Properties of the Type B Histone Acetyltransferase Hat1

H4 TAIL INTERACTION, SITE PREFERENCE, AND INVOLVEMENT IN DNA REPAIR

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The Hat1 histone acetyltransferase catalyzes the acetylation of H4 at lysines 5 and 12, the same sites that are acetylated in newly synthesized histone H4. By performing histone acetyltransferase (HAT) assays on various synthetic H4 N-terminal peptides, we have examined the interactions between Hat1 and the H4 tail domain. It was found that acetylation requires the presence of positively charged amino acids at positions 8 and 16 of H4, positions that are normally occupied by lysine; however, lysine per se is not essential and can be replaced by arginine. In contrast, replacing Lys-8 and -16 of H4 with glutamates reduces acetylation to background levels. Similarly, phosphorylation of Ser-1 of the H4 tail depresses acetylation by both Hat1p and the human HAT-B complex. These results strongly support the model proposed by Ramakrishnan and colleagues for the interaction between Hat1 and the H4 tail (Dutnall, R. N., Tafrov, S. T., Sternglanz, R., and Ramakrishnan, V. (1998) Cell 94, 427–438) and may have implications for the genetic analysis of histone acetylation. It was also found that Lys-12 of H4 is preferentially acetylated by human HAT-B, in further agreement with the proposed model of H4 tail binding. Finally, we have demonstrated that deletion of the hat1 gene from the fission yeast Schizosaccharomyces pombe causes increased sensitivity to the DNA-damaging agent methyl methanesulfonate in the absence of any additional mutations. This is in contrast to results obtained with a Saccharomyces cerevisiae hat1Δ strain, which must also carry mutations of the acetylatable lysines of H3 for heightened methyl methanesulfonate sensitivity to be observed. Thus, although the role of Hat1 in DNA damage repair is evolutionarily conserved, the ability of H3 acetylation to compensate for Hat1 deletion appears to be more variable.

During replication-coupled nucleosome assembly, newly synthesized histones H3 and H4 are deposited onto newly replicated DNA by the chromatin assembly factor CAF-1 (1). H3 and H4 form a heterodimer prior to deposition in both cytosolic and nuclear chromatin assembly complexes (2, 3). Notably, in species as divergent as humans, Drosophila, and Tetrahymena, newly synthesized H4 is acetylated in a conserved pattern at lysines 5 and 12 prior to its association with DNA (the sites are Lys-4 and -11 in Tetrahymena due to a deletion of the usual arginine residue at position 3) (4, 5). Deacetylation of newly synthesized H4 occurs gradually within 30–60 min following nucleosome assembly (6). The function of the acetylation of nascent H4 is currently unknown; however, when deacetylation is inhibited, newly replicated chromatin fails to mature properly (7).

A likely candidate for the enzyme that acetylates newly synthesized H4 is Hat1p, a type B histone acetyltransferase found in virtually all eukaryotic systems examined (8). Hat1 is recovered in cytosolic extracts, but there is evidence that it also resides in the nucleus (9–14). Hat1 specifically acetylates free (non-nucleosomal) H4 in vitro, generating the Lys-5/Lys-12 acetylation pattern of newly synthesized H4 (4, 9, 10, 15–18). In human cells, Hat1 functions as a member of the HAT-B complex, which also contains the small protein p46 (also known as Rbap46) (10). In budding yeast (Saccharomyces cerevisiae) the p46 orthologue is termed Hat2p (11, 16). The nuclear form of the yeast Hat1 complex contains an additional subunit, Hif1p (12, 13).

In S. cerevisiae, Hat1p has been demonstrated to be important for the proper establishment of telomeric silencing and for the repair of damaged DNA; in combination with mutations of the acetylatable lysines of histone H3, deletion of HAT1 causes loss of telomeric silencing and increased sensitivity to the DNA-damaging agent MMS (19, 20). Moreover, it has been shown that Hat1p is recruited to the sites of DNA double-stranded breaks, where it acetylates H4 at Lys-12 (21). It is possible that Hat1p participates in gene silencing and DNA repair by acetylating H4 during localized chromatin assembly.

The structure of Hat1p from S. cerevisiae has been solved, and a model has been proposed for the interaction of the H4 N-terminal domain with the catalytic site (there is as yet no Hat1p:H4 tail co-crystal) (22, 23) (see Fig. 1). The H4 tail is postulated to lie in a shallow channel on the concave face of the enzyme. When Lys-12 of H4, i.e. the lysine that is most readily acetylated by yeast Hat1p, is aligned adjacent to the acetyl-CoA moiety, the lysines at positions 8 and 16 come in close proximity to two acidic regions on the surface of the enzyme (Fig. 1, top). It has therefore been proposed that electrostatic interactions
between Lys-8/Lys-16 and the negatively charged patches serve to hold the H4 tail in place during the acetylation of Lys-12 (22).

