Human Histone Acetyltransferase 1 Protein Preferentially Acetylates H4 Histone Molecules in H3.1-H4 over H3.3-H4*5

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Background: Hat1-RbAp46 acetylates H4 lysine 5 and 12 residues. Results: Hat1-RbAp46 acetylates H4 in H3.1-H4 complex more efficiently than that in H3.3-H4 complex. Conclusion: Hat1-RbAp46 differentially acetylates H4 in H3.1-H4 and H3.3-H4 and impacts nucleosome assembly of H3.1 and H3.3 differently. Significance: Determination of how Hat1-RbAp46 impacts nucleosome assembly of H3.1 and H3.3 differently will help understand how chromatin states are inherited during S phase of the cell cycle.

In mammalian cells, canonical histone H3 (H3.1) and H3 variant (H3.3) differ by five amino acids and are assembled, along with histone H4, into nucleosomes via distinct nucleosome assembly pathways. H3.1-H4 molecules are assembled by histone chaperone CAF-1 in a replication-coupled process, whereas H3.3-H4 are assembled via HIRA in a replication-independent pathway. Newly synthesized histone H4 is acetylated at lysine 5 and 12 (H4K5,12) by histone acetyltransferase 1 (HAT1). However, it remains unclear whether HAT1 and H4K5,12ac differentially regulate these two nucleosome assembly processes. Here, we show that HAT1 binds and acetylates H4 in H3.1-H4 molecules preferentially over H4 in H3.3-H4. Depletion of Hat1, the catalytic subunit of HAT1 complex, results in reduced H3.1 occupancy at H3.1-enriched genes and reduced association of Importin 4 with H3.1, but not H3.3. Finally, depletion of Hat1 or CAF-1 p150 leads to changes in expression of a H3.1-enriched gene. These results indicate that Hat1 differentially impacts nucleosome assembly of H3.1-H4 and H3.3-H4.

In eukaryotes, DNA is compacted to form chromatin by intimate interactions between DNA and histone proteins. The fundamental repeating unit of chromatin is the nucleosome particle, which is composed of a hetero-octamer of histones enfolded by 147 bp of DNA. Each histone octamer consists of a conserved three-subunit complex, consisting of three polypeptides with apparent masses of 150, 60, and 48 kDa (6). In human cells, CAF-1 is essential for replication-coupled nucleosome assembly (7). In contrast, H3.3 is assembled into chromatin throughout the cell cycle in a replication-independent manner (5). H3.3 is enriched at genic regions including both actively transcribed and silent genes (9, 10). In addition, H3.3 has also been found at pericentric heterochromatin in mouse cells (9). The distinct localizations of H3.3 at chromatin are likely mediated by different histone chaperones that assemble H3.3-H4 into nucleosomes as histone chaperone HIRA is required for the localization of H3.3 at genic regions, whereas Daxx/ATRX is needed for the localization of H3.3 at pericentric heterochromatin (9, 11).

Mutational analysis indicates that three residues (87, 89, and 90) of H3.1 and H3.3 are necessary for specifying distinct chromatin localization and nucleosome assembly pathways of H3.1 and H3.3 (8, 9). Because of limited sequence divergence between H3.1 and H3.3, it is likely that other factors/proteins are also involved in the regulation of nucleosome assembly pathways of H3.1 and H3.3. Indeed, we and others have shown that acetylation of newly synthesized H3 plays an important role in regulating CAF-1-mediated nucleosome assembly following DNA replication and DNA repair in budding yeast (12–14). Furthermore, phosphorylation of histone H4 serine 47 promotes HIRA-mediated nucleosome assembly of H3.3-H4 in mammalian cells (15). Thus, modifications on new H3 and H4

*5 This work is supported, in whole or in part, by National Institutes of Health Grant GM81838.
5 This article contains supplemental Figs. S1–S6, Table 1, Experimental Procedures, and an additional reference.
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6 The abbreviations used are: CAF-1, chromatin assembly factor 1; H4K5,12ac, H4 acetylated at lysine residues 5 and 12; TSS, transcription start site; TTS, transcription terminal site; HIRA, histone cell cycle regulation-defective homolog A.
also contribute to specifying H3.1 and H3.3 into different assembly pathways.

