Histone H2B C-Terminal Helix Mediates trans-Histone H3K4 Methylation Independent of H2B Ubiquitination

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Received 29 July 2009/Returned for modification 28 August 2009/Accepted 22 April 2010

The trans-histone regulatory cross talk between H2BK123 ubiquitination (H2Bub1) and H3K4 and H3K79 methylation is not fully understood. In this study, we report that the residues arginine 119 and threonine 122 in the H2B C-terminal helix are important for transcription and cell growth and play a direct role in controlling H2Bub1 and H3K4 methylation. These residues modulate H2Bub1 levels by controlling the chromatin binding and activities of the deubiquitinases. Furthermore, we find an uncoupling of the H2Bub1-mediated coregulation of both H3K4 and -K79 methylation, as these H2B C-terminal helix residues are part of a distinct surface that affects only Set1-COMPASS (complex proteins associated with Set1)-mediated H3K4 methylation without affecting the functions of Dot1. Importantly, we also find that these residues interact with Spp1 and control the chromatin association, integrity, and overall stability of Set1-COMPASS independent of H2Bub1. Therefore, we have uncovered a novel role for the H2B C-terminal helix in the trans-histone cross talk as a binding surface for Set1-COMPASS. We provide further insight into the trans-histone cross talk and propose that H2Bub1 stabilizes the nucleosome by preventing H2A-H2B eviction and, thereby, retains the “docking site” for Set1-COMPASS on chromatin to maintain its stable chromatin association, complex stability, and processive methylation.

Posttranslational modifications of the four core histones (H2A, H2B, H3, and H4) play an important role in controlling nucleosome/chromatin structure for factor access (2, 22–23, 27). These covalent modifications include acetylation, phosphorylation, methylation, ubiquitination, sumoylation, and ADP-ribosylation. In histone methylation, each modified lysine exists as a monomethylated (me1), dimethylated (me2), or trimethylated (me3) form, adding yet another level of variation. Histone modifications are regulated during replication, transcription, recombination, and repair (7, 23, 37, 51). In turn, several modifications serve as “marks” for a cellular process and are recognized by proteins with specific interaction domains (2, 41). Additionally, these modifications regulate the establishment of other modifications within the same histone (in cis) (20) or on different histones at a distant site(s) (in trans) (10, 25), a phenomenon termed “histone cross talk.” One well-studied example of a trans-histone cross talk is the regulation of H3K4 and H3K79 methylation by H2BK123 monoubiquitination (H2Bub1) (35, 45, 53).

The primary components involved in this evolutionarily conserved, transcription-coupled, and regulatory cross talk are as follows. Rad6/Bre1 are the E2/E3 ubiquitin-conjugating/ligating enzymes, which add a single ubiquitin to K123 at the H2B C-terminal region. Dot1, a distributive, nonprocessive, and non-SET domain methyltransferase (12), catalyzes H3K79 methylation (3, 34). Set1-COMPASS (complex proteins associated with Set1), a multiprotein complex consisting of the methyltransferase (Set1) and seven regulatory subunits (Swd1, Swd2, Swd3, Bre2, Sdc1, Spp1, and Shg1), catalyzes processive H3K4 methylation. While Swd1 and Swd3 are essential for complex integrity, Bre2 and Sdc1 are needed for maintaining normal levels of all forms of H3K4 methylation, and Spp1 is important only for H3K4me3 (6, 31, 43). Regulation of the cross talk is seen from the diverse and drastic changes in H3K4 and -K79 methylation levels in the absence of H2Bub1 (5, 44). Initial models proposed to explain this cross talk suggested that H2Bub1 acts as a “wedge” to nonspecifically unfold chromatin for Set1 and Dot1 to gain access to their substrates or, alternatively, that it functions as a “bridge” to directly recruit them (16, 49). In a previous study, we discovered that H2Bub1 does not “open up” the chromatin; instead, it stabilizes the nucleosome by preventing H2A-H2B eviction (4). Further, we proposed nucleosome stabilization as a likely mechanism by which H2Bub1 promotes stable and prolonged association of the methyltransferases with chromatin and their substrates to catalyze high levels of H3K4 and -K79 methylation.

Two studies with conflicting conclusions have implicated Swd2, a Set1-COMPASS subunit, as the key link between H2Bub1 and H3K4 methylation. Whereas one study showed that H2Bub1 regulates the integrity of Set1-COMPASS by affecting Swd2 levels (26), the other revealed that the H2Bub1-dependent ubiquitination of Swd2 and the subsequent recruitment of Spp1 are important for H3K4 methylation (52). Therefore, regulation of Set1-COMPASS functions is still not fully understood. Additionally, Swd2 has also been implicated in the regulation of H3K79 methylation by recruiting Dot1 (26). Recently, the basic patch in the H4 N terminus was shown to play a role in the trans-histone pathway by controlling the chromatin binding and functions of Dot1 (9). However, binding sites for Set1-COMPASS on chromatin are not known. In this study, we report that the residues R119 and T122 in the H2B C-
terminal helix play a direct role in controlling both H2Bub1 and H3K4 methylation levels. Importantly, we find that these residues interact with Spp1 and modulate the chromatin association, integrity, and overall stability of Set1-COMPASS independent of H2Bub1. Collectively, our study uncovers a novel role for the H2B C-terminal helix in the trans-histone cross talk by serving as a “docking” surface for Set1-COMPASS, without affecting the functions of Dot1, and therefore reveals an uncoupling of the H2Bub1-mediated coregulation of H3K4 and -K79 methylation.

MATERIALS AND METHODS

Yeast strains. Saccharomyces cerevisiae strains used in this study were mostly derived from strain Y131 or YMH171 (39, 48). Epitope tags were introduced into the C-terminal region of individual Set1-COMPASS subunits Ubp10 and Rad6 at their endogenous loci following PCR, using either pY6M (containing 9 copies of Myc) or pYMI (containing 3 copies of hemagglutinin [HA]) as the template (21). Ubp8 was C-terminally tagged with 3 copies of Flag, amplified by PCR using pFLAG-KanMX as the template (15). SET1 was N-terminally tagged using the construct p9Myc-SET1::TRP1 following linearization with SnaBl (6). Y131-based deletion strains lacking RAD6, SWD1, SDC1, or SPP1 were created using PCR products containing the disrupted gene locus, including the inserted KanMX module amplified from the genomic DNA isolated from the respective BY4742-based yeast deletion strains (Open Biosystems). Detailed genotypes of all the yeast strains used in this study are listed in Table 1.

### Table 1. Yeast strains used in this study

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δ-transferase (GST) tags (HisGST, HTB1 containing R119A, T122D, or both mutations was PCR amplified and mobilized as an NdeI-BamHI fragment to replace the wild-type sequence in H2BP-6ET11a (kindly provided by Brad Cairns).

Expression and purification of recombinant proteins. Constructs containing SPP1 or SDC1 in pBG101 or the vector alone were transformed into Escherichia coli strain BL21(DE3)-RIL (Stratagene). Cells were grown to an optical density at 600 nm (OD600) of 0.6 at 37°C, induced with 0.1 mM IPTG (isopropyl-b-D-thiogalactopyranoside), and grown overnight at 16°C prior to harvesting. For the expression of Spp1, the media were supplemented with 0.15 mM ZnSO4. Cells were sonicated in NET buffer (25 mM Tris-Cl at pH 8.0, 50 mM NaCl, 5% glycerol, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride [PMSF], 1 μg/ml pepstatin A, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and incubated on ice for 30 min following addition of Triton X-100 (1%) to solubilize proteins.

