Acetylation of lysine 56 of histone H3 (H3-Lys-56) occurs in S phase and disappears during G2/M phase of the cell cycle. However, it is not clear how this modification is regulated during the progression of the cell cycle. We and others have shown that the histone acetyltransferase (HAT) Rtt109 is the primary HAT responsible for acetylating H3-Lys-56 in budding yeast. Here we show that Rtt109 forms a complex with Vps75 and that both recombinant Rtt109-Vps75 complexes and native complexes purified from yeast cells acetylate H3 present in H3/H4/H2A/H2B core histones but not other histones. In addition, both recombinant and native Rtt109-Vps75 HAT complexes exhibited no detectable activity toward nucleosomal H3, suggesting that H3-Lys-56 acetylation is at least in part regulated by the inability of Rtt109-Vps75 complexes to acetylate nucleosomal H3 during G2/M phase of the cell cycle. Further, Rtt109 bound mutant H3/H4 tetramers composed of histones lacking their N-terminal tail domains less efficiently than wild-type H3/H4 tetramers, and Rtt109-Vps75 complexes displayed reduced HAT activity toward these mutant H3/H4 tetramers. Thus, the N termini of H3/H4 tetramers are required for efficient acetylation of H3 by the Rtt109-Vps75 complex. Taken together, these studies provide insights into how H3-Lys-56 acetylation is regulated during the cell cycle.

In eukaryotic cells, nucleosomes comprising ~146 bp of DNA wrapped around histone octamers form the basic unit of chromatin (1, 2). In recent years, it has become clear that chromatin is dynamically regulated during the processes of transcription, DNA repair, and DNA replication (3). One of the primary means of regulating chromatin structure and function is mediated by posttranslational modification of histones, including acetylation, methylation, and ubiquitination (4–6).

Histone acetylation plays an important role in transcription, DNA replication, nucleosome assembly, and DNA repair (7–9).

These diverse functions are likely to be carried out by distinct histone acetyltransferases (HATs) that target different lysine residues on distinct histones. Although the acetylation of N-terminal lysine residues of histones H3/H4 is well known, lysine 56 of H3 (H3-Lys-56), a lysine residue in the core domain of H3, has only more recently been found to be acetylated (10–14). Further, in contrast to the acetylated form remaining constant throughout the cell cycle as it does for most H3/H4 lysine residues, acetylation of H3-Lys-56 occurs in S phase and disappears during G2/M phase of the cell cycle (10, 14). However, it is not clear how H3-Lys-56 acetylation is restricted to S phase of the cell cycle.

Two NAD-dependent histone deacetylases (HDACs), Hst3 and Hst4, have been found to deacetylate H3-Lys-56 (15, 16). Moreover, the expression of these two enzymes peaks at G2/M phase of the cell cycle when the levels of acetylated H3-Lys-56 are lowest, suggesting that H3-Lys-56 acetylation is regulated, at least in part, by the protein levels of H3-Lys-56 HDACs. Whether H3-Lys-56 HDACs are the only factors that regulate H3-Lys-56 acetylation during the progression of the cell cycle, however, is unknown.

Recently, we and others have discovered that in the yeast *Saccharomyces cerevisiae*, Rtt109 is a unique HAT, sharing no sequence homology to any known HATs discovered so far, that acetylates H3-Lys-56 (17, 18). In *vitro*, recombinant Rtt109 and Rtt109 complexes purified from yeast cells acetylate H3 but not H4 in H3/H4 tetramers, suggesting that Rtt109 specifically targets H3. Cells lacking Rtt109 lose H3-Lys-56 acetylation and are sensitive to DNA-damaging agents to a similar degree as cells expressing H3 mutants unable to be acetylated at lysine 56. In addition, Rtt109 genetically interacts with several genes involved in DNA replication as well as with genes involved in double strand break repair (17, 19–21). These genetic interactions suggest that H3-Lys-56 acetylation mediated by Rtt109 is involved in DNA replication. However, it is not known whether Rtt109 acetylates H2A and H2B, or H3 when H3 is assembled into nucleosomes. Thus, here we report further biochemical characterization of this novel HAT.

