ABSTRACT  Much of euchromatin regulation occurs through reversible methylation of histone H3 lysine-4 and lysine-36 (H3K4me and H3K36me). Using the budding yeast Saccharomyces cerevisiae, we previously found that levels of H3K4me modulated temperature sensitive alleles of the transcriptional elongation complex Spt6-Spn1 through an unknown H3K4me effector pathway. Here we identify the Rpd3S histone deacetylase complex as the H3K4me effector underlying these Spt6-Spn1 genetic interactions. Exploiting these Spt6-Spn1 genetic interactions, we show that H3K4me and H3K36me collaboratively impact Rpd3S function in an opposing manner. H3K36me is deposited by the histone methyltransferase Set2 and is known to promote Rpd3S function at RNA PolII transcribed open reading frames. Using genetic epistasis experiments, we find that mutations perturbing the Set2-H3K36me-Rpd3S pathway suppress the growth defects caused by temperature sensitive alleles of SPT6 and SPN1, illuminating that this pathway antagonizes Spt6-Spn1. Using these sensitive genetic assays, we also identify a role for H3K4me in antagonizing Rpd3S that functions through the Rpd3S subunit Rco1, which is known to bind H3 N-terminal tails in a manner that is prevented by H3K4me. Further genetic experiments reveal that the H3K4 and H3K36 demethylases JHD2 and RPH1 mediate this combinatorial control of Rpd3S. Finally, our studies also show that the Rpd3L complex, which acts at promoter-proximal regions of PolII transcribed genes, counters Rpd3S for genetic modulation of Spt6-Spn1, and that these two Rpd3 complexes balance the activities of each other. Our findings present the first evidence that H3K4me and H3K36me act combinatorially to control Rpd3S.

Unstructured N-terminal tails of histone proteins emanate from the nucleosome core and are sites of a diverse array of post-translational modifications (Strahl and Allis 2000). Methyllations of histone H3 on lysine-4 and lysine-36 (H3K4me and H3K36me) are among the most well studied histone post-translational modifications. Like all lysine methylations, H3K4 and H3K36 can be mono, di, or tri methylated (me1, me2, me3), and these forms have distinctive roles in regulation of proteins with domains that distinguish these states (Yun et al. 2011; Musselman et al. 2012; Rando 2012). Together with a plethora of other histone post-translational modifications, H3K4me and H3K36me contribute to a diverse chromatin landscape. A prediction of the histone code hypothesis states that chromatin effector complexes interpret this diverse landscape through their ability to distinguish multiple histone modification states (Strahl and Allis 2000). Indeed, chromatin effector complexes often contain multiple subunits with protein domains known to distinguish histone modification states (Doyon and Cote 2004; Li et al. 2007; Li et al. 2007; Wang et al. 2011). However, although this prediction has been satisfied with clear biochemical support (Li et al. 2007; McDaniel et al. 2016), there exists little genetic evidence specifically addressing this component of the histone code hypothesis.

Rpd3 is the catalytic subunit of the conserved histone deacetylase complexes Rpd3L and Rpd3S in the yeast S. cerevisiae (Rundlett et al. 1996; Bernstein et al. 2000; Kurdistani et al. 2002). Distinctive functions of Rpd3S and Rpd3L are enabled by their chromatin recruitment to discrete regions of RNA PolII transcribed genes, with Rpd3L being recruited to 5’ promoter-proximal regions (Carrozza et al. 2005a) while
Rpd3S is principally found downstream within the gene bodies (Carrozza et al. 2005b; Keogh et al. 2005). Both Rpd3S and Rpd3L share the three core subunits Rpd3, Sin3, and Ume1 and their differing localizations on chromatin are likely facilitated through additional protein subunits that distinguish these complexes. Rpd3L contains numerous additional protein subunits (Carrozza et al. 2005a), and controls meiotic progression in diploid cells as well as the transcriptional program of haploid cells entering quiescence (Lardenois et al. 2015; Mcknight et al. 2015). The Rco1 and Eaf3 proteins are specific for Rpd3S, which negatively regulates transcriptional elongation by RNA PolII in mitotically proliferating cells and prevents spurios intragenic transcription (Carrozza et al. 2005b; Joshi and Struhl 2005; Keogh et al. 2005; Quan and Hartzog 2010). Eaf3 possesses a biochemically confirmed capacity for binding to H3K36me3 (Li et al. 2007; Xu et al. 2008; Ruan et al. 2015; Steunou et al. 2016) and accordingly mediates Rpd3S activity at RNA PolII transcribed open reading frames where H3K36me3 is found (Carrozza et al. 2005b; Keogh et al. 2005). The function of Rpd3S also requires the Rco1 subunit, which binds the H3 N-terminal tail through its tandem PHD domains (Lee et al. 2013; Ruan et al. 2015; Mcdaniel et al. 2016). Interestingly, methylation of H3K4 prevents Rco1 binding to the H3 N-terminus in vitro (Mcdaniel et al. 2016), though the in vivo roles of H3K4me for Rpd3S function remain unknown.