Proteins were purified using glutathione-Sepharose 4B (GE Healthcare) by incubating the bacterial lysate with beads at 4°C for 2.5 h with end-over-end rotation. Bead-bound proteins were washed extensively with NET buffer containing 1 mM dithiothreitol (DTT), and any copurifying bacterial chaperones were removed by washing the beads once with Mg-ATP buffer (50 mM Tris-Cl at pH 7.4, 10 mM MgSO4, 2 mM ATP). Proteins were eluted from beads using 75 mM Tris-Cl at pH 8.0 and 15 mM glutathione and dialyzed overnight against binding buffer (50 mM Tris-Cl at pH 8.0, 150 mM NaCl, 1 mM DTT, 10% glycerol, 1 mM PMSF, 1 μg/ml pepstatin A, 1 μg/ml aprotinin, and 1 μg/ml leupeptin). The recombinant protein concentration was determined by Western blotting, using a gradient of serially diluted pure GST protein (4.8 mg/ml stock; Thermo Scientific) as the reference.

Histone binding assay. Constructs containing wild-type or mutant HTB1 were transformed into E. coli strain BL21(DE3)-RIL (Stratagene). Cells were grown to an OD600 of 0.6 at 37°C, induced with 0.1 mM IPTG, grown for 5 h at 37°C and lysed in binding buffer as described above. To detect histone binding, bacterial lysate (10 μg) was incubated with recombinant GST, HisGST, HisGST-Spp1, or HisGST-Sdc1 (15 μM) and binding buffer in a total volume of 0.25 ml for 1 h at 4°C. Glutathione-Sepharose 4B beads (10 μl) were added, and the incubation was continued for an additional hour. Beads were extensively washed with washing buffer containing 0.1% NP-40 and boiled in 2% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (30 μl). A major portion (80%) was resolved using SDS-PAGE, and binding was detected using anti-H2B (1:5,000; Active Motif), and the remaining aliquot was used to detect bead-bound GST-tagged proteins employing anti-GST (1:20,000; GE Healthcare).

Yeast extracts and Western blotting. Whole-cell extracts were prepared as described previously (14), with minor modifications. Briefly, 3 × 107 cells from log-phase yeast cultures were harvested, washed with water, and lysed by bead beating in 400 μl of SUME buffer (1% SDS, 8 M urea, 10 mM MOPS [morpholinepropanesulfonic acid] at pH 6.8, and 10 mM EDTA). Subsequently, NaCl was added (0.4 M, final) and vigorously mixed; the lysate was clarified by centrifugation at 16,100 × g for 20 min in an Eppendorf microcentrifuge. Nuclear extracts were prepared essentially as described previously (28), except the nuclear pellet was solubilized by brief sonication in SUME buffer and clarified by centrifugation. The protein concentration of the clarified lysates was determined using the Bio-Rad DC protein assay kit by following the manufacturer’s instructions. For detecting ubiquitination or sumoylation, cells (8 × 107) from log-phase cultures were collected, washed with water, and immediately boiled in 200 μl of SDS sample buffer (50 mM Tris-Cl at pH 7.5 and 2% SDS). After centrifugation, the supernatant was added to the clarified lysate (10%, final), and protein concentrations were determined as described above. Antibodies used in this study were purchased from Millipore unless specified, and their dilutions and sources are indicated in parentheses: anti-HA and anti-Myc (1:1,000; gifts from Ethan Lee), anti-Flag (1:5,000; Sigma), anti-Pak1 (1:5,000; Molecular Probes), anti-H3 (1:5,000; Active Motif), anti-H3K79me3 (1:5,000; a gift from Michael Grunstein), anti-H3K97me2 (1:3,000), anti-H3K4me1 (1:1,000), anti-H3K4me2 (1:7,500), anti-H3K4me3 (1:2,500; Active Motif), anti-H3K56ac (1:2,500), and anti-H4K16ac (1:1,000). As seen in Fig. 1 and 2, recent batches of antibodies from Millipore, especially anti-Flag, followed by elution with 3× Flag peptide.
A second round of immunoprecipitation (IP) was done using the eluted chromatin and an antibody that recognizes mono- and polyubiquitinated proteins (clone FK2; Biomol) (13). The IP DNA was recovered using the QIAquick PCR purification kit and eluted in 50 μl sterile water.

Real-time quantitative PCR (qPCR) was carried out using SYBR green premix (Bio-Rad) in Bio-Rad MyiQ thermal cyclers. Relative fold enrichment for factor occupancy or a histone modification was calculated using the threshold cycles (CT) obtained for IP and input DNA in qPCR employing the 2^-ΔΔCT method. The relative enrichment obtained for H3K4 methylation and H2Bub1 was also normalized to histone occupancy. Fold enrichment in H2Bub1 occupancy in the wild-type or H2B C-terminal helix mutants was calculated relative to H2B-K123R (set to 1), which lacks ubiquitination. As a negative control, chromatin obtained from a yeast strain without the Myc or HA tag (no-tag control) was used in ChIP to determine the nonspecific precipitation by anti-Myc or anti-HA, respectively. Following chromatin IP using anti-HA, successful enrichment relative to the no-tag control was obtained only for the 3HA-tagged Swd2. Significant enrichment of chromatin associated with Set1, Spp1, Sdc1, or Bre2 was obtained only upon using the 9Myc-tagged versions of these proteins. We investigated gene-specific occupancy and distribution of Spp1 and Sdc1, because their global chromatin-bound levels were reduced in H2B-R119A and H2B-T122D (see Fig. 6K and L). Even though global chromatin-bound Bre2 and Swd2 levels were not reduced, we chose to examine their occupancy because Bre2 directly interacts with Sdc1 and this heteromeric subcomplex is important for proper H3K4 methylation (47). Additionally, Swd2 has been shown to be an important link between H2Bub1 and H3K4/K79 methylation (26). In the single mutants, chromatin-bound levels of Swd1, Swd3, and Set1, three subunits essential for complex integrity (40), were not altered. Therefore, to directly assess the effects of H2B mutations on the gene-specific occupancy of the entire Set1-COMPASS, we evaluated only Set1 occupancy, as it is an essential and catalytic core component of the complex (40). Chromatin obtained from set1Δ was used in ChIP to assess nonspecific precipitation by antibodies that recognize H3K4me1, H3K4me2, and H3K4me3. To determine the nonspecific precipitation and fold enrichment using anti-H3, a control IP without addition of any antibody was performed. At least two to three independent experiments with triplicate PCRs were done for all the data obtained by ChIP and ChDIP assays. To compare and assess changes in the distribution and overall occupancy of a histone modification or Set1-COMPASS subunits across an open reading frame (ORF) in yeast strains harboring either wild-type or mutant H2B, the normalized occupancy obtained for all regions in the mutants and that obtained for the middle and 3' ORF regions in the wild type were calculated relative to the normalized occupancy obtained for 5' ORF region in the wild type, which was set to 1.