Newly synthesized H4 is acetylated at lysine residues 5 and 12 (H4K5,12ac), and this acetylation is conserved from yeast to human cells (16). Acetylation of H4K5,12 is catalyzed by the HAT1 lysine acetyltransferase (17). The HAT1 holoenzyme consists of two subunits, the catalytic subunit Hat1 and the RbAp46 subunit. RbAp46 shares sequence homology to the p48 subunit of CAF-1 (17). Because H4 co-purified with CAF-1 is acetylated at lysine residues 5 and 12, it has been proposed that this modification has a role in regulating CAF-1-mediated nucleosome assembly (18). However, how this modification regulates CAF-1-mediated nucleosome assembly remains unclear. In addition, it has been shown that Hat1-RbAp46 holoenzyme co-purified with both H3.1 and H3.3 (5). It was unknown whether HAT1 regulates nucleosome assembly of both H3.1 and H3.3. Here, we show that HAT1 binds H3.1-H4 tetramer preferentially over H3.3-H4 and acetylates H4 in H3.1-H4 complex more efficiently than H4 in H3.3-H4 complex. Depletion of Hat1 results in a reduced H3.1 occupancy at two H3.1-enriched genes tested. This reduction is likely due to reduced association of H3.1 with Importin 4, which in turn impacts the binding of CAF-1 to H3.1-H4 and cell cycle progression. These results indicate that Hat1 and H4K5,12ac differentially regulate nucleosome assembly of H3.1-H4 and H3.3-H4.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Transfection, and Infection—**293T and HeLa cells were grown in DMEM (Invitrogen) supplemented with 10% FCS and 1% penicillin/streptomycin. Stable cell lines (including those expressing e-H3.1, e-H3.3, each tagged with both the FLAG and HA epitopes, CAPE-1p60 tagged with both FLAG and His, were grown in the presence of 1 μg/ml puromycin. Cells were incubated at 37 °C with 5% CO₂. Transient transfection was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Lentivirus-expressing Hat1 shRNAs was packaged using 293T cells and infected into targeting cells following protocols provided by Sigma-Aldrich.

**Chromatin Immunoprecipitation Assay and Real-time PCR—**2 × 10⁶ cells were cross-linked with 1% (v/v) formaldehyde for 10 min at room temperature and quenched by addition of glycine to a final concentration of 125 mM. Cells were washed with 1×PBS (1× Tris-buffered saline) and then resuspended in 1 ml of lysis buffer (50 mM HEPES, pH 7.5, 1% Triton X-100, 140 mM NaCl, 1 mM EDTA, 0.1% (w/v) sodium deoxycholate, and protease inhibitors). The cell lysis was sonicated in a Bioruptor (Diagenode) to achieve a mean DNA fragment size of 0.5–1 kbp. After clarification by centrifugation, the supernatants were subjected to a melting curve analysis. Primer sequences for genes tested are listed in supplemental Table 1.

**RESULTS**

**HAT1 Binds and Acetylates H4 in H3.1-H4 Complex over H4 in H3.3-H4 Complex Preferentially—**Newly synthesized H4 is acetylated at lysine 5 and 12 by the HAT1 holoenzyme, consisting of Hat1 and RbAp46 subunit (17). To understand whether Hat1 and H4K5,12ac play any roles in nucleosome assembly of H3.1-H4 and H3.3-H4, we first purified e-H3.1 and e-H3.3, each tagged with both the FLAG and HA epitopes, from 293T cells and detected co-purified proteins by Western blotting. In agreement with published reports, histone chaperone CAF-1 bound preferentially with H3.1, whereas histone chaperone HIRA bound preferentially with H3.3 (5). Remarkably, we also observed that significantly more Hat1 and RbAp46 co-purified with e-H3.1 than e-H3.3. In addition, H4K5,12ac was enriched...
on H4 that co-purified with H3.1 compared with H4 co-purified with H3.3 (Fig. 1A). These results suggest that the HAT1 complex binds to H3.1-H4 and H3.3-H4 differently in vivo.

