boiled in Laemmli SDS sample buffer for 10 min and applied to 10% SDS-PAGE with subsequent Western blotting as described (39).

RESULTS

Identification of Yeast HDAC Complexes—Several yeast HAT complexes bind to Ni²⁺-agarose, which concentrates these activities from yeast whole cell extract for further purification (37). We tested if HDAC activities might also be found in the Ni²⁺-agarose eluant to determine if this material might also serve as a starting point for purifying HDAC multiprotein complexes. Whole yeast cell extract of a Sds3-HAp strain was prepared and bound to Ni²⁺-agarose. The flow-through and eluates from Ni²⁺-agarose were then subjected to Mono-Q chromatography followed by Superose 6 size exclusion chromatography (Fig. 1). We detected three HDAC activities by this procedure. These included an Rpd3-containing complex of approximately 0.6 MDA (data not shown), which was found in the Ni²⁺-agarose flow-through and is presumably related to that described by Rundlett and co-workers (26). We did not observe co-fractionation of Sds3-HAp with this Rpd3 complex (data not shown). Two additional HDAC complexes eluted together from the Ni²⁺-agarose but were separated on the subsequent Mono-Q column (Fig. 2A). Using Western blot analysis we found that Rpd3p, Sin3p, and Sds3-HAp co-fractionated with the HDAC complex eluting at approximately 0.35 M NaCl (Fig. 2A). A second HDAC activity eluted at approximately 0.25 M NaCl, and subsequent Western blot analysis indicated that it co-elutes with Hda1p (Fig. 2A).

Co-fractionation of Sds3p, Rpd3p, and Sin3p—To further test this, Rpd3p is an actual component of the Rpd3p-HDAC complex, we tested for co-elution of Sds3-HAp with Rpd3p, Sin3p, and HDAC activity by gel filtration chromatography. Mono-Q fractions containing the Rpd3p complex from the Sds3-HAp strain (fractions 28–34) were pooled, concentrated, and applied to a Superose 6 size exclusion column. The HDAC activity eluted at an apparent molecular mass of 1.2 MDA (Fig. 2B). This was consistent with our earlier findings showing the same
molecular weight for this particular HDAC complex from a wild type strain (data not shown). Aliquots of fractions 16–24 of Superose 6 size exclusion chromatography were applied to SDS-PAGE. Subsequent Western blotting with antibodies for Rpd3p, Sin3p, and HA (Sds3p) demonstrated co-elution of all three of these proteins with the deacetylase activity peak (Fig. 2B). The high molecular weight of this complex and the fact that it contains Sin3p and Rpd3p suggest that it is related to or identical to the complex described by Kasten and colleagues (28).

Co-immunoprecipitation of Sds3p, Rpd3p, Sin3p, and HDAC Activity—To confirm that Sds3p is a bona fide subunit of the 1.2-MDa Rpd3p complex, we tested for co-immunoprecipitation of these two proteins and HDAC activity from the Superose fractions. We used fraction 19 of the Superose 6 size exclusion chromatography of the Sds3-HA strain, which corresponds to the HDAC activity peak (Fig. 2B) and performed co-immunoprecipitation experiments. Samples were incubated with HA antibodies coupled to beads, the beads were pelleted, and the HDAC activity of supernatant and beads was tested. Fig. 3A shows that the HDAC activity was clearly immunoprecipitated with the HA antibodies, illustrating that Sds3p is part of the HDAC complex. As a control we incubated samples of a HDAC peak fraction of a Superose 6 size exclusion chromatography of a SDS3Δ (WT) strain with HA antibodies coupled to beads. Fig. 3B (group 1) shows that HDAC activity was not immunoprecipitated with the HA antibodies from this strain, ruling out any nonspecific interaction of HDAC activity with the Rpd3 complex. Moreover, the HDAC activity of the Rpd3p complex from both the Sds3-HA and wild type strains immunoprecipitated with Rpd3 antibodies coupled to beads (Fig. 3B, groups 2 and 3). To further confirm the co-immunoprecipitation of Sds3p and Rpd3p, Western blots of the immunoprecipitations with anti-HA were performed. In Fig. 3C (upper panel), Rpd3p and HA-Sds3p can be detected in the bead fraction using fraction 19 of the Superose 6 column of the Sds3p-expressing strain, whereas no signal is detectable in the supernatant. Lanes 2 and 3 of Fig. 3C (lower panel) show co-immunoprecipitation of Rpd3p with antibody against Rpd3 using Superose 6 peak fraction of the WT, untagged strain. By contrast, no Rpd3p protein was immunoprecipitated with antibodies for HA from this strain (Fig. 3C, lower panel, lanes 4 and 5).