In a previous study, we tested this hypothesis by performing in vitro HAT assays using variously acetylated H4 tail peptides. In support of the model, our results demonstrated that prior acetylation of the H4 tail at Lys-8 and -16 dramatically reduced the ability of recombinant yeast Hat1p and native human HAT-B to acetylate Lys-12 in vitro (24).

To further explore the interaction of the H4 N-terminal domain with Hat1, we have extended our analysis to include amino acid substitutions of the H4 tail at lysines 8 and 16. We have previously shown that Hat1 is the only HAT activity detected in HeLa S100 extracts (17). The reaction proceeded for 2.5 min at 37 °C and was terminated by placing the tube for 5 min into a slurry of dry ice in 95% ethanol. H4 was recovered by acid extraction and precipitated using 25% trichloroacetic acid (final concentration); pellets were washed in acidified acetone (0.05 M HCl) and then 100% acetone and dried (17, 25). Triton X-100/acid/urea-PAGE and Western blotting were performed as described previously (17, 27). Antibodies used were as follows: anti-histone H4 acetylated on Lys-5 (1:1000 dilution; Upstate Biotechnology). Antibody specificity was verified by Western blotting of acetylated histones in the presence or absence of competing, variously acetylated, H4 N-terminal peptides; both of the anti-acetylated H4 antibodies strongly recognized Lys-5/Lys-12-diacetylated H4, as determined by Western blotting of histones resolved in Triton X-100/acid/urea gels.

Deletion of the hat1 Gene from S. pombe—S. pombe yeast were routinely cultured on YEA medium (28). The genotypes of all yeast strains used in this study are given in Table 1. The gene encoding the S. pombe Hat1 protein (SPAC139.06; Uniprot accession number Q9UTM7) was deleted using the PCR-based one-step gene replacement technique described by Bahler et al. (29). PCR primers HAT-forward (5′-ATGAGTGCTGTTGA-TGAATGGGTACATAATGCCAATGAATGCATAGAAAT-3′) and HAT-reverse (5′-AAGATTGAGCAAGTTTTT-GGGTTTTTCGAGGGCAATCTTTCCCTTAAGCITGGTG-GCTTTTTGACAGAATTCGAGCTCGTTTAAAC-3′) were used to amplify the kanMX6 selectable marker from plasmid pFA6a-3HA-kanMX6. This PCR product, containing ~60 bp the dephosphorylation of Ser-1 in phosphorylated H4 tail peptides). Okadaic acid did not interfere with HAT activity. Peptide dephosphorylation reactions were performed at 37 °C using 2–6 μg of bacterial alkaline phosphatase (Sigma) per μg of peptide in 20 mM Tris- HCl, pH 8.0. Protocols for performing HAT assays on immobilized peptide substrates are given in detail elsewhere (25).

Acetylation of Recombinant H4, Triton X-100/Acid/Urea Gel Electrophoresis—S100 cytosolic extracts prepared from HeLa cells were immunodepleted of native H4 using antibodies that recognize H4 acetylated at lysines 5 and 12 (the pattern of acetylation found in cytosolic H4) as described previously (17). Treatment was overnight at 4 °C; immunodepletion was monitored by Western blotting. HAT reactions used 100 μl of immunodepleted S100 extract, 1.5 μg of recombinant H4 (Upstate Biotechnology), and 10 μl unlabeled acetyl-CoA. We have previously shown that Hat1 is the only HAT activity detected in HeLa S100 extracts (17). The reaction proceeded for 2.5 min at 37 °C and was terminated by placing the tube for 5 min into a slurry of dry ice in 95% ethanol. H4 was recovered by acid extraction and precipitated using 25% trichloroacetic acid (final concentration); pellets were washed in acidified acetone (0.05 M HCl) and then 100% acetone and dried (17, 25). Triton X-100/acid/urea-PAGE and Western blotting were performed as described previously (17, 27). Antibodies used were as follows: anti-histone H4 acetylated on Lys-5 (1:1000 dilution; Serotec); anti-total H4 (1:500 dilution; Cell Signaling Technology); and anti-histone H4 acetylated on Lys-12 (1:1000 dilution; Upstate Biotechnology). Antibody specificity was verified by Western blotting of acetylated histones in the presence or absence of competing, variously acetylated, H4 N-terminal peptides; both of the anti-acetylated H4 antibodies strongly recognized Lys-5/Lys-12-diacetylated H4, as determined by Western blotting of histones resolved in Triton X-100/acid/urea gels.

