The Hat1 histone acetyltransferase has been implicated in the acetylation of histone H4 during chromatin assembly. In this study, we have characterized the Hat1 complex from the fission yeast *Schizosaccharomyces pombe* and have examined its role in telomeric silencing. Hat1 is found associated with the RbAp46 homologue Mis16, an essential protein. The Hat1 complex acetylates lysines 5 and 12 of histone H4, the sites that are acetylated in newly synthesized H4 in a wide range of eukaryotes. Deletion of *hat1* in *S. pombe* is itself sufficient to cause the loss of silencing at telomeres. This is in contrast to results obtained with an *S. cerevisiae hat1*Δ strain, which must also carry mutations of specific acetylable lysines in the H3 tail domain for loss of telomeric silencing to occur. Notably, deletion of *hat1* from *S. pombe* resulted in an increase of acetylation of histone H4 in subtelomeric chromatin, concomitant with derepression of this region. A similar loss of telomeric silencing was also observed after growing cells in the presence of the deacetylase inhibitor trichostatin A. However, deleting *hat1* did not cause loss of silencing at centromeres or the silent mating type locus. These results point to a direct link between Hat1, H4 acetylation, and the establishment of repressed telomeric chromatin in fission yeast.

During nucleosome assembly, newly synthesized H4 is acetylated prior to its deposition onto DNA (2, 40, 71, 83). In humans, *Drosophila*, and *Tetrahymena*, the acetylation of new H4 takes place in a conserved pattern, at lysines 5 and 12 (the sites are K4 and K11 in *Tetrahymena*, due to a deletion of the usual arginine residue at position 3) (24, 76). Deacetylation of new H4 occurs over the next 30 to 60 min (40, 73) and is required for proper chromatin maturation (6). The acetylation of new H4 may facilitate the import of H3/H4 dimers into the nucleus (5, 15, 21, 26, 31, 87). Moreover, recent studies using *Physarum* as a model system have provided evidence that the K5/K12 diacyetlation of H4 is required for efficient nucleosome assembly in that system (26). It therefore seems likely that the rigorous conservation of the diacetylation of nascent H4 reflects an important role in the import/assembly process.

The most likely candidate for the enzyme that acetylates newly synthesized H4 is Hat1 (Kat1), a type B histone acetyltransferase (HAT) (7, 60). Hat1 acetylates free H4 at lysines 5 and 12 in vitro, consistent with the acetylation pattern of new H4 (22, 24, 46, 64, 68, 75, 87). In many organisms Hat1 is associated with p46 (termed Hat2p in *Saccharomyces cerevisiae*), which stimulates its enzymatic activity (59, 72, 82). Although it was long thought of as predominantly a cytoplasmic enzyme, it is now clear that Hat1 is also present in nuclei (1, 51, 63, 68, 72, 82). In *S. cerevisiae*, the nuclear Hat1 complex also contains the protein Hif1p (1, 63).

Several lines of evidence have indicated that Hat1 is involved in DNA damage repair (12, 14, 66) and can be recruited to the sites of DNA double-strand breaks (67). Moreover, in combination with mutations of the acetylable lysines of histone H3, deletion of *HAT1* causes loss of telomeric silencing in budding yeast (44, 63). However, deletion of *HAT1* without mutating H3 has no effect on either silencing or DNA repair in that system (44, 66). This suggests that the acetylation of H3 and H4 can act redundantly during silencing and repair, possibly during chromatin assembly.

Although they are both unicellular organisms classified as fungi, *S. cerevisiae* and *Schizosaccharomyces pombe* are estimated to be separated by approximately one billion years of evolution (39). Significant differences between them include the manner of cell division (budding as opposed to fission), the structure of centromeres (in *S. cerevisiae* the kinetochore consists of a single nucleosome; in *S. pombe*, centromeres are much more mammalian-like and are up to 100 kb long), and the presence of introns in ~45% of *S. pombe* genes (~5% of the genes in *S. cerevisiae* have introns [85]). In addition, the yeasts differ dramatically in silencing mechanisms. Unlike *S. pombe*, *S. cerevisiae* does not exhibit the methylation of histone H3 at K9 (or use RNA interference [RNAi] or an Swi6/HP1 homologue) for silencing at telomeres, centromeres, or the mating type loci. *S. pombe* uses all these methods at all these sites (19, 20). In light of these differences and of their evolutionary separation, it is not necessarily expected that budding and fission yeasts share a requirement for Hat1 to effect telomeric silencing.

In previous work we have shown that, in contrast to results obtained for *S. cerevisiae*, deletion of *hat1* in the fission yeast *S. pombe* causes heightened sensitivity to DNA damage in the absence of concurrent mutations of histone H3 (14). To further explore possible differences in Hat1 function between these two evolutionarily distant fungal systems, we have purified and analyzed the Hat1 complex from fission yeast. We find that Hat1 is associated with Mis16 (an orthologue of RbAp46 and Hat2p), and we confirm that, unlike Hat2 in budding yeast, *mis16* is an essential gene (38). As with most eukaryotes, the *S. pombe* Hat1 complex acetylates lysines 5 and 12 of histone H4. However, deletion

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**Schizosaccharomyces pombe** Hat1 (Kat1) Is Associated with Mis16 and Is Required for Telomeric Silencing

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of hat1 in and of itself causes loss of telomeric silencing in *S. pombe*, without concurrent mutations of the H3 N-terminal domain. Loss of Hat1 did not reduce silencing at centromeres or the mating type locus. Surprisingly, deletion of *hat1* caused an increase in the acetylation of H4 at telomeres, rather than the reverse. Our results demonstrate that Hat1 is essential for the establishment of telomeric silencing and organization in fission yeast, and they suggest that the proper diacetylation of H4 during chromatin assembly is required to foster the generation of heterochromatin at telomeres in *S. pombe*.