Rtt109 co-purifies with Vps75, a previously uncharacterized protein, from yeast cells (17, 22). We have reconstituted recombinant Rtt109-Vps75 complexes, and these recombinant com-
plexes display HAT activity toward H3. Moreover, both native Rtt109-Vps75 complexes purified from yeast cells and recombinant Rtt109-Vps75 complexes purified from bacteria acetylate only H3, but not other histones, and do so only when H3 is not assembled into nucleosomes. These results suggest that the reduction of H3-Lys-56 acetylation during G2/M phase of the cell cycle is mediated, at least in part, by the inability of Rtt109 to acetylate nucleosomal H3. Furthermore, the N-terminal tail domains of H3/H4 tetramers were found to be required for efficient binding of Rtt109 to histones and H3-Lys-56 acetylation by Rtt109-Vps75 complexes. Thus, these studies provide insights into the substrate requirements of Rtt109 as well as the cell cycle regulation of H3-Lys-56 acetylation.

**EXPERIMENTAL PROCEDURES**

**Generation of rtt109Δ and vps75Δ Strains**—The rtt109Δ deletion strain was generated as described (17). Standard procedures were followed to delete the VPS75 gene through homologous recombination using the KanMX6 cassette.

**Western Blot Analysis**—Western blot analysis was performed as described previously using antibodies that specifically recognize acetylated H3-Lys-56 (14).

**Purification of Rtt109-Vps75 Complexes from Yeast Cells**—Whole-cell extracts were prepared using 2 liters of yeast cells expressing Rtt109 or Vps75 fused at the C terminus with the tandem affinity purification (TAP) tag, and the proteins were purified as described (17).

**Purification of Recombinant Histone H3/H4 Tetramers, H3/H4/H2A/H2B Core Histones, and Nucleosomes**—Recombinant *Drosophila* H3/H4 tetramers were purified as described (23). Sequences for generating the mutant “tailless” H3/H4 tetramers were from *Xenopus laevis*. The tailless H3 comprises amino acids 27–135, and the tailless H4 comprises amino acids 20–102 (24). The H3 and H4 mutants were expressed from the plasmid pST39GSTAsf1H3H4 (J. K. Tyler, University of Colorado, Aurora, CO) and purified following procedures similar to those used to purify full-length H3/H4 tetramers (23). H3/H4/H2A/H2B core histones were purified from 293 cells using procedures described by Ausio and van Holde (25).

Mononucleosomes and mixtures of mono- and dinucleosomes were purified from 6 liters of 293 cells using methods similar to those for purifying core histones (25), except that the hydroxyapatite chromatography step was not applied. Mononucleosomes were separated from other nucleosome species using sucrose gradient fractionation, and fractions containing predominantly mononucleosomes or mixed populations of mono- and dinucleosomes were pooled together (supplemental Fig. S4).

**Generation of Rtt109-Vps75 Complex Expression Constructs and Purification of Recombinant Proteins**—GST-Rtt109 was cloned and expressed as described (17). The VPS75 gene was cloned using genomic DNA as template for PCR. Since the VPS75 gene contains an intron, the DNA sequence corresponding to the intron was deleted using site-directed mutagenesis. Conventional chromatography methods for protein purification were used to purify Rtt109-Vps75 complexes.

