Hat2p recognizes the histone H3 tail to specify the acetylation of the newly synthesized H3/H4 heterodimer by the Hat1p/Hat2p complex

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Post-translational modifications of histones are significant regulators of replication, transcription, and DNA repair. Particularly, newly synthesized histone H4 in H3/H4 heterodimers becomes acetylated on N-terminal lysine residues prior to its incorporation into chromatin. Previous studies have established that the histone acetyltransferase (HAT) complex Hat1p/Hat2p mediates this modification. However, the mechanism of how Hat1p/Hat2p recognizes and facilitates the enzymatic activities on the newly assembled H3/H4 heterodimer remains unknown. Furthermore, Hat2p is a WD40 repeat protein, which is found in many histone modifier complexes. However, how the WD40 repeat proteins facilitate enzymatic activities of histone modification enzymes is unclear. In this study, we first solved the high-resolution crystal structure of a Hat1p/Hat2p/CoA/H4 peptide complex and found that the H4 tail interacts with both Hat1p and Hat2p, by which substrate recruitment is facilitated. We further discovered that H3 N-terminal peptides can bind to the Hat2p WD40 domain and solved the structure of the Hat1p/Hat2p/CoA/H4/H3 peptide complex. Moreover, the interaction with Hat2p requires unmodified Arg2/Lys4 and Lys9 on the H3 tail, suggesting a novel model to specify the activity of Hat1p/Hat2p toward newly synthesized H3/H4 heterodimers. Together, our study demonstrated the substrate recognition mechanism by the Hat1p/Hat2p complex, which is critical for DNA replication and other chromatin remodeling processes.

[Keywords: acetyltransferase; Hat1p/Hat2p; histone modification]

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As the key building blocks of chromatin, histones are subjected to a variety of post-translational modifications that have critical roles in replication, transcription, heterochromatin maintenance, and DNA repair [Nakayama et al. 2001; Grewal and Moazed 2003; Kouzarides 2007; Bannister and Kouzarides 2011]. Histone acetylation, which represents the transfer of an acetyl moiety from acetyl-coenzyme A [AcCoA] to the ε-amino group of target lysine residues, is catalyzed by histone acetyltransferase (HAT) families [Brownell and Allis 1996; Wu et al. 2012]. Hat1p, the first HAT identified in yeast, was originally isolated from cytosolic extract and therefore characterized as a type B HAT [Parthun et al. 1996], although further studies showed that Hat1p is also present in the nucleus [Verreault et al. 1998; Imhof and Wolffe 1999; Poveda et al. 2004]. As its unique feature, Hat1p is specific for the acetylation of newly synthesized H4 at Lys5 and Lys12 residues before the incorporation of H3/H4 heterodimer into nucleosomes [Kleff et al. 1995; Parthun et al. 1996]. Genetic deletion of Hat1p in yeast or its
homolog, Hat1, in mice resulted in the loss of acetylation on H4K5 and H4K12 on the nascent histone (Parthun et al. 1996; Nagarajan et al. 2013), supporting that Hat1p is the sole enzyme responsible for this evolutionarily conserved histone modification.

Several binding partners of Hat1p have been identified, including Hat2p and Hif1 [Hat1-interacting factor 1]. Hat2p and its human homolog, retinoblastoma-binding protein RbAp46, significantly enhanced the enzymatic activity of Hat1p by facilitating H4 association with Hat1p (Parthun et al. 1996). Furthermore, Hat2p can modulate the substrate specificity of Hat1p, i.e., while the recombinant Hat1p acetylated both H4K5 and H4K12 in vitro, the Hat1p/Hat2p complex purified from yeast extracts was highly specific for H4K12 (Parthun et al. 1996). Therefore, it has been suggested that Hat1p/Hat2p forms a holoenzyme complex [Ai and Parthun 2004]. In addition, Hif1, which is exclusively a nuclear protein, has been shown to bind the Hat1p/Hat2p complex and function as a histone chaperone to facilitate chromatin assembly (Ai and Parthun 2004; Poveda et al. 2004).