Further confirmation that Sds3 associates with the Rpd3-Sin3 complex came from immunoprecipitation experiments performed in crude extracts. Antibodies directed against either Rpd3 or Sin3 immunoprecipitated Sds3 from whole cell extract (Fig. 3D) (35). As expected, antibody directed against Rpd3 was also able to immunoprecipitate Sin3 from the same extract (data not shown). These results indicate that, in addition to highly fractionated preparations, Rpd3, Sin3, and Sds3 associate within cell extracts.

Deletion of SDS3 Alters the Chromatographic Behavior of the Rpd3-Sin3 Complex—To investigate if the integrity of the 1.2-MDa HDAC complex was dependent on SDS3, we prepared whole cell extracts from a sds3Δ strain and applied it to Ni2+-agarose followed by Mono-Q chromatography. The HDAC activity in the fractions normally containing the WT 1.2-MDa Rpd3p complex (28–32) was decreased (Fig. 4A, HDAC activity of sds3Δ) compared with that of wild type (compare with Fig. 2A, HDAC activity of the Sds3-HAp-expressing strain). Subsequent Western blotting of fractions 19–32 of this Mono-Q column showed a different elution profile of Sin3p and Rpd3p. Both proteins eluted at a lower salt concentration from the column compared with wild type (0.2–0.3 M NaCl instead of 0.35 M NaCl). Rpd3p and Sin3p eluted in the range of Hda1p. Hda1p peaked with the main HDAC activity at fractions 22–24, which was comparable to the wild type elution pattern for Hda1p.

The HDAC Activity of the Rpd3 Complex Depends on SDS3—To determine if the deletion of SDS3 affects not only the elution profile of Sin3p and Rpd3p, but also the integrity and activity of the complex, we pooled fractions 20–32 of the Sds3Δ Mono-Q chromatography, concentrated the material, and applied it to Superose 6 size exclusion chromatography. The HDAC activity was examined as described, and corresponding aliquots were analyzed by SDS-PAGE and subsequent Western blotting. Fig. 4B shows that the main HDAC activity eluted at approximately 0.6 MDa and corresponds to Hda1p, indicating that the deletion of SDS3 does not affect a 0.6-MDa Hda1p complex. However, the remaining Rpd3 com-
plex had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.

Deletion of SDS3 Decreases the Interaction between Sin3p and Rpd3p—The elution of Sin3p from the Superose 6 column (Fig. 4B) overlapped with that of Rpd3p. However, these proteins had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.

Deletion of SDS3 Decreases the Interaction between Sin3p and Rpd3p—The elution of Sin3p from the Superose 6 column (Fig. 4B) overlapped with that of Rpd3p. However, these proteins had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.

Deletion of SDS3 Decreases the Interaction between Sin3p and Rpd3p—The elution of Sin3p from the Superose 6 column (Fig. 4B) overlapped with that of Rpd3p. However, these proteins had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.

Deletion of SDS3 Decreases the Interaction between Sin3p and Rpd3p—The elution of Sin3p from the Superose 6 column (Fig. 4B) overlapped with that of Rpd3p. However, these proteins had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.

