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Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5′ transcribed regions

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Summary

Co-transcriptional histone methylations by Set1 and Set2 have been shown to affect histone acetylation at promoters and 3′ regions of genes, respectively. While histone H3K4 trimethylation (H3K4me3) is thought to promote nucleosome acetylation and remodeling near promoters, we show here that H3K4 dimethylation (H3K4me2) by Set1 leads to reduced histone acetylation levels near 5′ ends of genes. H3K4me2 recruits the Set3 complex via the Set3 PHD finger, localizing the Hos2 and Hst1 subunits to deacetylate histones in 5′ transcribed regions. Cells lacking the Set1-Set3 complex pathway are sensitive to mycophenolic acid and have reduced polymerase levels at a Set3 target gene, suggesting a positive role in transcription. We propose that Set1 establishes two distinct chromatin zones on genes: H3K4me3 leads to high levels of acetylation and low nucleosome density at promoters, while H3K4me2 just downstream recruits the Set3 complex to suppress nucleosome acetylation and remodeling.

Introduction

Eukaryotic transcription is dynamically regulated by post-translational modifications of histones that include phosphorylation, acetylation, ubiquitylation, and methylation (Kouzarides, 2007; Li et al., 2007a). Histone lysine methylation has important roles in regulation of transcription by RNA polymerase II (RNApII), X-chromosome inactivation, heterochromatin formation, and gene silencing. Methylations at specific histone residues are often correlated with either activation or repression of transcription. For example, H3K4, K36, and K79 methylations are enriched at actively transcribed regions, while transcriptionally inactive regions have higher levels of H3K9, H3K27, and H4K20 methylation. Lysines can be modified by one, two, or three methyl groups and these different methylation states can have distinct functions.

In budding yeast, H3K4 and K36 are methylated by Set1 and Set2, respectively (Ng et al., 2003; Strahl et al., 2002; van Leeuwen et al., 2002). These methylations are co-transcriptionally targeted to active genes through interactions between the methyltransferases and specific phosphorylated forms of the RNApII subunit Rpb1 C-terminal domain (CTD). CTD phosphorylation at serine 5 by the kinase subunit of TFIIH recruits the Set1-COMPASS complex to 5′ ends of genes, resulting in a peak of H3K4me3 around promoters (Krogan et al., 2003; Liu et al., 2005; Ng et al., 2003; Pokholok et al., 2005). Interestingly, H3K4me2 is highest

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just downstream of transcription start sites while mono-methylation is more dispersed throughout genes (Liu et al., 2005; Pokholok et al., 2005), leading to the suggestion that different levels of H3K4 methylation may have different functions. In the elongation phase of transcription, phosphorylation of CTD serines 2 and 5 by Ctk1 creates a binding site for Set2, resulting in H3K36me2 and me3 throughout transcribed regions but peaking in 3′ parts of genes (Kizer et al., 2005; Krogan et al., 2003; Li et al., 2002; Schaft et al., 2003; Xiao et al., 2003). Levels of these methylations may also be regulated by specific histone demethylase. The co-transcriptional methylation patterns and the enzymes that create them appear to be largely conserved between yeast and mammals (Barski et al., 2007; Bernstein et al., 2005; Liu et al., 2005; Pokholok et al., 2005).

The primary function of histone methylation appears to be to recruit downstream effector proteins. Known methyl-lysine binding domains include the chromodomain, the tudor domain, and PHD fingers. These domains appear in many proteins that affect transcription and chromatin (Taverna et al., 2007). For example, the chromodomain protein Eaf3 and PHD finger protein Rco1 are subunits of the Rpd3C(S) deacetylase complex (Carrozza et al., 2005; Keogh et al., 2005). The Eaf3 chromodomain binds to H3 tails methylated at K36 by Set2 and, together with the Rco1 PHD finger, is crucial for the association of Rpd3C(S) with chromatin (Li et al., 2007b). The Set2-Rpd3C(S) pathway deacetylates histones within transcribed regions to repress cryptic RNApII promoters (Carrozza et al., 2005; Li et al., 2007c). This pathway also negatively regulates transcription elongation: deleting the genes for Set2 or Rpd3C(S) bypasses the requirement for positive elongation factor Bur1 and confers resistance to elongation inhibitors 6-azauracil (6-AU) and mycophenolic acid (MPA) (Keogh et al., 2005).

The functions of H3K4 methylation are less well understood and apparently more complex than H3K36 methylation. In mammalian systems, H3K4 methyl binding proteins include the chromodomain protein Chd1 (a chromatin remodeler), PHD finger protein BPTF (a subunit of the NURF chromatin remodeling complex), the ING PHD finger proteins (associated with histone acetyltransferase and deacetylase complexes), and double tudor domain protein JMJD2A (a JmjC histone demethylase that also contains two PHD fingers) (Huang et al., 2006; Li et al., 2006; Pena et al., 2006; Shi et al., 2006; Sims et al., 2005; Sims and Reinberg, 2006; Taverna et al., 2007; Wysocka et al., 2006).

In Saccharomyces cerevisiae, genetic experiments indicate that H3K4 methylation functions in diverse cellular processes that include RNApII transcription, DNA repair, meiotic differentiation, and silencing at telomeres and rDNA (Dehe and Geli, 2006). Loss of H3K4 methylation by *set1Δ* results in changes in transcription patterns (Boa et al., 2003), but the downstream effector proteins are still unclear. Despite some contradictory evidence, it appears unlikely that yeast Chd1 interacts with H3K4me3 (Flanagan et al., 2007; Okuda et al., 2007; Pray-Grant et al., 2005). The PHD finger protein Yng1 recruits the HAT complex NuA3 to H3K4me3, but loss of the NuA3 complex has only a marginal effect on RNApII transcription (Taverna et al., 2006). Therefore, additional factors are likely to recognize H3K4 methylation to mediate downstream effects. In budding yeast, multiple PHD finger proteins can bind to methylated H3K4 peptides in vitro (Shi et al., 2007), but the functional significance of these interactions remains to be demonstrated.

Interestingly, the Set3 protein contains a PHD finger that can bind to methylated H3K4 in vitro (Shi et al., 2007). Although Set3 contains a SET domain, no methyltransferase activity has yet been reported. Set3 was genetically identified as a repressor of meiosis and interacts physically with two histone deacetylases, the Rpd3-like protein Hos2 and the sirtuin Hst1. Other members of the complex (Set3C) include Snt1, Sif2, Hos4, and Cpr1 (Pijnappel et al., 2001). Another study indicates that Set3 and Hos2 can contribute positively to efficient transcription by RNApII; Set3C regulates histone acetylation levels in the coding regions of *GAL* genes and

INO1 under activating conditions (Wang et al., 2002). How this deacetylation is targeted has been unclear.

Here we show that H3K4 dimethylation by Set1 recruits Set3C to promoter-proximal regions. The Hos2 and Hst1 subunits of Set3C deacetylate histone H4 and H3 near 5′ ends of genes. Deacetylation of nucleosomes just downstream of the promoter may promote efficient elongation by RNApII because mutants for the Set1-Set3 complex pathway affect RNApII levels at a target gene and are sensitive to MPA. Our results indicate the H3K4me2 and H3K4me3 are distinct marks with very different effects on histone acetylation.

Results

Deletion of *SET1* or *SET2* increases histone acetylation in distinct regions of genes

Histone methylations are most often linked to repression of transcription. Even the co-transcriptional methylation of H3K36 by Set2, a modification positively correlated with gene expression, acts to repress cryptic initiation and elongation within transcribed regions of genes. In apparent contrast, Set1-dependent methylation of H3K4 is generally thought to positively affect gene expression. To further explore the downstream effects of H3K4 and other co-transcriptional histone methylations, histone acetylation patterns were studied at several actively transcribed genes in wild type, *set1Δ*, and *set2Δ* strains. Acetylation of both histone H4 (using an antibody that recognizes tetra-acetyl H4) and H3 (using an antibody recognizing K9ac and K14ac) were assayed by chromatin immunoprecipitation (ChIP). Levels of acetylation were normalized to total histone content as measured by H3 crosslinking.

The *set1Δ*, but not *set2Δ*, strain showed increased acetylation at a telomere-proximal region of chromosome VI (TEL), which is used as a non-transcribed internal control (Fig 1A–C and E). Increased telomere acetylation was also observed in *dot1Δ* cells (Fig S1). This is consistent with reports that H3K4 and K79 methylation are required for silencing at telomeres (Fingerman et al., 2005; van Leeuwen et al., 2002). The increased acetylation in these mutants likely leads to increased DNA accessibility and derepression of genes near telomeres.

As expected, in wild-type cells the promoters of *YEF3* and *PMA1* had relatively low levels of nucleosomes, but the H4 and H3 histones present were highly acetylated (Fig 1A, B). Lower histone levels were also seen at 3′ ends of these genes and the remaining H4 and H3 had higher levels of acetylation than transcribed regions, probably due to the promoters of adjacent genes (Fig 1A, B, primer pairs 5). As previously observed (Carrozza et al., 2005; Keogh et al., 2005; Li et al., 2007c), a *set2Δ* strain had increased levels of H4 and H3 acetylation in the 3′ transcribed regions of *YEF3* and *PMA1* (Fig 1A, B, primer pairs 2–4). Interestingly, this strain also exhibited slightly reduced levels of H4 acetylation at promoters of these genes, possibly reflecting the role of Eaf3 in the NuA4 complex (Reid et al., 2004). Surprisingly, increased acetylation and lower overall histone density in coding regions was also observed in the absence of *SET1*. This increase was strongest in the 5′ transcribed region of the gene near and just downstream of the promoter (Fig 1A, B, primer pairs 1 and 2). These changes were overlapping but clearly different from the changes seen in the strain lacking Set2.

Since the Set2-Rpd3C(S) pathway preferentially affects histone acetylation at relatively long genes (Li et al., 2007c), we also analyzed two shorter genes, *PYK1* and *ADH1*. Lack of *SET2* increased acetylation of both histones H3 and H4 in 3′ transcribed regions of *YEF3* and *PMA1* (Fig 1A, B) but not at *PYK1* or *ADH1* (Fig 1C). In contrast, deletion of *SET1* increased acetylation of H3 and H4 in the 5′ regions of *PYK1* and *ADH1* as well as *PMA1* and *YEF3* (Fig 1A–D). Therefore, the Set1 effects on acetylation are distinct from those seen in cells lacking the Set2-Rpd3C(S) pathway.