In support of its critical roles on chromatin structure, Hat1 deficiency in mice caused neonatal lethality associated with an array of developmental defects, especially of the lungs and skeleton (Nagarajan et al. 2013). In addition, Hat1 knockout mouse embryonic fibroblasts (MEFs) displayed a significant increase in genomic instability as well as defects in DNA repair. Moreover, not only the acetylation on H4, the direct target of Hat1, but the acetylation on histones H3 were also altered during replication-coupled chromatin assembly in cells, possibly due to the potential cross-regulation of distinct histone modifications (Nagarajan et al. 2013). Similarly, although the genetic deletion of Hat1p alone revealed a relatively modest phenotype in yeast, Hat1p deletion combined with mutations of H3 N-terminal tails resulted in the loss of telomeric silencing and also sensitivity to DNA-damaging agents (Kelly et al. 2000; Qin and Parthun 2002).

Although studies have begun to elucidate the roles of Hat1p/Hat2p in chromatin remodeling processes, several important questions remain to be addressed. First, human RbAp46 can bind to the α helix immediately following the N-terminal tail of H4, and it has been suggested that such interaction might facilitate the association between human Hat1 and its substrate [Verreault et al. 1998; Vermaak et al. 1999; Furuyama et al. 2006]. However, it is unclear whether this H4/RbAp46 interaction is evolutionarily conserved and, more importantly, whether this interaction could function in the context of the Hat1p/Hat2p catalytic complex. Furthermore, it was proposed that the methylation of the H4 tail (e.g., on H4K8 and H4K16) could interfere with binding to the catalytic cleft on Hat1p and therefore help to control the substrate specificity of Hat1p toward the newly synthesized H4 protein [Dutnall et al. 1998; Makowski et al. 2001; Benson et al. 2007]. How specificity is achieved for newly synthesized H3/H4 heterodimers and how Hat2p contributes to this selectivity remain unknown.

Results

Overall structure of the Hat1p/Hat2p/CoA/H4 complex

To unravel those questions, we solved the crystal structure of a Hat1p/Hat2p/CoA/H4 peptide complex at 2.0 Å resolution (Fig. 1; Supplemental Table S1). In the structure, each Hat1p/Hat2p/CoA/H4 heterotetrameric complex contains four components, with the stoichiometry of 1:1:1:1 (Fig. 1A). Although the segment of yeast histone H4 used for crystallization contained residues 1-48, only residues 7-46 could be well defined in the structure (Supple-
of hydrogen bonds, salt bridges, and hydrophobic interactions. The Hat1p and Hat2p interaction is stabilized by a series of hydrogen bonds, salt bridges, and hydrophobic interactions. Deletion of the LP1 segment (ΔLP1) of Hat1p abolished the interaction between Hat1p and Hat2p in vitro (Fig. 2E; Supplemental Fig. S3), which suggested that some other protein might involve in the interaction of Hat1p and Hat2p. Indeed, consistent with the structural observation, the N terminus of histone H4 might function as a "linker" protein that interacts with both Hat1p and Hat2p (Fig. 1), which could spatially interact with each other even without the contribution of LP1 (Fig. 2E). For higher organisms, the Hat1p/Hat2p complex formation may be more complicated than in yeast. Consistent with this hypothesis, although the W197E and W199E mutants of Hat1p would abolish the interaction between the Hat1p and Hat2p heterodimeric formation in vitro, the W197E and W199E alleles of HAT1 were able to complement the DNA damage sensitivity in the hat1Δ yeast, suggesting that the Hat1p/Hat2p interaction is likely to be stabilized by multiple interactions or other proteins in vivo (Supplemental Fig. S4).

On the other hand, the mutations of Y37A, A163Y, Y194A, Y196A, and A202D of Hat2p showed little effect on the interaction in vitro (Fig. 2B,C). Similarly, we also tested a set of mutants of Hat2p and found that the L266E of Hat2p lost its ability to bind Hat1p, while other mutations showed little effect (Fig. 2F,G).