Chromatin association assay. Chromatin fractionation was done using purified nuclei by following the procedures described previously (1, 28). Two log-phase yeast cultures (4 × 10^8) were treated with 0.1% sodium azide, harvested, and washed twice with water. The cells were incubated at room temperature for 10 min in prespheroplasting buffer {100 mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)]-KOH at pH 9.4, 10 mM DTT, 0.1% sodium azide}. Cells were then resuspended in spheroplasting buffer (50 mM potassium phosphate buffer, 600 mM sorbitol, 10 mM DTT) and incubated in a 30°C water bath shaker for 1 h following addition of Quantzyme (100 to 150 units; MP Biomedicals). Spheroplasts were washed with wash buffer (50 mM HEPES-KOH at pH 7.6, 100 mM KCl, 2.5 mM MgCl₂, 400 mM sorbitol, protease inhibitors [Pis; 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A], 5 mM FIG. 2. H2B C-terminal residues R119 and T122 control H2Bub1 levels, H3K4 methylation, and telomeric gene silencing. (A and D) Western blots for H2Bub1, as described in the legend to Fig. 1B. (B and E) Western blots for H3K4 and -K79 methylation. (C) Ribbon representations of the H2B C-terminal helix generated in the UCSF Chimera package using coordinates from the solved yeast nucleosome structure (Protein Data Bank [PDB] accession no. 1ID3) (54). Relevant amino acids are shown in ball and stick format. The diagonal white line demarcates the two phases of the helix. (F) Tenfold serial dilutions of yeast cultures were spotted on synthetic complete medium (SC) or medium-containing 5FOA and grown at 30°C for 2 days. WT, wild type.
N-ethylmaleimide [NEM]) prior to further processing. Following being washed, spheroplasts were resuspended in buffer A (10 mM HEPES-KOH [pH 7.6], 10 mM KCl, 1.5 mM MgCl₂, 10% glycerol, 0.34 M sucrose, 1 mM DTT, 1× complete EDTA-free PI cocktail [Roche], 5 mM NEM) and lysed with 0.1% Triton X-100 by incubation on ice for 5 min with mixing. After centrifugation, the nuclear pellet was resuspended in 1 ml purification buffer (PB; 10 mM Tris-Cl at pH 7.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, 1× complete EDTA-free PI cocktail, 5 mM NEM). The two nucleus-containing suspensions were combined and diluted to a total volume of 7.5 ml with PB. The diluted suspension was mixed with an equal volume of 66% Percoll (GE Healthcare) prepared in PB and centrifuged at 30,000 g at 4°C for 5 min. The layer containing pure nuclei was collected, diluted to 12 ml with recovery buffer (PB without glycerol), and centrifuged at 20,000 × g and at 4°C for 15 min. The nucleus-containing pellet was resuspended in 1 ml recovery buffer, transferred to a 1.5-ml tube, and centrifuged.

To obtain chromatin, pure nuclei were lysed in a hypotonic solution (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, 1× complete EDTA-free PI cocktail, 5 mM NEM) and incubated on ice for 30 min. The pellet was lysed again in the hypotonic solution for 15 min following centrifugation (15,000 × g at 4°C for 5 min). The chromatin-containing pellet was solubilized in SUME buffer using a brief sonication, and insoluble material was removed by centrifugation. Initially, the chromatin amounts obtained from the different strains were normalized based on the H3 levels evaluated by Western blotting. Normalized chromatin was then subjected to Western blotting to assess the changes in factor occupancy.

**Gene expression analysis.** Yeast cells (10⁷) obtained from a mid-log phase culture were harvested, and total RNA was extracted using the MasterPure yeast RNA purification kit (Epitexus) by following the manufacturer’s instructions and further purified using the Oligoex mRNA kit (Qiagen) to isolate mRNA. The Transcriptor first-strand cDNA synthesis kit (Roche) was used to reverse transcribe mRNA (100 ng) to obtain cDNA by following the protocol provided by the manufacturer. Changes in transcript levels of ACT1, ACT2, or Set1-COMPASS subunits were assessed using standard PCR and agarose gel electrophoresis essentially as described previously (49) or, alternatively, by employing qPCR, which was carried out using SYBR green premix (Bio-Rad) in Bio-Rad MyQ thermal cyclers and primers that anneal to the 3′ regions of PMA1 or DMA2. The fold changes in transcript levels were calculated using the 2^(-ΔΔCT) method relative to the transcript level of ACT1. The fold changes in normalized transcript levels in the mutants were then calculated relative to the wild type (set to 1). Three independent biological replicates and triplicate PCR were performed.

**RESULTS**

The amino acid composition following H2B residue K123 is not important for H2B ubiquitination and H3K4 methylation. Previously, to test whether the SUMO moiety can substitute for ubiquitin at the H2B C terminus to mediate the trans-histone cross talk (4), we created a chimeric H2B by introducing two consensus sumoylation sites to replace H2B residues T122 and K123 [H2B(124-129A)] (Fig. 1A) to determine their role in controlling H2Bub1 and H3K34 methylation. H2B(124-129A) had no apparent effect on H2Bub1 and H3K4 methylation (Fig. 1C and D). However, eliminating all the residues following K123 [H2B-Y124stop; Fig. 1A] resulted in a severe decrease in both of the histone modifications (Fig. 1C and D). Therefore, even though most of the residues following K123 form an unstructured C-terminal “tail” domain (54), they are still essential for maintaining normal levels of H2Bub1 and H3K4 methylation, but their exact amino acid sequences are not important for these processes.

Yeast strains used in this study contain N-terminally Flag epitope-tagged H2B or its derivatives and were derived from parental histone shuffle strain Y131 (39). Importantly, this strain does not contain any extra mutation that suppresses the effect of the H2B-K123R mutant on H3K4- and K79 methylation (11, 33). Moreover, previously reported H2A and H2B mutations with or without a Flag epitope exerted the same effect on H3K4 methylation and H2Bub1 in Y131 and in other independent yeast strains (32, 33).

**Mutations in H2B T122 differentially affect H2Bub1 and H3K4, but not H3K79, methylation.** Since the amino acid composition following H2B residue K123 is not important for H2Bub1 and H3K4 methylation, the decrease in H3K4me3 in H2B(2SU)-K2L (Fig. 1B) is likely due to the threonine-to-isoleucine substitution at position 122. Therefore, we tested whether the residues preceding K123 play any role in establishing H2Bub1 and H3K4 methylation. We replaced T122 in H2B with the following differently charged residues, an alanine (T122A), arginine (T122R), or aspartate (T122D), and assessed their effects on H2Bub1 and H3K4 methylation. Compared to wild-type levels, H2Bub1 levels were reduced in H2B-T122R but remained unaffected in H2B-T122A (Fig. 2A). In contrast, the H2Bub1 level was enhanced in H2B-T122D. A 4.6-fold increase in H2Bub1 levels relative to unmodified H2B was observed in this mutant compared to that in the wild type (Fig. 2A).

Next, we tested whether mutations in T122 also affected H3K4 methylation. Similar to its effect on H2Bub1 levels, H2B-T122A did not alter H3K4 methylation (Fig. 2B). H2B-T122R led to a slight reduction in H3K4me3 (Fig. 2B), although it caused a significant decrease in H2Bub1 levels. This observation is similar to the finding that bur1 and certain rad6 mutants cause a severe reduction in H2Bub1 levels but maintain near normal levels of H3K4 methylation (4, 24, 49). Given the increase in H2Bub1 levels in H2B-T122D, we anticipated an increase in H3K4 methylation. Surprisingly, H3K4me1 and -me2 were not affected, and the levels of H3K4me3 were reduced in this mutant (Fig. 2B). This decrease in H3K4me3 even in the presence of large amounts of H2Bub1 reveals a novel and distinct role for the H2B C-terminal helix in modulating the function of Set1-COMPASS.

H2Bub1 also regulates Dot1-mediated H3K79 methylation (3, 34). However, both H2B-T122D and H2B-T122R had no effect on H3K79me2 or -me3 (Fig. 2B). Therefore, these mutations specifically affect H3K4me3. To the best of our knowledge, this is the first demonstration of an uncoupling of the H2Bub1-mediated coregulation of H3K4 and -K79 methylation by specific H2B mutations.