**MATERIALS AND METHODS**

**S. pombe strain construction.** *S. pombe* was cultured and maintained in YEA medium (37). The genotypes of the strains are listed in Table 1. The PCR and DNA sequencing.

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| 975    | h*, wild type | 50                  |
| 972    | h*, wild type | 50                  |
| FY336  | h- ade6-210 leu1-32 ura4-DS/E TM1::ura4 | 3                   |
| FY496  | h- ade6-210 leu1-32 ura4-DS/E imr1L (dg-glu) Ncold::ura4 oriL | 3                   |
| FY648  | h- ade6-210 leu1-32 ura4-DS/E otr1R (dg-glu BamHI-Spel fragment) Sphl::ura4 | 3                   |
| FYW72  | h*- ade6-210 leu1-32 ura4-DS/E orfRSpht::ades6 TEL2L-ura4 | 54                  |
| mis16-myc | h- leu1 orfRspht::mis16-myc [ura4*] | 38                  |
| FW093  | h- ura4::bpfl-lacZ leu1-32 ade6-210 | C. Hoffman          |
| LPB6   | h+ hat1D::kan | 14                  |
| KTP7   | h+/h+ mis16'/mis16::kan ade6-M216/ade6-M210 leu1+/leu1-32 | This study          |
| KTP22  | h+ ura4 hat1-4sPACPTAP | This study          |
| KTP25  | h+ hat1D::kan ade6-210-32 ura4::bpfl-lacZ | This study          |
| KTP29  | h+ hat1D::kan ade6-210-32 ura4::DS/E imr1L (dg-glu) Ncold::ura4 oriL | This study          |
| KTP30  | h+ hat1D::kan ade6-210-32 ura4::DS/E otr1R (dg-glu BamHI-Spel fragment) Sphl::ura4 | This study          |
| KTP33  | h+ hat1D::kan ade6-210-32 ura4::DS/E TM1::ura4 | This study          |
| KTP35  | h+ hat1D::kan ade6-210-32 ura4::DS/E tRNA Phe-otr1L (Xhol-BamHI fragment) HpaI::ura4 | This study          |
| KTP36  | h*+ hat1D::kan ade6-210-32 ura4::DS/E otr1 Rspht::ades6 TEL2L-ura4 12C | This study          |
| KTP40  | h+ hat1-kaPACTAP mis16-myc | This study          |
| CHI070  | h*+ ura4::DS/E leu1-32 ade6-M210 kinte:ura4+ hat1D::kan | This study          |
| CHI0709 | h*+ ura4::DS/E leu1-32 ade6-M210 kinte:ura4+ | This study          |

**Tandem affinity purification.** Tandem affinity purification of the Hat1 complex was performed using published protocols (65, 69).

**Mass spectrometry.** Protein samples were subjected to electrophoresis through approximately 2 cm of a 10% SDS-polyacrylamide gel. Lanes were excised above the dye front and fixed for 30 min with a solution containing 50% methanol and 5% acetic acid. Gel fragments were washed with distilled water and analyzed using an LCQ Deca ion trap mass spectrometer (Tapalin Biological Mass Spectrometry Facility at Harvard University).

**MMS assays.** The analysis of the sensitivities of wild-type and mutant *S. pombe* strains was performed as previously described (66). Five-fold serial dilutions were made and spotted on Edinburgh minimal medium (EMM) plates (57) containing 0.01% methyl methanesulfonate (MMS) (Sigma-Aldrich). Plates were incubated for 3 to 4 days at 30°C.

**Gel electrophoresis and immunoblotting.** To separate the subunits of the Hat1 complex, purified extracts were subjected to SDS-PAGE in 10% to 12.5% polyacrylamide gels. Immunoblotting was performed according to the Western-Star system (Applied Biosystems). Anti-c-myc (sc-40; Santa Cruz Biotechnology) was diluted 1:500 in blocking buffer; secondary antibodies conjugated to alkaline phosphatase were diluted 1:5,000.