**H4 interacts with both Hat1p and Hat2p**

The binding of the N-terminal segment of Histone H4 with Hat1p/Hat2p could be divided into three regions [residues 8–18, 19–30, and 31–45] (Fig. 3A–C). The N-terminal region [residues 7–18] of H4 was embedded in the cave between Hat1p and Hat2p and mainly interacted with Hat1p, which was similar to the reported Hat1p/H4 structure (Kumar et al. 2012). Lys20 in the second region [residues 19–30] formed a salt bridge with the Asp357 from LP2 of Hat2p (Fig. 3A). The C-terminal region [residues 29–45], which formed the helix 1 of H4, inserted into the groove formed by the Hat2p LP2 extension, the N-terminal helix 1, and the C terminus (Fig. 3B,C), which is consistent with the structural observation between H4 and RbAp46/8 (the human homologs of Hat2p) (Fig. 3D; Murzina et al. 2008). Overall, the interface areas of the N-terminal and C-terminal regions of H4 covered ~608 Å² and 802 Å², respectively. The interaction between H4 and the Hat1p/Hat2p complex was strongly stabilized by salt bridge bonds formed by the positively charged residues of H4, such as Lys16, Lys20, Arg35, Arg39, Arg40, and Lys44, with the conserved acidic residues (Asp335, Asp336, Glu338, and Asp339) of Hat2p LP2 (Fig. 3B; Supplemental Fig. S5).

Previous structural and biochemical studies suggested that the Lys12 of H4 (H4K12) inserts into the active site of Hat1p to gain access to AcCoA (Dutnall et al. 1998; Wu et al. 2012). In our structure, H4K12 is aligned in the active site, with the CoA molecule coming into a concave groove from the opposite side of H4K12, which enables...
the ε-amino group of H4K12 and the SH group of CoA to contact each other (Fig. 3A; Supplemental Fig. S6). Our structure was consistent with the catalytic mechanism that had been proposed with the structure of human Hat1 (Wu et al. 2012). Hat1p/Hat2p complex purified from yeast extracts specifically acetylated H4K12 residue, while the recombinant yeast Hat1p was not only able to acetylate H4K12 but also exhibited low activity to H4K5 (Parthun et al. 1996). In vitro acetylation assays showed that the recombinant Hat1p/Hat2p complex specifically
acetylated the H4K12 residue, in agreement with the idea that Hat2p regulates the substrate specificity of Hat1p (Supplemental Fig. S7). It seems that Hat2p could exert such an effect potentially through binding to helix 1 of H4, which helps to preferentially stabilize H4K12 in the active site.

Previous studies of Hat1p using a variety of histone substrates identified a putative glycine-rich consensus sequence that Hat1p targets for acetylation (Parthun et al. 1996). We constructed mutations in this consensus sequence in the context of GST-H4 tail fusions to determine whether these residues influence binding of the Hat1p/Hat2p complex to its substrate. As seen in Figure 3, E and F, the glycine residues in the −1 and −3 position could influence Hat1p/Hat2p complex binding, while the +2 glycine has little effect. Acetylation of Lys16 is a highly abundant chromatin mark (Dang et al. 2009). In the structure, a salt bridge could be observed between H4K16 and E338 of Hat2p (Fig. 3A). The isothermal titration calorimetry (ITC) results show that the acetylation of Lys16 could decrease the binding affinity between the Hat1p/Hat2p complex and GST-tagged mutant H4 peptides was evaluated by quantifying the intensity of the bands in SDS-PAGE using the AlphaEaseFC software and normalizing it to the wild type (WT). The presented data are the average of three experiments. The error bars correspond to one standard deviation. The mutants are labeled as in E.
the Hat1p/Hat2p complex and the H4 peptide (Supplemental Fig. S8), which indicates that the Hat1p/Hat2p complex prefers to bind newly synthesized H4.

**Hat2p could bind the different methylated states of H3**

Our structure of the Hat1p/Hat2p/CoA/H4 complex demonstrated that the Hat2p has a critical role in facilitating the binding of H4 and the enzymatic specificity of Hat1p (Supplemental Fig. S7). However, previous studies suggested that the newly synthesized H3 and H4 proteins form a heterodimer before the acetylation of H4, and it was believed that the free form of the H4 protein was only transiently present after translation (Ruiz-Carrillo et al. 1975, Jackson et al. 1976). Therefore, it is very important to understand how Hat1p/Hat2p recognizes newly assembled H3/H4 heterodimers and, in particular, whether H3 has any function in this process.