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Mutations in H2B R119 affect only H2Bub1 and H3K4 methylation without affecting H3K79 methylation. The increased H2Bub1 level observed in H2B-T122D is reminiscent of the effect observed in yeast strains lacking H2Bub1-specific deubiquitinases (UBP8 and/or UBP10). Since H3K4 methylation was increased in ubp8Δ and/or ubp10Δ (14, 17, 46), the reduced H3K4me3 in H2B-T122D is not due to the elevated levels of H2Bub1. Instead, it suggests a role for the H2B C-terminal helix in controlling the function of Set1-COMPASS. Next, we postulated that in addition to T122, the other residue(s) preceding K123 might also be important for this trans-histone pathway. To test this hypothesis, we mutated the arginine (R) residue at position 119. The H2B R119 residue was targeted because it is spatially adjacent to, and resides on the same phase of the H2B C-terminal helix as, T122 and K123 (Fig. 2C). Western analyses revealed that the levels of H2Bub1 and H3K4 methylation were severely reduced in the H2B-R119D mutant (Fig. 2D and E). In H2B-R119K, the levels of H2Bub1, H3K4, and -K79 methylation were similar to those in the wild type (data not shown). Importantly, similar to H2B-T122D, H2B-R119A and -R119K enhance H2Bub1 levels (~2.4-fold change) (Fig. 2D) and severely reduces H3K4me3 (Fig. 2E). A slight reduction in H3K4me2 was also observed in H2B-R119A (Fig. 2E). Moreover, H3K79 methylation was not reduced in H2B-R119D and H2B-R119A (Fig. 2E), further demonstrating an uncoupling of the H2Bub1-mediated coregulation of H3K4 and -K79 methylation. The effect of H2B-R119A and H2B-T122D appears to be highly selective toward H3K4 methylation, as they do not affect H3K56 or H4K16 acetylation (data not shown). Additionally, the reduced H3K4 methylation in these H2B mutants is not due to enhanced demethylation, since global Jhd2 levels are not altered by the H2B-R119A or H2B-T122D mutant (data not shown).

The increase in H2Bub1 levels observed in H2B-R119A and H2B-T122D is not due to any increase in overall H2B transcript levels, and these H2B mutations do not induce transcription of the extraneous GAL1/GAL10 promoter-driven copy of HTA2-HTB2 (data not shown), which is present in the yeast strain used in this study (Y131) (33). Additionally, the effects of both mutations on H2Bub1 and H3K4 methylation were reproducibly observed in an Y131-derived strain (FGY8), which lacks the GAL1/GAL10 promoter-driven HTA2-HTB2, and in a completely independent strain background (FY406) (data not shown). Collectively, we conclude that the R119 and T122 residues preceding H2BK123 play an important role in modulating both H2Bub1 and H3K4 methylation levels.

H2B-R119A and H2B-T122D confer a telomeric silencing defect. H3K4 methylation is important for maintaining telomeric gene silencing in yeast (49). Therefore, we tested whether the H2B C-terminal helix mutations induce expression of the silent URA3 gene integrated at the left-end telomere of chromosome VII (49). Since H2B-R119D caused a severe reduction in H3K4 methylation (Fig. 2E), it failed to grow on 5-fluoroorotic acid (5FOA)-containing medium, similar to H2B-K123R (Fig. 2F). Moreover, H2B-R119A and H2B-T122D, which reduced only H3K4me3, also conferred a severe telomere-silencing defect similar to that of H2B-K123R and H2B-R119D (Fig. 2F). This finding confirms a role for H3K4me3 in maintaining telomeric silencing, as reported previously (8). However, it conflicts with the findings from another study (43), which showed that H3K4me3 is not required for telomeric silencing. Indeed, H2B-T122R also caused a reduction in H3K4me3, albeit to a lesser degree than H2B-R119A and H2B-T122D (Fig. 2B and E), but it did not confer 5FOA sensitivity (Fig. 2F). Therefore, our findings suggest that a certain overall threshold level of H3K4me3 is needed for maintaining telomeric silencing.

H2B-R119A and H2B-T122D increase H2Bub1 levels by hindering deubiquitination. H2B-R119A and H2B-T122D yield ~2.4- and ~4.6-fold increases in H2Bub1 levels, respectively (Fig. 2B and H2, H2B-R119A, and H2B-T122D in the absence of deubiquitinases (ubp8Δ ubp10Δ) to circumvent any contributions from an altered deubiquitination. Western analysis showed that the overall H2Bub1 levels in the H2B mutants were similar to those in wild-type H2B in ubp8Δ ubp10Δ (Fig. 3A, right). Notably, the ~2-fold difference in H2Bub1 levels between H2B-R119A and H2B-T122D, which was evident in the wild-type strain (Fig. 2B and 3A, left), was not observed in ubp8Δ ubp10Δ (Fig. 3A, right). This result suggests that the increased H2Bub1 levels in the H2B-R119A or H2B-T122D mutant (in the UBP8 UBP10 background) is likely not due to a differential stimulation of Rad6-mediated ubiquitination, but instead, these levels might be due to defects in the function of deubiquitinases.

Next, we examined the global chromatin-bound levels of Rad6, Ubp8, and Ubp10 in strains harboring wild-type or mutant H2B using standard chromatin fractionation analysis (1, 28, 55). Briefly, nuclei isolated from the wild type or mutants were lysed using a hypotonic solution to obtain chromatin. An equal amount of chromatin from each strain was subjected to Western analyses. To ensure equal loading of chromatin, we examined the levels of H3. Compared to the levels in the wild type, a similar fold reduction in the chromatin-bound levels of Rad6 and Ubp8 was observed in H2B-R119A and H2B-T122D, and Ubp10 levels remained unchanged (Fig. 3B). This finding suggests that the augmentation of H2Bub1 levels in both of the mutants is not due to the increased chromatin-bound Rad6 level; instead, it is due to the reduced chromatin association of Ubp8 and defects in deubiquitination.

To directly test the effects of H2B-R119A and H2B-T122D in maintaining H2Bub1 levels, we examined the ability of these mutations to affect deubiquitination in vitro. When isolated under denaturing conditions, wild-type and mutant H2B showed similar H2Bub1 levels in ubp8Δ ubp10Δ (Fig. 3A). Therefore, we used these strains for the experiment, as they contained equal amounts of H2Bub1 but differed only in their H2B sequences. Since H2Bub1 is a very labile modification and is rapidly removed by deubiquitinases, initially we prepared extracts from the ubp8Δ ubp10Δ strain expressing either wild-type or mutant H2B under native conditions and monitored the differences in the loss of H2Bub1 due to nonspecific removal by other yeast deubiquitinases. As shown in Fig. 3C, wild-type and mutant H2B retained similar H2Bub1 levels, even when isolated under native conditions (moc, 0 min) and following prolonged incubation (mock, 10 min and 40 min). These data confirm that Ubp8 and Ubp10 are the major H2Bub1-specific deubiquitinases in yeast. Therefore, we incu-
bated extracts from the \textit{ubp8} \textit{ubp10} strain harboring wild-type or mutant H2B with an extract from a wild-type yeast strain, which contains intact Ubp8 and Ubp10. All extracts were prepared under native conditions to preserve active deubiquitinases. H2Bub1 levels remained unchanged in extracts containing H2B-R119A or H2B-T122D, even after 40 min of incubation with extracts containing Ubp8 and Ubp10 (Fig. 3C). This is in striking contrast to near-complete loss of H2Bub1 in extracts containing wild-type H2B under the same condition. This finding demonstrates that H2B-R119A and H2B-T122D impede the ability of Ubp8 and Ubp10 to remove H2Bub1. Collectively, we conclude that the increased H2Bub1 levels in H2B-R119A and H2B-T122D are the result of decreased chromatin association and reduced enzymatic action of deubiquitinases, especially Ubp8.

