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Report

The Rpd3 Core Complex Is a Chromatin Stabilization Module

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Summary

The *S. cerevisiae* Rpd3 large (Rpd3L) and small (Rpd3S) histone deacetylase (HDAC) complexes are prototypes for understanding transcriptional repression in eukaryotes [1]. The current view is that they function by deacetylating chromatin, thereby limiting accessibility of transcriptional factors to the underlying DNA. However, an Rpd3 catalytic mutant retains substantial repression capability when targeted to a promoter as a LexA fusion protein [2]. We investigated the HDAC-independent properties of the Rpd3 complexes biochemically and discovered a chaperone function, which promotes histone deposition onto DNA, and a novel activity, which prevents nucleosome eviction but not remodeling mediated by the ATP-dependent RSC complex. These HDAC-independent activities inhibit Pol II transcription on a nucleosomal template. The functions of the endogenous Rpd3 complexes can be recapitulated with recombinant Rpd3 core complex comprising Sin3, Rpd3, and Ume1. To test the hypothesis that Rpd3 contributes to chromatin stabilization in vivo, we measured histone H3 density genomewide and found that it was reduced at promoters in an Rpd3 deletion mutant but partially restored in a catalytic mutant. Importantly, the effects on H3 density are most apparent on RSC-enriched genes [3]. Our data suggest that the Rpd3 core complex could contribute to repression via a novel nucleosome stabilization function.

Results and Discussion

Rpd3S Contains H3K36me3-Independent Histone Chaperone and Nucleosome Stabilization Functions

The Rpd3 HDAC is the prototype for understanding gene repression on chromatin [4]. HDACs function by removing acetyl marks placed on histone tails by histone acetyltransferases such as SAGA and NuA4 [5]. Acetylated histones decondense chromatin directly [6] and/or serve as targets for ATP-dependent remodeling enzymes including SWI/SNF [7] and RSC [8]. Bromodomains within these enzymes recruit them to acetylated chromatin and enhance their remodeling function [9]. In yeast, Rpd3L and Rpd3S share three subunits: Rpd3, Sin3, and Ume1 [10, 11]. Rpd3L contains numerous additional subunits [12] and is targeted to promoters by sequence-specific DNA binding proteins like Ume6 [13, 14]. Importantly, the Rpd3 HDAC activity contributes to but is not essential for repression on promoters when targeted as a LexA fusion [2]. The Rpd3S complex contains two additional subunits, Rco1 and Eaf3, which target it to H3K36 trimethylated (H3K36me3) nucleosomes in the ORF [15]. Set2 catalyzes H3K36me3 and associates with Pol II [16]. Rpd3S maintains a hypoacetylated state in the ORF and suppresses cryptic transcription [10, 11, 17]. Recently, Rpd3S was reported to interact with elongating Pol II, and its recruitment to transcribed regions was dependent on phosphorylation of the carboxy-terminal domain of Rpb1 [18].

Several aspects of Rpd3 function were of interest to us. First, the in vivo roles of both Rpd3S and Rpd3L were consistent with a nucleosome stability function. Second, in addition to Rpd3, the two complexes share two other subunits, Ume1 and Sin3. In yeast and mammalian cells, Sin3 has a long history of correlating with repression of transcription on chromatin [19]. Finally, the observation that Rpd3 catalytic mutants retain some repression capabilities when targeted via LexA fusions suggested that some other aspect of the protein was contributing to its function [2]. This is not to say that the deacetylase is unessential, only that other aspects of Rpd3 complexes may cooperate with the HDAC to ensure full repression. To explore the HDAC-independent functions of Rpd3, we considered the possibility that it might affect nucleosome remodeling. For example, a previous study by Kingston and colleagues revealed that human SWI/SNF ATPases copurified with a Sin3/HDAC complex and that their remodeling activities were compromised by the HDAC [20].

We initiated our study with Rpd3S because of our continuing interest in the mechanism of Pol II elongation on nucleosomal templates, which in our system requires nucleosome remodeling and octamer eviction by RSC. Because we began with Rpd3S, we also asked whether H3K36me3 would affect nucleosome remodeling. Tandem affinity purification (TAP) was employed to purify the RSC and Rpd3S proteins from *S. cerevisiae* (Figure 1A) [21]. H3K36me3 histones were generated with the methyl-lysine analog (MLA) technology [22]. H3K36 was first mutated to cysteine (H3K36C) and then alkylated with (2-bromoethyl) trimethylammonium bromide to form a methyl-lysine analog or MLA (H3K36C-me3). We will refer to the MLA as H3K36me3 for convenience. The MLA is recognized in a western blotting experiment by an H3K36me3 antibody (Figure S1A available online). Subsequently, unmethylated (naive) or H3K36me3 mononucleosomes were reconstituted on a ^{32}P-labeled DNA fragment containing the 601 nucleosome positioning sequence [23, 24]. Rpd3S bound to both nucleosomes in an EMSA assay and displayed a higher affinity for H3K36me3 nucleosomes as shown previously [25] (Figure S1B).