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**TABLE 1**

| Strain | Genotype | Reference or Source |
|--------|----------|---------------------|
| 975    | h⁺       | 48                  |
| FWP2   | h⁺ leu1–32 ade6-M210 | C. Hoffman |
| FWP6   | h⁺ leu1–32       | C. Hoffman |
| FWP39  | h⁺ ade6-M216    | C. Hoffman |
| LBP6   | h⁺ hat1Δ::kan   | This study         |
| LBP7   | h⁺ hat1Δ::kan   | This study         |
| LBP8   | h⁺ leu1–32 hat1Δ::kan | This study |

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**FIGURE 1. Proposed model for binding of the H4 tail to yeast Hat1p.** The histone H4 tail is proposed to lie in a shallow channel on the Hat1p concave face (22, 23). Top, when Lys-12 is aligned adjacent to the acetyl-CoA moiety, Lys-8 and -16 are positioned to interact with acidic patches on the enzyme surface. Bottom, when Lys-5 is aligned next to acetyl-CoA, Arg-3 can potentially interact with the acidic patch as shown. In this case, Ser-1 is brought close to the same acidic region. (After Dutnall et al. (Fig. 4 in Ref. 23); copyright Cold Spring Harbor Laboratory Press; used by permission.)
of sequence homology flanking the hat1+ gene, was used to transform a diploid strain (constructed by mating FWP2 and FWP39 and selecting for Ade+ diploid cells due to intragenic complementation of the ade6-M210 and ade6-M216 alleles) to G418 resistance. Deletion of the hat1+ gene was confirmed by PCR. Tetrad dissection of the diploid transformant resulted in a 2:2 pattern of G418 resistant to G418-sensitive progeny, indicating that the hat1Δ deletion progeny are viable. Diagnostic PCR of the progeny revealed that the PCR product associated with the hat1Δ deletion co-segregates with G418 resistance. The hat1+ gene was subsequently deleted from strain 975 (h/H11001) by homologous recombination using a PCR product containing the hat1::kan locus amplified from one of the G418-resistant progeny to create strains LBP6 and LBP7. This gene deletion was also confirmed by PCR analysis.

The analysis of the MMS sensitivity of wild-type and mutant yeast was performed as previously described (20). Sensitivity to UV light was tested at 80 and 120 joules, using a UVC 500 UV cross-linker (GE Healthcare).

RESULTS

Acetylation by Hat1 Requires Positively Charged Amino Acids at Positions 8 and 16 of the H4 Tail—In a previous study, we determined that prior acetylation of H4 tail peptides at Lys-8 and/or Lys-16 significantly reduced the ability of both the human HAT-B complex and recombinant yHat1p to acetylate lysines 5 and 12 of H4 (24). This was interpreted as supporting the model of the Hat1-H4 tail interaction proposed by Dutnall et al. (22); however, questions still remained concerning the requirement for (unacetylated) lysine at positions 8 and 16. One possibility was that a positive charge was itself sufficient and not lysine per se. Alternatively, it could be that lysine was uniquely required at these sites for Hat1 to recognize and acetylate the H4 tail. To test these alternatives, H4 tail peptides, containing either glutamine (to mimic acetylation) or arginine (to provide a nonspecific positive charge), were synthesized and tested in HAT assays using recombinant yHat1p. (It is important to emphasize that Lys-5 and Lys-12, the substrate lysines of Hat1, are unaltered in these experiments and thus remain potentially acetylatable.) The results are presented in Fig. 2A.

Consistent with our previous results, substituting glutamine for lysine at Lys-8 and Lys-16 dramatically reduced the ability of yHat1p to acetylate the H4 tail peptide (Fig. 2, A and B). This confirms the importance of lysine and/or a positive charge at Lys-8 and Lys-16 for acetylation to occur. Surprisingly, substituting arginine at positions 8 and 16 resulted in acetylation as great or greater than that observed when the normal lysine residues were present at these sites (Fig. 2, A and B). Similar
results were obtained for the native HeLa HAT-B complex (Fig. 2C). These results provide additional support for the proposed model of H4 tail binding and help further refine our understanding of the Hat1-H4 tail interaction. Clearly lysine is not uniquely required at positions 8 and 16 for Hat1 to recognize and acetylate the H4 tail. Nevertheless, it is of considerable importance to retain positive charges at these sites.