\textbf{H2B-R119A and H2B-T122D alter the occupancy of H2Bub1 and H3K4 methylation on transcribed genes.} Next, we used chromatin immunoprecipitation (ChIP) assays to assess overall occupancy and distribution of H2Bub1 and H3K4 methylation on chromatin in H2B-R119A and H2B-T122D. We looked at the relative enrichment of these histone modifications at the 5', middle, and 3' ORF regions of constitutively expressed genes \textit{PMA1} and \textit{DMA2} (Fig. 4A). To evaluate the occupancy of H2Bub1, we carried out a chromatin double-immunoprecipitation assay (4, 49). In parallel ChIP assays, we used methylation-specific antibodies to directly isolate chromatin containing different methylated forms of H3K4.

A uniform distribution of H2Bub1 across the ORFs of \textit{PMA1} and \textit{DMA2} was observed in the wild type, and this distribution pattern was not disrupted in H2B-R119A and H2B-T122D (Fig. 4B). However, the overall occupancy of H2Bub1 in both genes was considerably increased in the mutants. Also, the level of H2Bub1 occupancy was generally higher in H2B-T122D than in H2B-R119A. These findings correlate well with the increase in steady-state H2Bub1 levels in these mutants (Fig. 2A and D). Taken together, these findings demonstrate that the H2B C-terminal helix residues R119 and T122 play an important role in modulating H2Bub1 levels during transcription.

Unlike H3K4me3, the occupancy and distribution of
H3K4me1 and -me2 on PMA1 and DMA2 in the H2B-R119A and H2B-T122D mutants showed a few surprising differences. In general, changes in the levels of H3K4me1 and -me2 on PMA1 in the two mutants closely resemble the changes in the global levels of these modifications (Fig. 2B and E), albeit with minor region-specific differences. The occupancy of H3K4me2 on PMA1 was severely reduced in H2B-R119A compared to H2B-T122D and the wild type (Fig. 4D, left). Also, H3K4me1 occupancy on PMA1 remained unchanged in the mutants, except for a reduction in the 3′ region (Fig. 4C, left). However, both the distribution and occupancy of H3K4me1 and -me2 on DMA2 were dramatically altered in the H2B mutants relative to the wild type (Fig. 4C and D, right), and they differ from PMA1 and the results of Western analyses (Fig. 2B and E). Occupancy of H3K4me2 was reduced in the middle and 3′ regions of DMA2 in H2B-R119A and H2B-T122D. In contrast, an increase in H3K4me2 occupancy at the 5′ region of DMA2 was observed in both mutants (Fig. 4D, right). Compared to the wild type, the occupancy of H3K4me1 on DMA2 in the mutants showed a decrease in the 3′ region, but an increase in 5′ region, with a dramatic 6-fold increase in H2B-R119A (Fig. 4C, right). Therefore, these findings suggest that H2B R119 and T122 play an important role in modulating all forms of H3K4 methylation during transcription. Importantly, since H2B-R119A and H2B-T122D affect both global and local levels of H3K4 methylation even in the presence of high levels of H2Bub1 (Fig. 2 and 4), our findings further suggest that these H2B mutations might modulate the functions of Set1-COMPASS independent of H2Bub1.

H2B-R119A and H2B-T122D affect Set1-COMPASS-mediated H3K4 methylation independent of H2Bub1. To test whether H2B-R119A and H2B-T122D affect Set1-COMPASS functions independent of H2Bub1, we examined H3K4 methylation levels in yeast strains harboring the wild-type or mutant H2B but lacking the deubiquitinases Ubp8 and Ubp10. The ubp8Δ ubp10Δ strain harboring either wild-type or mutant H2B showed very high but similar levels of H2Bub1 (Fig. 3A). However, H3K4me3 levels were still reduced in strains harboring mutant H2B (Fig. 5A). This finding supports the hypothesis that H2B residues R119 and T122 can affect Set1-COMPASS independent of H2Bub1.

To gain insight into the mechanism by which H2B-R119A and H2B-T122D affect H3K4 methylation, we tested whether enhancing the Set1 activity could restore H3K4me3 in these mutants. To this end, we used a dominant Set1 allele (Set1D-G990E), which increases all forms of H3K4 methylation in the wild type and has the ability to partially restore them even in the absence of H2Bub1 (42). Expression of Set1D-G990E did not restore H3K4me3 levels in H2B-R119A and H2B-T122D to those present in the wild type (Fig. 5B, compare lane 1 to lanes 6 and 9). Additionally, in both mutants, the ability of Set1D-G990E to produce H3K4me3 is reduced (Fig. 5B, compare lane 3 to lanes 6 and 9).

Since H2B-R119A and H2B-T122D cause an increase in H2Bub1 levels (Fig. 2), the reduced H3K4me3 by Set1 and Set1D-G990E in these mutants might be due to the following two reasons: H2B C-terminal helix mutations might debilitate the stimulation of methyltransferase activities by H2Bub1, or H2B mutations might directly affect the function of Set1 or Set1D-G990E independent of H2Bub1. To test these possibilities, Set1 or Set1D-G990E was expressed in strains lacking H2Bub1 (rad6Δ) and containing either wild-type or mutant H2B. Consistent with a previous finding (42), Set1D-G990E was able to partially restore H3K4me1 and -me2 in rad6Δ (Fig. 5C, lane 4). However, its ability to catalyze H3K4me1 was reduced in the rad6Δ H2B-R119A and rad6Δ H2B-T122D strains (Fig. 5C, lanes 7 and 10). Importantly, Set1D-G990E-
mediated H3K4me2 and -me3 were completely abolished by the H2B mutations (Fig. 5C, lanes 7 and 10). These results further confirm our novel finding that the H2B C-terminal helix residues R119 and T122 can modulate the function of methyltransferases (Set1 and Set1D-G990E) independent of H2Bub1.

In the absence of H2Bub1, Set1 produces low levels of H3K4me1, but it cannot catalyze H3K4me2 and -me3 (5, 44) (Fig. 5C, lane 2). Interestingly, the low level of H3K4me1 seen in rad6Δ is reduced in the rad6Δ H2B-R119A and rad6Δ H2B-T122D strains (Fig. 5C; compare lane 2 to lanes 5 and 8). This result shows that H2B-R119A and H2B-T122D affect Set1 functions even in the absence of H2Bub1. Collectively, our findings clearly establish that the H2B C-terminal residues R119 and T122 can modulate Set1-COMPASS independent of H2Bub1.

H2B-R119A and H2B-T122D reduce the chromatin-bound levels of Set1-COMPASS components Sdc1 and Spp1. Next, we investigated how the H2B C-terminal region might directly modulate the function of Set1-COMPASS. Both Set1 and Set1D-G990E are present as holoenzymes, copurifying with all COMPASS components, and their activities are dependent on Bre2 (42). Both enzymes are also dependent on other COMPASS components Swd3, Sdc1, and Spp1 for their activities (Fig. 5D). Since COMPASS subunits modulate Set1 activity, this puts forth a possibility that H2B-R119A and H2B-T122D might affect the association of COMPASS subunits with chromatin and cause changes in H3K4 methylation by Set1, especially a decrease in H3K4me3.