**Assays for Histone Acetyltransferase Activity**—The histone acetyltransferase activities of Rtt109-containing complexes were determined as described previously, with minor modifications (17). Briefly, samples were incubated at 30 °C for 2 h in 15-μl reaction mixtures that contained 50 mM Tris-HCl, pH 8.0, 5% (w/v) glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, 5 mM phenylmethylsulfonyl fluoride, and 6 pmol of [3H]acetyl-CoA (4.3 mCi/mmol; Amersham Biosciences). When indicated, 1 μg of recombinant *Drosophila* H3/H4 tetramers (dhH3/H4), H2A/H2B dimers (H2A/H2B), H3/H4/H2A/H2B core histones, tailless H3/H4 tetramers (shH3/H4), mononucleosomes, or mixed populations of mono- and dinucleosomes was added as substrate. One-half of each reaction mixture, 7.5 μl, was then spotted onto P-81 phosphocellulose paper filters (Upstate Biotechnology) and air-dried. Paper filters were washed five times for 5 min each with 100 ml of 50 mM NaHCO₃, pH 9.0, buffer and once with 50 ml of acetone. The radioactivity of air-dried filters was measured using a liquid scintillation counter. To detect which protein was acetylated, proteins from each reaction mixture were first resolved using 15% SDS-PAGE, and then gels were dried and exposed to films. To detect whether H3-Lys-56 was acetylated, samples were incubated with unlabeled acetyl-CoA at 30 °C for the indicated time periods, and Western blot analysis using antibodies that recognized H3 acetylated at lysine 56 was performed.

**GST Pull-down Assays**—GST-Rtt109 (10 μg) or GST-REGα (10 μg) bound to glutathione-Sepharose was incubated with different amounts of H3/H4 tetramers in a buffer containing 25 mM Tris, pH 7.5, 1 mM EDTA, 0.01% Nonidet P-40, and 200 mM NaCl overnight at 4 °C. After washing away unbound proteins, proteins bound to GST-Rtt109 or GST-REGα were eluted using 1× SDS sample buffer, resolved using SDS-PAGE, and revealed by staining with Coomassie Brilliant Blue (CBB) or Western blot analysis using antibodies against H3.

**DNA Damage Sensitivity Assays**—Yeast strains were grown overnight at 30 °C in rich YPD medium and initially diluted to 6 × 10⁶ cells/ml. Subsequently, cells were 10-fold serially diluted and spotted onto medium containing one of the indicated DNA-damaging agents: camptothecin (1 μg/ml), hydroxyurea (50 mM), or methylmethane sulfonate (0.005%). To test for UV sensitivity, cells spotted onto YPD medium were irradiated by UV using a cross-linker.

**RESULTS**

**Vps75 Is a Component of the Rtt109 HAT Complex**—Using a genome-wide screening technique in *S. cerevisiae* to identify proteins involved in acetylation of H3-Lys-56, we recently identified the unique HAT Rtt109 (17). In addition, we confirmed an observation made by others that Vps75, a previously uncharacterized protein, co-purifies with Rtt109 from yeast cells (22). Indeed, this Rtt109-Vps75 complex displays Rtt109-dependent HAT activity toward H3 (Fig. 1, A and B). However, the vps75Δ mutant was not among those identified in our genome-wide screen as affecting H3-Lys-56 acetylation (17), suggesting that Vps75 is not required for H3-Lys-56 acetylation in *vivo*. To test this idea, we deleted the VPS75 gene from yeast cells and analyzed H3-Lys-56 acetylation by Western blot. As shown in Fig. 1C, H3-Lys-56 acetylation was not altered to a significant degree in the vps75Δ mutant cells as compared with wild-type cells. Furthermore, the vps75Δ mutant cells were not sensitive...
to DNA-damaging agents (supplemental Fig. S1), a phenotype displayed by cells with altered H3-Lys-56 acetylation (10, 17). Thus, Vps75 is not required for H3-Lys-56 acetylation in yeast cells or for Rtt109 to acetylate H3-Lys-56 in vitro (17, 18).

As Vps75 is not required for H3-Lys-56 acetylation by Rtt109, possibly Vps75 is just a contaminating protein that co-purifies with Rtt109 and is not part of the Rtt109 HAT complex. To determine whether Vps75 directly forms a complex with Rtt109, we attempted to express and purify a recombinant Rtt109-Vps75 complex. Indeed, when Vps75 and Rtt109 were co-expressed in Escherichia coli, a complex of Rtt109 and Vps75 was present throughout all chromatography steps (Fig. 1D). Moreover, these recombinant complexes displayed HAT activity toward H3 (Fig. 1, E and F). Thus, these results demonstrate that Vps75 is a component of the Rtt109-Vps75 HAT complex, with Rtt109 as the catalytic subunit.