The assembled nucleosomes were incubated with RSC in the presence or absence of Rpd3S and analyzed by native gel electrophoresis. RSC mobilized the histone octamer as indicated by the faster migration of the 601 nucleosome on a native gel. However, Rpd3S did not significantly inhibit this activity (Figure 1B). Similar effects were observed on H3K36me3 nucleosomes (Figure S1C). We conclude that

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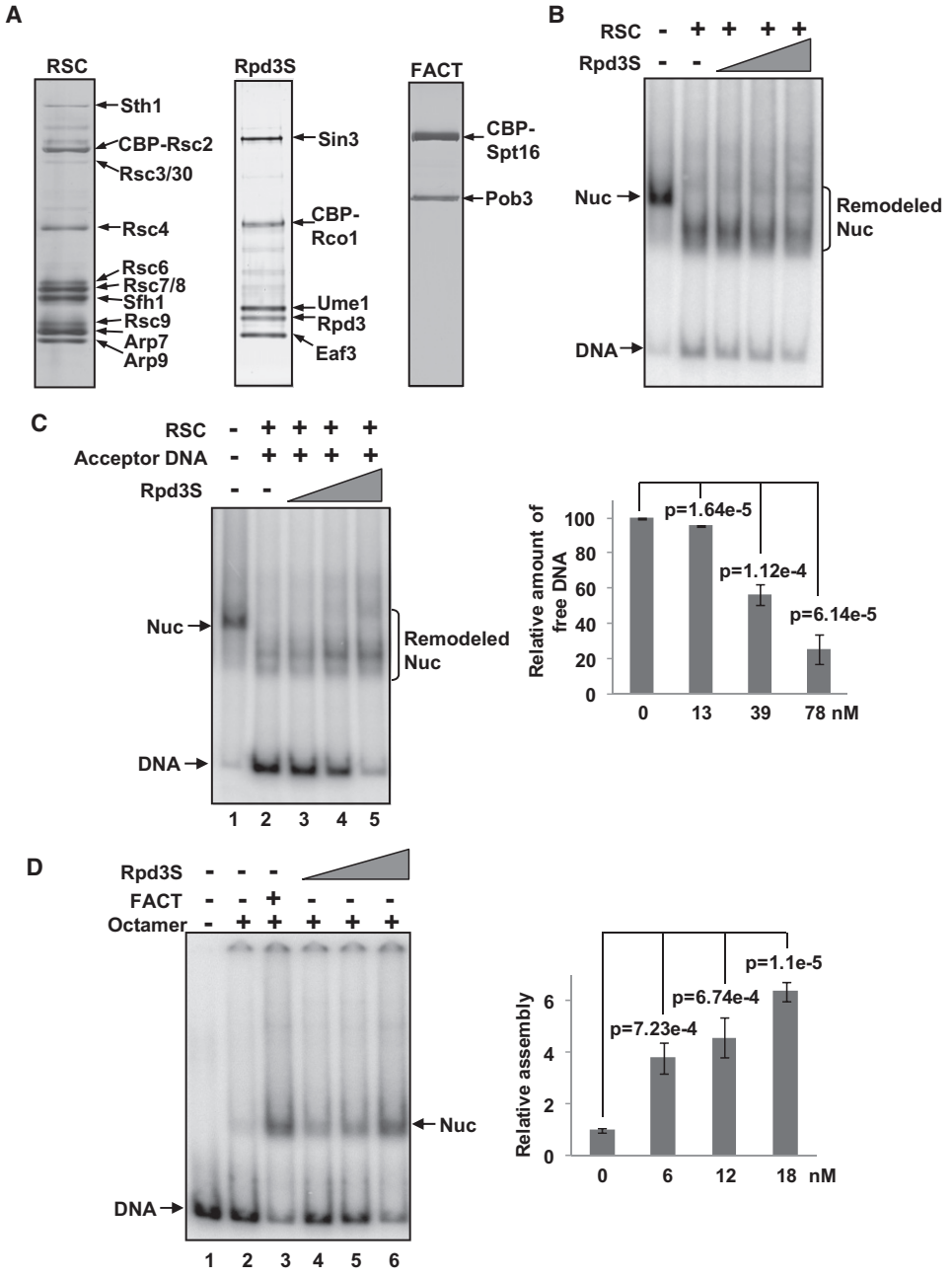

Figure 1. Rpd3S Inhibits RSC-Dependent Nucleosome Eviction and Promotes Nucleosome Assembly In Vitro

(A) Silver stain gel of TAP-purified RSC2, Rpd3S, and FACT complexes.

