Regulation of Histone H3 Lysine 56 Acetylation in Schizosaccharomyces pombe

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In Saccharomyces cerevisiae, acetylation of lysine 56 (Lys-56) in the globular domain of histone H3 plays an important role in response to genotoxic agents that interfere with DNA replication. However, the regulation and biological function of this modification are poorly defined in other eukaryotes. Here we show that Lys-56 acetylation in Schizosaccharomyces pombe occurs transiently during passage through S-phase and is normally removed in G2. Genotoxic agents that cause DNA double strand breaks during replication elicit a delay in deacetylation of histone H3 Lys-56. In addition, mutant cells that cannot acetylate Lys-56 are acutely sensitive to genotoxic agents that block DNA replication. Moreover, we show that Spbc342.06cp, a previously uncharacterized open reading frame, encodes the functional homolog of S. cerevisiae Rtt109, and that this protein acetylates H3 Lys-56 both in vitro and in vivo. Altogether, our results indicate that both the regulation of histone H3 Lys-56 acetylation by its histone acetyltransferase and histone deacetylase and its role in the DNA damage response are conserved among two distantly related yeast model organisms.

Histone acetylation corresponds to the covalent attachment of an acetyl group to a lysine residue. This modification is mediated by histone acetyltransferases (HATs)7 and is reversible as the acetyl group can be removed through the action of histone deacetylases (HDACs). Lysine acetylation on histones is best characterized for its role in transcriptional regulation, but evidence for a crucial function during replication and DNA damage tolerance is accumulating (1–3). Indeed, newly synthesized histones that are deposited throughout the genome during replication are transiently acetylated at several lysine residues (4, 5). These include sites of acetylation in the N-terminal tails of both histones H3 and H4 (6–8). Recently, two novel sites of acetylation in the globular domains of newly synthesized histone molecules were uncovered by mass spectrometry of Saccharomyces cerevisiae histones, lysine 91 (Lys-91) of histone H4 (9) and lysine 56 (Lys-56) of histone H3 (10–15).

H3 Lys-56 acetylation (H3 Lys-56-Ac) shows a particular link between DNA replication and DNA damage tolerance. In budding yeast, this modification occurs concomitantly with DNA replication as virtually all the newly synthesized histone H3 molecules deposited throughout the genome are Lys-56-acetylated, whereas in the G2/M-phase of the cell cycle the vast majority of H3 Lys-56 is deacetylated (11, 15–17). However, in response to DNA breaks during replication, histone H3 Lys-56 acetylation is maintained in a DNA damage checkpoint-dependent manner. In S. cerevisiae this modification plays a key role in surviving DNA damage as cells in which histone H3 cannot be acetylated at Lys-56 are acutely sensitive to genotoxic agents that interfere with DNA replication (10–13).

The deacetylation of H3 Lys-56 requires the Sir2-related HDACs Hst3 and Hst4 (16, 17). Hst3 and Hst4 are cell cycle regulated with peak expression occurring in G2/M and M/G1, respectively. Interestingly, the persistence of H3 Lys-56-Ac in response to damage is dependent upon the DNA damage checkpoint, which represses the transcription of the HST3/HST4 genes thereby maintaining high levels of H3 Lys-56-Ac (11, 17). In cells lacking both Hst3 and Hst4, histone H3 is almost fully acetylated at Lys-56 (98%) during the entire cell cycle. This results in thermosensitivity, severe sensitivity to genotoxic agents, increased levels of mitotic

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The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; H3 Lys-56-Ac, H3 Lys-56 acetylation; WCE, whole-cell extracts; WT, wild-type; Cpt, camptothecin; MMS, methylmethane sulfonate; pol, polymerase.
chromosome loss, and a high incidence of spontaneous DNA damage (16, 17).

Until recently, the HAT responsible for H3 Lys-56 acetylation was unknown leaving a gap in fully understanding the regulation of this mark. This was because of the fact that yeast mutants of enzymes with recognizable HAT motifs are not defective in H3 Lys-56 acetylation (12). Very recently, yeast mutants of enzymes with recognizable HAT motifs are known to be defective in H3 Lys-56 acetylation (18–21). Deletion of Rtt109, a gene previously described as a regulator of Ty1 transposition, results in the absence of H3 Lys-56-Ac (13, 16, 18, 19, 21, 22). Taken together, these data suggest an intimate, but poorly understood, functional relationship between Rtt109, Asf1, and H3 Lys-56-Ac.