**HAT assays.** In *vitro* histone acetyltransferase (HAT) filter binding assays using H4 peptides were performed as previously described (14). For the acetylation of H4 peptides and recombinant H4, Hat1p was affinity purified (tobacco etch virus [TEV] protease eluate) from KTP1. For 100-nl reaction mixtures, the following were combined: 100 mM sodium butyrate (pH 7.2) to a 5 mM concentration, 10-mg/ml acetylated bovine serum albumin (GE Healthcare) to a 1-mg/ml concentration, 0.5 μg re-

**Table 1 S. pombe strains**

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5-fold serial dilutions of the suspensions were made and incubated for 2 to 4 days at 30°C. Cells were grown for 2 to 3 days. To test the effect of histone hyperacetylation on telomeric silencing, 3-mI cultures were seeded with 6.2 × 10⁶ cells/ml in 50 ml of YEL. The culture was fixed with 3% paraformaldehyde for 2 to 4 days at 30°C (27). Cells were grown for ~44 h (~3 doublings in TSA; methanol-treated cells were reseeded if overgrown). Cells were then counted and resuspended at 2 × 10⁵ cells/ml in the absence of TSA, and then 2.5- to 5-fold serial dilutions of the suspensions were made and 5 µl of each dilution was spotted onto one of the following: YEA, YEA containing 0.04% 5-FOA, EMGG, or EMGG-ura plates (57). Plates were incubated at 30°C for 2 to 3 days. To test the effect of histone hyperacetylation on telomeric silencing, 3-mI cultures were seeded with 6.2 × 10⁵ cells/ml treated with 50 μg/ml trichostatin A (TSA) (Wako) or methanol (vehicle) in YEL at 30°C (27). Cells were grown for ~44 h (~3 doublings in TSA; methanol-treated cells were reseeded if overgrown). Cells were then counted and resuspended at 2 × 10⁵ cells/ml in the absence of TSA, and then 2.5- to 5-fold serial dilutions of the suspensions were made and 5 µl of each dilution was spotted onto EMMG (minimal glutamate), EMGG-ura, and 5-FOA (1 g/liter) plates, all in the absence of TSA. Plates were incubated for 2 to 4 days at 30°C.

Chromatin immunoprecipitation (ChIP). Cells were grown to 1 × 10⁸ cells/ml in 50 ml of YEL. The culture was fixed with 3% paraformaldehyde for 30 min at 30°C with gentle shaking. Glycine was added to a 0.125 M concentration. After centrifugation for 5 minutes at 3,000 rpm, cells were washed three times with 1 ml ice-cold phosphate-buffered saline (PBS) containing 50 mM sodium butyrate. Pelleted cells were resuspended in 50 mM Tris (pH 8.0)–1% SDS and treated with proteinase K for 2 hours at 37°C. The input fraction was extracted twice with phenol-chloroform, precipitated, adjusted to 50 mM Tris (pH 8.0), 10 mM EDTA, and 1% SDS, and then acetone. Protein pellets were vacuum dried, resuspended in 20 μl Tris-EDTA (TE). Immunoprecipitations, were performed with either anti-acH4 K12 (Millipore), anti-acH4 K5/12, or anti-acH4 K8/16 antibodies (13), using protein A-Sepharose beads. Rabbit IgG (Bethyl Laboratories) and anti-rabbit immunoprotein were used for control immunoprecipitations. Input fractions were removed from the lysate prior to immunoprecipitation, adjusted to 50 mM Tris (pH 8.0), 10 mM EDTA, and 1% SDS, incubated overnight at 65°C, cooled to room temperature, and then precipitated with ethanol and vacuum dried. The pellet was resuspended in 50 mM Tris (pH 8.0)–1% SDS and treated with proteasome K for 2 h at 37°C. The input fraction was extracted twice with phenol-chloroform, precipitated, and resuspended in 20 μl Tris-EDTA (TE). Immunoprecipitated chromatin beads (bound fraction) were washed and DNA extracted as described above.

Real-time PCR. To amplify immunoprecipitated ura4 DNA sequence, real-time PCR was performed using primers Ura4Chip-F (5'-GAACGGCC TCAAAGAGTGGT-3') and Ura4Chip-R (5'-GATGATATGCTTAC GCAG-3'). To amplify immunoprecipitated fus1 DNA sequence, real-time PCR was performed using primers Fus1F (5’-AGAGCACAACCCC GTCC-3’) and Fus1R (5’-TTTGGCTAGTGGATCTCGGC-3’).

Analysis of real-time PCR data was performed according to published methods (58). Triplicate threshold cycle (CT) values for input and bound fractions were averaged from chromatin immunoprecipitations (ChIPs) using antisense or nonimmune serum. Average net CT values for ura4 and fus1 primers were calculated by subtracting the average input CT from the average bound CT for the two primers. For each individual primer, the average net CT for FY1872 was subtracted from the average net CT of KTP36 to give the average net CT difference between the hat1Δ and wild-type strains. The negative of this value was used as an exponent for the base 1.9 to calculate the relative level of immunoprecipitated acetylated histones at the ura4 or fus1 DNA sequence. The calculations are summarized by the following equations: average net CTura4 = average bound CTura4 – average input CTura4, average net CTFus1 = average bound CTFus1 – average input CTFus1; average net CTura4 difference = average net KTP36 CTura4 – average net FY1872 CTura4; average net CTFus1 difference = average net KTP36 CTFus1 – average net FY1872 CTFus1; relative level of IP histones at ura4 in hat1Δ mutant over wild type = 1.9^-average net CTura4 difference, and relative level of IP histones at fus1 in hat1Δ mutant over WT = 1.9^-average net CTFus1 difference.