H3K4 dimethylation leads to reduced histone acetylation at 5′ ends of genes

It was important to ask whether the increased promoter proximal acetylation in *set1Δ* was due to loss of H3K4 methylation, and if so, which level of methylation. ChIP analysis of histone acetylation in a strain in which histone H3K4 was mutated to alanine increased acetylation in 5′ regions similarly to *set1Δ* (data not shown), making it likely that the Set1 effect was mediated through H3K4 methylation. The process of histone H3K4 methylation is subject to complex regulation by multiple factors that contribute specifically to H3K4 di- or trimethylation (Laribee et al., 2005; Lee et al., 2007; Mueller et al., 2006; Shahbazian et al., 2005; Wood et al., 2005). We took advantage of various mutants to manipulate the methylation level of H3K4.

The Rad6-Bre1 complex ubiquitylates K123 of histone H2B and this modification is specifically required for multiple methylations at H3K4 (Dehe et al., 2005). In agreement with earlier studies, our ChIP results showed that deletion of *RAD6* or *BRE1* caused a complete loss of H3K4me3 and H3K4me2, but H3K4me1 levels were unaffected (Fig 2A). Interestingly, these deletions lead to increased histone acetylation in the 5′ regions of *YEF3* and other genes tested, similar to the patterns seen in *set1Δ* (Fig 2A, B and data not shown). Therefore, monomethylation of H3K4 is not sufficient to reduce acetylation levels just downstream of promoters. H2B ubiquitination by Rad6-Bre1 is also required for H3K79 methylation by Dot1 (Shahbazian et al., 2005). However, deletion of *DOT1* had no effect on 5′ region histone acetylation levels (Fig S1) so these effects cannot be due to loss of H3K79 methylation. Based on these results, we can infer that loss of H3K4me2 or me3 leads to increased histone acetylation at 5′ ends of genes.

Genome-wide chromatin immunoprecipitation experiments have shown that H3K4me3 has a sharp peak at promoters while H3K4me2 peaks slightly further downstream (Barski et al., 2007; Bernstein et al., 2005; Liu et al., 2005; Pokholok et al., 2005). This can be seen at the *YEF3* gene, where H3K4me3 peaks near the transcription start site but H3K4me2 is strongly enriched at the 5′ end of the transcribed region (Fig 2A). As this region overlaps the site of increased acetylation in *set1Δ*, we hypothesized that H3K4me2 might direct decreased acetylation of histones. The Set1 protein contains both SET and RNA Recognition Motif (RRM) domains. The role of the RRM domain is not yet clear, but its deletion specifically reduces H3K4me3 but not H3K4me2 (Fingerman et al., 2005; Schlichter and Cairns, 2005). In our hands, expression of Set1 lacking the RRM domain caused a complete loss of H3K4me3 and only a slight reduction of H3K4me2 at the 5′ end of *YEF3* and other genes tested (Fig 2C, D). Despite the lack of H3K4me3, no increase in H3 acetylation and only slightly increased H4 acetylation was observed in this mutant (Fig 2C, D). These results indicate that H3K4me2 is sufficient to promote decreased histone acetylation near 5′ ends of genes.

Loss of H3K4me3 results in increased acetylation at telomeres

Although histone methylations at H3K4 and H3K79 are correlated positively with transcription, their respective methyltransferases Set1 and Dot1 are important for silencing at telomeres (Fingerman et al., 2005; van Leeuwen et al., 2002). It is not clear how these two methyl marks regulate silencing, but histone acetylation at telomeres is strongly increased in the absence of *SET1* or *DOT1* (Figs 1E and S2A). To determine which level of H3K4 methylation contributes to suppression of telomere acetylation, various additional mutants were tested. Cells lacking *rad6Δ* or *bre1Δ* also show increased telomere acetylation, arguing that H3K4me1 is not sufficient to maintain lower acetylation levels (Fig S2A, B). Deletion of the Set1 RRM domain, where levels of H3K4me2 and K4me1 are maintained, also resulted in high levels of telomere acetylation (Fig 2C, S2A, B). Therefore, H3K4me3, but not H3K4me2 or H3K4me1, is required to promote lower levels of acetylation at telomeres, in agreement with the specific role of H3K4me3 in telomeric silencing (Fingerman et al., 2005). This contrasts with the specific role for H3K4me2 in acetylation near 5′ ends of genes and argues

that regulation of acetylation levels by Set1 at these two locations occurs by distinct mechanisms.

The Set3 complex deacetylates histones at 5′ ends of genes

A recent study showed that several PHD finger proteins in budding yeast can bind to methylated K4 of histone H3 in vitro (Shi et al., 2007). Likely candidates that could affect histone acetylation levels are *RXT1*, *PHO23*, and *SET3*. Rxt1 and Pho23 are components of the Rpd3C (L) histone deacetylase complex (Carrozza et al., 2005; Keogh et al., 2005). Set3 is part of a complex containing the two histone deacetylases Hos2 and Hst1 (Pijnappel et al., 2001). To test whether H3K4 methylation might direct histone deacetylation by either of these HDAC complexes, strains deleted for *SET3*, *RXT1*, or *PHO23* were tested for increased histone acetylation in transcribed regions.

Although deletion of *PHO23* slightly increased acetylation at the *YEF3* promoter, neither *rxt1Δ* nor *pho23Δ* affected histone acetylation in 5′ transcribed regions (Fig 3A). In contrast, *set3Δ* led to significantly increased histone acetylation in this location, a pattern closely resembling the changes seen in a *SET1* deletion (Fig 3A). Similar effects of *set3Δ* were also observed at the *PMA1*, *PYK1*, *ADH1*, and *RPS13* genes (Fig 3B). One notable difference between *set1Δ* and *set3Δ* is that *set3Δ* did not increase acetylation levels at telomeres. Since loss of methylation at either H3K4 or K36 increases acetylation in transcribed regions, levels of these modifications were also tested in a *set3Δ* strain. No difference was seen in H3K4me2, H3K4me3, or H3K36me3 levels between *set3Δ* and wild-type strains (Fig S3). Therefore, the increased acetylation in *set3Δ* is not indirectly due to loss of H3K4 or K36 methylation.

Set3 is part of a complex (Set3C) containing the histone deacetylases Hos2 and Hst1, as well as Cpr1, Snt1, Sif2, and Hos4 (Pijnappel et al., 2001; see Fig S4). ChIP experiments were carried out to determine histone acetylation levels in the absence of each subunit of Set3C. Deletions of any of the Set3C subunits increased acetylation downstream of the promoter region, with *hos2Δ*, *snt1Δ*, *sif2Δ*, and *hos4Δ* showing the same pattern as *set1Δ* (Figs 3C, S5, and S6). Deletions of two subunits differed somewhat. In the *cpr1Δ* strain the increase in H3 acetylation was similar to *set3Δ*, but H4 acetylation was also significantly higher at 3′ ends and telomeres (Figs 3C and S6). Therefore, Cpr1 likely has other functions in addition to its role in Set3C. Interestingly, *hst1Δ* had a smaller effect on H4 acetylation, while the increase in H3 acetylation level in *hst1Δ* was larger than that seen with other mutants for the Set3 complex (Fig 3C). This result could suggest that, within Set3C, Hst1 primarily deacetylates H3 while Hos2 functions at H4. Alternatively, the difference could result from the fact that Hst1 is also part of an additional complex containing Sum1 (Xie et al., 1999). Taken together, the ChIP experiments indicate that Set3C deacetylates histones near the 5′ ends of genes.

H3K4 methylation recruits the Set3 complex

Given that loss of Set1 or Set3C resulted in higher acetylation levels in 5′ transcribed regions, we hypothesized that H3K4 methylation might recruit the Set3 complex via binding to the Set3 PHD finger domain. To test whether Set3C binding to nucleosomes in vivo requires H3K4 methylation, the complex was purified from extracts using TAP-tagged Hos2 or Set3. Precipitates were analyzed by immunoblot analysis with anti-H3 antibody. In strains containing Set1 (*SET1*), both Set3 and Hos2 interact with histone H3. In contrast, Set3C from cells lacking Set1 (*set1Δ*) had no detectable H3 association (Fig 4A).

Mutation of a key tryptophan residue (W140A) in the Set3 PHD finger abrogates association of Set3 with methylated H3K4 in vitro (Shi et al., 2007). To test the role of the Set3 PHD finger, chromatin fragments were isolated (via an Hhf2-TAP protein) from *SET1* or *set1Δ* strains and then incubated with extracts from cells expressing epitope-tagged wild-type (Set3-HA) or

mutant Set3 (Set3-HA(W140A)). The precipitates were analyzed by immunoblot analysis with anti-HA antibody. Again, only chromatin from Set1-containing cells could bind to Set3C, and this interaction was completely dependent upon having a functional Set3 PHD finger (Fig 4B).

To localize the interaction of Set3C with nucleosomes along genes, ChIP of Hos2-TAP was performed. In low resolution experiments, Hos2 has previously been reported to crosslink to transcribed regions (Wang et al., 2002). We also observed that Hos2 localizes to transcribed regions, but specifically biased to 5′ ends of *YEF3* and other genes (Fig 4C and data not shown). Importantly, deletion of Set1 (Fig 4C) or the PHD finger mutant Set3(W140A) (Fig 4D) caused loss of Hos2 crosslinking, strongly arguing that Set3C recruitment requires interaction between Set3 PHD finger and methylated H3K4.

Next, histone acetylation levels were assayed in cells expressing the PHD finger mutant Set3 (W140A). As shown in Fig 4E, the Set3(W140A) mutation caused an increase in histone acetylation at the 5′ transcribed region that was indistinguishable from that seen in a *set3Δ* strain. The mutant Set3 protein was expressed at levels comparable to wild type (Fig 4B), so the increased acetylation is not due to defects in protein stability. Based on these in vitro and in vivo results, we conclude that the Set3 PHD finger links H3K4 methylation to histone deacetylation by Set3C at 5′ ends of genes.