(B) The effect of Rpd3S on RSC-dependent nucleosome remodeling. 2 nM RSC was incubated with 0.3 nM ^{32}P-labeled mononucleosome and 0, 13, 39, or 78 nM Rpd3S. The remodeling products were fractionated by native PAGE. A phosphorimage of the gel is shown. See also Figures S1A–S1C for the effect of H3K36me3 on Rpd3S in binding and RSC-mediated nucleosome remodeling reactions.

(C) The effect of Rpd3S on RSC-dependent nucleosome eviction. Left, 6 nM RSC was incubated with 0.3 nM mononucleosome and 0, 13, 39, or 78 nM Rpd3S in the presence of 10 ng of pGEM3Z601R acceptor DNA. Bar graph on the right represents quantitation by ImageQuant TL (GE) of the three independent experiments. The relative amounts of free DNA generated by eviction were plotted as a bar graph normalized to that generated by 6 nM RSC alone, which was assigned a value of 100. The error bars show ±standard deviation (SD). The p value is calculated by Student's t test. See also Figure S1D for the effect of H3K36me3 on Rpd3S in RSC-mediated nucleosome eviction.

(D) Rpd3S-mediated chromatin assembly assay. Left, the reaction contained 18 nM FACT, or 6, 12, 18 nM of Rpd3S, respectively, with recombinant *Xenopus* octamers and a ^{32}P-labeled 601 DNA fragment. A phosphorimage of a native gel is shown. Graph on the right represents quantitation of the amounts of assembled nucleosomes by Rpd3S relative to no Rpd3S control (i.e., octamers alone). The free DNA and assembled nucleosome are indicated. The error bars show ±standard deviation (SD). The p value is calculated with Student's t test. For chaperone assays see also Figure S1E for the effect of H3K36me3 on Rpd3S, Figure S1F for the effect of Rpd3S mutants, and Figure S1G for the effect of trichostatin.

Rpd3S does not inhibit nucleosome remodeling under the experimental conditions tested in our assays.

As reported previously [26], RSC has the ability to transfer a histone octamer from one DNA molecule to another, often termed an acceptor. This octamer transfer capability, referred to as eviction, is important for RSC's function [8]. Upon addition of an unlabeled supercoiled acceptor DNA to our remodeling reactions, RSC transferred the majority of octamers from the labeled DNA probe to the unlabeled DNA, thereby generating substantial amounts of free ^{32}P-DNA (Figure 1C, lane 2). Importantly, the amount of eviction, as measured by accumulation of free DNA, decreased significantly with increasing doses of Rpd3S (Figure 1C, lanes 3–5 and accompanying bar graph), while the amount of remodeled nucleosome increased. Similar effects on remodeling and eviction were observed at similar Rpd3S concentrations on H3K36me3 chromatin (Figures S1C and S1D). We conclude that Rpd3S inhibits RSC-mediated octamer eviction in an H3K36me3-independent manner in vitro.

Inhibition of ATP-dependent octamer eviction is an activity that may act to maintain nucleosome stability and histone density over a regulatory region and gene. Another activity that could serve a similar role would be the ability of Rpd3S to act as a chaperone by assembling histones onto DNA. Histone chaperones such as FACT, Nap1, Asf1, and Spt6 play important roles in transcription regulation in yeast [27, 28]. To address this possibility, Rpd3S was first incubated with unmodified, naive octamers to allow protein-protein interactions, followed by incubation with a ^{32}P-labeled DNA bearing the 601 positioning sequence. Nucleosome formation was analyzed on a native gel (Figure 1D). To verify the efficiency of our in vitro system, we compared Rpd3S with the well-studied histone chaperone FACT (Figure 1A) [27], which is known to specifically load histones onto DNA. As reported previously [29], FACT strongly stimulated nucleosome formation (Figure 1D, lane 3). Importantly, Rpd3S also promoted nucleosome assembly in a dose-dependent manner (Figure 1D, lanes 4–6). Surprisingly, the concentration of Rpd3S necessary to assemble nucleosomes was measurably lower than that required to inhibit eviction. H3K36me3 did not enhance and even inhibited the chaperone function of Rpd3S (Figure S1E). Moreover, an Rpd3S mutant lacking the PHD domain of Rco1 and the chromo domain (CHD) of Eaf3, both of which target Rpd3S to H3K36me3 [25], displayed similar relative nucleosome assembly activity as did the wild-type protein. H3K36me3 negatively affected the mutant's chaperone function similar to its effect on wild-type Rpd3S (Figure S1F). We conclude that the Rpd3S complex acts as a histone chaperone to promote histone deposition onto DNA in vitro in an H3K36me3-independent manner.