Native and Recombinant Rtt109-Vps75 Complexes Acetylate Non-nucleosomal H3—We have shown that Rtt109-Vps75 complexes purified from yeast cells acetylate H3, but not H4, when recombinant H3/H4 tetramers are used as substrates (17). Thus, we next sought to determine the substrate requirements for Rtt109-Vps75 complexes using both native complexes purified from yeast cells and recombinant complexes purified from E. coli. First, we tested the ability of Rtt109-Vps75 complexes to acetylate H3/H4/H2A/H2B core histones purified from 293 cells. Rtt109-Vps75 complexes purified from yeast cells acetylated H3 present in core histone complexes, as determined using both a filter binding assay (Fig. 2A) and autoradiography (Fig. 2B), although less efficiently than when H3 was present as a component of recombinant H3/H4 tetramers. Moreover, the complex acetylated H3, but not H2A, H2B, or H4 (Fig. 2B and supplemental Figs. S2 and S3A), suggesting that Rtt109-Vps75 complexes can confer histone specificity in vitro. In addition, the recombinant Rtt109-Vps75 complexes exhibited a similar degree of HAT activity as native complexes toward H3 when H3/H4 tetramers or H3/H4/H2A/H2B core histones were used as substrate (Fig. 2, A, B, D, and E).

After analyzing the acetylation of H3 by Rtt109-Vps75 complexes when H3 is present as a mixture of four core histones, we next determined how Rtt109-Vps75 complexes acetylate H3-Lys-56. Surprisingly, although global acetylation of H3 by Rtt109-Vps75 complexes was more efficient when H3 was presented as H3/H4 tetramers, both recombinant and native complexes

FIGURE 1. Vps75 is a component of the Rtt109 HAT complex but is not required for acetylation of lysine 56 of histone H3. A and B, Rtt109 is required for the HAT activity toward H3 exhibited by complexes that co-purify with Vps75 from yeast cells. Vps75-TAP was purified from wild-type or rtt109 mutant cells, and the purified proteins were incubated with recombinant dH3/H4 tetramers in the presence of [3H]acetyl-CoA. The incorporated [3H]acetate was detected by scintillation counting (A), or alternatively, proteins labeled by [3H]acetate were resolved using SDS-PAGE followed by staining with CBB to detect total proteins and autoradiography to detect [3H]-acetylated proteins (B). C, Vps75 is not required for H3-Lys-56 acetylation in yeast cells. Western blot analysis was performed to examine H3-Lys-56 acetylation levels in whole-cell extracts from wild-type (WT), rtt109Δ, and vps75Δ cells using antibodies against histone H3 (H3) or acetylated lysine 56 of H3 (H3-K56Ac). D, Vps75 co-purifies with Rtt109 in E. coli. Vps75-Rtt109 complexes were purified from E. coli, resolved using SDS-PAGE, and visualized by CBB. E and F, recombinant Rtt109-Vps75 complexes display HAT activity toward H3. Experiments were performed as described in A and B, except using recombinant Rtt109-Vps75 complexes purified from E. coli. REGΔ, a proteasome-binding protein, was used as a negative control. Twenty-five ng of native Rtt109 and 5 ng of recombinant Rtt109-Vps75 complexes were used for each 15-μl HAT assay.