F-tests were performed to determine if the variances from the ChIPs using antisense were significantly different (<0.05) from those from the ChIPs using nonimmune serum. t-tests (two-sample unequal variances or two-sample equal variances) were performed to determine if the difference between the ChIPs using antisense was significantly different (<0.05) from that between the ChIPs using nonimmune serum.

RESULTS

S. pombe Hat1 is associated with Mis16. The native Hat1 complex was affinity purified by means of TAP tagging. We have previously shown that the loss of Hat1 in S. pombe causes sensitivity to DNA-damaging agents (14). To verify that TAP-tagged Hat1 remains functional, cells in which Hat1–TAP replaced native Hat1 were tested for normal MMS resistance (see Fig. S1 in the supplemental material).

There are several genes in S. pombe that code for homologues of RbAp46/48, including mis16, prw1, and pf3 (SPAC25H1.06) (25). Analysis of the affinity-purified Hat1 complex by mass spectrometry identified only one other major protein in addition to Hat1 itself: Mis16 (14 peptides detected). Mis16 is an S. pombe orthologue of p46/Hat2.
(Cnp1) loading in fission yeast (38, 79). It is required for kinetochore function and histone deacetylation at S. pombe centromeres (38). The association of Hat1 with Mis16 was confirmed by isolating Hat1 from a Hat1-TAP yeast strain that also contained myc-tagged Mis16. Western blotting verified the presence of Mis16-myctag in the purified Hat1 complex (see Fig. S2 in the supplemental material). As first demonstrated by Hayashi et al. (38), deletion analysis confirmed that mis16 is an essential gene (see Fig. S3 in the supplemental material). This is in contrast to the case for the HAT2 gene in S. cerevisiae (59, 70).

The Hat1 complex acetylates lysines 5 and 12 of histone H4.

The acetylation activity of the Hat1 complex was then examined. As a first approach, in vitro HAT assays were performed using H4 N-terminal peptides as the substrates. As we have previously shown for Hat1 from budding yeast and human (HeLa) cells (14, 52), Hat1 from S. pombe was able to readily acetylate an unacetylated H4 tail peptide but not a peptide previously acetylated at lysines 5 and 12 (the predicted Hat1 substrate lysines) (Fig. 2). The acetylation of lysines 5 and 12 was then directly tested by means of HAT assays using recombinant H4 as a substrate. Western blotting confirmed that lysines 5 and 12 were robustly acetylated by S. pombe Hat1 (Fig. 3). A weak activity at lysine 8 was also detected; a similar weak activity at this site has also been observed for Hat1 from budding yeast and human (HeLa) cells (14, 75) (see Discussion).

Hat1 is essential for telomeric, but not all, silencing in fission yeast.

To study the role of Hat1 in telomeric silencing, a hat1Δ yeast strain that possessed as its sole copy of ura4 a gene inserted at a subtelomeric region was generated. When ura4 is silent, growth on the counterselective agent 5-FOA can occur. Conversely, expression of ura4 will cause cell death on 5-FOA but permit growth on medium lacking uracil. As expected, wild-type cells and the expression of ura4 on the counterselective agent 5-FOA can occur. Conversely, ex-

FIG 2 Acetylation of H4 N-terminal peptides by S. pombe Hat1 in vitro. Unacetylated (UN) and K5/K12-diacyetylated (5/12) H4 N-terminal peptides were incubated in vitro for 30 min at 37°C with S. pombe Hat1 and [3H]acetyl-CoA. Reactions were also performed without added peptide (NP) and with the unacetylated peptide minus Hat1 (UN-Hat1). Results are expressed as a percentage of radioactivity incorporated into the unacetylated peptide.

vector restored telomeric silencing (see Fig. S4 in the supplemental material). In contrast, deleting hat1 did not cause the loss of silencing at any centromeric region tested (Fig. 5). In fact, a slight increase in silencing at the central core region was consistently observed (strain KTP33 in Fig. 5). Despite this increase in silencing, loss of Hat1 did not significantly alter the mitotic stability of minichromosome Ch16, as determined by the half-red colony sector assay of Allshire et al. (3) (data not presented). Moreover, deleting hat1 did not reduce silencing at the silent mating type

FIG 3 Acetylation of recombinant H4 by S. pombe Hat1. Recombinant H4 was incubated with Hat1 and unlabeled acetyl-CoA. Proteins from the reaction were resolved by electrophoresis and analyzed by Western blotting using antibodies that recognize total H4 (A) or H4 acetylated at K5 and/or K12 (B), K5 (C), K12 (D), or K8 (E). Acetylation at K16 was not detected above background (not shown).

FIG 4 hat1 deletion results in the loss of telomeric silencing in S. pombe. (A) Wild-type and experimental yeast strains were cultured on YEA plates in the presence (5FOA) or absence (YEA) of 5-FOA. Spot cultures represent 5-fold dilutions. Cells were grown for two (YEA) or three (5-FOA) days at 30°C. FY1872, ura4-tel; KTP36, ura4-tel hat1Δ; KTP24, ura4 disrupted, hat1Δ; LBP6, hat1Δ. (B) Wild-type and experimental yeast strains were cultured on EMMG plates in the presence (EMMG) or absence (EMMG–ura) of added uracil. Spot cultures represent 5-fold dilutions. Cells were grown for two (EMMG) or three (EMMG–ura) days at 30°C. FY1872, ura4-tel; KTP36, ura4-tel hat1Δ; KTP24, ura4 disrupted, hat1Δ; LBP6, hat1Δ.
locus (see Fig. S5 in the supplemental material), as judged by comparing 5-FOA resistance in hat1/H11001 and hat1/H9004 strains bearing the kint2::ura4 reporter (34).