The inhibition of chaperone function by H3K36me3 was quite interesting given that Rpd3S normally targets this modification for binding within an ORF. However, the effect was also observed in a mutant of Rpd3S lacking the targeting domains suggesting that H3K36me3 inhibits a specific aspect of the chaperone function. For example, H3K36me3 also negatively affected the chaperone activity of FACT (data not shown). Collectively, the chaperone and eviction data suggest that Rpd3S possesses a chromatin stabilization function, which is independent of the specific H3K36me3 targeting function. Although our assays utilized unacetylated histones, it remained a remote possibility that the HDAC function of Rpd3 might somehow contribute. However, Rpd3S promoted nucleosome formation at the same efficiency in the absence or presence of trichostatin concentrations sufficient to inhibit 90% of Rpd3's HDAC activity (Figure S1G).

The 3-Subunit Core of Rpd3L and Rpd3S Mediates the Nucleosome Stabilization Function

We next asked whether the Rpd3L complex exhibited similar properties as Rpd3S because the two enzymes share a set of three core subunits [10, 11]. To address this question, we TAP purified the Rpd3L complex (Figure 2A) and tested its effect on RSC-dependent remodeling (data not shown) and octamer eviction (Figure 2B). Like Rpd3S, Rpd3L had little effect on remodeling but significantly inhibited octamer eviction as indicated by the reduced amounts of free DNA observed with increased amounts of protein (Figure 2B). Moreover, like Rpd3S, Rpd3L stimulated nucleosome assembly with naive octamers (Figure 2C).

Because Rpd3L shares a 3-subunit core complex (3-core) with Rpd3S, we next asked whether this module contributes to the nucleosome stabilization function. The 3-subunit core complex was reconstituted by coexpression of *S. cerevisiae* Ume1, Rpd3, and Sin3 in Sf9 cells via a baculovirus system. Sin3 was tagged with the FLAG epitope and the complex was purified with a two-step procedure involving an anti-FLAG immuno-affinity column followed by gel filtration chromatography. The final products were relatively pure except for an unknown protein that copurified (Figure 2D). We observed a significant and dose-dependent inhibition of RSC-mediated octamer eviction by the 3-subunit core complex (Figure 2E). The core complex also enhanced nucleosome formation in a chaperone assay, similar to Rpd3S and Rpd3L (Figure 2C).

In an attempt to identify the subunit responsible for nucleosome stabilization, we used FLAG-affinity chromatography to purify each of the individual subunits (Figure S2A). In side-by-side purifications of similar scale and yield, only Ume1 purified to near homogeneity as a single species. Sin3 was degraded slightly and Rpd3 copurified with several higher molecular weight bands. Nevertheless, the amounts of full-length subunits were sufficient for testing. Surprisingly, no individual subunit inhibited RSC-mediated octamer eviction (Figure S2B) or assembled nucleosomes to any significant extent (Figure S2C). We conclude that the entire Rpd3 core complex (Sin3, Ume1, and Rpd3) is necessary and sufficient for the chromatin stabilization function in vitro.

The Nucleosome Stabilization Function Inhibits Nucleosomal Transcription In Vitro

To further study the nucleosome stabilization function of Rpd3 HDACs, we performed in vitro transcription. Previously, we established a system to study Pol II transcription through a nucleosome by using a "C-tail" template bearing a single-stranded stretch of dC ligated to a DNA fragment encompassing the 601 positioning sequence (Figure 3A) [30]. Pol II employs the C-tail as a promoter and elongates into the 601 nucleosome. RSC was shown to stimulate Pol II elongation through the nucleosomal barrier [30]. Our current view is that RSC stimulates transcription by evicting the nucleosome. We wished to determine whether the nucleosome stabilization function of Rpd3 was potent enough to prevent RSC-mediated transcription. Consistent with our previous study, transcription with TAP-purified yeast Pol II generated only small amounts of full-length (FL) transcripts (Figure 3B, lane 1). Pol II arrested at discrete locations and short transcripts were produced. In the presence of ATP and acceptor DNA,