Set3C is recruited by H3K4 dimethylation

Our results suggest that H3K4me2 leads to reduction of histone acetylation in 5′ transcribed regions via Set3C. However, it was reported that a GST-Set3 PHD finger fusion specifically bound in vitro to H3K4me3 but not K4me2 (Shi et al., 2007). To assay binding preference in a more physiological context, TAP-purified Set3C (Fig 5A) was incubated with beads coupled to the same H3 tail peptides used by Shi et al. (generously provided by Or Gozani). Set3C showed no binding to unmethylated peptide. Importantly, native Set3C bound preferentially to H3K4me2 peptide relative to tri- or monomethylated K4 (Fig 5B). Similar results were obtained using whole cell extracts expressing a tagged Hos2 (Fig 5C). No such binding was observed in extracts containing the PHD finger mutant Set3(W140A), indicating that the Set3 PHD finger mediates interaction between Set3C and H3K4me2 (Fig 5D).

To test whether H3K4me2 mediates Set3C recruitment in vivo, *HOS2* crosslinking was assayed in *set1ΔRRM* and *rad6Δ* mutant strains. Hos2 was unaffected in the Set1ΔRRM mutant, indicating that H3K4me3 is not required for Set3C recruitment (Fig 5E). However, the Hos2 ChIP signal was gone in the *rad6Δ* strain that lacks both H3K4me2 and H3K4me3 (Fig 5F). Therefore, as seen for 5′ deacetylation (Fig 2), H3K4me2 but not H3K4me3 is the relevant mark for Set3C recruitment in vivo.

The Set3 complex promotes transcription

Cells lacking Set3 grow normally and are not sensitive to DNA damaging agents such as MMS. EMS, UV irradiation, bleomycin, or hydroxyurea (Pijnappel et al., 2001). The available genetic evidence suggests Set3, like Set1 and Set2, could be involved in transcription. Since *set1Δ* cells are sensitive to the drugs 6-AU and MPA, which affect transcription elongation by reducing nucleotide pools, we tested whether deleting Set3C genes causes sensitivity to either of these chemicals. Unlike *set1Δ*, mutants for Set3C are not sensitive to 6-AU. However, deletions of *SET3*, *HOS2*, *SNT1*, or *SIF2* are sensitive to MPA (Fig 6A). Interestingly, this is not true for deletions of *HST1* or *CPR1*, the two subunits that differ from the rest of the complex in their effects on acetylation (Fig 3C and Fig S6). Recently, it was shown that the Set3C core subunits Set3, Hos2, Sif2, and Snt1, but not Hst1 and Cpr1, were required for normal growth in the presence of tunicamycin (Cohen et al., 2008). Deletions of *HST1* or *CPR1* also show a distinct pattern of genetic interactions from other Set3C subunits in high-throughput E-MAP

assays (Krogan et al., 2006). Therefore, Hst1 and Cpr1 may have somewhat different functions than those of the other Set3C subunits. To determine if MPA sensitivity is related to Set3C binding to methylated H3K4, cells expressing PHD finger mutant Set3(W140A) were tested. Set3(W140A) could not rescue the MPA sensitivity of a *set3Δ* cell (Fig 6B), indicating that nucleosome binding is essential for Set3C function in vivo.

It was previously shown that Set3 and Hos2 are required for efficient transcription of *GAL* genes (Wang et al., 2002), so ChIP was used to monitor *GAL1* under inducing conditions. As seen at other genes, deletion of *SET3* led to increased acetylation at 5′ end of *GAL1* (Fig 6C and S7A). In contrast, RNApII signals in *set3Δ* were reduced throughout *GAL1* (Fig 6D and S7B). RNApII was also assayed after glucose shut off of *GAL1* transcription. After two minutes, both WT and *set3Δ* strains lost promoter-associated RNApII, indicating that repression is rapid. By three minutes, *set3Δ* had reduced levels of RNApII at the 3′ end while levels remained high in WT. RNApII was completely dissociated from *GAL1* gene in both strains after five minutes (Fig 6D and S7B). *GAL1* induction was assayed by monitoring mRNA levels after addition of galactose. *GAL1* transcripts rapidly accumulated in cells expressing wild type Set3, while lower levels were seen in *set3Δ* and PHD finger mutant Set3(W140A) cells (Fig 6E). No changes in transcript levels were seen at several other genes tested (Fig S8). These results suggest that Set3C can affect gene expression by promoting the interaction of RNApII with a subset of genes that includes *GAL1*. Whether Set3C has different effects on other genes remains to be seen.

DISCUSSION

Methylation of histone H3K4 correlates with active transcription, but it is not clear how this modification affects gene expression. Here we show that one function of H3K4 methylation is to recruit an HDAC complex to 5′ ends of genes via the PHD domain of the Set3 protein. Set3C contains two histone deacetylase subunits, Hos2 and Hst1. This pathway is distinct from the pathway used further downstream, where Set2 methylation of H3K36 targets histone deacetylation by the Rpd3C(S) complex (Carrozza et al., 2005; Keogh et al., 2005). We propose that transcribed genes can be divided into at least three distinct chromatin zones (Fig 7). Promoter regions are marked by high acetylation levels and H3K4me3, but low nucleosome density. A region characterized by H3K4me2 lies just downstream, where acetylation levels are suppressed by Set3C. More distal transcribed regions have high H3K36 methylation levels and Rpd3C(S) deacetylation activity. How the boundaries between these regions are established and whether they are sharp or fluid will be important to explore.

Both H3K4me3 and H3K4me2 localize near transcription start sites, but H3K4me2 peaks slightly downstream of H3K4me3 (Barski et al., 2007; Bernstein et al., 2005; Liu et al., 2005; Pokholok et al., 2005). The transition between these modifications appears to be regulated by multiple factors and our findings indicate that these two methylation levels define distinct chromatin states. Deletion of the Set1 RRM, which specifically abrogates H3K4me3 while preserving H3K4me2, does not increase 5′ acetylation levels and has no effect on Set3C recruitment. In contrast, cells lacking both H3K4me2 and me3 (*rad6Δ* or *bre1Δ*) lose Set3C binding and have increased levels of 5′ acetylation. Therefore, H3K4me2 must be the relevant mark for recruiting Set3C. Recently, loss of the Bur1-Bur2 kinase or PAF complex were shown to cause increased histone acetylation at 5′ ends of genes independently of the Set2/Rpd3C(S) pathway (Chu et al., 2007). Both the Bur and PAF complexes promote H2B ubiquitylation and thereby H3K4me2 and me3 (Laribee et al., 2005; Wood et al., 2005), so their effects on acetylation may be mediated by Set3C.

Multiple findings suggest the physiological role of Set3C is in transcription regulation. Set3C gene deletions show synthetic negative genetic interactions with deletions of many

transcription related genes, including those for Set2, the SWR/Htz1 complex, Rpd3(L)C, and many components of the SAGA, THO, PAF, and basal transcription complexes (Collins et al., 2007; Krogan et al., 2003). Loss of Set3C derepresses meiotic gene transcription (Pijnappel et al., 2001) but impairs efficient transcription of *GAL* and *INO1* genes (Wang et al., 2002). Deletion of *SET3* reduces RNApII crosslinking to *GAL1* (Fig 6D) and loss of Set3C or a Set3 PHD finger mutation results in inefficient induction of *GAL1* (Fig 6E). These mutations also confer sensitivity to MPA, a phenotype often correlated with inefficient transcription elongation (Fig 6A). The experiments presented here strongly suggest that Set3C functions near 5′ ends of genes to affect transcription.

At the molecular level, deacetylation of 5′ transcribed regions could affect transcription in several ways. One important function of the Set1-Set3C pathway may be to limit histone acetylation from spreading beyond promoters into transcribed regions. Transcription activators bound upstream recruit HAT and chromatin remodeling complexes to promoters to remove or displace nucleosomes that would otherwise occlude the promoter. The Set1-Set3C pathway may “sharpen” this zone of nucleosome acetylation and remodeling. In the absence of Set1 or Set3C, we observe increased acetylation at 5′ ends that is often accompanied by lower histone H3 density (Figs 1, 2, 3). Although Set3C is not essential for laboratory growth, it may be critical for expressing particular genes or under more stringently selective growth conditions. An increasing number of genes exhibit regulation at the level of RNApII escape into elongation phase (so called “paused” polymerases, see Margaritis and Holstege, 2008). It is interesting to speculate that early stages of elongation could be regulated through modifications of the first transcribed nucleosome (Morillon et al., 2005).

Another function for the Set1-Set3C pathway could be to repress transcription initiation from cryptic promoters within transcribed regions. The Set2-Rpd3C(S) pathway deacetylates histones near 3′ ends of genes and this inhibits transcription initiation from cryptic promoters in that region (Carrozza et al., 2005; Keogh et al., 2005). However, this pathway appears to be biased towards cryptic promoters in long, infrequently transcribed genes (Li et al., 2007c). Loss of Set3 did not lead to the same internal initiations reported for loss of Rpd3C(S) (data not shown). Unlike loss of the Set1-Set3C pathway, deletion of *SET2* does not affect acetylation at two shorter genes, *PYK1* and *ADH1* (Fig 1C). The Set1-Set3C pathway may specifically be used for repression of cryptic transcription initiation near 5′ ends of long genes and at relatively short genes, but so far we have not observed such cryptic transcripts.

If H3K4me2 recruits Set3C, what is the role of H3K4me3? Cells expressing Set1ΔRRM lack H3K4me3 (Fingerman et al., 2005; Schlichter and Cairns, 2005) and have increased histone acetylation near telomeres (Fig S2), consistent with a previous report that H3K4me3 is required for telomeric silencing (Fingerman et al., 2005). This effect was not seen in cells lacking Set3C, arguing that telomeric deacetylation is carried out by another HDAC, most likely Sir2/3/4. How H3K4me3 promotes telomeric deacetylation is unclear, but it appears that loss of H3K4me3 allows Sir2/3/4 to bind to other regions of the genome, thereby titrating it away from telomeres (Santos-Rosa et al., 2004; van Leeuwen and Gottschling, 2002; Venkatasubrahmanyam et al., 2007). H3K4 methylation levels at telomeres are much lower than that seen around transcribed regions (Fig 2), making it unlikely that H3K4me3 directly promotes telomeric silencing.