It has been suggested that the biochemical activity of the Set1 complex purified from the total cell population does not reflect the events on chromatin in vivo (30). Therefore, we used a combinatorial approach of fractionation and ChIP assays to examine the global and gene-specific changes in Set1-COMPASS subunits on chromatin, respectively. The global chromatin associations of Set1 (Fig. 6G, top), Swd1, Swd2, Swd3, and Bre2 (Fig. 6I to L, respectively) in H2B-R119A and H2B-T122D were similar to that of the wild type. Also, steady-state levels of Set1 and all other COMPASS components in whole-cell extracts were not affected by these mutations (Fig. 6A to F). However, the chromatin-bound Spp1 and Sdc1 levels were reduced in both mutants (Fig. 6G and H). The levels of chromatin-bound Sdc1 are lower in H2B-R119A than in H2B-T122D (Fig. 6G), correlating well with the observed decrease in the global H3K4me2 and -me3 levels in H2B-R119A (Fig. 2).

H2B-R119A and H2B-T122D affect the integrity of Set1-COMPASS on chromatin during transcription. Next, we examined the Set1-COMPASS integrity on chromatin by evaluating the distribution and occupancy of some of the regulatory subunits on constitutively expressed genes PMA1 and DMA2 using ChIP assays. In general, the overall distribution for most factors remained unchanged in both H2B-R119A and H2B-T122D compared to that of the wild type (Fig. 7). Nevertheless, a gene- and region-specific decrease in factor occupancy was observed, further confirming that both mutations affect the integrity of Set1-COMPASS on chromatin. Set1 occupancy was affected by H2B-T122D in the 3′ regions of both genes (Fig. 7A). Swd2 occupancy was reduced in all regions of PMA1 in H2B-R119A but was affected only in the 5′ region of DMA2 (Fig. 7B). Bre2 occupancy remained mostly unchanged in both mutants (Fig. 7C). Although overall chromatin-bound Sdc1
levels were reduced in H2B mutants (Fig. 6G), the occupancy of Sdc1 was reduced only in the middle and 3’/H11032 regions of DMA2 in H2B-T122D but remained unaffected in most regions of both genes (Fig. 7D). The following two possibilities can be envisaged for the discrepancy in the chromatin-bound Sdc1 levels observed in fractionation and ChIP assays. First, Sdc1 occupancy is reduced on other genes, but not on PMA1 and DMA2. Second, the interaction of Sdc1 with Set1-COMPASS and/or chromatin may be weakened by H2B-R119A and H2B-T122D. This weakened intermolecular interaction causes dissociation of Sdc1 during fractionation, culminating in the apparent reduced levels on chromatin (Fig. 6G). However, formaldehyde cross-linking stabilizes these weakened interactions and prevents the detection of changes in chromatin interaction of Sdc1 in ChIP assays. Consistent with the reduction in overall chromatin-bound Spp1 levels in fractionation (Fig. 6H), Spp1 occupancy was reduced in almost all regions of

FIG. 6. H2B-R119A and H2B-T122D reduce chromatin-bound levels of Sdc1 and Spp1. Whole-cell extracts (A to F) and the chromatin fraction (G to L) obtained from indicated strains were used for Western blotting to determine changes in the levels of the Set1-COMPASS subunits. The levels of Pgk1 and H3 serve as loading controls.

FIG. 7. H2B-R119A and H2B-T122D affect the occupancy of Set1-COMPASS subunits on transcribed genes. (A to E) Distribution and occupancy of the indicated Set1-COMPASS subunits in 5’, middle, and 3’ ORF regions of PMA1 and DMA2 in the indicated strains determined by ChIP assay, as described in the legend to Fig. 4. Error bars show standard errors of the means obtained from at least three independent experiments. An asterisk denotes a statistically significant difference in factor occupancy at a given region between the mutant and the wild type, as determined by Student’s t test (P < 0.05).
PMA1 and in some regions of DMA2 in both H2B mutants (Fig. 7E). Collectively, our findings from chromatin fractionation and ChIP assays suggest that the H2B-R119A and H2B-T122D reduce H3K4 methylation levels by affecting the Set1-COMPASS integrity on chromatin, probably by disrupting the direct chromatin association or the binding of some of the regulatory subunits.

Spp1 interacts with H2B, and this interaction can be disrupted by H2B-R119A/T122D. We used GST pulldown assays to test whether components of Set1-COMPASS bind to the H2B C-terminal helix. Since the chromatin binding of Spp1 and Sdc1 was reduced by H2B-R119A and H2B-T122D (Fig. 6G and H), we purified recombinant Spp1 and Sdc1 expressed in bacteria containing N-terminal hexahistidine and GST tags (His6GST) and incubated them with a bacterial lysate containing wild-type or mutant H2B. Only His6GST-Spp1, but not His6GST-Sdc1, was able to bind H2B before binding to glutathione-Sepharose beads. Only His6GST-Spp1, but not His6GST-Sdc1, was able to bind H2B (Fig. 8A). Next, we tested whether residue R119 or T122 play a role in this interaction between Spp1 and H2B. To this end, we incubated His6GST-Spp1 with bacterial lysates containing either wild-type or mutant H2B. Compared to wild-type H2B, only H2B-T122D caused a slight reduction in Spp1 binding (Fig. 8B). Therefore, we tested whether a double-site mutant H2B containing both R119A and T122D might compromise Spp1 binding. Indeed, H2B-R119A/T122D showed severely reduced binding to Spp1 (Fig. 8B). Collectively, our findings show that Spp1 interacts with H2B via the R119 and T122 residues and suggest that disruption of this interaction results in changes in H3K4 methylation and reduced chromatin binding of Set1-COMPASS in H2B-R119A and H2B-T122D.

Importantly, our findings provide mechanistic insight into the role of the H2B C-terminal helix in the trans-histone cross talk independent of H2Bub1 by modulating the Set1-COMPASS association with the nucleosome.

H2B-R119A/T122D affects H2Bub1, H3K4 methylation, cell growth, and transcription. Since H2B-R119A/T122D binds poorly to Spp1 (Fig. 8B), we tested the effect of double mutation on overall H2Bub1, H3K4, and H3K79 methylation levels. The steady-state H2Bub1 levels were dramatically increased in H2B-R119A/T122D relative to the wild type (Fig. 9A) and the single-site H2B mutants (Fig. 9A, bottom). Furthermore, H2B-R119A/T122D showed a very severe reduction in all forms of H3K4 methylation compared to the wild type (Fig. 9B) and single mutants (Fig. 2B and E). However, no change in H3K79 methylation was observed (Fig. 9B). These findings show that H2B-R119A/T122D affects only Set1-COMPASS, but not Dot1, function independent of H2Bub1. The effect of H2B-R119A/T122D on Set1-COMPASS function is also evident from the severe reduction in the low levels of H3K4me1 present in the rad6Δ strain (Fig. 9B; long exposure blot), further demonstrating the H2Bub1-independent direct modulation of Set1-COMPASS by the H2B C-terminal helix residues.

Next, we examined the changes in distribution and overall occupancy of H3K4 methylation on PMA1 and DMA2 in H2B-R119A/T122D by ChIP assay. Consistent with Western analyses (Fig. 9B), both H3K4me2 and H3K4me3 were severely reduced in all regions of both genes in H2B-R119A/T122D compared to the wild type (Fig. 9C) and single-site mutants (Fig. 4D and E). Distribution and occupancy of H3K4me1 showed no change in PMA1 but was altered in DMA2 in H2B-R119A/T122D (Fig. 9C), similar to those in the single mutants (Fig. 4C). Collectively, these findings suggest that the drastic reduction in H3K4 methylation is likely to due to a severe debilitation of chromatin-association of Set1-COMPASS in H2B-R119A/T122D.