Deleting hat1 causes increased acetylation of subtelomeric chromatin. We then asked whether the loss of Hat1 affects histone acetylation at the subtelomeric ura4 locus. ChIP analyses were performed on hat1 wild-type and hat1Δ cells containing the subtelomeric ura4 marker, using antibodies specific for various acetylated states of histone H4. Quantitative real-time PCR was then carried out to measure the immunoprecipitation efficiency of ura4 in comparison to that of the fus1 gene (which is located more than 750 kb from the end of chromosome 1). Because fus1 is transcribed only during nitrogen starvation (62), it is highly useful as a baseline locus to monitor global changes in histone acetylation.

Deletion of hat1 significantly raised the degree of histone acetylation in subtelomeric chromatin at lysine 12 of H4; no change occurred at the fus1 locus (Fig. 6). Increased acetylation at the ura4 gene was also observed at lysines 8 and 16 in the H4 tail domain (Fig. 7). Thus, the loss of ura4 silencing, as measured by the inability to grow in the presence of 5-FOA, is accompanied by alterations of histone posttranslational modifications indicative of a more “active” chromatin configuration. To further test the relationship between histone acetylation and telomeric silencing, S. pombe cells possessing the telomeric marker but wild type for hat1 were grown for several generations in the presence of the deacetylase inhibitor trichostatin A (TSA). Cells were then washed and plated in the absence of TSA. Strikingly, TSA treatment caused a loss of telomeric silencing similar to that seen in hat1Δ cells, which persisted in the absence of the deacetylase inhibitor (Fig. 8). TSA has also been shown to cause derepression at centromeres (27) and the mating type loci (36) in S. pombe, further underscoring the antagonism between silencing and histone hyperacetylation. Our results now represent the first evidence obtained from any organism that Hat1 can independently regulate telomeric silencing, and they suggest an intimate link between the acetylation of new H4 and the proper assembly of heterochromatin.

DISCUSSION

Our results demonstrate that, as in other systems (51, 59, 82), the Hat1 acetyltransferase in fission yeast is associated with an orthologue of RbAp46/48, which in the case of S. pombe is Mis16. Mis16 can also form a separate complex with Mis18 to effect CENP-A loading at centromeres (38). Unlike HAT2 in S. cerevisiae, mis16 is an essential gene (this report and reference 38). Given that hat1Δ cells are viable, the lethality of mis16Δ is most likely due to the role of Mis16 in centromere assembly and chromosome segregation (38). RbAp46 and/or p48 is also involved in centromere assembly in human cells (38), as well as in Drosophila (where p48 partici-
pates in the deposition of the centromeric H3 variant CenH3.

The acetylation of lysines 5 and 12 by Hat1 in vitro has long been established (8, 14, 22, 24, 46; reviewed in references 61, 68, 75, and 82). More recent experiments in vertebrate (chicken) cells have indicated that Hat1 acetylates "cytosolic" histone H4 in vivo, in the conserved K5/K12 pattern (12). There is also evidence that Hat1 from S. cerevisiae acetylates both sites in vivo (64), in contrast to results obtained in vitro with the budding yeast holoenzyme, which acetylates only K12 (59). The Hat1 complex from S. pombe acetylated H4 at lysines 5 and 12. A low level of acetylation at lysine-8 (but not lysine-16) was also detected when recombinant H4 was used as a substrate. It is presently uncertain whether this reflects a true Hat1 activity in vivo. However, it is worth noting that H4 associated with native CAF-1 from human cells shows a low degree of acet-
ylation at lysine-8, in addition to acetylation at K5 and K12 (81). Almost all of the acetyl-K8 was in the diacetylated H4 isoform (81), which may explain why the K5/K12-diacetylated peptide is not appreciably acetylated by Hat1 in vitro.

Deleting the gene coding for Hat1 has minimal effects on cell growth and/or chromatin assembly (12, 14, 45, 59, 70), although a slight increase in G1 cells has been observed (87). However, the loss of Hat1 has been linked to defects in DNA double-strand break repair (12, 14, 66, 67) and the abrogation of telomeric silencing in yeast (references 44 and 63 and this report). In S. cerevisiae, derepression at telomeres is observed only when the deletion of HAT1 is accompanied by mutations of specific acetylatable lysines in the H3 N-terminal domain (44, 63). In S. pombe, loss of Hat1 is itself sufficient to cause a dramatic decrease in telomeric silencing. Thus, the functional redundancy between H3 acetylation and Hat1 observed in S. cerevisiae is absent in S. pombe. The fact that deleting or mutating CAF-1 subunits reduces telomeric silencing in budding yeast (28, 41, 47) provides evidence that replication-coupled histone de-
position is integral to the maintenance of silent chromatin. Our results now suggest that the proper diacetylation of new H4 is a critical element in this process. Notably, acetylation of H4 at lysine-12, a hallmark of Hat1 function, has been linked to gene silencing in yeast and Drosophila (17, 44, 74, 80).