The role of H3K4me3 in transcription remains unclear. We did not observe major changes in acetylation of genes in Set1ΔRRM cells. Several groups (Fingerman et al., 2005; Schlichter and Cairns, 2005) saw no transcription defects at several test genes in this mutant. While one analysis of gene expression in a *set1Δ* strain indicated a widespread role in transcription (Boa et al., 2003), a recent microarray analysis found that loss of Set1 only resulted in partial derepression of genes near telomeres (Venkatasubrahmanyam et al., 2007). This study

suggested that Set1 functions redundantly with Htz1 to antagonize ectopic silencing of euchromatic genes by Sir2/3/4 (Venkatasubrahmanyam et al., 2007). H3K4me3 may directly block binding of the Sir2 complex to nucleosomes or instead antagonize repression by recruiting HATs and remodelers to promoters.

Several HAT complexes could fit this second model (Fig 7). NuA3 complex contains a PHD finger protein called Yng1 that binds to H3K4me3, helping recruit the complex to 5′ ends of genes where the Sas3 subunit acetylates H3K14 (Taverna et al., 2006). NuA3 contains a second PHD finger protein called Nto1, which binds methylated H3K36 in vitro (Shi et al., 2007), that is also necessary for NuA3 recruitment (Martin et al., 2006). A second HAT that may bind H3K4me3 is NuA4, which contains two chromodomain proteins, Esa1 and Eaf3, and a PHD finger protein, Yng2. The specific binding site for the Esa1 chromodomain is unknown, but Eaf3 and Yng2 bind to methylated H3K36 and H3K4, respectively. For NuA3 and NuA4, both H3K4 and H3K36 methylation could promote recruitment either together or separately. Interestingly, a slight decrease in promoter acetylation is seen upon deletion of *SET2* (Fig 2) or *EAF3* (Reid et al., 2004), indicating that H3K36 methylation may be more important near promoters than current models predict.

Given the overall conservation of co-transcriptional H3K4 and H3K36 methylation, it is likely that higher eukaryotes also contain a deacetylase complex that is recruited by H3K4me2. Based on computational analysis of protein sequence and domain architecture, the mammalian protein that most closely resembles Set3 is MLL5, which also has a single PHD finger and SET domain. Little is known about MLL5, but it is located in a region of chromosome 7 that is often deleted in myeloid leukemias (Emerling et al., 2002). It has also been suggested that the Set3 complex may be related to the NcoR-SMRT complexes, both of which contain an Rpd3-like protein (HDAC3 in mammals, Hos2 in yeast), a WD40 protein (TBL1 in mammals, Sif2 in yeast), and a SANT domain protein (NcoR/SMRT and yeast Snt1) (Pijnappel et al., 2001; Yang and Seto, 2008). It is also possible that a non-homologous complex performs an analogous role to the yeast Set3C. The mammalian ING1 and ING2 proteins contain PHD fingers and are subunits of mSin3A histone deacetylase complexes (Yang and Seto, 2008). These ING proteins can bind to methylated H3K4, but it remains to be seen whether they target histone deacetylation to specific transcribed regions of genes.

In yeast and mammalian genomes, there are multiple SET domain and PHD finger proteins that remain to be characterized. Given the connections between the aberrant gene expression in cancer cells and the complexes containing Set1/MLL and Set2, understanding the functions of Set3 and the other Set/MLL proteins remains an important goal.

Experimental Procedures

Antibodies

Histone antibodies used: Anti-H3K4me1 (Upstate 07-473), anti-H3K4me2 (Upstate 06-030), anti-H3K4me3 (Upstate 07-473), anti-acetyl H4 (Upstate 06-598), anti-acetyl H3 (Upstate 06-599), anti-H3 (Abcam 1791), and anti-H3K36me3 (Abcam 9050). Anti-Rpb3 was from Neoclone.

Yeast Strains and Plasmids

Yeast strains used are listed in Sup Table S1. To generate pRS415-*SET3*-3XHA, the *SET3* promoter and ORF region was amplified by PCR using primers creating terminal NotI/BamHI sites. This PCR fragment was ligated into the corresponding sites of pRS415-*JHD1*-3XHA (Kim and Buratowski, 2007) to produce an in frame fusion expressing triple-HA epitope tagged protein. The *SET3* W140A mutant was constructed by PCR mutagenesis of pRS415-

SET3-3XHA The sequences of oligonucleotides used are listed in Sup Table S2. Spotting analyses for sensitivity to 6-azauracil (6-AU; 75 or 150 μg/ml) or mycophenolic acid (MPA; 15 or 30μg/ml) were performed as previously described (Kim and Buratowski, 2007).

TAP purification

Strains were grown in YPD medium to an optical density at 600 nm of 0.7. Extracts were made by glass bead disruption in lysis buffer (10 mM Tris-HCl (pH 7.9), 350 mM NaCl, 10% glycerol, 2 mM dithiothreitol, 0.1% NP-40, 2mM EDTA) containing protease inhibitors. Purification was as described (Gould et al., 2004). Purified proteins were eluted from the calmodulin column with buffer containing 20 mM EGTA and analyzed by SDS-PAGE. In Fig 5A, 40μl of purified Set3C was used for silver staining.

Peptide interaction assays

For Fig 5D, peptide binding assays were performed as described (Shi et al., 2007) with 1 μg of biotinylated histone peptides and 30μl of purified Set3 complex Components were incubated inbinding buffer (50 mM Tris-HCl (pH 7.5), 0.1% NP-40) plus 300 mM NaCl at 4°C overnight. For Fig 5C and D, whole cell extracts made by glass bead lysis in binding buffer containing 500mM NaCl and protease inhibitors. Extracts were diluted to 300mM NaCl and 500 μg of extracts were incubated with 1 μg of biotinylated histone peptides at 4°C overnight. 60μl of streptavidin coupled Dynabeads (Invitrogen) were added to precipitate complexes. After 1 hour incubation at 4°C, beads were washed four times with 1.5 ml of binding buffer and resolved by SDS-PAGE followed by immunoblot analysis.

Chromatin binding assay

Chromatin pull-down assay was carried out as previously described (Kim and Buratowski, 2007). For Fig 4A, Hos2-TAP or Set3-TAP from a *SET1* or *set1Δ* background was precipitated with IgG Sepharose. The bead-bound proteins were washed four times with lysis buffer (10 mM Tris-Cl (pH 8.0), 150 mM NaCl, 0.1% Nonidet P-40, and protease inhibitors) and resolved by SDS-PAGE followed by immunoblot analysis with anti-TAP or anti-H3 antibody. For Fig 4B, chromatin fragments were isolated from whole cell extracts by IgG Sepharose precipitation of Hhf2-TAP. Bead-bound nucleosomes were incubated with whole cell extracts from cells containing tagged Set3-HA proteins. After overnight incubation at 4°C, complexes were washed four times with lysis buffer and resolved by SDS-PAGE followed by immunoblot analysis.

Chromatin Immunoprecipitations

Chromatin immunoprecipitations were done as previously described (Kim and Buratowski, 2007). 0.5μl of anti-H3, anti-acetyl H4, anti-H3K4me3, anti-H3K4me2, or anti-H3K36me3; 1.0μl of anti-acetyl H3; or 2.0μl of anti-H3K4me1 were bound to Protein A-agarose beads and used to precipitate chromatin. For all antibodies except for anti-H3K4me1 and anti-H3, binding was done overnight in FA lysis buffer containing 1 M NaCl. Precipitates were washed with the same buffer, once with FA lysis buffer containing 1.5 M NaCl, once with buffer containing 10 mM Tris-HCl (pH 8.0), 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% Na Deoxycholate, and once with TE (10 mM Tris-HCl(pH8.0), 1 mM EDTA). For anti-H3K4me1 and anti-H3 antibodies, binding was done overnight in FA lysis buffer containing 275 mM NaCl and the precipitates were washed with the same buffer, and once with FA lysis buffer containing 500 mM NaCl. PCR conditions were 60 sec at 94°C, followed by 23~25 cycles of 30 sec at 94°C, 30 sec at 55°C, and 45 sec at 72°C, followed by 2 min at 72°C. The sequences of oligonucleotides used in this study are listed in Sup Table S2. Signals for histone modifications were normalized to total H3.

RT-PCR

RNA was extracted from cells with hot phenol. First-strand cDNA was prepared using 1μg total RNA, Superscript II reverse transcriptase (Invitrogen), and gene specific primers (Sup Table 2). 1/50 of the cDNA and 0.6μCi [α-32P] dATP were used for PCR amplification (60 sec at 94°C, followed by 25 cycles of 30 sec at 94°C, 30 sec at 55°C, and 45 sec at 72°C, followed by 2 min at 72°C). PCR signals were quantitated by Fujix PhosphoImager.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. Cell 2007;129:823–837. [PubMed: 17512414]

Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, McMahon S, Karlsson EK, Kulbokas EJ 3rd, Gingeras TR, et al. Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 2005;120:169–181. [PubMed: 15680324]

Boa S, Coert C, Patterton HG. Saccharomyces cerevisiae Set1p is a methyltransferase specific for lysine 4 of histone H3 and is required for efficient gene expression. Yeast 2003;20:827–835. [PubMed: 12845608]

Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP, Workman JL. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 2005;123:581–592. [PubMed: 16286007]

Chu Y, Simic R, Warner MH, Arndt KM, Prelich G. Regulation of histone modification and cryptic transcription by the Bur1 and Paf1 complexes. Embo J 2007;26:4646–4656. [PubMed: 17948059]

Cohen TJ, Mallory MJ, Strich R, Yao TP. Hos2p/Set3p deacetylase complex signals secretory stress through the Mpk1p cell integrity pathway. Eukaryot Cell 2008;7:1191–1199. [PubMed: 18487345]

Collins SR, Kemmeren P, Zhao XC, Greenblatt JF, Spencer F, Holstege FC, Weissman JS, Krogan NJ. Toward a comprehensive atlas of the physical interactome of Saccharomyces cerevisiae. Mol Cell Proteomics 2007;6:439–450. [PubMed: 17200106]

Dehe PM, Geli V. The multiple faces of Set1. Biochem Cell Biol 2006;84:536–548. [PubMed: 16936826]