To understand the functional implications of the preferential association of HAT1 with H3.1, we examined how the e-Hat1, exogenously expressed and purified from 293T cells (supplemental Fig. S1A), acetylates histone H4 in H3.1-H4 and H3.3-H4 tetramers in vitro. e-Hat1 acetylated H4K5,12 preferentially in H3.1-H4 over H3.3-H4 tetramers as detected by Western blot analysis of H4K5,12ac (Fig. 1B) and by analyzing 3H-labeled proteins using either a scintillation counter (Fig. 1C) or an autoradiography (Fig. 1D). Thus, HAT1 purified from human cells exhibits activity preferentially for H4 complexed with H3.1 over H3.3. To confirm this result, we purified recombinant Hat1 or Hat1-RbAp46 complex from insect cells (supplemental Fig. S1B). Both recombinant Hat1 and the Hat1-RbAp46 complex exhibited significant more activity toward H3.1-H4 over H3.3-H4 (Fig. 1, E and F). Moreover, recombinant RbAp46 and Hat1-RbAp46 complexes bound to H3.1-H4 preferentially over H3.3-H4 in vitro (Fig. 1G). Finally, relatively less H3-H4 bound to recombinant Hat1 compared with Hat1-RbAp46 complex (Fig. 1G). Because H3.1-H4 and H3.3-H4 were purified through the same procedures and we have previously shown that Pak2 phosphorylates H4 in H3.1-H4 and H3.3-H4 similarly (15), the differences in the ability of HAT1 to acetylate H3.1-H4 and H3.3-H4 complexes are not likely due to differences in contaminated proteins in H3.1-H4 and H3.3-H4.

Supporting this idea, the Ada2-Ada3-Gcn5 complex, which was purified as described under supplemental “Experimental Procedures” acetylated H3.1-H4 and H3.3-H4 equally well (supplemental Fig. S2). Together, these results show that both e-Hat1 purified from human cells and recombinant Hat1-RbAp46 complex bind and acetylate H3.1-H4 molecules preferentially over H3.3-H4.

Hat1 Depletion and Expression of H4K5,12R Mutant Alter H3.1 Occupancy at Candidate Gene Loci—The results described above suggest that Hat1-mediated H4K5,12ac may play a more important role in nucleosome assembly of H3.1-H4. To test this idea, we used a chromatin immunoprecipitation (ChiP) assay to determine how depletion of Hat1 affects H3.1 occupancy at the H3.1-enriched genes. As reported previously, in HeLa cells, e-H3.1 was enriched at the genome regions flanking the transcription start site (TSS) of TRIM42 and the transcription terminal site (TTS) of CSRP3 compared with TM4SF1 and TP53TGI, two H3.3-enriched genes (Fig. 2B) (10, 15). In addition, compared with normal HeLa cells that did not express epitope-tagged H3.1, significantly more H3.1 was detected at the two H3.3 genes in the e-H3.1 cell line (supplemental Fig. S3A). The enrichment of H3.1 at the H3.3-enriched genes may be due to deposition of H3.1 at these H3.3-enriched genes during S phase of the cell cycle. Importantly, depletion ofCAF-1 p150, the large subunit ofCAF-1, resulted in a dramatic reduction in p150 protein levels (Fig. 2A) and reduced e-H3.1 occupancy at the TSS of TRIM42 and TTS of CSRP3 (Fig. 2B). Thus, the H3.1 occupancy at these two inactive genes depends on CAF-1. This result is consistent with the fact that CAF-1 is involved in nucleosome assembly of H3.1-H4 and indicates that this ChiP assay can be used to determine factors involved in nucleosome assembly of H3.1 in vivo. Using this method, we asked how depletion of Hat1 affects H3.1 occupancy at these gene loci. Depletion of Hat1 led to reduced protein levels of Hat1 (Fig. 2C) and reduced H3.1 occupancy at the TSS of TRIM42 and TTS of CSRP3 (Fig. 2D). Interestingly, we also observed a reduction of H3.1 ChiP signal at TP53TGI after CAF-1p150 depletion (Fig. 2B) and at TM4SF1 after Hat1 depletion (Fig. 2D). These two genes are H3.3-enriched genes. These results are consistent with the idea that H3.1 detected at these gene loci may be due to deposition of H3.1 during S phase of the cell cycle.