In contrast to the effects on telomeric silencing, deletion of
hat1 did not cause loss of silencing at the silent mating type locus or at any centromeric region tested. In fact, silencing in hat1Δ mutants became slightly more pronounced at the central core region. It is possible that the loss of Hat1 frees a greater proportion of Mis16 to associate with Mis18 (as we find no evidence for the association of Mis18 with Hat1), thereby facilitating CENP-A deposition at the central core region (23, 38, 78). Silencing at S. pombe centromeres involves several mechanisms, including histone H3 methylation, RNAi, and the deposition of Swi6 (HP1) (11, 18, 20, 35, 49, 56). Multiple elements also contribute to the assembly and maintenance of telomeres and adjacent subtelomeric regions (including telomerase, CAF-1, telomere-specific and heterochromatin proteins, RNAi, and the regulation of histone modifications [4, 16, 28, 29, 41, 47, 55, 77, 84]). Our results provide the first evidence that the steps required for silencing centromeric chromatin act independently of Hat1 and that herein lies a fundamental difference between the telomeric and centromeric silencing pathways in S. pombe.

In a recent report it was shown that in human cells Hat1 preferentially acetylates H4 in H3.1/H4 dimers (relative to H3.3/H4 dimers) and that Hat1 depletion affects the association of H3.1/H4 with importin 4 (87). However, in another study it was observed that Hat1 depletion in HeLa cells did not cause accumulation of H3/H4 dimers in the cytoplasm, indicating a redundancy in import processes (21). In this regard it is worth noting that in both budding and fission yeasts, the sole H3 subtype is equivalent to the replacement variant H3.3 (53), which, unlike H3.1, can be deposited through the HIRA pathway (33, 42, 86). Our own results establish that Hat1 deletion in S. pombe does not depress silencing at centromeres or the silent mating type locus or alter histone modifications at the fus1 gene, arguing against the global disruption of chromatin organization.

Loss of Hat1 caused a significant increase in the acetylation of subtelomeric chromatin at multiple acetylatable sites in the H4 N-terminal domain. Although perhaps counterintuitive, this is consistent with the loss of transcriptional silencing and with the encroachment of an active chromatin structure into the subtelomere. In line with this, telomeric silencing was also lost by pretreatment with the deacetylase inhibitor trichostatin A (Fig. 8). A similar loss of silencing was previously observed at S. pombe centromeres and the mating type loci after TSA treatment (27, 36). Other studies have shown that the MYST family histone acetyltransferase Mst2 helps to negatively regulate telomeric silencing in S. pombe (32) and that Esa1 (another MYST member and a component of the NuA4 complex) actively acetylates telomeric H4 in budding yeast (88).

It remains formally possible that the loss of Hat1 causes an increase in nucleosome density preferentially at telomeres (and not globally), thereby accounting for the specific rise in acetylation that we describe. However, this would be inconsistent with our observed increase in transcription of the subtelomeric ura4 marker, as gene activation in S. pombe correlates with decreased nucleosome occupancy, especially at promoter regions (10, 48). Moreover, Hat1Δ deletion has no effect on the nucleosome repeat length (i.e., histone density) of newly replicated chromatin in vertebrate cells (12). It is therefore not unreasonable to propose that the loss of Hat1 provides an opportunity for the anomalous hyperacetylation of subtelomeric chromatin, which interferes with the normal silencing pathway. In future studies it will be of interest to define the other elements involved in the derepression of telomeric chromatin and the increase in telomeric acetylation.