Dehe PM, Pamblanco M, Luciano P, Lebrun R, Moinier D, Sendra R, Verreault A, Tordera V, Geli V. Histone H3 lysine 4 mono-methylation does not require ubiquitination of histone H2B. J Mol Biol 2005;353:477–484. [PubMed: 16185711]

Emerling BM, Bonifas J, Kratz CP, Donovan S, Taylor BR, Green ED, Le Beau MM, Shannon KM. MLL5, a homolog of Drosophila trithorax located within a segment of chromosome band 7q22 implicated in myeloid leukemia. Oncogene 2002;21:4849–4854. [PubMed: 12101424]

Fingerman IM, Wu CL, Wilson BD, Briggs SD. Global loss of Set1-mediated H3 Lys4 trimethylation is associated with silencing defects in Saccharomyces cerevisiae. J Biol Chem 2005;280:28761–28765. [PubMed: 15964832]

Flanagan JF, Blus BJ, Kim D, Clines KL, Rastinejad F, Khorasanizadeh S. Molecular implications of evolutionary differences in CHD double chromodomains. J Mol Biol 2007;369:334–342. [PubMed: 17433364]

Gould KL, Ren L, Feoktistova AS, Jennings JL, Link AJ. Tandem affinity purification and identification of protein complex components. Methods 2004;33:239–244. [PubMed: 15157891]

Huang Y, Fang J, Bedford MT, Zhang Y, Xu RM. Recognition of histone H3 lysine-4 methylation by the double tudor domain of JMJD2A. Science 2006;312:748–751. [PubMed: 16601153]

Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin K, Punna T, Thompson NJ, et al. Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell 2005;123:593–605. [PubMed: 16286008]

Kim T, Buratowski S. Two Saccharomyces cerevisiae JmjC domain proteins demethylate histone H3 Lys36 in transcribed regions to promote elongation. J Biol Chem 2007;282:20827–20835. [PubMed: 17525156]

Kizer KO, Phatnani HP, Shibata Y, Hall H, Greenleaf AL, Strahl BD. A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K36 methylation with transcript elongation. Mol Cell Biol 2005;25:3305–3316. [PubMed: 15798214]

Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705. [PubMed: 17320507]

Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, et al. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature 2006;440:637–643. [PubMed: 16554755]

Krogan NJ, Kim M, Tong A, Golshani A, Cagney G, Canadien V, Richards DP, Beattie BK, Emili A, Boone C, et al. Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol Cell Biol 2003;23:4207–4218. [PubMed: 12773564]

Laribee RN, Krogan NJ, Xiao T, Shibata Y, Hughes TR, Greenblatt JF, Strahl BD. BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. Curr Biol 2005;15:1487–1493. [PubMed: 16040246]

Lee JS, Shukla A, Schneider J, Swanson SK, Washburn MP, Florens L, Bhaumik SR, Shilatifard A. Histone Crosstalk between H2B Monoubiquitination and H3 Methylation Mediated by COMPASS. Cell 2007;131:1084–1096. [PubMed: 18083099]

Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007a;128:707–719. [PubMed: 17320508]

Li B, Gogol M, Carey M, Lee D, Seidel C, Workman JL. Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science 2007b;316:1050–1054. [PubMed: 17510366]

Li B, Gogol M, Carey M, Pattenden SG, Seidel C, Workman JL. Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. Genes Dev 2007c;21:1422–1430. [PubMed: 17545470]

Li H, Ilin S, Wang W, Duncan EM, Wysocka J, Allis CD, Patel DJ. Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. Nature 2006;442:91–95. [PubMed: 16728978]

Li J, Moazed D, Gygi SP. Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation. J Biol Chem 2002;277:49383–49388. [PubMed: 12381723]

Liu CL, Kaplan T, Kim M, Buratowski S, Schreiber SL, Friedman N, Rando OJ. Single-nucleosome mapping of histone modifications in S. cerevisiae. PLoS Biol 2005;3:e328. [PubMed: 16122352]

Margaritis T, Holstege FC. Poised RNA polymerase II gives pause for thought. Cell 2008;133:581–584. [PubMed: 18485867]

Martin DG, Grimes DE, Baetz K, Howe L. Methylation of histone H3 mediates the association of the NuA3 histone acetyltransferase with chromatin. Mol Cell Biol 2006;26:3018–3028. [PubMed: 16581777]

Morillon A, Karabetsou N, Nair A, Mellor J. Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. Mol Cell 2005;18:723–734. [PubMed: 15949446]

Mueller JE, Canze M, Bryk M. The requirements for COMPASS and Paf1 in transcriptional silencing and methylation of histone H3 in Saccharomyces cerevisiae. Genetics 2006;173:557–567. [PubMed: 16582434]

Ng HH, Robert F, Young RA, Struhl K. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol Cell 2003;11:709–719. [PubMed: 12667453]

Okuda M, Horikoshi M, Nishimura Y. Structural polymorphism of chromodomains in Chd1. J Mol Biol 2007;365:1047–1062. [PubMed: 17098252]

Pena PV, Davrazou F, Shi X, Walter KL, Verkhusha VV, Gozani O, Zhao R, Kutateladze TG. Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. Nature 2006;442:100–103. [PubMed: 16728977]

Pijnappel WW, Schaft D, Roguev A, Shevchenko A, Tekotte H, Wilm M, Rigaut G, Seraphin B, Aasland R, Stewart AF. The S. cerevisiae SET3 complex includes two histone deacetylases, Hos2 and Hst1, and is a meiotic-specific repressor of the sporulation gene program. Genes Dev 2001;15:2991–3004. [PubMed: 11711434]

Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolfe PA, Herbolsheimer E, et al. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell 2005;122:517–527. [PubMed: 16122420]

Pray-Grant MG, Daniel JA, Schieltz D, Yates JR 3rd, Grant PA. Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature 2005;433:434–438. [PubMed: 15647753]

Reid JL, Moqtaderi Z, Struhl K. Eaf3 regulates the global pattern of histone acetylation in Saccharomyces cerevisiae. Mol Cell Biol 2004;24:757–764. [PubMed: 14701747]

Santos-Rosa H, Bannister AJ, Dehe PM, Geli V, Kouzarides T. Methylation of H3 lysine 4 at euchromatin promotes Sir3p association with heterochromatin. J Biol Chem 2004;279:47506–47512. [PubMed: 15280381]

Schaft D, Roguev A, Kotovic KM, Shevchenko A, Sarov M, Shevchenko A, Neugebauer KM, Stewart AF. The histone 3 lysine 36 methyltransferase, SET2, is involved in transcriptional elongation. Nucleic Acids Res 2003;31:2475–2482. [PubMed: 12736296]

Schlichter A, Cairns BR. Histone trimethylation by Set1 is coordinated by the RRM, autoinhibitory, and catalytic domains. Embo J 2005;24:1222–1231. [PubMed: 15775977]

Shahbazian MD, Zhang K, Grunstein M. Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. Mol Cell 2005;19:271–277. [PubMed: 16039595]

Shi X, Hong T, Walter KL, Ewalt M, Michishita E, Hung T, Carney D, Pena P, Lan F, Kaadige MR, et al. ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 2006;442:96–99. [PubMed: 16728974]

Shi X, Kachirskaia I, Walter KL, Kuo JH, Lake A, Davrazou F, Chan SM, Martin DG, Fingerman IM, Briggs SD, et al. Proteome-wide analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J Biol Chem 2007;282:2450–2455. [PubMed: 17142463]

Sims RJ 3rd, Chen CF, Santos-Rosa H, Kouzarides T, Patel SS, Reinberg D. Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J Biol Chem 2005;280:41789–41792. [PubMed: 16263726]

Sims RJ 3rd, Reinberg D. Histone H3 Lys 4 methylation: caught in a bind? Genes Dev 2006;20:2779–2786. [PubMed: 17043307]

Strahl BD, Grant PA, Briggs SD, Sun ZW, Bone JR, Caldwell JA, Mollah S, Cook RG, Shabanowitz J, Hunt DF, Allis CD. Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol Cell Biol 2002;22:1298–1306. [PubMed: 11839797]

Taverna SD, Ilin S, Rogers RS, Tanny JC, Lavender H, Li H, Baker L, Boyle J, Blair LP, Chait BT, et al. Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. Mol Cell 2006;24:785–796. [PubMed: 17157260]

Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 2007;14:1025–1040. [PubMed: 17984965]

van Leeuwen F, Gafken PR, Gottschling DE. Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 2002;109:745–756. [PubMed: 12086673]

van Leeuwen F, Gottschling DE. Genome-wide histone modifications: gaining specificity by preventing promiscuity. Curr Opin Cell Biol 2002;14:756–762. [PubMed: 12473351]

Venkatasubrahmanyam S, Hwang WW, Meneghini MD, Tong AH, Madhani HD. Genome-wide, as opposed to local, antisilencing is mediated redundantly by the euchromatic factors Set1 and H2A.Z. Proc Natl Acad Sci U S A 2007;104:16609–16614. [PubMed: 17925448]

Wang A, Kurdistani SK, Grunstein M. Requirement of Hos2 histone deacetylase for gene activity in yeast. Science 2002;298:1412–1414. [PubMed: 12434058]

Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. Mol Cell 2005;20:589–599. [PubMed: 16307922]

Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, et al. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature 2006;442:86–90. [PubMed: 16728976]

Xiao T, Hall H, Kizer KO, Shibata Y, Hall MC, Borchers CH, Strahl BD. Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. Genes Dev 2003;17:654–663. [PubMed: 12629047]

Xie J, Pierce M, Gailus-Durner V, Wagner M, Winter E, Vershon AK. Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in Saccharomyces cerevisiae. Embo J 1999;18:6448–6454. [PubMed: 10562556]

Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008;9:206–218. [PubMed: 18292778]