Deletion of SDS3 Decreases the Interaction between Sin3p and Rpd3p—The elution of Sin3p from the Superose 6 column (Fig. 4B) overlapped with that of Rpd3p. However, these proteins had a smaller molecular weight compared with wild type complex (Fig. 2B). Western blot analysis revealed a signal for Rpd3 at fractions 20 and 21, which corresponds to an approximate molecular mass of 0.9 MDa. Importantly, this 0.9-MDa Rpd3 breakdown did not have HDAC activity.
applied to Superose 6 on the SMART system at 0.5 M NaCl. Rpd3 eluted in the range of 0.7–0.5 MDa, which corresponds to the measured HDAC activity. Hda1p eluted at approximately 0.6 MDa, corresponding to an approximate molecular mass of 0.9 MDa. No HDAC activity was detected in this molecular range. Sin3p was separated into distinct subcomplexes in the absence of Sds3p. Sin3p was chromatographed from the control for ing strain were analyzed on the Superose 6 column at 0.5 M NaCl using the SMART system. Mono-Q fractions 20–36 (Fig. 2B) were applied to Superose 6 on the SMART system. Two HDAC peaks were separated corresponding to the 1.2-MDa Sin3p, Rpd3p, and Sds3-HAp complex, and the 0.6-MDa Hda1p complex, respectively.

To determine whether Sds3p is part of the Rpd3p-Sin3p complex, we reintroduced an HA-tagged SDS3 gene into the sds3Δ strain. The reintroduced gene rescued the complex. We used this strain to partially purify the complex and used Western blot analysis to illustrate co-elution of Sin3p, Rpd3p, and Sds3-HAp in the absence of Sin3p (20). Disruption of SAP30 in yeast shows phenotypes comparable to disruption of SIN3 and RPD3, suggesting that it works in the same genetic pathway. Moreover, Sap30p and Rpd3p have been shown to co-immunoprecipitate with antibodies against Rpd3p (40).

Deletion of another yeast gene, SDS3, also showed similar phenotypes to deletions of SIN3 and RPD3. The yeast SDS3 gene was originally identified in a screen for mutations that cause increased silencing of a crippled HMR silencer in a rap1 mutant background (33, 41). This screen identified more than 20 other genes, including SIN3 and RPD3. Subsequent analysis showed that SDS3 shares several transcription regulation properties with SIN3/RPD3, although epistasis tests of the silencing effect suggested that SDS3 might differ in function, at least subtly, from RPD3/SIN3 (35). However, more recent work has shown that sds3 mutants have phenotypes very similar to those seen in sin3 and rpd3 mutants, with transcriptional regulation of the same set of genes affected in all three mutants (35). The changes in transcriptional regulation seen in rpd3/sds3 and sin3/rpd3 double mutants are no more severe than the single mutants. This genetic analysis indicates that SDS3 is in the same functional pathway as RPD3 and SIN3, and this idea is supported by co-immunoprecipitation experiments showing that Sds3p can associate with Sin3p (35).

**Fig. 5. Separation of Rpd3p and Sin3p breakdown complexes from the sds3Δ strain.** A. Mono-Q fractions 20–32 (Fig. 4A) were applied to Superose 6 on the SMART system at 0.5 M NaCl. Rpd3 eluted at 1.2 MDa, corresponding to a molecular mass of 0.9 MDa. No HDAC activity was detected in this molecular range. Sin3p eluted in the range of 0.7–0.5 MDa. Hda1p eluted at approximately 0.6 MDa, which corresponds to the measured HDAC activity (A). B. As a control for A, the corresponding fractions from the Sds3-HAp expressing strain were analyzed on the Superose 6 column at 0.5 M NaCl using the SMART system. Mono-Q fractions 20–32 (Fig. 2B) were applied to Superose 6 on the SMART system. Two HDAC peaks were separated corresponding to the 1.2-MDa Sin3p, Rpd3p, and Sds3-HAp complex, and the 0.6-MDa Hda1p complex, respectively.