Previous studies have reported that the proteins that contain the WD40 domain could bind to histone tails, such as WDR5 and NURF55 (Couture et al. 2006; Han et al. 2006; Schmitges et al. 2011), but in the Hat1p/Hat2p/CoA/H4 complex, no direct interface was observed between the hole of the WD40 domain of Hat2p and the H4 tail. We propose that the Hat2p WD40 domain might be able to bind to the H3 tail in H3/H4 heterodimer. Indeed, our in vitro ITC analyses indicated that the H3 peptide containing the first 15 amino acids could interact with the Hat1p/Hat2p/H4 peptide complex with a ratio of 1:1 and with an apparent affinity with a Kd of ~0.34 μM (Supplemental Fig. S9).

We next set out to cocrystallize the H3 peptide (residues 1–15) with the Hat1p/Hat2p/H4 complex and CoA molecule and solved the structure of the Hat1p/Hat2p/H3/H4/CoA complex at a resolution of 2.5 Å (Fig. 4A,B). The omit Fo–Fc electron density clearly defined the first 12 amino acids of the H3 peptide in the structure (Supplemental Fig. S1B), which is located in a narrow channel on the surface of the WD40 domain (Fig. 4C,D). Arg2 of H3 inserts into the small hole of the WD40 domain of Hat2p. The interaction between H3 and Hat2p is stabilized through highly conserved residues in Hat2p by forming a series of hydrogen bonds and salt bridges (Fig. 4C; Supplemental Fig. S5). The overall binding surface is negatively charged on Hat2p, corresponding with the positively charged N-terminal tail of H3 (Fig. 4D). Of note, the structure of the Hat1p/Hat2p/H3/H4/CoA complex closely resembles the structure of Hat1p/Hat2p/H4/CoA, with an RMSD of 0.663 Å for Hat1p and 0.227 Å for Hat2p, suggesting that the binding of the H3 N-terminal tail to Hat2p does not induce significant conformational change on the Hat1p/Hat2p complex (Supplemental Fig. S10).

It is quite interesting that the side chain of H3R2 inserts into the small hole of the WD40 domain of Hat2p. The Arg2 forms hydrogen bonds with Asn212 and Asp213 of Hat2p. In addition, the side chains of Arg123 and Arg125 of Hat2p show a large conformational change upon binding with H3 (Supplemental Fig. S11), which might help to make more space for the side chain of H3R2. H3R2 can be subjected to symmetric or asymmetric dimethylation in cells, which regulates transcriptional activity (Kirmizis et al. 2007). The structure reveals that the methylation of Arg2 might disrupt the corresponding interactions and also result in the spatial constraint for insertion into the pocket. The size of the small hole appears to be conserved among different species (Supplemental Fig. S12). As predicted, in contrast to the unmethylated H3 peptide, symmetric or asymmetric dimethylation of Arg2 abolished the interaction between H3 and the Hat1p/Hat2p/H4 complex in the ITC assay (Fig. 4E).

The different methylation states on H3K4 and H3K9 are common events that occur in cells and have different biological functions for regulating the gene transcription and chromatin assembly (Stewart et al. 2005, Sims and Reinberg 2006). Our structure reveals that the side chain of H3K4 forms two hydrogen bonds with the side chains of Asn162 and Glu120 of Hat2p, and the side chain of H3K9 forms a salt bridge with the side chain of Glu69 of Hat2p (Fig. 4C), which indicates that the modified H3K4 or H3K9 might influence the reorganization of H3 with Hat2p. Indeed, the ITC results showed that H3K4me1, H3K4me2, and H3K4me3 have binding affinity to the Hat1p/Hat2p/H41–48 complex with a Kd of 2.48, 4.78, and 11.62 μM, and H3K9me1, H3K9me2, and H3K9me3 bind to the Hat1p/Hat2p/H41–48 complex with a Kd of 0.29, 1.30, and 2.45 μM, respectively (Fig. 4F,G; Supplemental Fig. S13). Compared with the free H3 tail binding to the Hat1p/Hat2p/H41–48 complex with a Kd ~0.34 μM, the trimethylation on H3K4 destabilizes the interaction between H3 and Hat2p, and methylation of H3K9 has a weaker effect on the binding affinity. Previous studies have reported that the newly synthesized histone H3 has the monomethylation on H3K9 (Lovallo et al. 2006). Our results suggest that Hat2p could recognize the histone H3 tails with/without this modification (Fig. 4G), which indicates that the H3K9 might be monomethylated either before or after the H4K12 acetylation on the newly synthesized histone H3/H4 heterodimer. Definitely, this needs further investigation to know the sequential orders. Together, our data indicated that the interaction of H3 with Hat2p depends on the modification status of its tail.