Given the dramatic change in H3K4 methylation on chromatin (Fig. 9C), we examined the effect of H2B-R119A/T122D on the transcription of PMA1 and DMA2. Measurement of the steady-state mRNA levels showed that PMA1 transcripts were reduced in H2B-R119A and H2B-R119A/T122D, whereas the DMA2 transcript levels were not reduced in any mutant (Fig. 9D). These findings reveal the intrinsic differences in the regulation of PMA1 and DMA2. Importantly, they suggest that the
control of Set1-COMPASS-mediated H3K4 methylation by the H2B C-terminal helix may not be tightly coupled to ongoing transcription.

Consistent with reduced H3K4 methylation (Fig. 9B and C), $H2B$-$R119AT122D$ conferred the silencing defect (data not shown), similar to the single mutants (Fig. 2F). Importantly, $H2B$-$R119AT122D$ conferred a severe slow-growth defect compared to the wild type or single mutants (Fig. 9E). Altogether, our results demonstrate that the H2B C-terminal helix plays an important role in maintaining cell growth, in controlling the active transcription of certain genes, and in the establishment of H3K4 methylation by Set1-COMPASS.

Set1-COMPASS integrity is compromised by $H2B$-$R119AT122D$. To test how $H2B$-$R119AT122D$ affects the chromatin association of Set1-COMPASS, we examined the distribution and occupancy of individual subunits on $PMA1$ and $DMA2$ using ChIP assays. In general, a drastic reduction in the occupancy of Swd2, Bre2, Sdc1, and Spp1 was observed in almost all regions of both genes (Fig. 10B to E). Set1 occupancy remained unchanged in $PMA1$ but was reduced in

![Figure 9](image-url)
DMA2 (Fig. 10A). Unlike the single mutations (Fig. 7), the reduced occupancy of many subunits of COMPASS in almost all regions of PMA1 and DMA2 suggests that the chromatin binding of the entire Set1-COMPASS is disrupted by H2B-R119AT122D.

Next, we performed fractionation to determine the effect of H2B-R119AT122D on the overall chromatin association of Set1-COMPASS. Chromatin-bound levels of Set1, Swd1, and Swd2 were reduced in H2B-R119AT122D (Fig. 10F and H). Since Set1 and Swd1 are essential for the complex integrity (40), our finding suggests that the H2B-R119AT122D reduces not only the binding but also the Set1-COMPASS integrity on chromatin. The chromatin-bound Sdc1 levels are nearly absent in the H2B-R119AT122D mutant (Fig. 10F). This result further confirms the finding that H2B residues R119 and T122 are important for the association of Sdc1 with chromatin, as seen from the partial loss of chromatin-bound Sdc1 in the single mutants (Fig. 6G). Importantly, the chromatin-bound levels of Spp1 were decreased (Fig. 10F), consistent with the reduced binding of H2B-R119AT122D with Spp1 in GST pulldown assays (Fig. 8B). Surprisingly, in control experiments, we found a reduction in the global, steady-state levels of Set1-COMPASS subunits even in whole-cell extracts, especially at a near total loss of Sdc1 (Fig. 10G). However, the transcript levels of all Set1-COMPASS subunits in H2B-R119AT122D were similar to those in the wild type (Fig. 10I). Therefore, the reduced chromatin and global levels of Set1-COMPASS subunits in H2B-R119AT122D demonstrate, for the first time, that the H2B C-terminal helix residues...
R119 and T122 are important for chromatin binding, integrity, and stability of Set1-COMPASS.

**DISCUSSION**

In this study, we have gained further insights into the trans-histone pathway by finding that the H2B C-terminal helix acts as a binding platform in modulating the dynamics of H2Bub1 and H3K4 methylation.

**Regulation of H2Bub1 by the H2B C-terminal helix.** Identification of Rad6/Bre1 and deubiquitinases (DUBs, Ubp6, Ubp8, and Ubp10) has provided insight into the mechanisms that control overall H2Bub1 levels (38, 53), but how these enzymes bind and act on chromatin remains unexplored. Here, we find novel roles for the residues adjacent to H2B residue K123 in modulating H2Bub1 levels; even the “tail” region after K123 is needed for maintaining normal H2Bub1 levels, but its primary sequence is dispensable (Fig. 1). Additionally, H2B C-terminal helix residues can alter the dynamics of ubiquitination and deubiquitination by affecting the chromatin binding of Rad6 and Ubp8 (Fig. 3B) and/or by directly affecting the enzymatic functions. For example, the chromatin binding-competent Ubp10 failed to deubiquitinate H2Bub1 from the mutants **in vitro** (Fig. 3C). Further, our mutagenesis study reveals that maintaining H2Bub1 levels is a charge-based phenomenon. For instance, R119A and R119D differentially affect H2Bub1 levels (Fig. 2D), and only R119K maintains wild-type levels (data not shown). Indeed, a positive charge at this position is present in H2B of higher eukaryotes, indicating a conserved mechanism likely exists to modulate H2Bub1 levels.

**Modulation of Set1-COMPASS functions by H2Bub1 and the H2B C-terminal helix.** Intrinsic instability and both transcription-dependent and transcription/replication-independent eviction of the H2A-H2B dimer contribute to overall nucleosome destabilization in yeast (19, 36, 54). Previously, we showed that H2Bub1 stabilizes the nucleosome by preventing eviction and retaining H2A-H2B on chromatin (4) (Fig. 11A) and proposed that stable nucleosomes likely stabilize and stimulate Set1 and Dot1 functions on chromatin.

In this study, we found that Set1-COMPASS binds H2B via an interaction of Spp1 with residues R119 and T122. Importantly, these residues are required only for Set1-COMPASS functions but do not affect Dot1-mediated H3K79 methylation, regardless of the H2Bub1 levels (Fig. 2 and 9). This leads to the question, why is Dot1 not affected? Given its distributive nature (12) and since H2Bub1 reconstituted into a chemically defined nucleosome **in vitro** showed a direct stimulation of Dot1-mediated H3K79 methylation via an allosteric mechanism (29), it is likely that the presence of H2Bub1 alone is sufficient to support Dot1 functions. Additionally, it is evident that R119 and T122 reside on a helical phase that faces away from H3K79 (Fig. 11A). Therefore, an uncoupling is achieved, as these residues might not affect Dot1 binding but instead serve as a distinct “docking site” for only Set1-COMPASS. Altogether, based on these findings, we propose a simple model for the trans-histone cross talk (Fig. 11B); H2Bub1 stabilizes the nucleosome by enhancing intranucleosomal interactions or by preventing H2A-H2B eviction. In turn, this leads to the retention of the “docking site” in the H2B C-terminal helix for Set1-COMPASS on chromatin, promoting both complex integrity and processive methylation. Additionally, the stable nucleosome or presence of H2Bub1 alone promotes prolonged association and allosteric changes in Dot1, resulting in high levels of H3K79 methylation.

We find that **H2B-R119AT122D** reduces the global and chromatin-bound level of Set1-COMPASS subunits even in the presence of high levels of H2Bub1 and that it also adversely affects the low levels of H3K4me1 in the absence of H2Bub1 (Fig. 9). Furthermore, Spp1 binds very poorly to **H2B-R119AT122D** **in vitro** (Fig. 8B). Collectively, these findings clearly establish that the “docking site” comprised of residues R119 and T122 in the H2B C-terminal helix, which is required for Spp1 binding, is also critical for chromatin association and the overall stability of Set1-COMPASS independent of H2Bub1. Further, it can be envisaged that the disruption of Spp1-H2B binding destabilizes Set1-COMPASS on chromatin, causing dissociation and/or poor association of a few individual subunits, as seen in the single mutants (Fig. 6 and 7). Since transcript levels of Set1-COMPASS subunits are not reduced in **H2B-R119AT122D** (Fig. 10I), the severe disruption of Spp1-H2B interaction in this mutant probably causes a drastic destabilization of Set1-COMPASS on chromatin. Our findings provide mechanistic explanations for the observed H2Bub1-independent association of Spp1 with chromatin (50) and, importantly, for the reduced steady-state levels of Set1, an integral component of the complex, in **spp1Δ** (6).