Native and Recombinant Rtt109-Vps75 Complexes Acetylate Non-nucleosomal H3—We have shown that Rtt109-Vps75 complexes purified from yeast cells acetylate H3, but not H4, when recombinant H3/H4 tetramers are used as substrates (17). Thus, we next sought to determine the substrate requirements for Rtt109-Vps75 complexes using both native complexes purified from yeast cells and recombinant complexes purified from E. coli. First, we tested the ability of Rtt109-Vps75 complexes to acetylate H3/H4/H2A/H2B core histones purified from E. coli. Next, we tested the ability of Rtt109-Vps75 complexes to acetylate H3 when presented as H3/H4/H2A/H2B core histones acetylated H3-Lys-56 to a similar degree irrespective of whether H3/H4 tetramers or H3/H4/H2A/H2B core histones were used as substrates (Fig. 2, C and F). The difference in the ability of Rtt109-Vps75 complexes to acetylate H3 when present in H3/H4 tetramers versus H3/H4/H2A/H2B core histones does not appear to be due to differences in salt concentrations (supplemental Fig. S3A). Possibly, the observed high HAT activity of Rtt109-Vps75 complexes toward H3/H4 tetramers as compared with core histones is due to acetylation of H3 lysine residues other than H3-Lys-56 that are readily accessible for acetylation when presented as H3/H4 recombinant tetramers in vitro but not when H3 is assembled with core histones. Alternatively, these putative additional H3 lysine residues acetylated by Rtt109-Vps75 complexes when H3 is a component of H3/H4 tetramers may already be modified in H3/H4/H2A/H2B core histones by acetylation and/or methylation in 293 cells, and as such, these lysine residues would no longer be available for acetylation by Rtt109-Vps75 complexes.

As Rtt109-Vps75 complexes exhibited reduced HAT activity toward core histones, of which nucleosomes are composed, we next determined whether Rtt109-Vps75 complexes could acetylate nucleosomal H3. To this end, we purified mononucleosomes as well as mixed populations of mono- and dinucleosomes from 293 cells (supplemental Fig. S4) and used these as substrates for HAT assays. Remarkably, neither native nor
recombinant Rtt109-Vps75 complexes exhibited detectable HAT activity toward H3 present in mononucleosomes or mixed populations of mono- and dinucleosomes (Fig. 2, A, B, D, and E and supplemental Fig. S3A). Because Rtt109-Vps75 complexes could acetylate H3 when recombinant H3/H4 tetramers were mixed with mononucleosomes or mixed populations of mono- and dinucleosomes, the inability of Rtt109 to acetylate H3 when H3 is present as a component of nucleosomes is not likely due to inhibitors co-purified with mononucleosomes or antibodies. The experiments were performed as described above, except that recombinant Rtt109-Vps75 complexes purified from E. coli were used for the HAT assays. The additional band detected by the anti-H3 antibodies in C and F in the context of mononucleosomes and mixed populations of mono- and dinucleosomes most likely represents an H3 degradation product. REGx was used as a negative control. Twenty-five ng of native Rtt109 and 5 ng of recombinant Rtt109-Vps75 complexes were used for each 15-µl HAT assay.

The N-terminal Tail Domains of H3 and H4 Are Required for Efficient Acetylation of H3-Lys-56 by Rtt109—The differences in HAT activities exhibited by Rtt109-Vps75 complexes toward H3, depending on whether H3 was a component of H3/H4 tetramers, core histones, or nucleosomes, suggest that the manner in which H3 is assembled is important for Rtt109-mediated acetylation. Further, specific domains of H3 outside of the core domain may affect acetylation of lysine 56. Indeed, native Rtt109-Vps75 complexes could not acetylate lysine 56 to a detectable degree when presented simply as an H3 peptide comprising residues surrounding lysine 56 (data not shown).