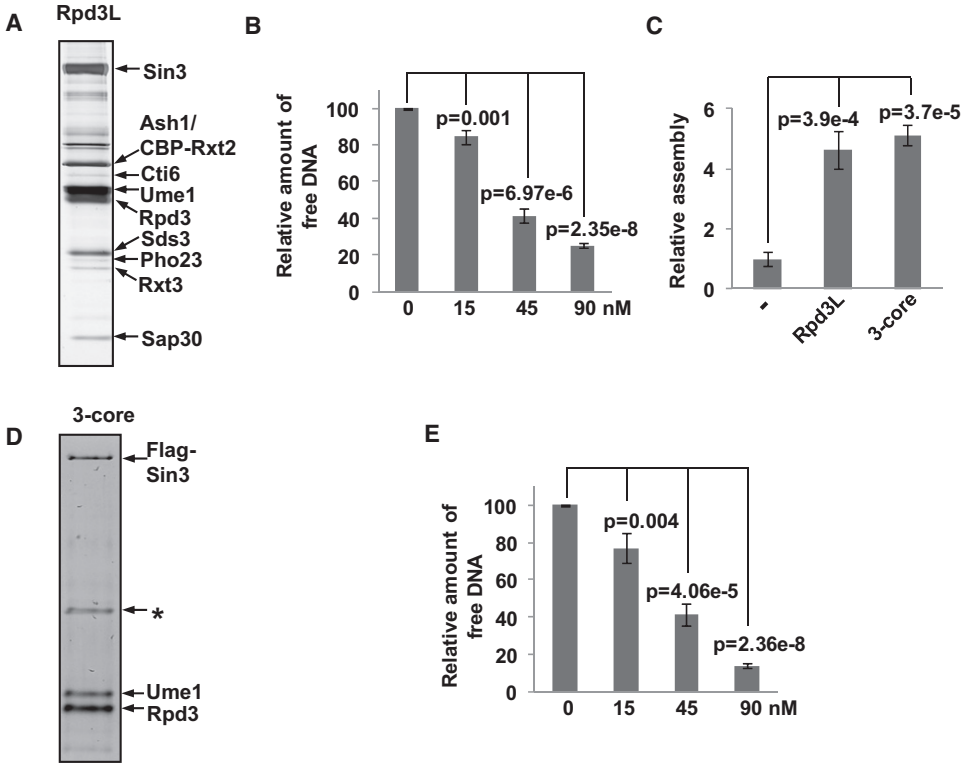

Figure 2. Rpd3L and 3-Subunit Core Complex Prevent RSC-Dependent Nucleosome Eviction and Promote Nucleosome Assembly In Vitro

(A) Silver stain gel of TAP-purified Rpd3L.

(B) The effect of Rpd3L on RSC-dependent nucleosome eviction. 6 nM RSC was incubated with 0, 15, 45, 90 nM Rpd3L, respectively, and analyzed as described in Figure 1C legend.

(C) Nucleosome assembly with *Xenopus* octamers and 18 nM Rpd3L or 3-subunit core complex, respectively, as in Figure 1D legend.

(D) Silver stain gel of recombinant 3-subunit core complex. The asterisk indicates an unknown protein that copurified with the 3-subunit core complex.

(E) The effect of 3-subunit core complex on RSC-dependent nucleosome eviction. 6 nM RSC was incubated with 601 nucleosome and 0, 15, 45, 90 nM of recombinant core complex, respectively, and analyzed as in Figure 1C legend. See also Figure S2A for silver-stained gels of the individual subunits, Figure S2B for their effect on RSC-mediated nucleosome eviction, and Figure S2C for chaperone assays.

RSC strongly stimulated transcription as reflected by the decrease in arrested transcripts and by increased production of full-length (FL) transcripts (Figure 3B, lane 2). The addition of the Rpd3 HDACs, in the form of either Rpd3S, Rpd3L, or the 3-subunit core complex, all diminished transcription in a dose-dependent manner on naive chromatin (Figure 3B, lanes 3–8). However, the presence of the chaperone FACT, which has no effect on RSC eviction (data not shown), did not affect the production of full-length transcripts (Figure 3B, lanes 9–10). The inhibition of transcription by Rpd3 HDACs was specific to a nucleosomal template as shown by the fact that they did not inhibit transcription on naked DNA (Figure 3C). Because Rpd3S displayed a higher affinity for H3K36me3 nucleosomes [25], we compared the inhibitory effect of Rpd3S and 3-subunit core complex on transcription with naive or H3K36me3 nucleosomal template. The H3K36me3 nucleosomes enhanced repression by Rpd3S but not by the 3-subunit core complex (Figure 3D). The results are consistent with the higher affinity of Rpd3S for H3K36me3 nucleosomes. These data indicate that the Rpd3 complexes antagonize RSC-mediated stimulation of Pol II transcription elongation through a nucleosome in vitro. The inhibition of transcription elongation by Rpd3S is consistent with an in vivo study showing that deletion of Rpd3 bypasses the requirement of positive elongation factor Bur1/Bur2 [11].

Our approach does not indicate whether Rpd3S, for example, can block an elongating Pol II molecule in vivo. It is not known how Pol II elongation occurs in living cells and whether there are situations where it would encounter Rpd3S-bound nucleosomes. Indeed, the current model, for which there is little experimental support, suggests that Rpd3S-bound nucleosomes accumulate behind Pol II [15]. Nevertheless, our assay provides a measure of the stability of the nucleosome conferred by Rpd3 in the presence of the strong ATP-dependent remodeling activity of RSC and the potent NTP-dependent DNA translocase activity of Pol II.