**Discussion**

Histone acetylation is a highly dynamic post-translational modification that critically regulates replication, transcription, and heterochromatin silencing (Shahbazian and Grunstein 2007). Hat1p, which is the founding member of type B HATs identified in *Saccharomyces cerevisiae*, functions with its cofactor, Hat2p, to acetylate N-terminal lysine residues of histone H4 before its incorporation into chromatin (Ai and Parthun 2004). In the present study, we revealed that a hydrophobic core at the interface of Hat1p and Hat2p, formed by an array of conserved residues, contributes most to the interaction between the two proteins. Furthermore, the mutations of W197E, Y199E, and F205E on Hat1p, and L266E on Hat2p, which disrupt this hydrophobic interaction, abolished the formation of the Hat1p/Hat2p complex (Figs. 1, 2).

Previous studies suggested that residues 5–19 of H4 could bind directly to the active site of Hat1p (Wu et al. 2012). Our data further showed that the N-terminal portion of H4 not only binds to Hat1p but also directly...
Figure 4. Hat2p could bind different methylation statuses of histone H3. (A) Overall structure of the Hat1p/Hat2p/H3/H4/CoA complex. The protein structure is shown in cartoon, and CoA is shown in stick representation, with Hat1p, Hat2p, the H3 peptide, and the H4 peptide colored in blue, cyan, orange, and purple, respectively. (B) The H3 peptide and H4 peptide in the structure of the Hat1p/Hat2p/H3 peptide/H4 peptide/CoA complex. Hat1p and Hat2p are shown in surface mode, with Hat1p colored blue, and Hat2p colored cyan. (C) Close-up interview of the binding sites of the H3 peptide on the Hat2p surface. The interaction residues of Hat2p and the H3 peptide are labeled in chartreuse and orange stick representation, respectively, and the salt bridges are shown as dashed lines. (D) Electrostatic potential surface of the H3-binding sites on Hat2p. Negatively and positively charged surfaces are colored in red and blue, respectively. (E) ITC curves of free H3\textsubscript{1–15} and methylation of H3R2 (H3\textsubscript{1–15}R2me2s and H3\textsubscript{1–15}R2me2a) peptides titrated into the Hat1p/Hat2p/H4 peptide complex. (ND) Not detectable. The first peak in the thermogram has not been used for analysis. (F,G) ITC fitting curves of the Hat1p/Hat2p/H4 peptide complex with histone H3 peptides of different K4 methylation states (F) and different K9 methylation states (G). Peptide sequences and complete thermodynamic parameters are listed in Supplemental Table S3. The first peak in the thermogram has not been used for analysis.
interacts with Hat2p, especially through residues 29–41 [Fig. 3]. This region, which is normally unfolded (Dutnall et al. 1998; Wu et al. 2012), folds into a helix upon interaction with Hat2p. Therefore, Hat2p contributes to the substrate specificity for Hat1p/Hat2p by stabilizing the access of H4K12 to the active site of Hat1p. Consistent with this notion, while recombinant human and yeast Hat1 can acetylate both H4K5 and H4K12, we showed that the complex of recombinant Hat1p/Hat2p specifically acetylates H4K12 [Supplemental Fig. S7].