Interestingly, histone H3 Lys-56 acetylation has been previously described as a regulator of Ty1 transposition, results in the absence of H3 Lys-56-Ac (13, 16, 18, 19, 21, 22). Taken together, these data suggest an intimate, but poorly understood, functional relationship between Rtt109, Asf1, and H3 Lys-56-Ac.

Interestingly, histone H3 Lys-56 acetylation has been reported in embryo-derived Drosophila S2 cells as well as transcriptionally active polytene chromosomes (14, 20), but paradoxically not in human HeLa cells (12, 14). To determine whether histone H3 Lys-56 acetylation is conserved in other eukaryotes, we investigated the presence and the regulation of this modification in Schizosaccharomyces pombe, which is estimated to have diverged from S. cerevisiae between 320 and 420 million years ago, making fission and budding yeast as distantly related to each other as mammals are to either yeast (23). In this manuscript, we demonstrate that histone H3 Lys-56 acetylation in S. pombe occurs transiently during S-phase, but is maintained in response to genotoxic agents that interfere with DNA replication. We also provide evidence that H3 Lys-56-Ac is catalyzed by a fission yeast orthologue of S. cerevisiae Rtt109 and that the absence of Rtt109 or the H3 K56R mutation confers sensitivity to genotoxic agents. Thus, both the cell cycle and DNA damage regulation of histone H3 Lys-56 acetylation are conserved in S. pombe.

**EXPERIMENTAL PROCEDURES**

**Fission Yeast Strains, Plasmids, Media, and Methods**—The S. pombe strains used in this study are listed in Table 1. Growth, maintenance, and standard genetic methods for fission yeast strains were as described (49). Details about strain construction are available upon request.

**Synchronization Conditions**—Cells exponentially grown in YES medium at 33 °C were synchronized using lactose gradients as described previously (24), except that three consecutive lactose gradient centrifugations were performed. Small G2 cells were resuspended in pre-warmed YES medium at 33 °C at an initial concentration of 2.5 × 10^7 cells/ml.

**Preparation of S. pombe Whole-Cell Extracts (WCE) and Western Blot Analysis**—2 × 10^7 cells were harvested, washed with ice-cold water, and frozen at −80 °C until WCE were prepared. The pellets were thawed on ice, washed in 20% trichloroacetic acid, and resuspended in 200 μl of 20% trichloroacetic acid. After cells were broken with glass beads, 400 μl of 5% trichloroacetic acid was added, and the cell lysate was spun into a new test tube. Following centrifugation at 13,000 rpm for 10 min, the supernatant was discarded, and the pellet was resuspended in 80 μl of loading buffer and heated for 5 min at 95 °C. 10 μl of WCE was separated in SDS-15% polyacrylamide gels and analyzed by Western blotting using the indicated rabbit primary antibodies and a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (Amersham Biosciences). The anti-H3-Lys-56-Ac and anti-γ-H2A antibodies are described previously (11, 16). A rabbit polyclonal antibody against the unmodified histone H3 N-terminal tail was raised against a synthetic peptide (H3N, residues 1–41 of S. cerevisiae histone H3) coupled to maleimide-activated keyhole limpet hemocyanin (Perbio) via an N-terminal cysteine residue. Antibodies against the H3 N-terminal domain were isolated from the crude serum by affinity purification over a SulfoLink column (Perbio) to which the H3N peptide was covalently linked via the N-terminal cysteine. Antibodies were eluted from the H3N peptide column using gentle Ag/Ab elution buffer (Perbio), dialyzed against phosphate-buffered saline and stored at −20 °C in the presence of 10% glycerol.

**HAT Activity Assay**—Experiments were performed as described previously (18). SpRtt109 was amplified by PCR from
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**RESULTS**

*Fission Yeast Histone H3 Is Acetylated at Lys-56*—The amino acid residues flanking Lys-56 in histone H3 are highly conserved (Fig. 1A). Thus, it seemed likely that antibodies raised against Lys-56-acetylated H3 from *S. cerevisiae* might also be used to explore the presence of this modification in other species. In *S. pombe* whole-cell lysates, this antibody detected a single polypeptide in the presence of a 1000-fold molar excess of H3 (52–64) peptides that were either acetylated or not modified at Lys-56. C. WCEs were prepared from WT cells (PB10) or cells containing a single copy of histone H3 gene encoding Lys-56 Arg (SPBX84), or Gln (SPBX85) at residue 56. Western blots were probed with the α H3-Lys-56-Ac or the α N-terminal H3 antibody as a loading control. The asterisk indicates a cross-reacting band.