Proteins did not co-elute to the extent that they did from the wild type strain (Fig. 2B). This suggests that the association of Sin3p and Rpd3p could be chromatographically separated in the sds3Δ strain, we applied the concentrated Mono-Q pool (fractions 20–32, see Fig. 4A) to an additional Superose 6 size exclusion column, using the Amersham Pharmacia Biotech SMART (Sensitive Methods And Recovery Technology) system, at 0.5 M NaCl. HDAC activity was measured, and Western blot analysis was performed (Fig. 5A). The remaining HDAC activity eluted at 0.6 MDa and corresponded to Hda1p. Rpd3p was detected in fractions 24 and 25, which correspond to a molecular mass of approximately 0.9 MDa. Sin3p was now detected in the molecular range of 0.7–0.5 MDa. Thus, under these chromatographic conditions, Rpd3p and Sin3p were separated into distinct subcomplexes in the absence of Sds3p (Fig. 5A). As a control, the concentrated Mono-Q pool of the Sds3-HA strain (fractions 20–32, see Fig. 2A) was applied to Superose 6 on the SMART system under the same conditions. Fig. 5B shows that two HDAC activities were separated. One activity corresponded to a 1.2-MDa complex, containing Sin3p, Rpd3p, and Sds3p, whereas the second activity, eluting at 0.6 MDa, corresponded to the Hda1p complex. This confirmed that the higher salt concentration used on this column did not cause a disruption of the WT Rpd3p-Sin3p-Sds3p complex.

**DISCUSSION**

To understand the detailed roles of Rpd3p-HDAC complexes, it will be necessary to identify the functions of other subunits of these multiprotein complexes. It was shown previously that Sin3p targets Rpd3p-dependent HDAC activity through interaction with the repressor protein Ume6 to certain promoters (19, 29). Therefore, it is very likely that other subunits are required for the regulation of the enzymatic activity of HDAC complexes. Sap30, for example, was identified as part of Sin3p-HDAC and Rpd3p-HDAC complexes in mammals and yeast (20, 40). It appears to be required for the normal function of these complexes. Furthermore, Sap30 is capable of repressing transcription when tethered to DNA. This might indicate that Sap30 facilitates interactions of Sin3p-HDAC and Rpd3p-HDAC and might even recruit HDAC activity in the absence of Sin3p (20). Disruption of SAP30 in yeast shows phenotypes comparable to disruption of SIN3 and RPD3, suggesting that it works in the same genetic pathway. Moreover, Sap30p and Rpd3p have been shown to co-immunoprecipitate with antibodies against Rpd3p (40).

Sds3p Is an Essential Component of the Sin3-Rpd3 HDAC Complex
Sin3p with the Rpd3p complex. At low salt concentration some Sin3p and Rpd3p co-elute in the absence of Sds3p and they can be co-immunoprecipitated from extract made from an SDS3 deletion strain. Finally, in the absence of Sds3p the Rpd3p complex lacked HDAC activity. Thus, the association of Sds3p, Sin3p, or another subunit that might dissociate in the absence of Sds3p is required for the ability of Rpd3p to act as a histone deacetylase. It is important to note, however, that the second Rpd3p-containing complex (0.6 MDa) identified in this study, which does not contain Sds3p, is fully active on histone substrates. Thus, in a different context, the HDAC activity of Rpd3p apparently functions in the absence of Sds3p. Alternatively, a different protein in the smaller complex may replace the function of Sds3p.

In conclusion, our observations indicate that Sds3p is necessary to maintain the function of a yeast Sin3p-Rpd3p complex. Sds3p facilitates protein-protein interactions within the complex to maintain its structure and enzymatic activity. Sds3p might provide part of the linkage between the co-repressor region of the complex (Sin3p) and the deacetylase function (Rpd3p). Furthermore, disruption of SDS3 abrogates the ability of the 1.2-MDa Rpd3-Sin3 complex to function as a histone deacetylase.

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