Involvement of Ser-1 in Acetylation of the H4 Tail by Hat1—In an early study of post-translational modifications of newly synthesized histones, it was suggested that new H4 may be phosphorylated at the N-terminal serine residue (30). It has also been reported that the NuA4 histone acetyltransferase is negatively regulated by phosphorylation of H4 at Ser-1 (31). It was therefore of interest to examine the effect of phosphorylation of H4 at Ser-1 on HAT-B activity. HAT assays were performed on an H4 N-terminal peptide phosphorylated at Ser-1 (Fig. 3A, S1P) (see supplemental Fig. S1 for a time course curve). Consistent with the findings of Utley et al. (31) concerning NuA4, an H4 peptide phosphorylated at Ser-1 was a relatively poor substrate for human HAT-B. Dephosphorylation of the S1P peptide with alkaline phosphatase restored acetylation (Fig. 3A), whereas treatment with bovine serum albumin had no effect (data not shown).

To determine whether phosphorylation of Ser-1 would affect the acetylation of Lys-5 or Lys-12 differently, additional HAT-B assays were performed on dimodified H4 peptides that were phosphorylated at Ser-1 and also acetylated at either Lys-5 or Lys-12 (Fig. 3B). As expected and in agreement with our previous results (24), prior acetylation at Lys-5 or Lys-12 reduced the incorporation of [3H]acetate by ≥50% (note that in these cases one of the Lys-5/Lys-12 substrate sites is already acetylated). Notably, phosphorylation of Ser-1 further reduced the acetylation of H4 peptides acetylated at Lys-5 or Lys-12 (Fig. 3B). Indeed, phosphorylation of a peptide already acetylated at Lys-12 reduced further acetylation by HeLa HAT-B to background levels, indicating that acetylation at Lys-5 was essentially eliminated under these conditions. Identical results were obtained with yeast Hat1p, although in this case acetylation of the Lys-12ac peptide was itself much reduced relative to acetylation by the human enzyme (a reflection for the strong preference of yHat1p for this site (Fig. 3C) (16, 26). To better visualize the effect of phosphorylation of the H4 tail on yHat1p activity, the results of Fig. 3C were replotted by setting acetate incorporation into the Lys-5ac and Lys-12ac peptides at 100% (Fig. 3D). From this it can be seen that phosphorylation of S1P dramatically reduced acetylation of the H4 tail by yHat1p at both substrate sites.

Acetylation of Immobilized Peptide Substrates—It remained formally possible that the observed reduction in acetylation of the Lys→Gln and S1P peptides was caused by a failure of these altered substrates to adhere properly to filters in preparation for scintillation counting. To eliminate this potential artifact, HAT assays were also performed on peptide substrates that were immobilized on agarose beads, as we have previously described (25). Immobilized peptides yielded results that were in full agreement with those obtained from conventional HAT assays (supplemental Fig. S2). It is therefore concluded that lysines 8 and 16 (and potentially Ser-1) of the H4 N-terminal domain can modulate the Hat1-H4 tail interaction.

Order of Acetylation of H4 by Hat1—The human HAT-B complex acetylates lysines 5 and 12 of H4 exclusively (10, 17). To determine the order of acetylation of these sites, recombinant H4 was briefly incubated with HeLa HAT-B in vitro in the presence of unlabeled acetyl-CoA, and the reaction was rapidly quenched in a dry ice bath. The products were then separated in a Triton X-100/acid/urea gel (to resolve acetylated isoforms) and analyzed by Western blotting using antibodies that recognize H4 acetylated at either Lys-5 or Lys-12 (Fig. 4).

Lys-12 is the preferred substrate of human HAT-B. When approximately half of newly acetylated H4 was still monoacetylated at Lys-12 (Fig. 4, lane 7), acetylation at Lys-5 was observed predominantly in the diacetylated isoform (lane 3). Thus, Lys-12 is typically acetylated first by HeLa HAT-B followed by
Substrate Interaction and in Vivo Function of Hat1

![Image](image_url)