*S. pombe* genomic DNA and cloned into the N-terminal His$_6$ tagging bacterial expression vector pET28a. Proteins were purified as described (18). Recombinant ScRtt109 and ScAsf1 were kindly provided by the Jackson laboratory. Reactions contained 100 ng of recombinant histone H3 and either 2000 ng of SpRtt109, 600 ng of ScAsf1, 1200 ng of SpRtt109, or a mock control. H3 alone and ScRtt109 plus 800 ng of bovine serum album were also analyzed for controls. Reactions were incubated for 45 min at 30 °C. HAT activity assays were performed as stated in the legend of Fig. 3.

**FIGURE 1. Fission yeast histone H3 is acetylated at lysine 56.** A, sequence alignment of the αN helices of histone H3 derived from *S. cerevisiae* (Sc), *S. pombe* (Sp), *D. melanogaster* (Dm), and *H. sapiens* (Hs). In Drosophila and human cells, Lys-56 is present in both replication-dependent (H3.1) and replication-independent histone H3 (H3.3). B, WCE from WT fission yeast cells (PB10) were analyzed by Western blotting with the α H3-Lys-56-Ac antibody in the presence of a 1000-fold molar excess of histone H3 (52–64) peptides that were either acetylated or not modified at Lys-56. C. WCEs were prepared from WT cells (PB10) or cells containing a single copy of histone H3 gene encoding Lys-56 Arg (FY4640), Arg (SPBX84), or Gln (SPBX85) at residue 56. Western blots were probed with the α H3-Lys-56-Ac or the α N-terminal H3 antibody as a loading control. The asterisk indicates a cross-reacting band.

(Fig. 1A). The fact that our antibody raised against an *S. cerevisiae* peptide readily detects H3 Lys-56 acetylation in *S. pombe* argues that the presence of a tyrosine residue at position 54 does not preclude detection of Lys-56 acetylation in Western blots. Thus, because the sequence flanking Lys-56 is identical in *S. pombe* and human cells, our failure to detect the acetylation in HeLa cells (data not shown) cannot be merely because of the substitution of the *S. cerevisiae* phenylalanine by a tyrosine. Therefore, if H3 Lys-56 acetylation does exist in human cells, it must be present at significantly lower stoichiometry than in *S. pombe* and *S. cerevisiae*. Alternatively, H3 Lys-56 acetylation-specific antibodies may be impeded by modification of adjacent residues or Lys-56 itself. For instance, methylation of H3 Lys-56 has been reported in murine cells (25), and a mono-methylation of histone H3 Arg-52, Arg-53, or Arg-56 has been detected by mass spectrometry in bovine histones (26). However, there was no attempt to prove that this methylation occurred in H3 Lys-56 as opposed to the arginine residues.

Lys-56 Histone H3 Acetylation Is Restricted to S-phase in Normal Cells but Persists in Response to Camptothecin Treatment—Having established its specificity, we used our antibody to determine the regulation of histone H3 Lys-56 acetylation during the cell cycle. A population of small G2 cells was isolated by lactose gradient centrifugation (24). These cells were returned to rich medium at 33 °C, and the seption index was monitored by microscopy to determine when cells entered S-phase (Fig. 2B). A sharp increase in H3 Lys-56 acetylation occurred between 30 and 45 min, concomitant with a rise in the fraction of septated cells (Fig. 2A). This acetylation was transient and subsequently declined between the 90- and 105-min time points, coinciding with the disappearance of septated cells. Progression through S-phase was also monitored using an antibody against histone H2A phosphorylated at Ser-128 (γ-H2A) that, even in untreated cells, detects spontaneous damage during normal passage through S-phase (16, 27). The Lys-56 acetylation signal coincided with a transient γ-H2A signal in untreated cells (Fig. 2, γ-H2A, long exposure). Thus, as observed in *S. cerevisiae* (11, 15), H3 Lys-56 acetylation is confined to S-phase during normal cell cycle progression in *S. pombe*. To induce S-phase damage, we treated cells with camptothecin (Cpt), a cancer therapeutic that functions to stabilize the covalent intermediate between topoisomerase I and DNA, generating single strand breaks that can be transformed into double strand breaks during replication (28). When cells were treated with 15 μM Cpt 30 min after release from G2 (Fig. 2A), Lys-56 acetylation and γ-H2A persisted for at least 120 min, long after these histone modifications had vanished from control cells that were not treated with Cpt (Fig. 2A). This was also observed when cells were synchronized using a *cdc10ts* mutation (29) and treated with the alkylating agent methylmethane sulfonate (MMS), which also induced replicative DNA damage (supplemental Fig. S1). Furthermore, when WT cells were treated with MMS, levels of H3 Lys-56 acetylation were increased (Fig. 3B). Thus, the persistence of H3 Lys-56 acetylation seems to be a physiological response to DNA damage during replication.
markers of S-phase progression. The 27% identity with ScRtt109 (Fig. 3 increased the levels of H3 Lys-56-Ac in WT cells (Fig. 3 mutant, even after MMS-induced DNA damage, which showed loss of H3 Lys-56 acetylation in the showing that this gene was non-essential. Western blot analysis relationship between this gene and H3 Lys-56 acetylation. Tar-