We previously showed that the Spt6-Spn1 histone chaperone complex was genetically engineered in an H3K4me3-dependent manner through the opposing roles of the H3K4 methyltransferase and demethylase enzymes Set1 and Jhd2 (Lee et al. 2018). Mutations in SET1 and other genes that cause reduced H3K4me3 enhance the growth defects caused by temperature sensitive alleles of SPN1 and SPT6, while mutation of JHD2, which causes increased H3K4me3, suppress Spt6-Spn1. These and other results supported our conclusion that H3K4me3 levels impacted Spt6-Spn1, though the pathways underlying Spt6-Spn1 regulation by H3K4me remained opaque (Lee et al. 2018).

Here we identify Rpd3S as the H3K4me effector pathway impacting Spt6-Spn1 and provide genetic evidence for the biochemically predicted, though untested, model that H3K4me collaborates with H3K36me to combinatorially control the function of Rpd3S through its Eaf3 and Rco1 subunits. We find that mutating components throughout the Set2-H3K36me-Rpd3S pathway suppress Spt6-Spn1 mutations, suggesting that activation of Rpd3S through H3K36me opposes Spt6-Spn1. Using sensitive epistasis experiments, we show that in opposition to this known H3K36me Rpd3S activating role, H3K4me negatively impacts Rpd3S. Further genetic experiments suggest that H3K4me opposes Rpd3S by inhibiting Rco1 binding to H3 N-terminal tails. Our genetic findings are in good agreement with the biochemically characterized specificities of the chromatin binding domains within Rco1 and Eaf3, and suggest that these binding specificities fine-tune the action of Rpd3S on chromatin.

**RESULTS**

**Opposing roles of H3K4me and H3K36me in control of Spt6-Spn1**

We previously found that deletion of JHD2 (jhd2Δ) suppressed TS alleles of SPT6 and SPN1 and this was attributable to increased H3K4me levels caused by jhd2Δ (Lee et al. 2018). Because set2Δ was previously found to suppress TS alleles in the functionally related FACT complex (Biswa et al. 2006), we asked if set2Δ similarly suppressed Spt6-Spn1 TS mutations. We found that set2Δ strongly suppressed the temperature sensitive growth defects caused by the spt6Δ and spn1Δ-K192N mutations (Figure 1A and 1B). The magnitude of suppression caused by set2Δ was greater than that caused by jhd2Δ and no additive effects on suppression were observed (Figure 1A and 1B). All strains described here were engineered using genetic crosses and tetrads dissection. For all results shown, we isolated at least 2 independently constructed strain replicates through tetrads dissection. Though replicates and WT control strains are not always shown in the interest of space, all results we report here are upheld in these replicates. Strain fitness was assessed using spot assays to compare growth rates at varied temperatures. Though also not shown in the interest of space, we always observed complete loss of growth of our TS strains at 38.5°C, confirming that the TS alleles were intact and no bypass suppression occurred.

**MATERIALS AND METHODS**

**Yeast Strains**

Standard yeast genetic methods were used for construction of all strains and deletion mutants were obtained from the gene deletion collection (Giaever et al. 2002). The GAL inducible JHD2 alleles were constructed using PCR based integration as described (Longtine et al. 1998). The JHD2(H427A) mutation was constructed using the delitto perfetto method (Storici and Resnick 2003). Yeast strains used in this study are listed in Table S1. All strains were constructed through genetic crosses followed by dissections in the BY4742 background.