Both global and chromatin-bound levels of Sdc1 are severely reduced compared to those of Spp1 and all other COMPASS subunits in **H2B-R119AT122D** (Fig. 10F and G). Since Spp1 is not known to directly interact with Sdc1, this finding puts forth a possibility that the perturbations to chromatin structure induced by H2B C-terminal helix mutations might affect the association of Sdc1 with COMPASS and/or chromatin in an Spp1-independent manner. This raises the question, how does the H2B C-terminal helix affect Sdc1? Since Sdc1 does not bind H2B **in vitro**, it is conceivable that the H2B C-terminal helix might control the binding of Sdc1 to some other region of chromatin or affects Sdc1 by altering the chromatin binding of a Set1-COMPASS subunit other than Spp1. Alternatively, differences seen in the chromatin binding of Sdc1 in fractionation and ChiP assays allude to yet another possibility. The decrease in chromatin-bound levels of Sdc1 seen in **H2B-R119A** and **-T122D** might be due to weakened intermolecular interaction of Sdc1 with other COMPASS subunits. The weak intermolecular interaction of Sdc1 might render it labile to dissociation during fractionation, resulting in its reduced levels on chromatin (Fig. 6G). Modest or no loss of Sdc1 on chromatin in ChiP assays in these mutants (Fig. 7D) might be due to the addition of formaldehyde, which cross-links and stabilizes the weak interactions and retains Sdc1 on chromatin. Therefore, our findings suggest that the H2B C-terminal helix plays a role in stabilizing the intermolecular interactions of Sdc1 with other COMPASS subunits on chromatin. Collectively, based on our findings, we propose that the H2B C-terminal helix controls the stability, integrity, and processivity of Set1-COMPASS by affecting the chromatin association of its subunits, at least in part, by directly affecting the binding of Spp1.

The association of Swd2 with Set1-COMPASS is dependent on H2Bub1, and it has been proposed to be the key link
involved in mediating the cross talk between H2Bub1 and H3K4 and -K79 methylation (26). Interestingly, we find that H2B-R119AT122D contains very high levels of H2Bub1, which are greater than those in the wild type and single mutants (Fig. 9A), yet the chromatin-bound Swd2 levels are reduced in this mutant (Fig. 10). While these reduced Swd2 levels correlate well with the decreased H3K4 methylation, they do not cause a reduction in H3K79 methylation levels (Fig. 9B). This finding demonstrates an uncoupling of the Swd2-dependent coregulation of H3K4 and -K79 methylation mediated by H2Bub1 and strongly suggests that Swd2 is an important factor needed for maintaining normal H3K4 methylation levels, but it is likely not the only critical factor needed for the trans-histone cross talk. Therefore, based on our findings from this and previous study (4), we propose that in the absence of H2Bub1 (Fig. 11B), H2A-H2B eviction or weakened intranucleosomal interactions might debilitate the direct contact between Spp1 and the H2B C-terminal helix. Further, the intersubunit interactions within Set1-COMPASS might also be affected, resulting in partial complex assembly or dissociation. Indeed, Bre2 and Swd2 have been shown to dissociate from Set-COMPASS in the absence of H2Bub1 (26). Additionally, the absence of H2Bub1 might augment the unfavorable allosteric changes in Set1 conformation, for example, persistence or promotion of the interaction between the autoinhibitory region and the SET domain that prevents Set1 processivity (42). Together, these defects might allow Set1-COMPASS to catalyze very low levels of H3K4me1 in the absence of H2Bub1 (Fig. 2 and 5). Therefore, we further propose that ubiquitin conjugation onto H2B stabilizes the nucleosome by retaining the “docking site” on chromatin that favors increased Spp1-H2B interaction and, thus, increased chromatin association and stability of Set1-COMPASS (Fig. 11B, right). In turn, this leads to the production of high levels of H3K4me1, -me2, and -me3.

A role for the H2B C-terminal helix in the regulation of transcription and cell growth. A simple explanation for the observed slow growth in H2B-R119AT122D (Fig. 9E) is that the robust increase in H2Bub1 levels might stabilize the nu-

FIG. 11. Trans-histone regulation of H3K4 and -K79 methylation by H2Bub1. (A) Model for the dynamic changes in yeast nucleosome stability. Location of the residues involved in Set1-COMPASS docking (H2B residues R119 and T122), site of H2B ubiquitination (K123), and H3 residues modified by methylation (K4 and K79) are shown on the yeast nucleosome. C-terminal G76 of ubiquitin (PDB accession no. 1UBQ) is placed close to H2B residue K123 to simulate H2Bub1. (B) Speculative model depicting the role of H2Bub1 and the H2B C-terminal helix in modulating the chromatin association, integrity, and stability of Set1-COMPASS. Dotted lines denote weak intermolecular interactions, subunit dissociation, and unfavorable conformation. CH₃ (gray), low levels of H3K4me1; CH₃ (black), high levels of H3K4me1, K4me2, and K4me3. See the text for details.
cleosome and likely impedes ongoing transcription, similar to that seen for ubp8Δ ubp10Δ (4). The H2B C-terminal helix appears to have a role in regulating transcription of PMA1 but not DMA2, as seen in the reduced PMA1 transcript levels (Fig. 9D). Constitutively expressed PMA1 exhibits a 200-fold-higher steady-state mRNA level and transcription rate (53 mRNA/min) than DMA2 (18). Therefore, it is tempting to speculate that the underlying differences in the rate of RNA polymerase II progression, local chromatin structure, and association/dissociation rate of regulatory factors in these two genes might contribute to the differences seen not only in transcription but also in the gene-specific and/or region-specific differences in the occupancy of H2Bub1 and H3K4 methylation (Fig. 4 and 9). This raises the question, is the role of the H2B C-terminal helix in regulating transcription connected to its (Fig. 4 and 9). Therefore, the role for the H2B C-terminal helix in regulating transcription and establishing transcription-coupled H2Bub1 and H3K4 methylation appears to be very complex and needs further investigation. Nevertheless, H3K4me1 occupancy is increased only in the 5′ region of DMA2, even though levels of Set1 and other subunits were reduced in the double mutant (Fig. 10A). This finding suggests that the H2B C-terminal helix controls the Set1-COMPASS composition differentially at different genes and at different regions of a gene. It has been proposed that partial Set1-COMPASS, with different combinations of its subunits, might exist on chromatin during distinct phases of transcription to establish various degrees of H3K4 methylation, and these partial complexes might be assembled on an as-needed basis depending on the dynamics and degree of H2Bub1 (6, 30). Therefore, our findings strongly support this possibility and further reveal a role for the H2B C-terminal helix in the dynamic modulation of Set1-COMPASS assembly.

ACKNOWLEDGMENTS

We thank David Allis, Brad Cairns, Vincent Géli, Michael Grunstein, Mary Ann Osley, Brian Strahl, Bill Tansey, Toshi Tsukiyama, Tony Weil, Fred Winston, and the Vanderbilt University Center for Structural Biology for kindly providing the reagents.

This work was supported by the Vanderbilt-Ingram Cancer Center, the Robert J. and Helen C. Kleberg Foundation, and grants from NCI SPORE in Breast Cancer (SPOCA098131) and the National Institutes of Heath (RO1CA109555).

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