Next, we examined the effect of CAF-1p150 and Hat1 depletion on the occupancy of H3.3 at these four genes. Remarkably, whereas depletion of CAF-1p150 resulted in significant increase of H3.3 occupancy at all these four genes tested, depletion of Hat1 had only minor effect on H3.3 occupancy at these four genes tested (supplemental Fig. S3, B and C). The differences in CAF-1p150 depletion and Hat1 depletion on the effect of H3.3 may be because depletion of CAF-1p150 may have a more pronounced effect on H3.1 deposition than Hat1 depletion, which in turn needs H3.3 to fill the void of H3.1. Nonetheless, these results suggest that Hat1 is important to regulate nucleosome assembly of H3.1-H4.

Finally, we determined how expression of exogenous H4 without any epitope tag (e-H4) or e-H4K5,12R and Q mutants affects H3.1 occupancy at these candidate genes (Fig. 2E). Although expression of e-H4 or e-H4K5,12Q resulted in a minor increase in H3.1 occupancy compared with empty vector control at both H3.1 and H3.3-enriched genes tested, expression of the e-H4K5,12R mutant led to a reduction of H3.1 occupancy compared with expression of the e-H4 or e-H4K5,12Q mutant (Fig. 2F). These results are consistent with the idea that H4K5,12ac catalyzed by the HAT1 holoenzyme regulates nucleosome assembly of H3.1.

Depletion of Hat1 Results in Reduced Association of H3.1-H4 with CAF-1 in Vivo—To understand how depletion of Hat1 and H4K5,12R affects H3.1 occupancy, we first examined how depletion of Hat1 affects the association of CAF-1 with H3.1-H4. To do this, we purified CAF-1 from 293T cells stably expressing CAF-1 p60 (e-p60) with or without Hat1 depletion and detected co-purified histones by Western blotting. Depletion of Hat1 resulted in a significant reduction of H3 and H4 co-purified with CAF-1 (Fig. 3A). In a reciprocal immunoprecipitation, less CAF-1 (as detected by both p150 and p60 subunits) co-purified with e-H3.1 from Hat1-depleted cells than those from control cells. In contrast, depletion of Hat1 had no apparent effect on the amounts of Daxx that co-purified with e-H3.3 (Fig. 3B). Daxx is another histone chaperone for histone H3.3. These results are consistent with the idea that Hat1 regulates nucleosome assembly of H3.1-H4 preferentially over H3.3-H4.

H4K5,12ac Negatively Regulates Association of H3.1-H4 with CAF-1 in Vitro—One explanation for the reduced association of CAF-1 for H3.1-H4 in Hat1-depleted cells is that H4K5,12ac increases the binding affinity of CAF-1 toward H3.1-H4. To test this idea, we determined how H4K5,12ac impacts the association of CAF-1 with H3.1-H4 and H3.3-H4. To obtain H3.1-H4 or H3.3-H4 with or without H4K5,12ac, recombinant H3.1-H4
FIGURE 1. HAT1 acetylates H4 in H3.1-H4 complex more efficiently than H4 in H3.3-H4. A, more HAT1 co-purified with H3.1 than H3.3 in vivo. H3.1 and H3.3 were immunoprecipitated from 293T cells stably expressing e-H3.1 and e-H3.3, each tagged with both FLAG and HA epitopes. As a negative control (Con), the same purification procedures were performed using normal 293T cells. Proteins in whole cell extract (Input) and immunoprecipitation (IP) were analyzed by Western blotting using the indicated antibodies.