FIGURE 2. Native and recombinant Rtt109-Vps75 complexes do not acetylate nucleosomal H3. A–C, native Rtt109-Vps75 complexes purified from yeast cells acetylate H3, including lysine 56 of H3, in the context of core histones but not when H3 is present as a component of mononucleosomes (Mono) or mixed populations of mono- and dinucleosomes (Mono + DI). Experiments were performed as described in the legend for Fig. 1, except that different histone substrates were used for the HAT assays. A, the HAT activities of native Rtt109-Vps75 complexes using different histone substrates were determined by scintillation counting. B, acetylation of H3 as a component of the indicated histone substrates by native Rtt109-Vps75 complexes was detected by autoradiography, and total proteins were visualized by CBB. C, acetylation of H3-Lys-56 by native Rtt109-Vps75 complexes in the context of the indicated histone substrates was analyzed by Western blot using antibodies against H3 acetylated at lysine (H3-K56Ac). To compare the levels of H3 protein used for the HAT assays, all samples were analyzed by Western blot using antibodies against H3. D–F, recombinant Rtt109-Vps75 complexes do not acetylate H3 present in mononucleosomes or mixed populations of mono- and dinucleosomes. The experiments were performed as described above, except that recombinant Rtt109-Vps75 complexes purified from E. coli were used for the HAT assays. The additional band detected by the anti-H3 antibodies in C and F in the context of mononucleosomes and mixed populations of mono- and dinucleosomes most likely represents an H3 degradation product. REGx was used as a negative control. Twenty-five ng of native Rtt109 and 5 ng of recombinant Rtt109-Vps75 complexes were used for each 15-µl HAT assay.
required for efficient acetylation of H3-Lys-56 by the Rtt109-Vps75 complex.

The N termini of H3 and H4 are far removed from lysine 56 of H3. However, it is possible that acetylation of lysine residues at the N terminus of H3 or H4 is required for H3-Lys-56 acetylation. Thus, we tested whether mutation of five lysine residues at the N terminus of H3 (Lys-9, Lys-14, Lys-18, Lys-23, and Lys-27) or three lysine residues at the N terminus of H4 (Lys-5, Lys-8, and Lys-12) to arginine affected H3-Lys-56 acetylation. As compared with wild-type cells, H3-Lys-56 acetylation was not affected to a detectable degree in either the H3 or the H4 N-terminal lysine-to-arginine mutant cells (supplemental Fig. S6), suggesting that acetylation of these lysine residues is not a prerequisite for H3-Lys-56 acetylation. Therefore, we next tested whether the N-terminal tail domains of H3/H4 tetramers might be required for the binding of Rtt109 with histones using a GST pull-down assay. As shown in Fig. 4A, GST-Rtt109 bound to H3/H4 tetramers, whereas under the same conditions, GST-REGu, a 20 S proteasome activator used as a control (26), did not. Using this assay, we then tested whether deletion of the N-terminal tail domains of H3/H4 affected the association of GST-Rtt109 with H3. As shown in Fig. 4B, GST-Rtt109 pulled down significantly more full-length H3 than tailless H3. Thus, these results together with those above indicate that the N-terminal tail domains of H3/H4 tetramers contribute to the association of Rtt109 with histones, and consequently, the efficient acetylation of H3 by Rtt109-Vps75 complexes.

DISCUSSION

In the present study, we have reconstituted Rtt109-Vps75 complexes using recombinant proteins and established that Vps75 is an integral component of the novel Rtt109-Vps75 HAT complex. Using both recombinant and native Rtt109-Vps75 complexes, we have shown that these HAT complexes acetylate H3 present in H3/H4/H2A/H2B core histones but not H3 present in nucleosomes. These results suggest that regulation of H3-Lys-56 acetylation during the cell cycle is mediated, in part, by the inability of Rtt109-Vps75 complexes to acetylate nucleosomal H3. Furthermore, we have shown that the N-terminal tail domains of H3/H4 tetramers contribute to the binding of Rtt109 to histones and the efficient acetylation of H3-Lys-56 by Rtt109-Vps75 complexes in vitro. Together, these results reveal insights into the substrate requirements for this novel HAT complex.