**Serial dilution assays**

Yeast strains were inoculated into several mL of YPD (1% yeast extract, 2% peptone, and 2% glucose) and grown overnight at room temperature (23°C). Each strain was diluted to an OD600 = 0.4, then 10-fold serially diluted five times and spotted on synthetic complete (SC) media (YNB media (Multicell Wisent) containing 5 g/L of ammonium sulfate and either 2% glucose or 2% galactose as described previously (Lee et al. 2018).

**Western blots**

Cells were grown to mid-logarithmic phase in synthetic complete (SC) medium + 2% raffinose and transferred to SC + 2% galactose medium. Aliquots of these cultures were taken 1 hr after transfer to galactose-containing media. Proteins were then extracted and processed for western blotting as described previously (Xu et al. 2012). Equal amounts of protein were electrophoresed on SDS-PAGE gels and transferred onto PVDF membranes. Immunoblot analysis was performed as described (Soloveychik et al. 2016). Visualization of the proteins was performed by exposing the membrane to light sensitive film. The PVDF membrane was stripped and re-probed with different antibodies. Stripping the PVDF membrane was accomplished by incubating the membrane for 30 min at 50°C with a mixture consisting of 100 mM 2-β-mercaptoethanol, 2% SDS and 62.5 mM Tris- HCl pH 6.7.

**Antibodies**

The following antibodies were used in this study and purchased from Abcam: Anti-Histone H3 antibody (ab1791), Anti-Histone H3K4me3 antibody (ab5850), and Anti-Histone H3K4me1 antibody (ab8895). The following antibody was used in this study and purchased from Millipore: Anti-Histone H3K4me2 (07-030).

**Data availability**

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. Strains are available upon request. Supplemental material available at Figshare: https://doi.org/10.25387/g3.7019552.
As the only known substrate of Set2 is H3K36, we hypothesized that the loss of H3K36me caused by set2Δ accounted for the suppression of Spn1-TS alleles. To test this, we engineered strains combining spn1-K192N with synthetic histone H3 alleles encoding histone H3 lysine 36 substituted with arginine (H3K36R) (Dai et al. 2008). The H3K36R substitution suppressed spn1-K192N temperature sensitivity compared with an isogenic strain expressing a wild type synthetic histone allele (Figure 1C). The magnitude of suppression by H3K36R substitution was equivalent to that of set2Δ and their combined influence was not additive (Figure 1C). This result supports the conclusion that suppression of spn1-K192N by set2Δ occurred through loss of H3K36me, showing that H3K36me somehow opposed Spn1 function.

To genetically deconvolute the impact of H3K4me and H3K36me on Spn1-TS, we engineered strains expressing the endogenous JHD2 gene under control of the GAL1-10 promoter (pGAL-JHD2), which leads to JHD2 overexpression in galactose medium. We found that growth of strains harboring pGAL-JHD2 in galactose containing media caused dramatic depletions of H3K4me3 and H3K4me2 (Figure 2A). As expected based on our previous findings, on media containing glucose, which leads to strong transcriptional repression of GAL1-10, pGAL-JHD2 phenocopied jhd2Δ and caused suppression of both spt6-14 and spn1-K192N (Figure 2C and 2D). To confirm that this epistatic relationship related specifically to H3K4 demethylation by Jhd2, we determined that set2Δ similarly suppressed the phenotypic enhancement of spn1-K192N caused by set1Δ (Figure 2E).

Collectively, these results demonstrate that Spn1-TS activity was opposed both by methylation of H3K36 and by hypo-methylation of H3K4 (denoted hereafter as “H3K4me0”). On media containing galactose however, we found that overexpression of JHD2 negatively affected the growth of spt6-14 and spn1-K192N (Figure 2C and 2D), consistent with our previous finding that mutations reducing H3K4me3 enhanced these Spn1-TS alleles (Lee et al. 2018). Indeed, the enhancement of Spn1-TS mutations caused by pGAL-JHD2 in the presence of galactose was reverted by mutation of Jhd2 histidine-427 to alanine (H427A), which renders Jhd2 catalytically inactive and unable to demethylate H3K4 (Ingvarsdottir et al. 2007; Liang et al. 2007) (Figure 2A and S1). Using the pGAL-JHD2 allele, we found that suppression of both spt6-14 and spn1-K192N by set2Δ was epistatic to the growth defect caused by JHD2 overexpression (Figure 2C and Figure 2D). To confirm that this epistatic relationship related specifically to H3K4 demethylation by Jhd2, we determined that set2Δ similarly suppressed the phenotypic enhancement of spn1-K192N caused by set1Δ (Figure 2E).