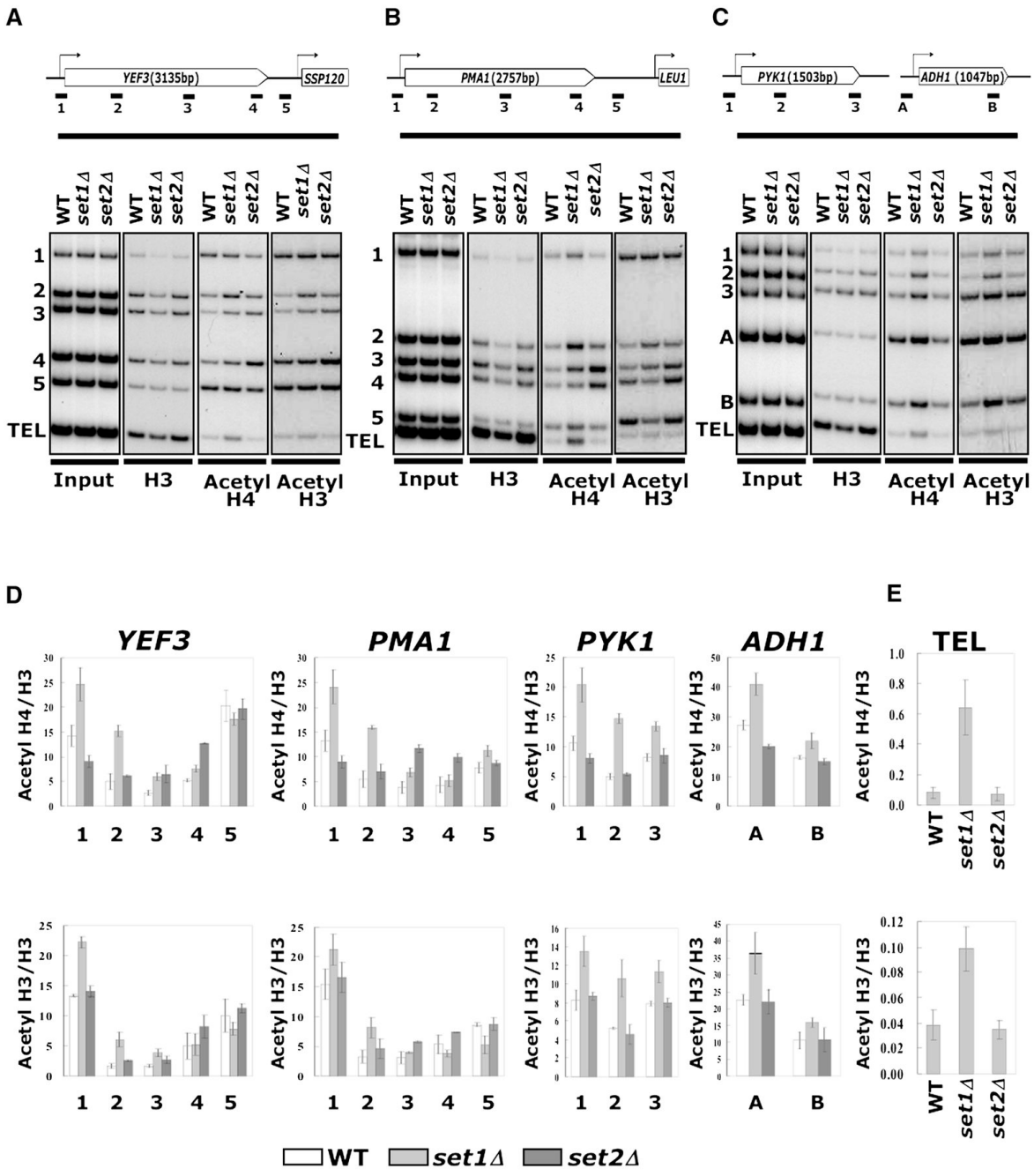

Fig 1. Set1 mediates a reduction in histone acetylation of 5′ regions

Cross-linked chromatin from wild-type, *set1Δ*, or *set2Δ* strains (indicated above each panel) was precipitated with anti-H3, anti-acetyl H4, or anti-acetyl H3 (indicated below panels). PCR analysis of the precipitated DNA was carried out on the *YEF3* (A), *PMA1* (B), and *PYK1* and *ADH1* (C) genes (numbered primer locations shown schematically at the top, PCR products below). TEL is from a non-transcribed region near the telomere of chromosome VI.

D. The signals for acetyl H4 or acetyl H3 were quantitated and normalized to the total H3 signal and the ratios were graphed (X axis shows primer number and Y axis shows ratio). Error bars show the standard deviation from at least three independent experiments.

E. Quantitation for the telomere primer pair was carried out as in D.

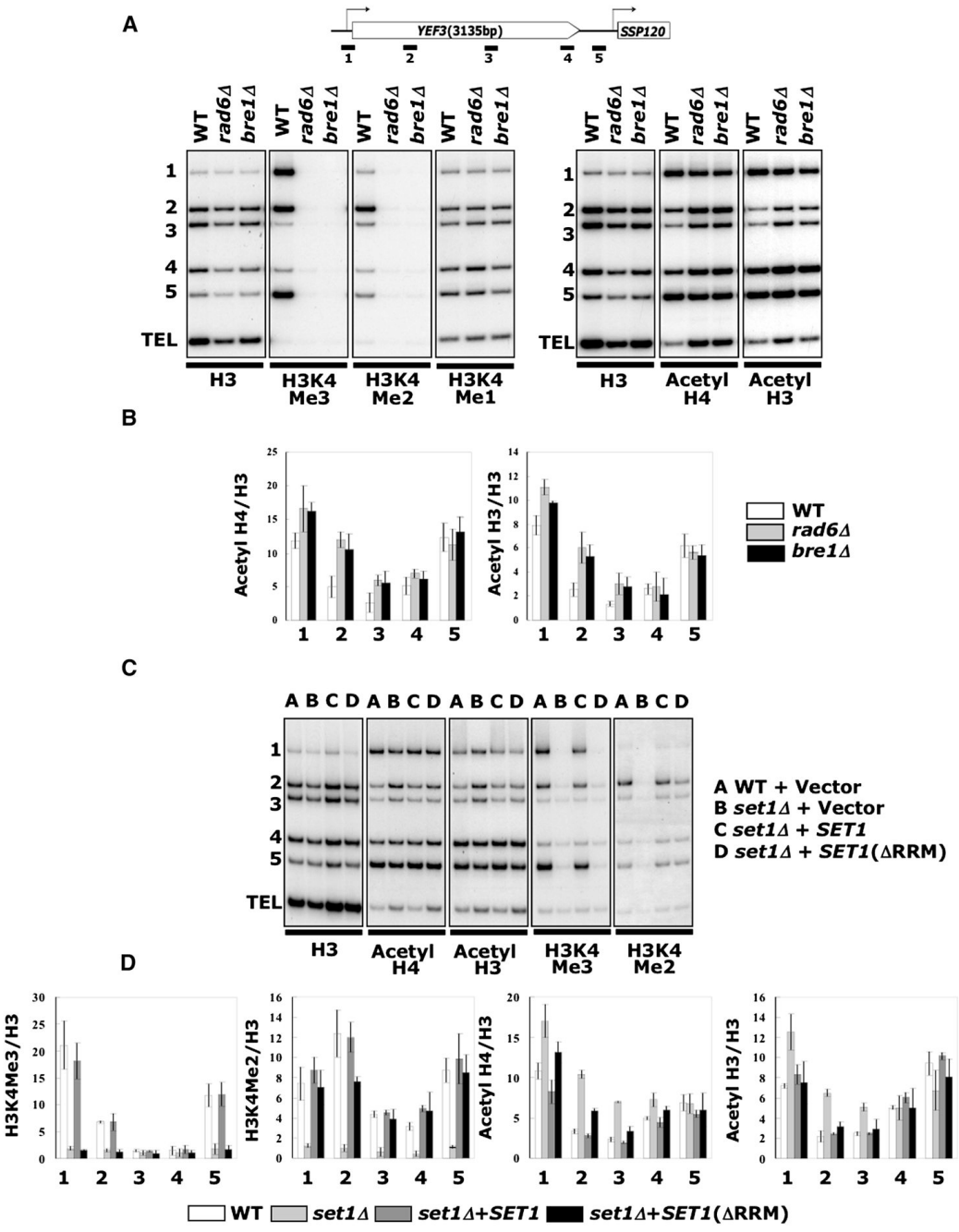

Fig 2. H3K4me2 mediates reduced histone acetylation of 5′ regions

A. ChIP from wild-type, *rad6Δ*, or *bre1Δ* strains using anti-H3, anti-H3K4me3, anti-H3K4me2, or anti-H3K4me1 (left panels) and anti-H3, anti-acetyl H4, or anti-acetyl H3 (right panels) were carried out on *YEF3*. Primer locations are shown schematically at top. Similar results were seen at all other genes tested.

B. The results from part A for acetyl H4 and acetyl H3 were normalized to the total H3 signal and the ratios were graphed. Error bars show the standard deviation from multiple experiments.

C. Vector or plasmids expressing wild-type Set1 or a mutant Set1 lacking the RRM domain (ΔRRM) were transformed into a *set1Δ* strain and ChIP for histone modifications was carried out as in parts A and B.