Although work describing the identification of Rtt109 as the Both in Vitro and in Vivo —Until very recently, the HAT responsible for acetylating H3 on Lys-56 was unknown. Although work describing the identification of Rtt109 as the HAT in budding yeast was ongoing (18), we searched the S. pombe genome data base for homologs of ScRtt109. One uncharacterized open reading frame, Spbc342.06cp, showed 27% identity with ScRtt109 (Fig. 3A). We sought to explore the relationship between this gene and H3 Lys-56 acetylation. Targeted gene deletion of Spbc342.06cp resulted in viable cells showing that this gene was non-essential. Western blot analysis showed loss of H3 Lys-56 acetylation in the spbc342.06cpD mutant, even after MMS-induced DNA damage, which increased the levels of H3 Lys-56-Ac in WT cells (Fig. 3B). We also analyzed cells deleted for hst4+, the putative homolog of the budding yeast Sir2-related HDACs HST3 and HST4, which target H3 Lys-56 for deacetylation. Indeed, loss of hst4+ resulted in hyperacetylation of H3 Lys-56-Ac, indicating that Hst4 function was conserved. Interestingly, MMS treatment of hst4Δ cells had little effect on H3 Lys-56-Ac; consistent with the finding that loss of HST3 and HST4 in budding yeast results in nearly complete acetylation of H3 Lys-56 throughout the cell cycle (16, 17).

To establish whether the effect of loss of Spbc342.06cp on H3 Lys-56-Ac was direct, we employed an in vitro HAT activity assay using recombinant Spbc342.06cp protein expressed in E. coli. As a positive control, we used recombinant ScRtt109, which has been shown to acetylate H3 Lys-56 in vitro (18, 19, 21). Like ScRtt109, the Spbc342.06cp protein was able to acet-

we examined the sensitivity of H3-K56R, rtt109Δ, and H3-K56R/rtt109Δ double mutant cells under a variety of DNA damaging conditions (Fig. 4B). The three mutant strains were not sensitive to lesions created by short wavelength ultraviolet light radiation and only mildly sensitive to ionizing radiation, which generated lesions that were mainly repaired during the G2 phase of the cell cycle in S. pombe. In contrast, each single mutant was sensitive to a number of genotoxic agents that cause both single and double strand DNA breaks during replication, including Cpt (data not shown), MMS, and hydroxyurea, an inhibitor of ribonucleotide reductase that stalls DNA replication forks by depleting deoxyribonucleoside triphosphates (30). Interestingly, the H3-K56R/rtt109Δ mutant was no more sensitive than either single mutant, showing that SpRtt109 and H3 Lys-56-Ac function in the same pathway for DNA damage tolerance. However, the rtt109Δ mutant was less sensitive to MMS than the H3-K56R or H3-K56R/rtt109Δ mutants. The simplest explanation is that the K56R mutation is more deleterious for MMS sensitivity than an unacetylated H3 Lys-56, for example by blocking another type of modification. Alternatively, there may be a low level of residual acetylation of H3 Lys-56 that is Rtt109-independent.