Collectively, these results demonstrate that Spn1-TS mutations as a tool enabling genetic interrogation of this prospective
H3K4me0/H3K36me regulatory pathway. Because spn1-K192N generally provided more robust genetic interactions compared with spt6-14, most of our studies used the spn1-K192N mutation and we show these here, though we always observed qualitatively equivalent interactions using spt6-14.

A parsimonious model explaining our results posits an effector complex that opposes Spt6-Spn1 which itself is combinatorially controlled by H3K4/36me states. The Rpd3S histone deacetylase complex possesses two requisite characteristics that satisfy this hypothesis: 1, Rpd3S is positively regulated through an interaction of its Eaf3 subunit with H3K36me (Li et al. 2007; Xu et al. 2008; Ruan et al. 2015); and 2, The Rco1 subunit of Rpd3S binds to H3 N-terminal tails, and methylation of H3K4 opposes Rco1 binding (Lee et al. 2007; Ruan et al. 2015; McDaniel et al. 2016). We determined that the temperature sensitive growth defects caused by spn1-K192N were suppressed by rpd3Δ and that suppression by rpd3Δ and jhd2Δ were not additive, similarly to what we found with jhd2Δ and set2Δ (Figure 3A). Supporting the model that a loss of Rpd3S function specifically accounted for this result, we found that rco1Δ and eaf3Δ equivalently suppressed spn1-K192N (Figure 3B and data not shown). Eaf3 is also found in the NuA4 histone acetyltransferase complex. To rule out a role for Eaf3 as a component of NuA4 in our findings, we interrogated genetic interactions of eaf7Δ, which causes a specific loss of Eaf3 from NuA4 while leaving Eaf3 incorporation into Rpd3S unperturbed (Rossetto et al. 2014). Deletion of EAF7 caused no detectable genetic interactions with spn1-K192N, further upholding the conclusion that perturbation of Rpd3S specifically accounted for our findings (Figure S2).

The activation of Rpd3S by H3K36me is well established. We propose that H3K4me0 acts collaboratively with H3K36me, though in a comparatively minor fashion, to promote Rpd3S function (Figure 3C). To test this, we again employed P GAL-JHD2 strains. Like set2Δ,
suppression of spn1-K192N by both eaf3Δ and rco1Δ was epistatic to the enhanced growth defect caused by JHD2 galactose overexpression (Figure 3D and 3E). As mutations in RCO1 and EAF3 disrupt Rpd3S while leaving the Rpd3L complex intact (Carrozza et al. 2005b), these findings are consistent with Rpd3S mediating the proposed H3K4me0/H3K36me effector pathway (Figure 3C).

**Figure 3** SPN1 mutants were suppressed by loss of Rpd3S. Plate spot assays (as described in Figure 1) were used to compare the growth of the indicated strains on synthetic media with 2% dextrose (DEX) or 2% galactose (GAL). (A) Genetic interactions of jhd2Δ and rpd3Δ with spn1-K192N. (B) Genetic interactions of jhd2Δ and rco1Δ with spn1-K192N. P_GAL-JHD2 (as described in Figure 2) is used in the following experiments. (C) Genetic model of Rpd3S regulation by H3K4me and H3K36me through the methyl-binding specificities of the Rpd3S subunits Rco1 and Eaf3. The heavier arrow indicates that H3K36me has a more crucial role in the regulation of Rpd3S than that of H3K4me0. (D) Genetic interactions of rco1Δ and P_GAL-JHD2 with spn1-K192N on GAL. (E) Genetic interactions of eaf3Δ and P_GAL-JHD2 with spn1-K192N on GAL.
In contrast to the well-characterized mechanistic role of H3K36me in activation of Rpd3S through Eaf3, the potential role of H3K4me in modulation of Rpd3S through Rco1 is unexplored. Rco1 binds to H3 N-terminal tails that are hypo-methylated on H3K4 through its tandem PHD domains, each of which is essential for interaction with the H3 N-terminus (Mcdaniel et al. 2016). To specifically evaluate the genetic consequences of loss of H3K4 binding by Rco1, we made use of an allele of Rco1 that lacks one of these PHD domains (rco1-PHDΔ) but encodes a stable protein that is assembled into Rpd3S in vivo (Li et al. 2007). We found that rco1-PHDΔ suppressed spn1-K192N equivalently to jhd2Δ, and that these phenotypes were not additive (Figure 4A). These results support our model that Jhd2 promoted Rpd3S function by decreasing the methylation state of H3K4, thereby enhancing the ability of Rco1 to activate Rpd3S through its H3 N-terminal tail binding activity (Figure 3C). A further prediction of this model is that rco1Δ should also suppress the enhanced spn1-K192N growth defects caused by the complete loss of H3K4me (Lee et al. 2018). We tested this using the H3K4R allele and found this was indeed the case: rco1Δ suppressed the growth defects of spn1-K192N H3K4R mutants (Figure 4B).