Rpd3 Affects H3 Density Preferentially at RSC-Bound Genes in Vivo

The ability of the two Rpd3 complexes to stabilize nucleosomes independent of HDAC activity suggested that they might play similar roles in vivo. To address this hypothesis, we prepared strains of yeast with the endogenous Rpd3 gene deleted but bearing an empty vector or vectors expressing either the wild-type or H150A catalytically inactive Rpd3 [2]. The mutant and wild-type Rpd3p were expressed at similar levels (Figure S3A). The H150A mutation appears to be completely defective for histone deacetylase activity as shown previously via an in vitro assay. However, it still interacts with Sin3 and partially retains the transcription repression

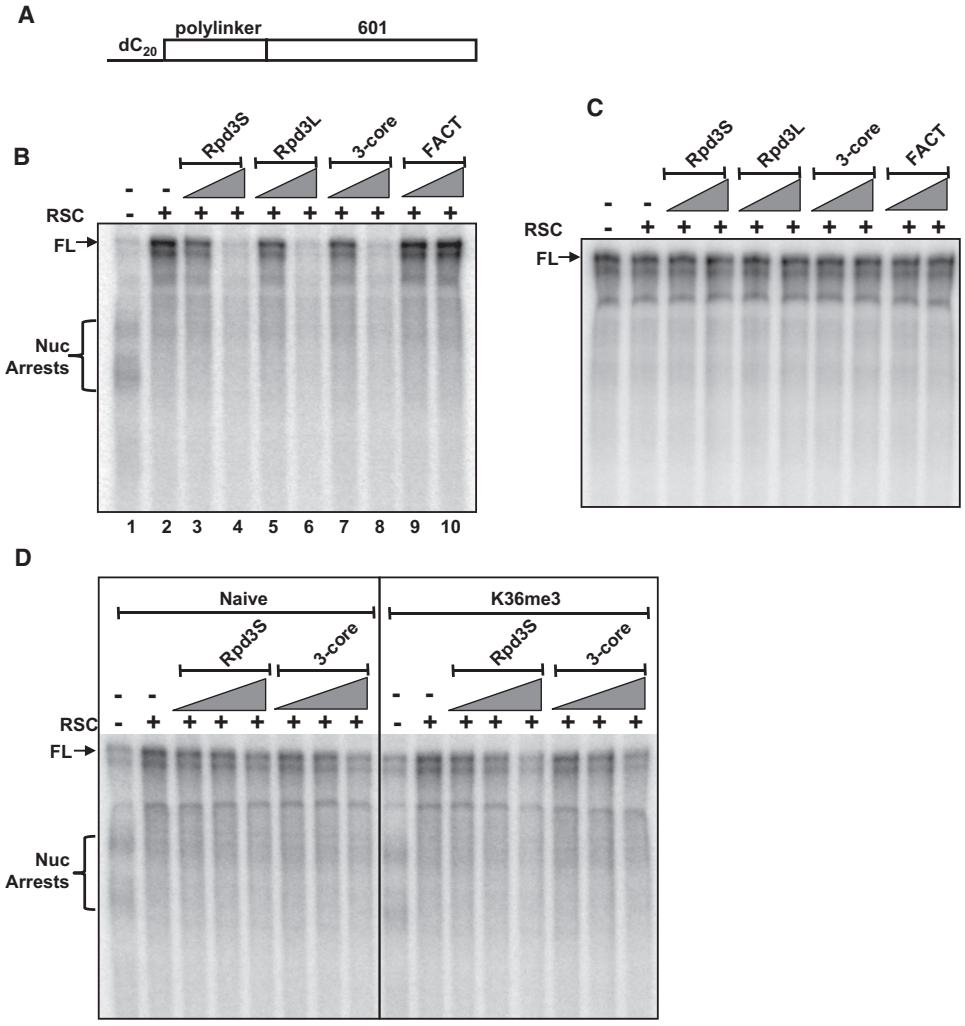

Figure 3. Rpd3 HDACs Inhibit RSC-Mediated Activation of Nucleosome Transcription

(A) Schematic of the C-tail template. The template contains the 601 positioning sequence and a 20-nucleotide single-stranded C-tail with an intervening polylinker from pGEM3Z601R. Pol II initiates from the C-tail.

(B) The template was assembled into a mononucleosome with naive recombinant *Xenopus* octamers and then preincubated with 3 nM RSC in the presence or absence of 30 or 60 nM Rpd3S, Rpd3L, 3-subunit core complex, or FACT for 1 hr at 30°C. Pol II, α-[^{32}P]CTP, NTPs, and RNase H were then added for 15 min at 30°C. The ^{32}P-labeled RNA products were fractionated on a 10% polyacrylamide/urea gel. A phosphorimage of the gel is shown.