We further analyzed the effect of mutants lacking H3 Lys-56-Ac on heterochromatin silencing. In fission yeast, the cen-

Spbc342.06cp Encodes Sprtt109 and Acetylates H3 Lys-56 Both in Vitro and in Vivo—To determine whether S. pombe cells require Lys-56-Ac to survive genotoxic agents,
assay silencing by the color of the colonies (Fig. 4A) (31). WT cells formed red colonies indicating repression of the inserted centromeric ade6 gene. However, H3-K56R, rtt109, and the H3-K56R/rtt109 mutant cells formed colonies of variable pink indicating a slight decrease in silencing at centromeres (Fig. 4A). This might indicate a cross-talk of H3 Lys-56 Ac with the establishment or the maintenance of other activating or repressing histone modifications required for proper centromeric heterochromatin formation.

**DISCUSSION**

In this manuscript, we demonstrate that histone H3 Lys-56 acetylation in *S. pombe* promotes cell survival in response to genotoxic agents that interfere with DNA replication (Fig. 4). Histone H3 Lys-56 acetylation occurs through *de novo* nucleosome assembly during normal passage through S-phase (Fig. 2) (11, 15). In normal cells, the acetylation is transient and removed during the G2 phase of the cell cycle. In both *S. pombe* and *S. cerevisiae*, the period of time in which H3 Lys-56 acetylation can be detected is extended in response to DNA breaks created by camptothecin (Fig. 2) and other DNA damaging agents (Fig. 3B and supplemental Fig. S1) (11, 17). This prolonged delay in deacetylation of H3 Lys-56 is likely to be important for increasing the window of time in which Lys-56 acetylation can perform its function in response to DNA damage. In *S. cerevisiae*, the DNA damage-induced persistence of H3 Lys-56 acetylation is achieved by the DNA damage checkpoint (11, 17). The checkpoint proteins act, at least in part, by preventing the activation of the HST3 and HST4 genes, which encode enzymes that promote deacetylation of H3 Lys-56 (17). This is conserved in fission yeast as deletion of *hst4*, the single gene homologous to *S. cerevisiae* HST3/HST4, results in hyperacetylation of H3 Lys-56 (Fig. 3B). This is consistent with the fact that expression of the *S. pombe hst4* gene suppresses the strong thermosensitive and telomeric silencing phenotypes of *S. cerevisiae* hst3hst4 mutants, which are known to result from hyperacetylation of H3 Lys-56 (16, 17, 32). This suggests that Hst4 promotes deacetylation of H3 Lys-56 in *S. pombe*. Our result showing that Cpt-induced DNA damage leads to a prolonged delay in H3 Lys-56 deacetylation suggests that the role of the DNA
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The discovery of the novel HAT, Rtt109, that acetylates H3 on Lys-56 in budding yeast and now in fission yeast, has completed the description of the enzymatic reactions that can regulate acetylation of H3 Lys-56 (18, 19, 21). Loss of ScRtt109 results in an increase in genomic instability as assayed by persistent Rad52 foci, Rad53 phosphorylation, and an increase in gross chromosomal rearrangements (18, 19). ScRtt109 is involved in surviving replicative DNA damage as rtt109 mutants in *S. cerevisiae* are sensitive to a wide array of DNA damaging agents that induce S-phase specific damage, but surprisingly not ionizing radiation, a treatment that mainly induces DNA breaks outside of S-phase. We also found that Sprtt109Δ mutants were sensitive to replicative stress (Fig. 4B) with the difference that Sprtt109Δ mutants were mildly sensitive to ionizing radiation. This might reflect a difference in cell cycle progression between the two yeasts. Fission yeast cells spend most of their time in G2, whereas budding yeast cells lack a discernable G2 phase of the cell cycle, suggesting that regulation of H3 Lys-56-Ac could be important in G2 in *S. pombe*. We find that H3-K56R and Sprtt109Δ mutants are moderately defective in silencing at pericentromeric heterochromatin, a biological process not found in *S. cerevisiae*. Thus, H3 Lys-56-Ac regulation appears to be involved in heterochromatin formation and/or stability in *S. pombe*. However, H3 Lys-56-Ac and Rtt109 are shown to be associated with some actively transcribed genes in *S. cerevisiae*, suggesting a link with transcriptional regulation (14, 20). Interestingly, RNA polymerase II-dependent transcription is required for initiating RNA interference-dependent silencing at the pericentromeric heterochromatin in *S. pombe* (33). Therefore, action between the two proteins has been found (18). In *S. pombe*, like in vertebrates, Cia1, the homolog of ASFI, is essential for viability (38, 39). Therefore, Cia1 appears to have a crucial function not shared by H3 Lys-56-Ac, as Sprtt109Δ cells are viable. In budding yeast, Asf1 binds the checkpoint kinase Rad53, but this function is not conserved in mammalian cells (39–41). Therefore, *S. pombe* may be a better model system to understand the essential role of Asf1/Cia1. Regardless, the finding that mutations in these genes are epistatic with the unacetylable H3 K56R mutation for surviving DNA damage (13, 18) suggests that the underlying cause of the phenotypes associated with rtt109Δ and asf1Δ is because of the inability of these strains to acetylate H3 at Lys-56.