B–D, HAT1 purified from 293T cells acetylates H3.1-H4 more efficiently than H3.3-H4. e-HAT1 purified from Escherichia coli in the presence of acetyl-CoA. The reaction mixtures were resolved on SDS-PAGE. H3 and acetylated H4 were detected by Western blotting using antibodies against H3 and H4K5,12ac, respectively (B). N.C., negative control without acetyl-CoA. The experiments in C and D were performed as described above except that [3H]acetyl-CoA was used in addition to titration of different amounts of H3.1-H4 or H3.3-H4. Half of each reaction mixture was used for scintillation counting (C), and the remaining half was resolved on SDS-PAGE, and the acetylated proteins were detected by autoradiography (D, upper panel) and total histones were detected by staining with Coomassie Brilliant Blue (CBB, D, lower panel).

E and F, recombinant Hat1 (E) or Hat1-RbAp46 (F) exhibits higher activity toward H3.1-H4 than H3.3-H4. The histone acetyltransferase assays were performed as described above except that recombinant proteins were used. G, Hat1 or Hat1-RbAp46 holoenzyme complex binds H3.1-H4 more efficiently than H3.3-H4. His6-tagged-Hat1-RbAp46 complex, His6-Hat1, or His6-RbAp46 was used to pull down two different amounts of H3.1-H4 or H3.3-H4, respectively. Western blotting was used to detect H3 and the His6 tag of each protein. N.C., negative control without addition of His-tagged proteins.
or H3.3-H4 tetramers were mixed with HAT1 with or without acetyl-CoA, the co-factor for acetylation. CAF-1 purified from human cells (supplemental Fig. S4) was used to bind H3.1-H4 in these reaction mixtures. As shown in Fig. 3C, CAF-1 preferentially bound H3.1-H4 tetramers over H3.3-H4 tetramers (compare lanes 5–6 with lanes 9–10), providing an explanation of why CAF-1 assembles H3.1-H4 into nucleosomes. Remarkably, acetylation of H4K5,12 by HAT1 reduced the binding of CAF-1 to H3.1-H4 in vitro (compare lanes 5–6 with lanes 7–8). This result suggests that H4K5,12ac negatively regulates the binding of CAF-1 with H3.1-H4 and that the reduction in the association of H3-H4 with CAF-1 observed in Hat1-depleted cells is not likely due to reduced binding affinity of CAF-1 for H3.1-H4 with unmodified H4K5,12 in Hat1-depleted cells. Thus, unlike acetylation at the H3 N terminus and acetylation of H3 lysine 56 that increase the binding affinity of CAF-1 with H3-H4 in vitro in budding yeast cells (13, 14), H4K5,12ac has a negative impact on the association of CAF-1 with H3-H4 in mammalian cells.

Depletion of Hat1 Results in Reduced Association of H.1-H4 with Importin 4—Newly synthesized H4 is acetylated at H4K5,12 before nuclear import (19). Moreover, this acetylation likely regulates nuclear import of H4 (20, 21). Therefore, the compromised association of CAF-1 with H3-H4 in Hat1-depleted cells could be due to the impaired nuclear import of H3.1-H4. To test these ideas, we first determined how Hat1 depletion affects the association of H3.1 with Importin 4, which is known to bind H3.1 and H3.3 (5). Significant less Importin 4 and Asf1a/Asf1b (another H3-H4 histone chaperone) co-purified with e-H3.1 from Hat1-depleted cells than control cells (compare lane 4 with lane 3). In contrast, Hat1 depletion had little detectable effect on the amounts of Asf1a and Importin 4 that co-purified with e-H3.3 (Fig. 4A). These results are consistent with the idea that Hat1 and possibly H4K5,12ac regulate the nuclear import of H3.1-H4, and thereby regulating nucleosome assembly of H3.1-H4.