D. Results from part C were quantitated and graphed as in part B.

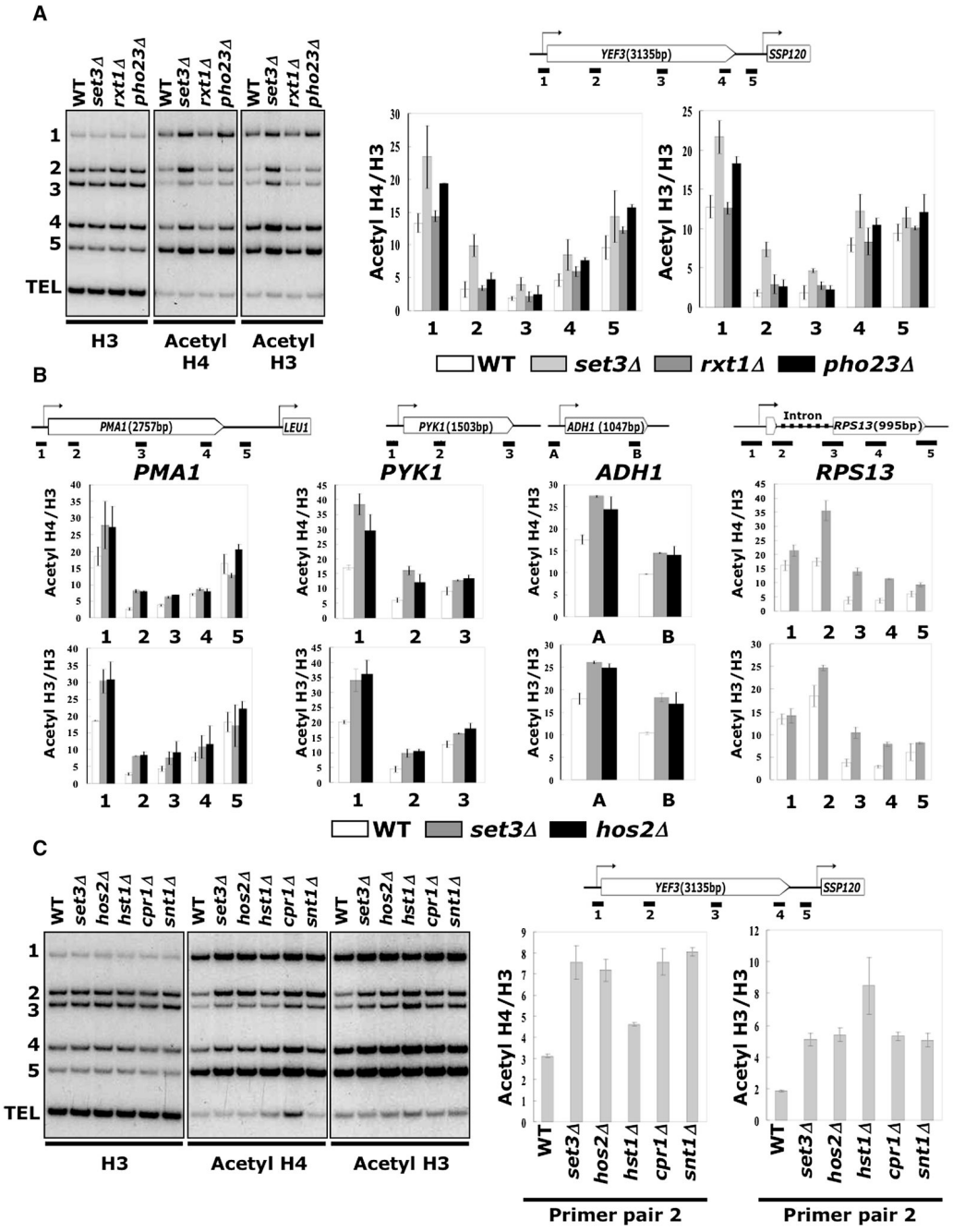

Fig 3. The Set3 complex regulates histone acetylation at 5′ ends of genes

A. ChIP from wild-type, *set3Δ*, *rxt1Δ*, or *pho23Δ* strains using anti-H3, anti-acetyl H4, or anti-acetyl H3 was performed on *YEF3*. The right panel shows quantification of the results from three repeats of the experiment. Error bars show the standard deviation from multiple experiments.

B. ChIP from wild-type, *set3Δ*, or *hos2Δ* strains using anti-H3, anti-acetyl H4, or anti-acetyl H3 was carried out on *PMA1*, *PYK1*, *ADH1*, or *RPS13*. Error bars show standard deviation.

C. ChIP from wild-type, *set3Δ*, *hos2Δ*, *hst1Δ*, *cpr1Δ*, or *snt1Δ* strains using anti-H3, anti-acetyl H4, or anti-acetyl H3 was carried out on *YEF3*. The right panel shows quantification of the

results from multiple experiments for primer pair 2. Error bars show standard deviation. Quantitation of the full primer set appears in Fig S6.

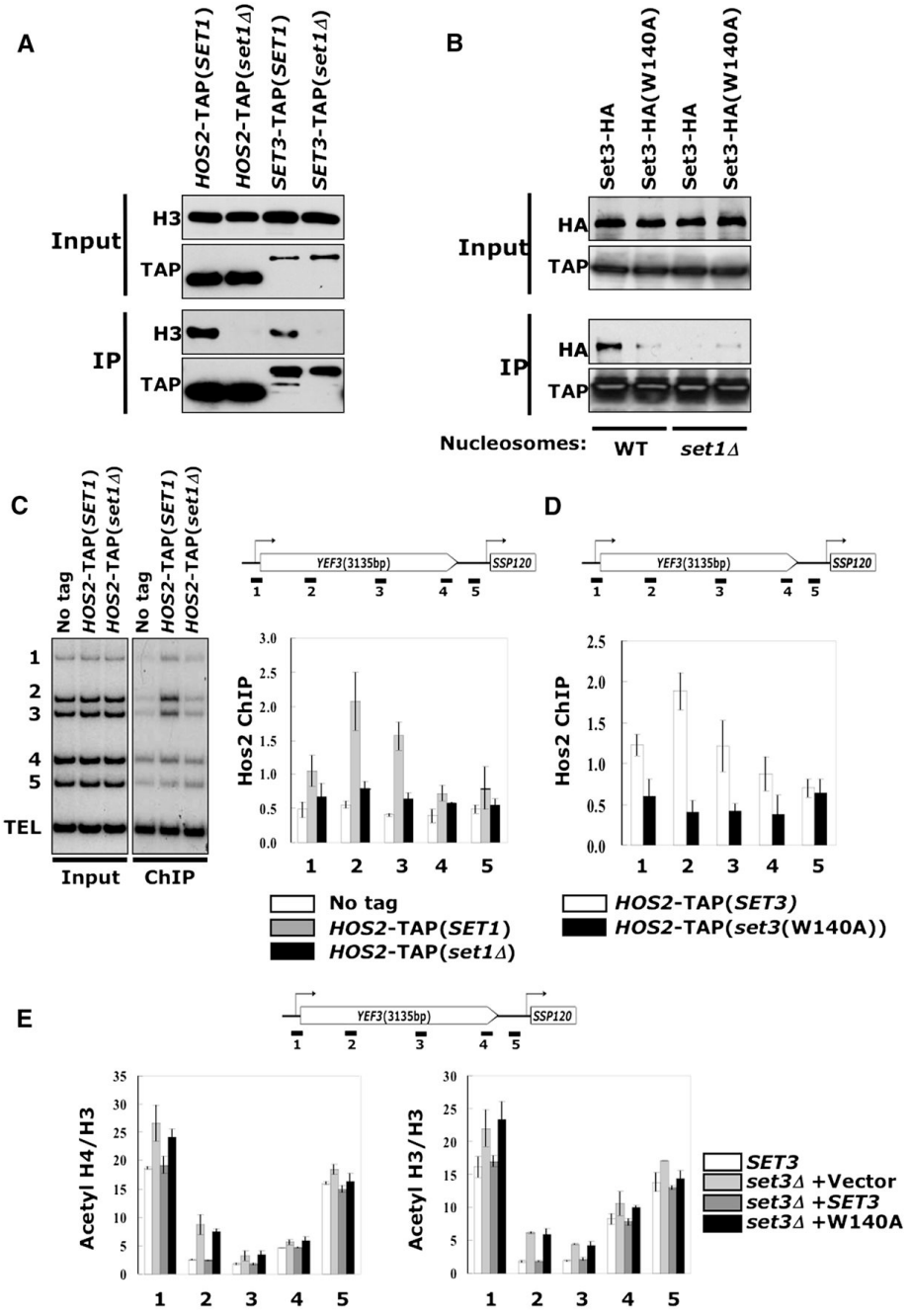

Fig 4. The interaction between the Set3 PHD finger and methylated H3K4 targets Set3C to 5′ ends of genes

A. Hos2-TAP or Set3-TAP was precipitated from *SET1* or *set1Δ* cells (indicated in parentheses) using IgG sepharose. The precipitates (IP) were analyzed by immunoblot analysis for TAP tagged proteins (TAP) and associated histones (H3).

B. Chromatin fragments were immobilized on beads via TAP-tagged Hhf2 (histone H4) from either *SET1* or *set1Δ* cells. Bead bound nucleosomes were incubated with whole cell extracts from wild-type Set3-HA or PHD finger mutant Set3-HA(W140A) strains. The precipitated proteins (IP) were analyzed by immunoblot analysis for TAP tagged histone H4 or epitope tagged Set3 (HA).

C. ChIP of Hos2-TAP was performed using chromatin from *SET1* or *set1Δ* cells. The left panel shows PCR products and right panel shows quantitation of multiple repeats of the experiment. Signals for *YEF3* regions were normalized to that of the telomeric probe (TEL) and input.
D. ChIP of Hos2-TAP was performed as in part C using chromatin from cells expressing wild type Set3 or mutant Set3(W140A).
E. Chromatin from *SET3*, *set3Δ*, or *set3Δ* cells with plasmid-borne *SET3*-HA or *set3*-HA (W140A) were precipitated with anti-H3, anti-acetyl H4, or anti-acetyl H3. PCR analysis of the precipitated DNA was carried out on the *YEF3*.