Like H3K4me, H3K36me is also subject to the opposing roles of methyltransferases and demethylases. Our model predicts that increased H3K36me levels caused by loss of H3K36 demethylation should show the opposite phenotype as set2Δ and enhance the temperature sensitive growth defect of spn1-K192N. Yeast possess two conformed H3K36 demethylases belonging to the Jumonji superfamily, Jhd1 and Rph1. Rph1 demethylates both H3K36 tri- and dimethyl substrates (Kim and Buratowski 2007; Klose et al. 2007) while Jhd1 targets di- and monomethyl H3K36 (Tsukada et al. 2006; Fang et al. 2007). We found no consequence for jhd1Δ in any of our experiments (data not shown). In contrast to jhd1Δ, we found that rph1Δ strongly exacerbated the spn1-K192N growth defects, and that this consequence was reverted by set2Δ, suggesting that Rph1-mediated H3K36 demethylation opposed Rpd3S function.

Figure 4 H3K4 and H3K36 methylation states collaboratively modulated Rpd3S. Plate spot assays (as described in Figure 1) were used to compare the growth of the indicated strains. rco1-PHDΔ is an allele of RCO1 that lacks the N-terminal PHD domain but encodes a stable protein that is assembled into Rpd3S in vivo. (A) Genetic interactions of jhd2Δ and rco1-PHDΔ with spn1-K192N. Growth of two independent isolates of each genotype is shown. (B) Genetic interactions of rco1Δ and H3K4R with spn1-K192N. Growth of two independent isolates of each genotype is shown. (C) Genetic interactions of rph1Δ and set2Δ with spn1-K192N. (D) Genetic interactions of jhd2Δ and rph1Δ with spn1-K192N.
(Figure 3C and 4C). Indeed, *spn1-K192N* enhancement by *rph1Δ* was also reversed by *rco1Δ* (Figure S3). Taking into consideration the preferred target of Rph1 demethylation, our results are consistent with previous findings revealing that it is the tri-methylated state of H3K36 (H3K36me3) that specifically activates Rpd3S through Eaf3 binding (Steuou et al. 2016). Finally, we found that *spn1-K192N* enhancement by *rph1Δ* was partially suppressed by *jhd2Δ* (Figure 4D). The partial suppression of *spn1-K192N* *rph1Δ* by *jhd2Δ* is consistent with the more predominant role we propose for H3K36me in Rpd3S activation compared with H3K4me0 (Figure 3C).

**Rpd3L complex availability counterbalanced Rpd3S function**

Surprisingly, we found that *rpd3Δ* suppressed *spn1-K192N* less robustly than *rco1Δ*, and that this less robust suppression was in fact epistatic to *rco1Δ*, suggesting that loss of other Rpd3 functions had detrimental effects on *spn1-K192N* (Figure 5A). The Rpd3L complex seemed like the best candidate for such a counterbalancing Rpd3 function. To assess the function of Rpd3L in our experiments, we utilized mutations in Rpd3L subunits that had varying effects on Rpd3L complex formation. Loss of the Pho23 subunit of Rpd3L is documented to have only minor consequences on complex integrity (Carrozza et al. 2005a). PHO23 deletion had no effect on *spn1-K192N* temperature sensitivity nor did it affect suppression of *spn1-K192N* by *jhd2Δ* (Figure 5B). In contrast to PHO23, we found that *dep1Δ*, which was previously shown to completely disrupt Rpd3L complex formation (Carrozza et al. 2005a), enhanced the temperature sensitive growth defect of *spn1-K192N* (Figure 5C). Thus, Rpd3L appeared to somehow promote *spn1* function in direct contrast to its Rpd3S counterpart.