(C) Same assay as performed in (B), except that naked C-tail DNA template was used instead of nucleosomal template.

(D) In vitro transcription was performed on naive or H3K36me3 nucleosomes. 3 nM RSC was incubated with 0, 5, 15, 45 nM Rpd3S or recombinant 3-subunit core complex, respectively.

function [2]. To test the effect in vivo, we chose two known targets of Rpd3, histone H3K18ac and H4K5ac [31]. The data demonstrate that the H150A and null Rpd3 mutants lead to similar levels of H3K18 and H4K5 hyperacetylation, suggesting that the point mutant is largely inactive in vivo (Figure S3B). Finally, we measured histone H3 density genome-wide in each of these three strains by using Agilent tiling arrays. Upon deletion of Rpd3, we observed a significant decrease in H3 density at intergenic/promoter regions genome-wide, while the coding regions/ORFs were less affected (Figure 4A) although still significant in some regions (data not shown). Importantly, however, the H150A derivative maintained significantly higher H3 density than in Rpd3-deletion cells (empty vector), although not as high as in Rpd3 wild-type cells. The data suggest a global role of Rpd3 in affecting histone density although the effect is more apparent in promoter regions.

Our biochemical studies showed that Rpd3 complexes prevent RSC from evicting histones on chromatin (Figures 1 and 2). We hypothesized that the observed changes in H3 density upon Rpd3 mutation might be more evident on genes that are bound by RSC in vivo. To test this hypothesis, we compared our data with that of the published genome-wide distribution of five subunits of the RSC1 and RSC2 complexes in *S. cerevisiae* [3]. We analyzed intergenic/promoter regions scored for high RSC binding by the authors (as measured via all five subunits; $p < 0.001$ for each region) and compared them with a similar number of targets displaying the least RSC binding (Figure 4B). We then analyzed the H3 density changes observed upon Rpd3 mutation in these same two subsets of targets. The deletion or mutation of Rpd3 minimally impaired H3 occupancy at low RSC-bound targets, while a more significant decrease was observed at

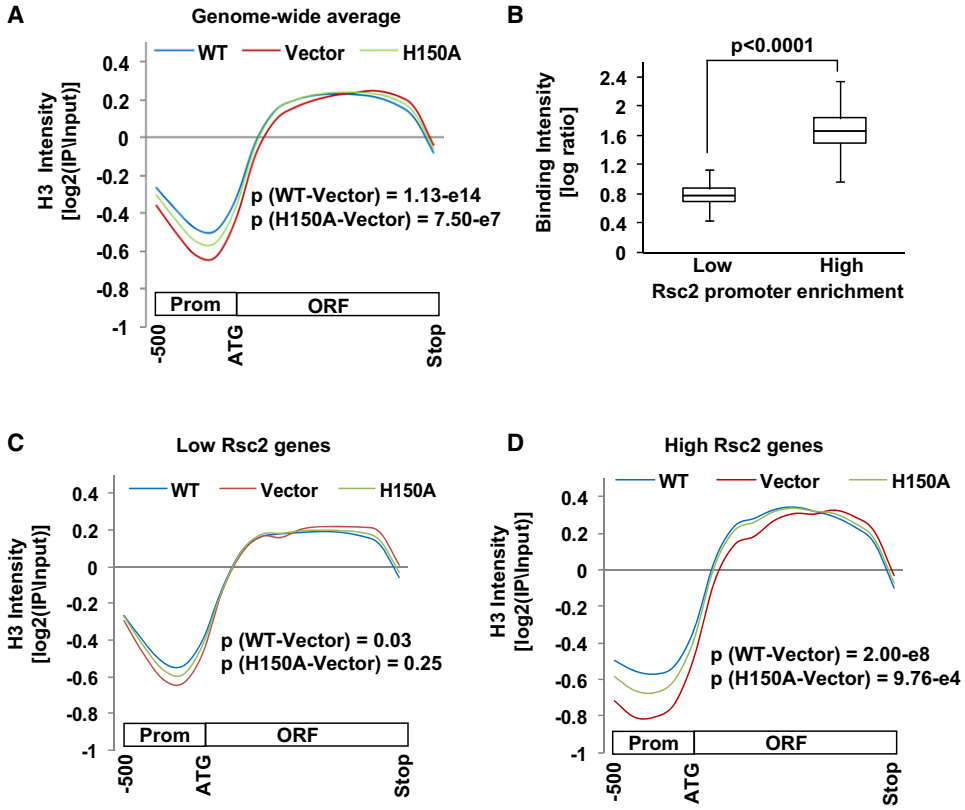

Figure 4. Rpd3 Complexes Stabilize Chromatin In Vivo

(A) H3 levels were measured by ChIP in wild-type (WT), *rpd3* H150A (H150A), and *rpd3Δ* (Vector) cells. ChIP DNA of Histone H3 and inputs were amplified, labeled, and hybridized to Agilent Tiling arrays. The average binding of 6,572 annotated genes and their upstream 500 bp regions are shown. Enrichment of H3 ChIP DNA is shown as the log2 ratios of ChIP versus input DNA. p values for the promoters were calculated with the Mann-Whitney test. See also Figure S3A for Rpd3 native versus H150A protein levels, Figure S3B for global acetylation levels of H3K18 and H4K5 in Rpd3 native, H150A, and null backgrounds, and Figures S3C and S3D for Rpd3 association with Pol II and H3.