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REFERENCES

1. Ai X, Parthun MR. 2004. The nuclear Hat1p/Hat2p complex: a molecular link between type B histone acetyltransferases and chromatin assembly. Mol. Cell. 14:195–205.
2. Allis CD, Chicoine LG, Richman R, Schulman IG. 1985. Deposition-related histone acetylation in micronuclei of conjugating Tetrahymena. Proc. Natl. Acad. Sci. U. S. A. 82:8048–8052.
3. Allshire RC, Nimmo ER, Ekwall K, Javerzat JP, Cranston G. 1995. Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. Genes Dev. 9:218–233.
4. Almeida R, Buscaino A, Allshire RC. 2006. Molecular biology: silencing unlimited.Curr. Biol. 16:R635–R638.
5. Alvarez F, et al. 2011. Sequential establishment of marks on soluble histones H3 and H4. J. Biol. Chem. 286:17714–17721.
6. Annunziato AT, Seale RL. 1983. Histone deacetylation is required for the maturational newly replicated chromatin. J. Biol. Chem. 258:12675–12684.
7. Annunziato AT, Hansen JC. 2000. Role of histone acetylation in the assembly and modulation of chromatin structures. Gene Expr. 9:37–61.
8. Annunziato AT. 2012. Assembling chromatin: the long and winding road. Biochim. Biophys. Acta 1819:96–210.
9. Bahler J, et al. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast 14:943–951.
10. Bai I, Morozov AV. 2010. Gene regulation by nucleosome positioning. Trends Genet. 26:476–483.
11. Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. Cell Res. 21:381–395.
12. Barman HK, et al. 2006. Histone acetyltransferase 1 is dispensable for replication-coupled chromatin assembly but contributes to recover DNA damages created following replication blockage in vertebrate cells. Biochim. Biophys. Res. Commun. 345:1547–1557.
13. Benson LJ, et al. 2006. Modifications of H3 and H4 during chromatin replication, nucleosome assembly, and histone exchange. J. Biol. Chem. 281:9287–9296.
14. Benson LJ, et al. 2007. Properties of the type B histone acetyltransferase Hat1-H4 tail interaction, site preference, and involvement in DNA repair. J. Biol. Chem. 282:836–842.
15. Blackwell JS, Wilkinson ST, Mosammaparast N, Pemberton LF. 2007. Mutational analysis of H3 and H4N termini reveals distinct roles in nuclear import. J. Biol. Chem. 282:20142–20150.
16. Blasco MA. 2007. The epigenetic regulation of mammalian telomeres. Nat. Rev. Genet. 8:299–309.
17. Braunstein M, Sobel RE, Allis CD, Turner BM, Broach JR. 1996. Efficient transcriptional silencing in Saccharomyces cerevisiae requires a heterochromatin histone acetylation pattern. Mol. Cell. Biol. 16:4349–4356.
18. Burrack LS, Berman J. 2012. Flexibility of centromere and kinetochore structures. Trends Genet. 28:204–212.
19. Cam HP, et al. 2005. Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. Nat. Genet. 37:809–819.
20. Cam HP, Chen ES, Grewal SIS. 2009. Transcriptional scaffolds for heterochromatin assembly. Cell 138:610–614.
21. Campos EI, et al. 2010. The program for processing newly synthesized histones H3.1 and H4. Nat. Struct. Mol. Biol. 17:1343–1351.
22. Chang L, et al. 1997. Histones in transit: cytoplasmic histone complexes and diacytation of H4 during nucleosome assembly in human cells. Biochemistry 36:469–480.
23. Chen ES, Saitoh S, Yanagida M, Takahashi K. 2003. A cell cycle-regulated GATA factor promotes centromeric localization of CENP-A in fission yeast. Mol. Cell. 11:175–187.
24. Chicoine LG, Schulman IG, Richman R, Cook RG, Allis CD. 1986. Nonrandom utilization of acetylation sites in histones isolated from *Tetrahymena*: evidence for functionally distinct H4 acetylation sites. J. Biol. Chem. 261:1071–1076.

25. Dolke K, et al. 2008. Fission yeast chromatin assembly factor 1 assists in the replication-coupled maintenance of heterochromatin. Genes Cells 13:1027–1043.

26. Eijlss-Lassallette A, Moqoud AR, Arnaud MC, Thiriet C. 2011. H4 replication-dependent diacetylation and Hat1 promote S-phase chromatin assembly in vivo. Mol. Biol. Cell 22:245–255.

27. Ekkall K, Olsson T, Turner BM, Cranston G, Allshire RC. 1997. Transient inhibition of histone deacetylation alters the structural and functional imprint at fusion yeast centromeres. Cell 91:1021–1032.

28. Enamoto S, Mccunezeriad PD, Gereimenid M, Sanders MA, Berman J. 1997. RLF2, a subunit of yeast chromatin assembly factor-I, is required for telomeric chromatin function in vivo. Genes Dev. 11:358–370.

29. Enamoto S, Berman J. 1998. Chromatin assembly factor 1 contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes Dev. 12:219–232.

30. Furuyma T, Dalal Y, Henikoff S. 2006. Chaperone-mediated assembly of centromeric chromatin in vitro. Proc. Nat. Acad. Sci. U. S. A. 103:6172–6177.

31. Głowczewski L, Waterborg JH, Berman JG. 1998. The replication-coupled maintenance of heterochromatin. Genes Cells 3:795–823.

32. Grewal SIS, Bonaduce MJ, Klar AJS. 2004. Histone acetyltransferase B complex mainly localized in the nucleus. J. Biol. Chem. 279:8732–8739.

33. Grewal SI. 2001. Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129–1133.

34. Gutz H, Heslot H, Leupold U, Loprieno N. 2004. Yeast chromatin assembly factor-I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes Dev. 12:219–232.

35. Glowczewski L, Waterborg JH, Berman JG. 1998. Chromatin assembly factor I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes Dev. 12:219–232.

36. Gutz H, Heslot H, Leupold U, Loprieno N. 1997. Schizosaccharomyces pombe, p 395–446. In King RC (ed), Handbook of genetics. Plenum Press, New York, NY.

37. Hayashi T, et al. 2004. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell. 118:715–729.

38. Heckman DS, et al. 2011. The tandem affinity purification (TAP) method: a quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

39. Parthun MR. 2006. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

40. Parthun MR, Widom J, Gottschling DE. 1996. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell 85:563–576.

41. Kaufman PD, Kobayashi R, Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.

42. Kaufman PD, Cohen JL, Osley MA. 1998. H4 histone acetyltransferases regulate epigenetic inheritance of transcriptional silencing and chromatin segregation in fission yeast. Genetics 150:563–576.