Unexpectedly, we discovered that Hat2p in the Hat1p/Hat2p complex can directly interact with the N-terminal tail of H3 [Fig. 4] in addition to stabilizing the association with substrate H4. The analysis of surface electrostatic potential on Hat2p showed that its H3-binding surface in the WD40 domain is highly negatively charged, which helps the interaction with the basic residues of the H3 N-terminal tail [Fig. 4C,D]. More interestingly, we observed that the binding of H3 to Hat2p depends on the methylation status of H3; i.e., dimethylation of H3R2 abolished the interaction and different methylation status of H3K4 and H3K9 and also affected the peptide-binding affinity [Fig. 4E–G]. Many histone modification enzyme complexes, such as the Nurd/Sin3, MLL/Set1, and PRC2 complexes, contain WD40 repeat proteins as core subunits. Several studies have reported that certain WD40 domains recognize methylated histone tails (Couture et al. 2006; Han et al. 2006; Margueron et al. 2009). It is possible that complementary amino acid changes in the catalytic and WD40 subunits can accommodate their robust association. Alternatively, other subunits in the enzyme complex may facilitate such association (Ahringer 2000; Schmitges et al. 2011; Dharmarajan et al. 2012). Our studies demonstrate that a subset of proteins with WD40 domains could function as a reader for histone methylation to regulate distinct chromatin-associated events. In addition to the methylation of H3R2 and H3K4, the N terminus of H3 can be acetylated in cells on multiple lysine residues [K9, K14, K18, K23, and K27]. Based on the charge complementarity, such acetylation would be predicted to disrupt the interaction between H3 and Hat2p. Therefore, it is tempting to speculate that by discriminating against unmethylated/unacetylated H3 N-terminal tails, Hat2p helps to establish the specificity of Hat1p/Hat2p toward newly synthesized H3/H4 heterodimers and away from heavily modified pre-existing histones. Such a mechanism could be essential for the proper maintenance of histone modification markers during chromatin assembly in DNA replication or transcription.

It is intriguing to note that in a model based on our structures, due to spatial constraint, H3/H4 in the Hat1p/Hat2p/H3/H4 complex cannot adopt the heterodimer conformation like that observed in nucleosomes [Fig. 5]. This is in agreement with the fact that Hat1p cannot acetylate histone H4 in nucleosomes [Klef et al. 1995; Parthun et al. 1996]. It is possible that upon their synthesis in the cytosol, H3/H4 might adopt a transition state conformation, and the association with certain histone chaperone proteins, such as Hif1, helps to stabilize the native heterodimer. Alternatively, Hat1p/Hat2p themselves might function as histone chaperones, and this hypothesis was supported by the fact that H3/H4 remains associated with Hat1p/Hat2p even after acetylation [Imhof and Wolffe 1999, Lusser et al. 1999, Vermaak et al. 1999, Ai and Parthun 2004, Poveda et al. 2004, Wu et al. 2012], and the Hat1p/Hat2p has been reported to interact with the origin replication complex [Suter et al. 2007; Ge et al. 2011]. After acetylation by the Hat1p/Hat2p complex, the H3/H4 heterodimer is then transferred to other chromatin-assembling factors that rearrange the H3/H4 conformation so that it becomes ready to be incorporated into chromatin. For instance, Asf1 has been shown to bind H3/H4 following acetylation and could cause a conformation change of the heterodimer [Zhou et al. 2011]. Further structural and biochemical analyses are required to address these possibilities.

In summary, with the high-resolution structure of the HAT Hat1p with its cofactor, Hat2p, and substrate, H4, we revealed that Hat2p is important for the substrate specificity of Hat1p. We further discovered that although H3 is not a direct substrate for Hat1p/Hat2p, the N-terminal tail of H3 binds with the WD40 domain of Hat2p, and this interaction depends on the different methylated status of H3. Based on the structure of the Hat1p/Hat2p/H3/H4 complex, we propose a model in which the simultaneous binding of H3 and H4 by Hat1p/Hat2p specifies acetylation toward newly synthesized H3/H4 heterodimers [Fig. 5], which could be critical for various chromatin-associated processes.

Materials and methods

Protein expression and purification

Yeast Hat2p (full length) with an engineered N-terminal 6xHis tag was cloned into vector pFastBac 1 (Invitrogen) and expressed...
in Hi-5 insect cells for 36 h at 28°C. After harvesting, Hat2p was initially purified using Ni-NTA (Novagen) and washed three times with buffer containing 25 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 15 mM imidazole. Yeast Hat1p [1–320] with a stop codon at the end was ligated into vector pET30a and expressed in *Escherichia coli* strain BL21 (DE3). The precleared cell extract with overexpressed Hat1p was loaded to the Ni column prepared before, which had bound Hat2p. After an extensive wash, the Hat1p/Hat2p complex was eluted with 25 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 250 mM imidazole and further fractionated by anion ion exchange column chromatography (Source-Q, Pharmacia). In order to obtain the Hat1p/Hat2p/H4 peptide complex, the purified Hat1p/Hat2p complex was applied to a column containing GST-H4_{1–48} bound to glutathione sepharose (Pharmacia), which was overexpressed in Rosetta (DE3).