Rtt109 can acetylate H3-Lys-56 in vitro and in yeast cells in the absence of Vps75. Thus, although Vps75 is a component of Rtt109-Vps75 complexes, it is not required for Rtt109-mediated acetylation of H3-Lys-56. Vps75 is homologous to the histone chaperone nucleosome assembly protein 1 (NAP1) and the human oncoprotein SET, a component of the inhibitor of acetyltransferases (INHAT) complex (data not shown). We envision at least two non-exclusive models to explain the function of Vps75 as a component of the Rtt109 HAT complex. First, Vps75 may function similar to NAP1 by interacting with histones, thus enhancing the binding affinity of Rtt109 for H3 and allowing Rtt109-Vps75 complexes to acetylate H3 more efficiently. Alternatively, Vps75 may function like INHAT. INHAT inhibits the HAT activity of several HATs by binding to histones and preventing a particular HAT from accessing its histone substrates (27). Thus, it is also possible that Vps75 binds to specific lysine residues of H3 and prevents Rtt109 from acetylating these lysine residues, thereby increasing the specificity of Rtt109 toward lysine 56. Future studies will address these two possibilities.

Acetylation of H3-Lys-56 occurs during the S phase of the cell cycle and disappears at G2/M phase (10, 14). However, how this acetylation is regulated during the progression of the cell cycle is not clear. Our studies presented here on the Rtt109-Vps75 complex along with two recent studies on the H3-Lys-56 HDACs Hst3 and Hst4 (15, 16) suggest that there are at least
two ways to regulate H3-Lys-56 acetylation during progression of the cell cycle. First, when H3 is assembled into nucleosomes, acetylation of H3-Lys-56 by Rtt109-Vps75 complexes is inhibited. Lysine 56 of H3 is located in the core domain of H3 at the end of an α-helix and is in close proximity to DNA when H3 is assembled into nucleosomes (2). Thus, it is possible that when H3 is assembled into nucleosomes, lysine 56 of H3 is inaccessible to Rtt109-Vps75 complexes. Alternatively, domains of H3/H4 in addition to those in close proximity to H3-Lys-56, such as their N-terminal tail domains, might be altered in nucleosomes such that the ability of Rtt109-Vps75 complexes to bind H3/H4, and consequently the ability of Rtt109 to acetylate H3-Lys-56, is reduced. Future studies may reveal additional potential mechanisms. Regardless, as Rtt109-Vps75 complexes could not acetylate nucleosomal H3, the presentation of H3 as a component of distinct complexes provides a way for regulating H3-Lys-56 acetylation during the cell cycle. Second, it has been proposed that H3-Lys-56 acetylation is regulated by the protein levels of two H3-Lys-56 HDACs, Hst3 and Hst4, because these two HDACs are highly expressed during G2/M phase of the cell cycle. Thus, the inability of Rtt109-Vps75 complexes to acetylate nucleosomal H3 combined with the high expression levels of Hst3 and Hst4 during G2/M phase of the cell cycle provide a dynamic means for regulating H3-Lys-56 acetylation during the cell cycle.

Recently, Schneider et al. (28) observed that Rtt109 is required for H3-Lys-56 acetylation and that this modification may be involved in transcriptional elongation. In addition, others have also reported that H3-Lys-56 acetylation is detected at active genes (12). However, acetylation of H3-Lys-56 occurs predominantly on newly synthesized histones (10), and indeed, several studies have indicated that the primary function of Rtt109 and H3-Lys-56 acetylation is likely involved in DNA replication and/or the DNA damage response (10, 17, 18, 21). Supporting this idea, we have shown that Rtt109-Vps75 HAT complexes exhibit no detectable activity toward H3 when H3 is assembled into nucleosomes. Together, these results suggest that the localization of H3-Lys-56 acetylation at active genes may reflect the S phase deposition of this modified form of H3 onto chromatin. Alternatively, this modification may be involved in transcription by replacing damaged histones with newly synthesized H3 acetylated at lysine 56. Future studies are needed to address the distinct roles of H3-Lys-56 acetylation mediated by Rtt109 in DNA replication and transcription.

Acknowledgments—We thank Dr. Heather M. Thompson for editing of the manuscript; Dr. Jessica Tyler for providing us with plasmids for the expression of mutant H3/H4 tetramers; and Drs. Song Tan, Sharon Dent, and Mark Parthun for plasmids.

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