43. Gutz H, Heslot H, Leupold U, Loprieno N. 1974. Schizosaccharomyces pombe. In King RC (ed), Handbook of genetics. Plenum Press, New York, NY.

44. Hayashi T, et al. 2004. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell. 118:715–729.

45. Berman JG. 1998. Chromatin assembly factor-I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. Genes Dev. 12:219–232.

46. Kaufman PD, Kobayashi R, Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.

47. Kaufman PD, Cohen JL, Osley MA. 1998. H4 histone acetyltransferases regulate epigenetic inheritance of transcriptional silencing and chromatin segregation in fission yeast. Genetics 150:563–576.

48. Gutz H, Heslot H, Leupold U, Loprieno N. 1974. Schizosaccharomyces pombe, p 395–446. In King RC (ed), Handbook of genetics. Plenum Press, New York, NY.

49. Hayashi T, et al. 2004. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell. 118:715–729.

50. Heckman DS, et al. 2011. The tandem affinity purification (TAP) method: a quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

51. Parthun MR. 2006. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

52. Parthun MR, Widom J, Gottschling DE. 1996. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell 85:563–576.

53. Kaufman PD, Kobayashi R, Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.

54. Kaufman PD, Cohen JL, Osley MA. 1998. H4 histone acetyltransferases regulate epigenetic inheritance of transcriptional silencing and chromatin segregation in fission yeast. Genetics 150:563–576.

55. Gutz H, Heslot H, Leupold U, Loprieno N. 1974. Schizosaccharomyces pombe, p 395–446. In King RC (ed), Handbook of genetics. Plenum Press, New York, NY.

56. Hayashi T, et al. 2004. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell. 118:715–729.

57. Heckman DS, et al. 2011. The tandem affinity purification (TAP) method: a quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

58. Parthun MR. 2006. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

59. Parthun MR, Widom J, Gottschling DE. 1996. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell 85:563–576.

60. Kaufman PD, Kobayashi R, Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.

61. Kaufman PD, Cohen JL, Osley MA. 1998. H4 histone acetyltransferases regulate epigenetic inheritance of transcriptional silencing and chromatin segregation in fission yeast. Genetics 150:563–576.

62. Gutz H, Heslot H, Leupold U, Loprieno N. 1974. Schizosaccharomyces pombe, p 395–446. In King RC (ed), Handbook of genetics. Plenum Press, New York, NY.

63. Hayashi T, et al. 2004. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell. 118:715–729.

64. Heckman DS, et al. 2011. The tandem affinity purification (TAP) method: a quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

65. Parthun MR. 2006. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *C. elegans* elegans. Nat. Protoc. 6:3698–709.

66. Parthun MR, Widom J, Gottschling DE. 1996. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell 85:563–576.

67. Kaufman PD, Kobayashi R, Stillman B. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.

68. Kaufman PD, Cohen JL, Osley MA. 1998. H4 histone acetyltransferases regulate epigenetic inheritance of transcriptional silencing and chromatin segregation in fission yeast. Genetics 150:563–576.
servation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proc. Natl. Acad. Sci. U. S. A. 92:1237–1241.
77. Sugiyama T, et al. 2007. SHREC, an effector complex for heterochromatic transcriptional silencing. Cell 128:491–504.
78. Takahashi K, Chen ES, Yanagida M. 2000. Requirement of Mis6 centromere connector for localizing a CENP-A-like protein in fission yeast. Science 288:2215–2219.
79. Takahashi K, Takayama Y, Masuda F, Kobayashi Y, Saitoh S. 2005. Two distinct pathways responsible for the loading of CENP-A to centromeres in the fission yeast cell cycle. Philos. Trans. R. Soc. B Biol. Sci. 360:595–606.
80. Turner BM, Birley AJ, Lavender J. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. Cell 69:375–384.
81. Verreault A, Kaufman PD, Kobayashi R, Stillman B. 1996. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell 87:95–104.
82. Verreault A, Kaufman PD, Kobayashi R, Stillman B. 1998. Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. Curr. Biol. 8:96–108.
83. Waterborg JH, Matthews JH. 1984. Patterns of histone acetylation in Physarum polycephalum: H2A and H2B acetylation is functionally distinct from H3 and H4 acetylation. Eur. J. Biochem. 142:329–335.
84. White SA, Allshire RC. 2008. RNAi-mediated chromatin silencing in fission yeast. Curr. Top. Microbiol. Immunol. 320:157–183.
85. Wood V, et al. 2002. The genome sequence of Schizosaccharomyces pombe. Nature 415:871–880.
86. Yamane K, et al. 2011. Asf1/HIRA facilitate global histone deacetylation and associate with HP1 to promote nucleosome occupancy at heterochromatic loci. Mol. Cell 41:56–66.
87. Zhang H, Han J, Kang B, Burgess R, Zhang Z. 2012. Human histone deacetyltransferase HAT1 preferentially acetylates H4 molecules in H3.1-H4 dimers over H3.3-H4 dimers. J. Biol. Chem. 287:6573–6581.
88. Zhou BO, et al. 2011. Histone H4 lysine 12 acetylation regulates telomeric heterochromatin plasticity in Saccharomyces cerevisiae. PLoS Genet. 7:e1001272, doi:10.1371/journal.pgen.1001272.