The disruption of Rpd3L was previously shown to greatly increase the amount of Rco1 protein in the cell (Biswas et al. 2008). We hypothesized that *dep1Δ* enhanced the temperature sensitivity of *spn1-K192N* via increased Rco1 protein levels and resulting increased Rpd3S function. To test this, we introduced *rco1Δ* into the *spn1-K192N* *dep1Δ* double mutant. Suppression of *spn1-K192N* by *rco1Δ* was epistatic to the enhancement caused by *dep1Δ* suggesting that the *dep1Δ* enhancement phenotype was due to increased Rpd3S function (Figure 5C). As our model posits that Jhd2 positively regulated Rpd3S through its demethylation of H3K4, *jhd2Δ* is predicted to therefore also alleviate the enhanced *spn1-K192N* growth defects caused by loss of Rpd3L. Consistent with this prediction, we found that *jhd2Δ* suppressed the growth defects of *spn1-K192N* *dep1Δ* mutants, but not to the same extent as *rco1Δ* did (Figure 5D). This less robust suppression of *spn1-K192N* by *jhd2Δ* compared with *rco1Δ* seems sensible, as Rpd3S is presumably still present in *jhd2Δ* cells while it is completely absent in *rco1Δ* cells (Carrozza et al. 2005a). As expected given the known role of Set2 in promoting Rpd3S chromatin recruitment, *set2Δ*, like *rco1Δ*, reverted the *spn1-K192N* *dep1Δ* growth defects. (Figure 5E).

**DISCUSSION**

We previously found that increased H3K4me3 levels suppressed TS alleles of Sp6-Spn1 while decreased H3K3me3 levels enhanced them (Lee et al. 2018). Here we show that perturbation of the Set2-H3K36me-Rpd3S pathway similarly suppresses TS alleles of Sp6-Spn1, revealing that Rpd3S function somehow opposes Sp6-Spn1 and permitting a series of genetic epistasis experiments investigating the contributions of H3K4me and H3K36me to Rpd3S functionality. Indeed, we provide genetic evidence consistent with the conclusion that H3K4me also modulates Rpd3S: *rco1Δ* and *rco1- PHΔ* suppressed *spn1-K192N* equivalently to and non-additively with *jhd2Δ*. Critically, *rco1Δ* also suppressed the exacerbated *spn1-K192N* growth defects caused by reduced H3K4me. Our subsequent genetic interrogations of both *RPH1* and of Rpd3L further support the model we present in Figure 3C. Functional insights into H3K4 and H3K36 demethylation in yeast remain relatively narrow compared with their corresponding methyltransferase enzymes, and our findings show that at least one role of Jhd2 and Rph1 is in fine-tuning H3K4me0/H3K36me3 activation of Rpd3S.

We attempted to use chromatin immunoprecipitation (ChIP) experiments with an epitope tagged allele of Rpd3 in mitotic cells to advance our model and were not able to attain reproducible Rpd3 chromatin association. Recent findings show that Rpd3 chromatin association is developmentally regulated and becomes much more robust and focused in haploid “Q” cells that have depleted their growth media and entered quiescence (McKnight et al. 2015). Perhaps relatedly, Rpd3 has prominent roles in meiotic development during which cells similarly transition into transcriptional dormancy in response to nutrient starvation (Xu et al. 2012; Yeheskey-Hayon et al. 2013; Lardenois et al. 2015). It seems plausible that the H3K4me0/H3K36me Rpd3S pathway we have identified using the sensitized Sp6-Spn1 TS allele background may have more relevant roles within these developmental contexts? It will be of interest to use more penetrating methods such as ChIP-Seq to advance our model in meiotic and/or Q cells. Of course, more accessible molecular methods such as RT-qPCR of select genes as well as ChIP to investigate H3K4me and H3K36me may also help to advance our model.