**FIGURE 4. Substrate preference of HeLa HAT-B.** Recombinant H4 was incubated with HeLa HAT-B extract in the presence of unlabeled acetyl-CoA (see “Experimental Procedures” for details). Reaction products were resolved by Triton X-100/acid/urea-PAGE and analyzed by Western blotting using antibodies that recognize total H4 (α-H4), H4 acetylated at Lys-5 (α-H4 K5ac), or H4 acetylated at Lys-12 (α-H4 K12ac). Lanes 1 and 6 contain HAT-B extract alone; lanes 2, 4, and 5 contain recombinant H4 alone; lanes 3 and 7 contain H4 after acetylation by HAT-B (equal loads of the same reaction). Lane M, acetylated marker histones from butyrate-treated HeLa cells. The positions of un-, mono-, and diacetylated H4 (0, 1, and 2, respectively) are indicated.

acetylation at Lys-5 to generate the diacetylated product. Scans of the Western blot indicated that there was a ~3-fold preference by HAT-B for Lys-12 over Lys-5, in agreement with a previous analysis of maize HAT-B (18), and consistent with the strong preference for Lys-12 by the yeast enzyme (16, 26).

**MMS Sensitivity of hat1Δ S. pombe Yeast—Deletion of the HAT1 gene from S. cerevisiae has no effect on cell growth under normal conditions (16, 26, 32).** However, when combined with specific mutations of the acetylatable lysines of histone H3, deletion of HAT1 in S. cerevisiae results in heightened sensitivity to the DNA-damaging agent MMS (20). To determine whether this phenotype was evolutionarily conserved, we deleted the hat1 gene from the fission yeast S. pombe. Wild-type and hat1Δ fission yeast (two independent strains of the latter) were then examined for MMS sensitivity. As expected, deleting hat1 from S. pombe had no effect on cell growth or doubling time (Fig. 5). Interestingly, hat1Δ S. pombe cells exhibited increased sensitivity to MMS in the absence of concomitant mutations of histone H3; moreover, introducing hat1 into hat1Δ cells restored MMS resistance (supplemental Fig. S3). It is therefore concluded that, in contrast to the case in budding yeast, Hat1 in S. pombe plays a role in DNA repair that is not fully redundant with the acetylation of histone H3. However, hat1Δ S. pombe cells did not have increased sensitivity to UV irradiation (similar to hat1Δ S. cerevisiae (20)) or to 10 mM hydroxyurea (data not shown).

**DISCUSSION**

In the original description of the crystal structure of yeast Hat1p, it was postulated that Lys-8 and Lys-16 of the H4 tail could engage in electrostatic interactions with two negatively charged regions adjacent to the catalytic site (Fig. 1) (22, 23). In a previous study, we initiated an examination of this hypothesis, by using H4 peptides acetylated at Lys-8 and/or Lys-16 as substrates in Hat1 assays (24). It was found that prior acetylation of Lys-8 or Lys-16 significantly reduced acetylation of Lys-5 and Lys-12 of H4 by recombinant yeast Hat1p or the human HAT-B complex and that Lys-8/Lys-16 diacetylation reduced enzyme activity to background levels (24). However, it remained unclear if this was indicative of a specific requirement for (unacetylated) lysine at these sites or of a more general need for positively charged amino acids. The present study resolves this question. By using peptide substrates in which Lys-8 and Lys-16 were altered to either arginine or glutamine, we have now determined that positively charged residues at positions 8 and 16 greatly stimulate acetylation of the H4 tail by Hat1; however, lysine is not a specific requirement. The ability of arginine to successfully substitute for lysine strongly supports the proposed alignment of the H4 N-terminal domain in the catalytic site, particularly regarding the postulated electrostatic interactions between the enzyme surface and the H4 tail (22, 23).

In our previous study (24), it was found that abrogating the positive charge of either Lys-8 or Lys-16 by monoacetylation of an H4 peptide significantly reduced (but did not eliminate) acetylation of the H4 tail by yeast Hat1p or HeLa HAT-B. Thus, both Lys-8 and Lys-16 are involved in Hat1-substrate recognition, as predicted by the model in Fig. 1; however, neither site is uniquely required. In S. cerevisiae, simultaneously mutating Lys-8 and Lys-16 to glutamine increases cell doubling time and the proportion of cells in G2/M phase (33), consistent with the effects of changing all four acetylatable lysines of the H4 tail to glutamine or alanine (34–37). Despite the slowed growth rates, clearly nucleosome assembly can occur in these mutants. However, in yeast strains lacking the H3 N-terminal domain, simultaneously altering Lys-5, -8, and -12 of H4 to glycine leads to cell death and defects in chromatin assembly (38).