To provide additional support to the idea that H4K5,12ac is important to regulate the association of Importin 4 and Asf1a/Asf1b, we replaced lysine 5 and 12 with arginine (H4K5,12R), which mimics the deacetylated form of H4K5,12, or glutamine (H4K5,12Q), and determined how these mutations affect the association of Importin 4 and Asf1a/Asf1b with H4. Significantly less Asf1b, Importin 4, and CAF-1 co-purified with e-H4K5,12R compared with the wild-type H4 control. In contrast, replacement K5,12 with glutamine had no apparent effect on the association of Importin 4, CAF-1, or Asf1 with H4 (Fig. 4B). In addition, the H4K5,12R mutant did not affect the association of H4 with Daxx to a significant degree. Because expression of the e-H4 or e-H4 mutants had no apparent effect on cell cycle progression (supplemental Fig. S5), we suggest that H4K5,12ac catalyzed by HAT1 directly regulates the interactions between Importin 4 with H3.1-H4, but not H3.3-H4, which in turn impacts the association of CAF-1 with H3.1-H4 in cells.

To determine the functional consequence of reduced association of H4K5,12R with Importin 4 and CAF-1, we transiently transfected HeLa with a plasmid for expression of e-H4,
e-H4K5,12R, or e-H4K5,12 Q and analyzed chromatin binding of these H4 proteins. e-H4K5,12R proteins exhibited a significant reduction in chromatin binding compared with e-H4, whereas the e-H4K5,12Q mutant exhibited slightly higher chromatin binding than e-H4 (Fig. 4, C and D). The differences in chromatin binding among e-H4, e-H4K5,12R, and
e-H4K5,12Q mutants were not likely due to the differences in their expression levels (Fig. 4E). These results are consistent with the idea that H4K5,12ac regulates nucleosome assembly via nuclear import.

**Effect of Depletion of Hat1 and CAF-1 on Gene Expression**—To determine whether depletion of Hat1 and CAF-1 affects gene expression, we first analyzed how depletion of Hat1 affects expression of three H3.3-enriched genes (TP53TG1, TM4SF1, and OSTF1) and two H3.1-enriched and silenced genes (CSRP3 and TRIM42). As controls, we also analyzed the expression of Hat1. Depletion of Hat1 resulted in a significant reduction of Hat1 mRNA (Fig. 5A) and had no apparent effect on the gene expression of the three H3.3-enriched genes tested. In contrast, depletion of Hat1 resulted in a 2-fold increase in expression of CSRP3, a H3.1-enriched gene (Fig. 5A). The effect of Hat1 depletion on TRIM42 expression was not detectable because of low expression of this gene (data not shown). Similarly, depletion of CAF-1p150 resulted in a 2-fold increase in expression of CSRP3 and had no apparent effect on the expression of the three H3.3-enriched genes tested (Fig. 5B). These results indicate that alterations in nucleosome assembly of H3.1 via Hat1 or CAF-1p150 depletion can lead to changes in gene expression of some, but not all H3.1-enriched genes.

**Depletion of Hat1 Affects Cell Cycle Progression**—The H3.1 is deposited during S phase of the cell cycle. We also determined whether depletion of Hat1 affects cell cycle progression. We observed significant more cells were accumulated at G1 with a concomitant reduction in S phase cells after Hat1 depletion in HeLa cells. For instance, 48 h after virus infection for Hat1 depletion, the percentage of G1 phase increased, whereas the percentage of S phase decreased in Hat1-depleted cells compared with nontargeting control cells (supplemental Fig. S6). Thus, like CAF-1 depletion (7), depletion of Hat1 also affects cell cycle progression.

**DISCUSSION**

Despite limited sequence divergence, H3.1 and H3.3 are assembled into nucleosomes by different histone chaperones, suggesting that nucleosome assembly of H3.1-H4 and H3.3-H4 must also be regulated by multiple means. Here, we report that the HAT1 holoenzyme is a factor that preferentially promotes nucleosome assembly of H3.1-H4 over H3.3-H4. We show that...
Role of HAT1 in Nucleosome Assembly

HAT1 preferentially binds H3.1-H4 and acetylates H3.1-H4 over H3.3-H4 in vivo and in vitro. These results indicate that the five H3.1 specific amino acids not only regulate the interactions between H3.1-H4 with CAF-1, but also contribute to specific recognition of H3.1 by the HAT1 holoenzyme. The HAT1 complex consists of two subunits, the catalytic subunit Hat1 and RbAp46. Our results suggest that RbAp46 contributes to the recognition of H3.1 over H3.3. RbAp46 shares 96% sequence identity with RbAp48 subunit of CAF-1 (17). Therefore, it is possible that although CAF-1p48 subunit is known to bind H4 (22, 23), the CAF-1p48 subunit may also contribute to the recognition of H3.1-specific residues.