*H3 K56R* mutant cells are acutely sensitive to several clastogens that, either directly or indirectly, generate DNA into double strand breaks during replication (Fig. 4B) (10–13). This sensitivity could result from defective homologous recombination, which is the major pathway for repairing replicative damage. Homothallic fission yeast cells switch their mating type by making use of a site-specific single-stranded lesion that is transformed into a double strand break during the passage of a unidirectional replication fork. This initiates a gene conversion event that replaces the mating type cassette at the active mating type locus (42–45). Therefore, we analyzed the effect of the absence of H3 Lys-56 acetylation in mating-type switching efficiency in *S. pombe*. Homothallic H3-K56R mutants did not show major defects in mating-type switching (data not shown) implying that H3 Lys-56 acetylation was not required for single gene conversions per se. This is in agreement with a previous finding that H3-K56R and rad52Δ mutations have additive effects on MMS sensitivity suggesting that the genes act in two separate genetic pathways in budding yeast (11). Furthermore, homologous recombination between sister chromatids is not
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severely impaired in asf1 mutants (35, 36, 46). Altogether the accumulated data strongly suggest that H3 Lys-56 acetylation is not required for homologous recombination.

During S-phase, Lys-56-acetylated H3 molecules are deposited behind replication forks throughout the genome (16). The acetylation of the Lys-56 side chain disrupts weak water-mediated contacts between histone H3 and DNA segments at the entry and exit points of the nucleosome core particle (47). Conceivably, this enhanced accessibility of short stretches of nucleosomal DNA could facilitate the action of proteins involved in replisome stabilization and/or the restart of stalled replication forks. Interestingly, several abnormal perturbations of the replisome occur when replication forks are blocked by dNTP depletion with hydroxyurea in S. cerevisiae asf1 mutants that lack H3 Lys-56 acetylation (48). In asf1 mutants, at least three key replication proteins (DNA polymerase ε, proliferating cell nuclear antigen, and RFC) dissociate from hydroxyurea-stalled forks. Conversely, DNA polymerase α abnormally accumulates at stalled forks (48). At least some of these replisome perturbations may lead to irreversible DNA damage and underlie the strong sensitivity of rtt109, asf1, and H3 K56R mutant cells to genotoxic agents that interfere with replication. Through a direct effect on chromatin structure, Lys-56 acetylation could, in principle, promote replication recovery without directly binding any protein. This might help to explain how a genome-wide modification such as H3 Lys-56 acetylation, can be exploited by cells to facilitate the repair of lesions that occur at stochastic sites during replication. However, the first double helical turns of DNA at the entry and exit points of the nucleosome rapidly bind to and dissociate from the histone surface even in the absence of Lys-56 acetylation (49, 50). Thus, it seems equally plausible that the function of H3 Lys-56 acetylation in response to DNA damage during replication might be exerted through the recruitment of effector proteins to sites of damaged replication forks.

Given that the acetylation of H3 Lys-56 by Rtt109, its deacetylation by sirtuin HDACs, and the role of H3 Lys-56 acetylation in the DNA damage response are conserved among two divergent yeast organisms, it is somewhat surprising that the modification has been detected in Drosophila but not in HeLa cells (12, 14). The acetylation of the N-terminal tails of newly synthesized histones is turned over very rapidly in human cells (4, 5). Therefore, the apparent absence of H3 Lys-56 acetylation in human cells could merely reflect technical limitations in detecting the small fraction of total histones that carry the modification. Additionally, because H3 Lys-56-Ac is found in Drosophila (14, 20), there must be an as-yet-undefined HAT responsible for this histone modification. However, Rtt109 from fission yeast may also exist in mammalian cells and that additional H3 Lys-56 acetyltransferases remain to be discovered.

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