When an H4 peptide was already acetylated at Lys-12, phosphorylation at Ser-1 virtually eliminated the ability of both yHat1p and HeLa HAT-B to acetylate Lys-5 (the remaining substrate lysine in H4). Dutnall et al. (22, 23) have proposed that Arg-3 of H4 engages in electrostatic interactions with an acidic patch on the enzyme surface when Lys-5 is aligned adjacent to acetyl-CoA (Fig. 1, bottom). Our results are fully consistent with this hypothesis. It seems reasonable to suggest that the phosphate group on phos-Ser-1 is repulsed by local negative charges, thereby preventing proper seating of the H4 peptide. A similar mechanism may explain the inhibitory effect of Ser-1 phosphorylation on acetylation of H4 by Esal/NuA4 (31); Esal (the HAT subunit of the NuA4 complex) and Hat1 share a structurally conserved core domain at the catalytic site (39). In synchronized HeLa cells, phos-Ser-H4 can be detected in nuclei during S phase (40), but there is as yet no evidence that human H4 is phosphorylated prior to deposition (to potentially regulate Hat1 activity).

The relative preference for Lys-12 over Lys-5 as a substrate now appears to be a general feature of Hat1 enzymes (18). This selectivity is particularly evident with S. cerevisiae Hat1p, which
acetylates Lys-5 very poorly in vitro and possibly not at all in vivo (16, 26). In contrast, we have previously demonstrated that the native human HAT-B complex acetylates both Lys-5 and Lys-12 (17), as does the human Hat1 subunit in vitro (10). The initial preference for Lys-12 is likely a consequence of the better fit of the H4 tail in the active site when Lys-12 is aligned adjacent to acetyl-CoA (Fig. 1). The finding that newly synthesized H4 in human cells is diacylated at Lys-5 and Lys-12 provides indirect evidence that HAT-B acetylates both sites in vivo (5).

Our results also bear upon genetic analyses of site-specific histone acetylation. An elegant approach to uncovering the consequences of acetylating specific histone lysines in S. cerevisiae has involved replacing these residues with either arginine (to preclude acetylation, and thus maintain constant positive charge), or with uncharged residues such as glycine and glutamine (to mimic continuous acetylation) (reviewed in Ref. 8). Such experiments have provided considerable insight into the function of acetylation in mating, transcription, and cell growth. An underlying assumption in these studies has been that amino acid substitutions will affect modifications predominantly (if not exclusively) at the altered sites. However, it is now clear that this assumption may not be valid in all cases and that certain lysine substitutions can directly influence the modification of other lysines in the same histone tail. Similarly, it has been shown that specific histone modifications can themselves be regulated by the acetylation, methylation, or phosphorylation of neighboring amino acids (24, 31, 41, 42) (discussed in Refs. 43 and 44) and that full acetylation of all four acetylatable lysines of the H4 tail requires Gcn5 (33), although H4 does not appear to be a target of Gcn5 in vivo. The interdependence of post-translational modifications likely provides responsiveness and flexibility to histone epigenetic signals (44).

Deletion of the hat1 gene in the fission yeast S. pombe results in an increased sensitivity to MMS (this study). Curiously, in the budding yeast S. cerevisiae, MMS sensitivity is only observed when the HAT1 deletion is combined with selected mutations of the acetylatable lysines of histone H3 (specifically, the simultaneous alteration of Lys-9, -18, and -27 to arginine) (20, 21). Thus, the functional redundancy between H3 acetylation and Hat1 observed in S. cerevisiae is absent in S. pombe. In budding yeast, loss of the histone chaperone Asf1 also causes DNA damage and sensitivity to MMS (20, 45) and there is genetic evidence that Hat1 and Asf1 may function in the same DNA double-stranded break repair pathway (20). It would therefore seem reasonable to propose that Hat1 facilitates DNA double-stranded break repair by acetylating H4 during histone deposition.

As the present report was being prepared for publication, Barman et al. (46) reported that HAT1−/− DT40 (chicken) cells also exhibit increased sensitivity to MMS in the absence of mutations of H3. These results indicate that the involvement of type B histone acetyltransferases in DNA damage repair is evolutionarily conserved. However, it is not clear why S. pombe and vertebrate cells should differ from S. cerevisiae with respect to the redundancy of Hat1 function and H3 acetylation. One possibility is that the requirement for deposition-related acetylation of H3 differs in the two yeast systems. Alternatively, compensatory chromatin assembly pathways may be more redundant in S. cerevisiae than in other model eukaryotes. In future experiments, we plan to further explore the relationships among histone modifications, chromatin assembly, and DNA repair in fission yeast.

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