We present several lines of evidence supporting the idea that Hat1 regulates nucleosome assembly of H3.1-H4 by, in part, regulating nuclear import of H3.1-H4. Supporting this conclusion, we show that depletion of Hat1 results in reduced binding of Importin 4 to H3.1, but not H3.3. Moreover, replacement of H4K5,12 with arginine results in a significant reduction in the association of Importin 4 with H4. While our study was under review, Alvarez et al. showed that acetylation of H4K5,12 is important for nuclear import of H4 (20). We suggest that preferential acetylation of H4K5,12 in H3.1-H4 complex over that in H3.3-H4 complex by HAT1 allows more efficient nuclear import of H3.1 and thereby preferentially regulating nucleosome assembly of H3.1-H4.

It has been shown that H4K5,12R, but not H4K5,12Q, promotes import of H4 in yeast cells. It is proposed that H4K5,12ac may promote discharge of Importin from H4 in the nucleus (24). This result appears to contradict to our results and others showing that H4K5,12ac promotes nuclear import of H4 in mammalian cells. The differential effect of H4K5,12ac on nuclear import may be due to the fact that yeast and mammalian cells contain different forms of H3. In budding yeast, there is only one form of histone H3, which is similar to H3.3 in sequence in mammalian cells. Thus, although H4K5,12ac is found on new H4 in both yeast and mammalian cells (16), how this modification regulates nucleosome assembly of H3-H4 may differ in yeast and mammalian cells.

Another unexpected observation we made is that HAT1 and H4K5,12ac negatively regulate the association of CAF-1 with H3.1-H4 in vitro. H4 co-purified with CAF-1 is known to contain different acetylated forms, including zero-, mono-, and diacetylation (18). However, the function of acetylated forms of H4 co-purified with CAF-1 was not clear. In vitro, CAF-1 purified from human cells binds H3.1-H4 less efficiently when H4 is acetylated by the HAT1 holoenzyme. We suggest that the reduced binding of CAF-1 to H3.1-H4 acetylated at H4K5,12ac may allow CAF-1 to dissociate from H3.1-H4 more readily once the CAF-1-H3.1-H4 complex is recruited to DNA, and thereby facilitating assembly of H3.1-H4 into nucleosomes. Consistent with this idea, it has been shown that CAF-1 dissociates from replicating chromatin before deacetylation of H4K5,12ac (25).

What is the functional consequence for a lack of regulation of nucleosome assembly of H3.1-H4 by Hat1? We show that depletion of Hat1 leads to reduced H3.1 occupancy and increased expression of CSRP3, a H3.1-enriched gene. Similar results were also obtained for depletion of CAF-1p150. Interestingly, although depletion of Hat1 and CAF-1 results in reduced H3.1 occupancy at TRIM42, the expression of this H3.1-enriched gene was not detectable, suggesting that Hat1 or CAF-1p150 depletion had not altered the expression of this H3.1-enriched gene significantly. These results suggest that Hat1 and CAF-1 are important to maintain gene expression state of some, but not all H3.1-enriched genes. Interestingly, it has been observed that H3.1 can complement H3.3 for regulation of gene transcription of some, but not all genes marked by H3.3 (26). Future studies are needed to determine why this is the case.
Role of HAT1 in Nucleosome Assembly

Acknowledgments—We thank Dr. Alain Verreault for various antibodies and plasmids used in this study and Dr. Bob Kingston for baculovirus for expression of Hat1 and RbAp46/p48. We also thank Dr. Song for the plasmid used for purification of the Ada2-Ada3-Gcn5 complex.

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