By what molecular mechanisms might H3K4me (and H3K36me) impact Rpd3S function? Histone modifications are typically suggested to impact chromatin effector complex function through their purported roles in recruitment of these complexes to specific regions of chromatin where these modifications reside. While considerable evidence exists for this, in the case of Rpd3S, focused studies suggest that the histone binding activities of Eaf3 and Rco1 play little/no role in chromatin recruitment and, rather, impact Rpd3S activity on chromatin allosterically (Drouin et al. 2010). Indeed, more recent findings illuminate a similar scenario in the function of the acetyltransferase complex NuA4, whose enzymatic action on chromatin is controlled through subunits with defined histone binding specificities that have no apparent role in chromatin recruitment of NuA4 (Steuou et al. 2016). It is thus attractive to speculate that H3K4me3 opposition of Rco1 histone binding prevents Rpd3S from deacetylating nucleosomes through allosteric inhibition of Rpd3S activity (as opposed to through Rpd3S chromatin association). Whether H3K4/36me regulation of Rpd3S acts through chromatin recruitment or allosteric modulation of Rpd3 activity (or both), our model predicts Rpd3S deacetylation should be restricted near the 5' ends of transcription units by H3K4me3, where this modification is typically found.

Finally, it is worth considering how reduced Rpd3S function suppresses Sp6-Spn1 TS mutations, as this interaction was so central to our studies. Our findings imply that increased acetylation might generically promote the function of histone chaperones such as Sp6-Spn1, a hypothesis consistent with the general view of acetylated nucleosomes being more loosely associated with DNA. Indeed, recent evidence suggest this is precisely the case for the related FACT complex (Sp16-Pob3), with histone acetylation promoting FACT localization on chromatin (Pathak et al. 2018). While this was not shown for Sp6-Spn1, it seems plausible that
the action of this complex on chromatin benefits as well from acetylated nucleosome substrates.

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Figure 5 Rpd3L complex availability counterbalanced Rpd3S function. Plate spot assays (as described in Figure 1) were used to compare the growth of the indicated strains. (A) Genetic interactions of rco1Δ and rpd3Δ with spn1-K192N. (B) Genetic interactions of jhd2Δ and pho23Δ with spn1-K192N. (C) Genetic interactions of dep1Δ and rco1Δ, with spn1-K192N. (D) Genetic interactions of dep1Δ and jhd2Δ with spn1-K192N. (E) Genetic interactions of dep1Δ and set2Δ with spn1-K192N. Growth of two independent isolates of each genotype is shown.

LITERATURE CITED
Bernstein, B. E., J. K. Tong, and S. L. Schreiber, 2000 Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA 97: 13708–13713. https://doi.org/10.1073/pnas.250477697
Biswas, D., R. Dutta-Biswas, M. Mitra, Y. Shibata, B. D. Strahl et al., 2006 Opposing roles for Set2 and yFACT in regulating TBP binding at promoters. EMBO J. 25: 4479–4489. https://doi.org/10.1038/sj.emboj.7601333
Biswas, D., S. Takahata, and D. J. Stillman, 2008 Different genetic functions for the Rpd3(L) and Rpd3(S) complexes suggest competition between NuA4 and Rpd3(S). Mol. Cell. Biol. 28: 4445–4458. https://doi.org/10.1128/MCB.00164-08
Xu, C., G. Cui, M. V. Botuyan, and G. Mer, 2008  Structural basis for the recognition of methylated histone H3K36 by the Eaf3 subunit of histone deacetylase complex Rpd3S. Structure 16: 1740–1750. https://doi.org/10.1016/j.str.2008.08.008

Xu, M., M. Soloveychik, M. Ranger, M. Schertzberg, Z. Shah et al., 2012  Timing of transcriptional quiescence during gametogenesis is controlled by global histone H3K4 demethylation. Dev. Cell 23: 1059–1071. https://doi.org/10.1016/j.devcel.2012.10.005

Yeheskely-Hayon, D., A. Kotler, M. Stark, T. Hashimshony, S. Sagee et al., 2013  The roles of the catalytic and noncatalytic activities of Rpd3L and Rpd3S in the regulation of gene transcription in yeast. PLoS One 8: e85088. https://doi.org/10.1371/journal.pone.0085088

Yun, M., J. Wu, J. L. Workman, and B. Li, 2011  Readers of histone modifications. Cell Res. 21: 564–578. https://doi.org/10.1038/cr.2011.42

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