After washing with 25 mM Tris-HCl (pH 8.0) and 150 mM NaCl, the Hat1p/Hat2p/H4_{1–48} complex was released by cleavage of histone H4 from GST using PreScission protease. The symmetry was further purified by size exclusion gel filtration chromatography (Superdex-200, Pharmacia). For crystallization, the complex was finally prepared in a buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 3 mM DTT.

The Hat1p/Hat2p/H4_{1–48}/H3_{1–15} complex was obtained by mixing Hat1p/Hat2p/H4_{1–48} with H3_{1–15} in 1:10 molar ratio.

**Crystallography, data collection, and structure determination**

Before crystallization, CoA was added to the Hat1p/Hat2p/H4_{1–48} complex and the Hat1/Hat2/H4_{1–48}/H3_{1–15} complex solutions with 2:1 molar ratio. Crystals of the Hat1p/Hat2p/H4_{1–48}/CoA complex or the Hat1p/Hat2p/H4_{1–48}/CoA complex were generated by mixing 1 μL of protein solution with 1 μL of well buffer using the hanging drop vapor diffusion method at 4°C. Crystals were obtained after 1 wk. The crystals were cryoprotected in reservoir solution containing 15–20% glycerol and flash-frozen in liquid nitrogen prior to data collection.

The Hat1p/Hat2p/H4_{7–46}/CoA complex structure was solved using molecular replacement with the Hat1 (PDB ID code 1BOB) structure and the RbpAp46/RbpAp48 (PDB ID code 3CFV or 3CSF) structure as search models. All of the data were collected at the Shanghai Synchrotron Radiation Facility BL17U and integrated and scaled using the HKL2000 package (Otwinowski and Minor 1997). Further processing was carried out using programs from the CCP4 suite (Collaborative Computational Project, Number 4 1994). Data collection statistics are summarized in Supplemental Table S1. The final model rebuilding was performed using COOT (Emsley and Cowtan 2004), and the protein structure was refined with PHENIX (Adams et al. 2002) using noncrystallographic symmetry restraints. The structure factors of the Hat1p/Hat2p/H4_{7–46}/CoA complex were deposited in the PDB (ID code 4PSW). The structure of the Hat1p/Hat2p/H4_{1–48}/CoA complex was solved using molecular replacement with the structure of the Hat1p/Hat2p/H4_{1–48}/CoA complex as a model, and the structures were deposited in the PDB (ID code 4PSX).

**In vitro acetylation assays and mass spectrometry (MS)**

In vitro HAT reactions were performed for 1 h at 30°C in a 50-μL reaction mixture containing ~8 μg of the Hat1p/Hat2p/H4_{1–48} complex, 1 mM AcCoA (Sigma), 5 mM nicotinamide (Sigma), 1 mM PMSE, and 1 mM DTT in HAT buffer (final 50 mM Tris-HCl at pH 7.5, 5% glycerol, 0.1 mM EDTA, 50 mM KCl) (Grant et al. 1997). For identification of acetylated lysine, samples were separated on one-dimensional SDS-PAGE under the reducing condition. The gel bands corresponding to the targeted protein were excised followed by incubation with 50 mM DTT for 30 min at 55°C, treated by 25 mM indole-3-acyclic acid (IAA) for 20 min at room temperature in the dark, and digested by the sequence grade modified trypsin (Promega) in 50 mM ammonium bicarbonate overnight at 37°C. The peptides were extracted twice with 0.1% trifluoroacetic acid in 50% acetonitrile aqueous solution for 30 min. The extractions were then centrifuged in a speedvac to reduce the volume. For liquid chromatography-tandem MS (LC-MS/MS) analysis, the digestion product was separated by a 65-min gradient elution at a flow rate of 0.250 μL/min by using the EASY-nLCII integrated nano-high-performance LC (nano-HPLC) system (Proxeon), which was directly interfaced with the Thermo LTQ-Orbitrap mass spectrometer. The analytical column was a homemade fused silica capillary column (75 μm inner diameter, 150 mm length, Upchurch) packed with C-18 resin (300 Å, 5 μm, Varian). Mobile phase A consisted of 0.1% formic acid, and mobile phase B consisted of 100% acetonitrile and 0.1% formic acid. The LTQ-Orbitrap mass spectrometer was operated in the data-dependent acquisition mode using Xcalibur 2.1 software, and there was a single full-scan mass spectrum in the Orbitrap (400–1800 m/z, 30,000 resolution) followed by 15 data-dependent MS/MS scans in the ion trap at 35% normalized collision energy. The MS/MS spectra from each LC-MS/MS run were searched against the selected database using a Proteome Discovery searching algorithm.