(B) Box and whisker plot for two subsets of targets that have high or low Rsc2 enrichment. Each subset has 495 and 506 targets, respectively. The p values are calculated by Student's t test.

(C and D) H3 levels of the low (C) and high (D) Rsc2 targets were measured in wild-type, *rpd3* H150A, and *rpd3Δ* cells. p values for the promoters were calculated by the Mann-Whitney test.

the RSC-enriched targets (Figures 4C and 4D). Importantly, the largest difference in H3 density between wild-type and either the null or H150A Rpd3 mutant was observed at RSC-enriched regions. The data suggest the possibility that the Rpd3 complex can somehow influence nucleosome stability at RSC-enriched promoter regions in vivo. It is unclear how this would affect gene expression because recent findings suggest that the nucleosome occupancy of Rpd3 targets is not always correlated with transcription frequency [32–34]. Additionally, Rpd3 affects transcription of full length and cryptic transcripts [10, 17], making it complicated to determine the true effect.

Conclusions and Perspectives

We have demonstrated that Rpd3S and Rpd3L possess a previously unrecognized capacity to promote nucleosome assembly like a histone chaperone. Additionally, both Rpd3 HDAC complexes prevent RSC-dependent histone eviction from nucleosomes, possibly through their histone chaperone activity. However, we note that the eviction and chaperone functions displayed different concentration dependence. We were also able to establish that the three common subunits of Rpd3S and Rpd3L formed a subcomplex, which contributed to the shared activities of the small and large Rpd3 HDACs. We speculate that the combination of a histone chaperone activity and inhibition of RSC eviction facilitate chromatin stability and complement the transcriptional repression function of the Rpd3 HDAC.

The diminished density of H3 in strains upon Rpd3 deletion or mutation was significant mainly on the promoter/intergenic regions. Despite the observation that transcription level is inversely correlated with H3 density [35], the levels of H3 in transcribed regions significantly exceed those in promoter regions. It remains a possibility that although Rpd3S is found primarily in ORFs, the normally high histone density masks its stabilization function. Histone density and chromatin stabilization in the ORF is known to involve numerous proteins, including chaperones such as Spt6 and FACT, many of which generate a cryptic transcription phenotype when mutated [36–38]. Hence the role of Rpd3's stabilization function in the ORF regions may be less apparent and require more sensitive assays or combinatorial mutations of other chaperones to become more evident. Alternately, the ability of H3K36me3 to inhibit Rpd3's chaperone function may disable that function in ORFs.

A prediction of the role of Rpd3, as either a chaperone or in chromatin stabilization, is that it should copurify with substantial amounts of H3. Indeed, we observed that Rpd3

complexes, purified with an Rpd3-TAP strain, contained a significant amount of H3 and less, but still detectable, amounts of Pol II. Stoichiometry measurements revealed that the ratio of Rpd3:H3:Pol II is 10:4.5:1 (Figures S3C and S3D). This result was obtained with concentrations of heparin and ethidium bromide, which are known to disfavor protein-DNA interactions. In the case of H3, these data are consistent with a chaperone function. In the case of Pol II, these data are consistent with the direct interaction of Pol II and Rpd3S proposed by Hinnebusch and colleagues [18].

It should be pointed out that our unpublished microarray data show that deletion of Rpd3 causes upregulation and downregulation of many genes as reported [39] (data not shown). Although it would be easy to dismiss the downregulated genes as indirect effects, the result belies a complex role of Rpd3 in transcription. For example, little is known about the mechanisms underlying global histone deacetylation and what role it plays. Additionally, Rpd3 has been shown to be directly required for activation of stress-inducible genes, and in some cases, the effect requires the catalytic activity [32–34]. In such scenarios, nucleosome density and the chromatin stabilization function may not always correlate with Rpd3 occupancy. Therefore, although our results reveal an additional function for Rpd3, which may have implications for the function of these proteins in higher eukaryotes, much remains to be learned of how this protein functions in genomic regulation.

Accession Numbers

The raw data have been deposited into the Gene Expression Omnibus (GEO) database (accession # GSE33829).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at doi:10.1016/j.cub.2011.11.042.

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