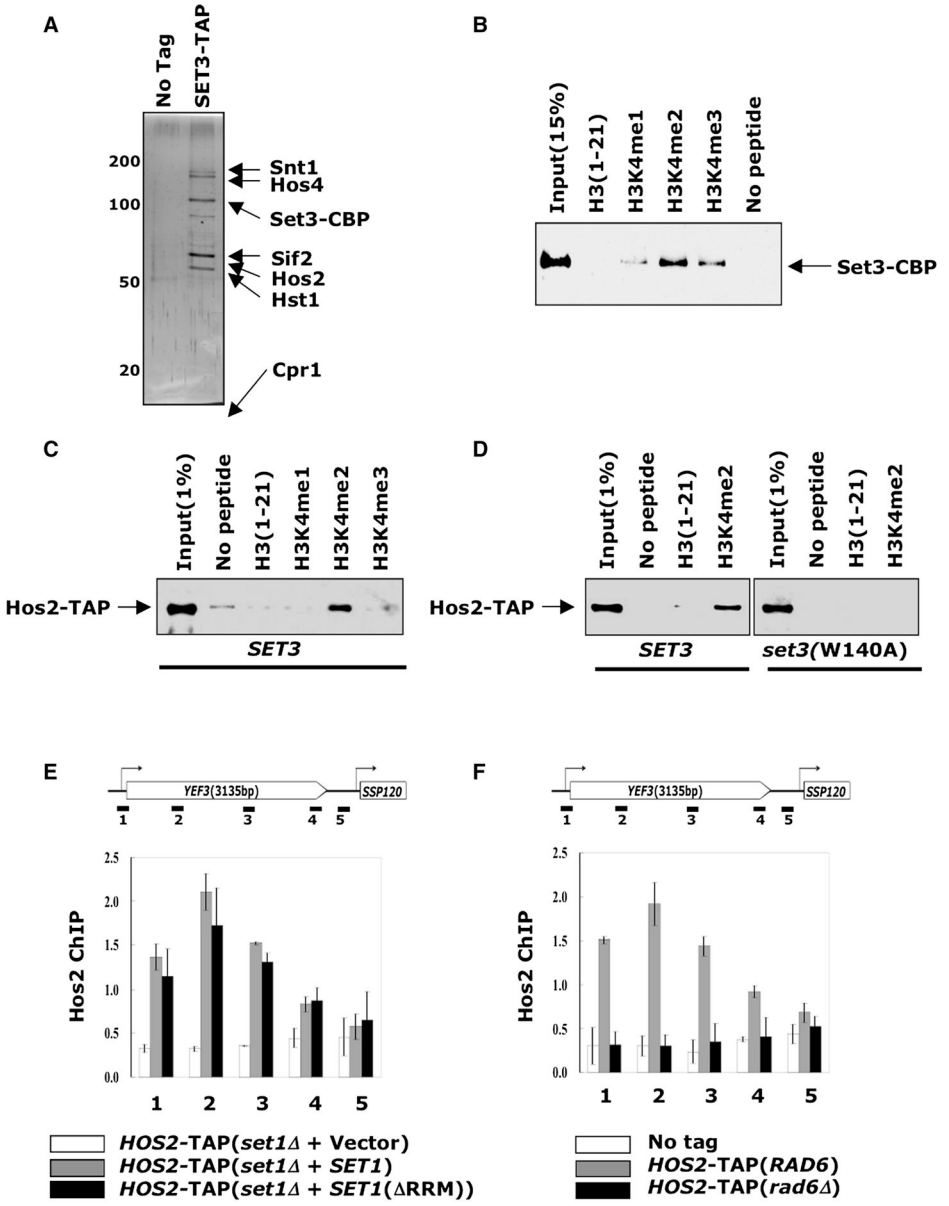

Figure 5. H3K4 dimethylation recruits Set3C

A. TAP-purified Set3 complex (40 μl) was visualized by silver staining.

B. Peptide binding assays were performed with 30μl purified Set3 complex and 1μg of the indicated histone peptides immobilized on beads. Precipitated protein was analyzed by immunoblot analysis with antibody recognizing the TAP tag.

C and D. Histone peptide binding assays were performed with whole cell extracts containing Hos2-TAP and either wild type Set3 or PHD finger mutant Set3(W140A). Precipitated Hos2 protein was analyzed by immunoblot analysis.

E. ChIP of Hos2-TAP was performed using chromatin from *set1Δ* cells containing empty vector, wild type *SET1*, or *SET1* (ΔRRM). PCR analysis of the precipitated DNA was carried out on *YEF3*. Error bars show standard deviation.

F. Chromatin from an untagged strain, *HOS2*-TAP, or *HOS2*-TAP(*rad6Δ*) cells were precipitated with IgG sepharose. PCR analysis of the precipitated *YEF3* DNA was performed and signals were normalized to a telomeric control region. Error bars show standard deviation.

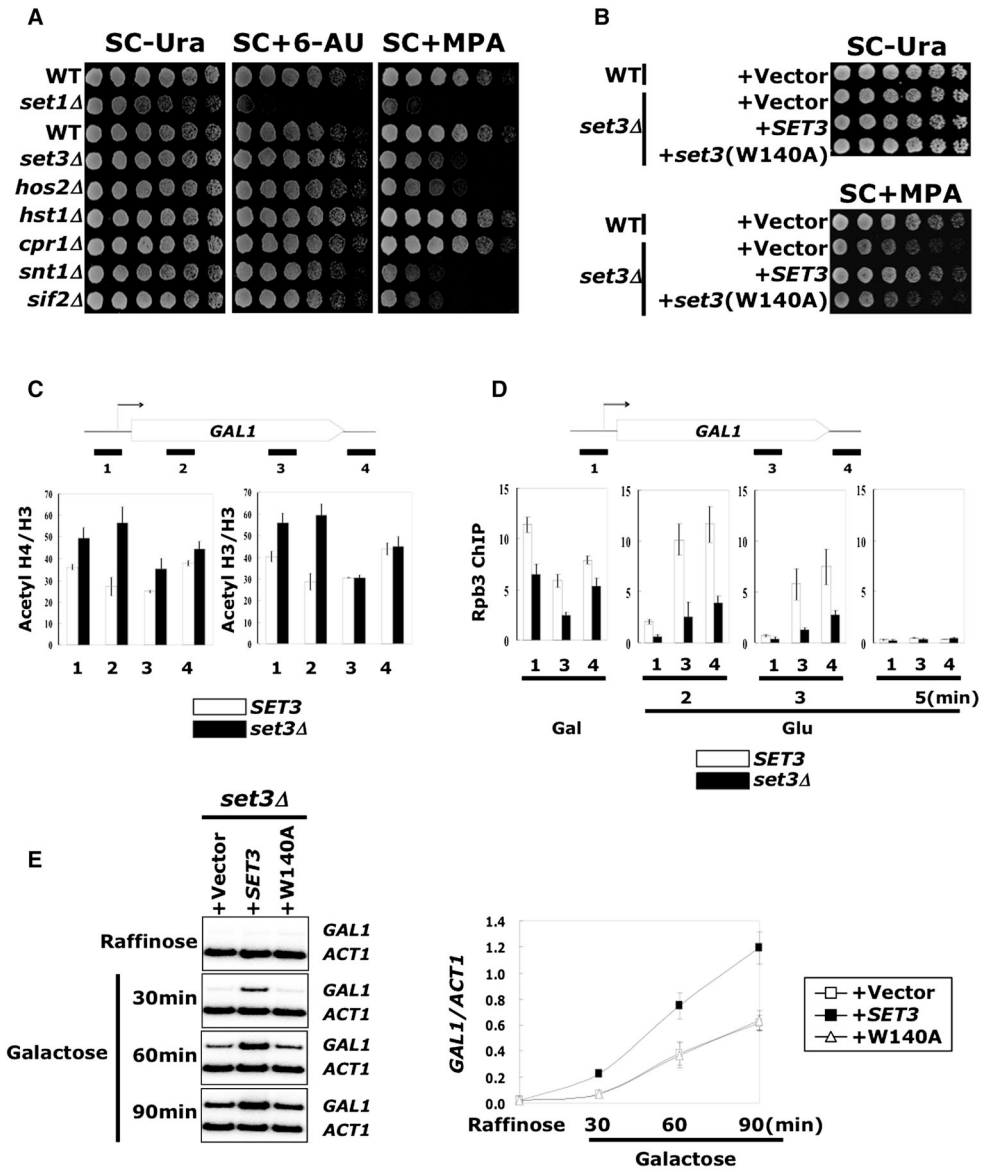

Figure 6. Set3C promotes transcription

A. The indicated deletion strains were spotted in 3-fold dilutions on Synthetic Complete media plates lacking uracil (SC-URA, 2 days growth shown) or SC-URA plates containing 6-AU or MPA (3 days).

B. A *set3Δ* strain was transformed with pRS415 (+Vector), pRS415-*SET3*-HA (+*SET3*), or pRS415-*set3*(W140A)-HA (+*set3*(W140A)) and spotted in 3-fold dilutions on SC plates (2 days) or SC plates containing MPA (3 days). For comparison, a *SET3* strain (WT) containing pRS415 (+Vector) is shown as a control.

C. Chromatin from *SET3* or *set3Δ* strains grown in galactose medium was precipitated with anti-H3, anti-acetyl H4, or anti-acetyl H3 and PCR carried out on *GAL1*. Error bars show standard deviation. PCR products are shown in Fig S7A.

D. ChIP from *SET3* or *set3Δ* strains using anti-Rpb3 was carried out on *GAL1*. Cells were grown in galactose and then shifted to glucose for the indicated times. The number shown is the ratio between the specific *GAL1* signal and the internal non-transcribed control. Error bars show standard deviation. PCR products appear in Fig S7B.

E. Cells with the indicated genotypes were grown in raffinose medium and then shifted to galactose medium for the indicated times. *GAL1* mRNA levels were determined by RT-PCR and normalized to *ACT1* mRNA. The left panel shows PCR products and right panel shows quantitation of multiple repeats of the experiment.

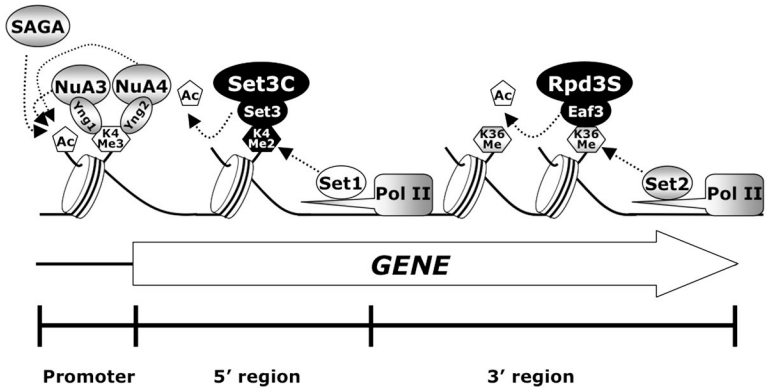

Figure 7. Model for regulation of histone acetylation by H3K4 and K36 methylation

At 5′ ends of genes, RNApII recruits the Set1-COMPASS complex, which methylates histone H3K4. HATs such as SAGA, NuA3, and NuA4 direct histone acetylation to promoters by interacting with upstream regulatory factors and/or trimethylated H3K4. Just downstream, H3K4me2 serves as a binding site for the Set3 PHD finger. The resulting association of Set3C facilitates histone deacetylation by the Hos2 and Hst1 subunits. Further downstream, interaction of Set2 with Ser2-phosphorylated RNApII leads to methylation of H3K36. This mark recruits Rpd3C(S) via the Eaf3 chromodomain, leading to deacetylation of histones in 3′ regions of genes.