**ITC**

Calorimetric experiments were conducted at 25°C with a MicroCal ITC200 instrument (GE Healthcare). The protein samples [Hat1p, Hat1pΔLP1, Hat2p, and the Hat1p/Hat2p/H4_{1–48} complex] were dialyzed against the buffer containing 150 mM NaCl and 20 mM HEPES (pH 7.5). The data were fitted using the Origin 7 software package (Microcal).

**Pull-down assay**

Hat1p, Hat2p, and H4 mutations were generated by two-step PCR. Hat1p and H4 mutants were cloned into the pGEX6p-1 (Novagen) vector and purified as wild type. Hat2p mutants were cloned into pFASTBAC-1 and purified as wild type. For GST pull-down experiments, GST-H4-bound glutathione sepharose beads (2.0 mg of protein each; GE Healthcare) were incubated with equal volumes of protein lysate. The gel bands corresponding to the targeted protein were excised followed by incubation with 50 mM DTT for 30 min at 55°C, treated by 25 mM indole-3-acyclic acid (IAA) for 20 min at room temperature in the dark, and digested by the sequence grade modified trypsin (Promega) in 50 mM ammonium bicarbonate overnight at 37°C. The peptides were extracted twice with 0.1% trifluoroacetic acid in 50% acetonitrile aqueous solution for 30 min. The extractions were then centrifuged in a speedvac to reduce the volume. For liquid chromatography-tandem MS (LC-MS/MS) analysis, the digestion product was separated by a 65-min gradient elution at a flow rate of 0.250 μL/min by using the EASY-nLCII integrated nano-high-performance LC (nano-HPLC) system (Proxeon), which was directly interfaced with the Thermo LTQ-Orbitrap mass spectrometer. The analytical column was a homemade fused silica capillary column (75 μm inner diameter, 150 mm length, Upchurch) packed with C-18 resin (300 Å, 5 μm, Varian). Mobile phase A consisted of 0.1% formic acid, and mobile phase B consisted of 100% acetonitrile and 0.1% formic acid. The LTQ-Orbitrap mass spectrometer was operated in the data-dependent acquisition mode using Xcalibur 2.1 software, and there was a single full-scan mass spectrum in the Orbitrap (400–1800 m/z, 30,000 resolution) followed by 15 data-dependent MS/MS scans in the ion trap at 35% normalized collision energy. The MS/MS spectra from each LC-MS/MS run were searched against the selected database using a Proteome Discovery searching algorithm.

**DNA damage sensitivity assay**

A fragment containing the *HAT1* ORF plus 1 kb of the upstream and downstream sequence was cloned into pRS426 [pRS426-*HAT1*]. *HAT1* alleles were constructed by site-directed mutagenesis using pRS426 as the template. The *HAT1* expression plasmids were transformed into UCC6584 (wild type) and MYP201 (hat1Δ) (Kelly et al. 2000). These strains also express an H3K9,14R allele as the sole copy of histone H3. Cells were
grown in liquid synthetic medium lacking uracil to retain the HAT1 expression vector, and an equal quantity of cells were harvested from each strain. Tenfold serial dilutions were made, and cells were spotted on synthetic medium in the absence or presence of methyl methane sulfonate [MMS].

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