The Histone Chaperone Anti-silencing Function 1 Stimulates the Acetylation of Newly Synthesized Histone H3 in S-phase*§

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Anti-silencing function 1 (Asf1) is a highly conserved chaperone of histones H3/H4 that assembles or disassembles chromatin during transcription, replication, and repair. We have found that budding yeast lacking Asf1 has greatly reduced levels of histone H3 acetylated at lysine 9. Lysine 9 is acetylated on newly synthesized budding yeast histone H3 prior to its assembly onto newly replicated DNA. Accordingly, we found that the vast majority of H3 Lys-9 acetylation peaked in S-phase, and this S-phase peak of H3 lysine 9 acetylation was absent in yeast lacking Asf1. By contrast, deletion of ASF1 has no effect on the S-phase specific peak of H4 lysine 12 acetylation; another modification carried by newly synthesized histones prior to chromatin assembly. We show that Gcn5 is the histone acetyltransferase responsible for the S-phase-specific peak of H3 lysine 9 acetylation. Strikingly, overexpression of Asf1 leads to greatly increased levels of H3 on acetylation on lysine 56 and Gcn5-dependent acetylation on lysine 9. Analysis of a panel of Asf1 mutations that modulate the ability of Asf1 to bind to histones H3/H4 demonstrates that the histone binding activity of Asf1 is required for the acetylation of Lys-9 and Lys-56 on newly synthesized H3. These results demonstrate that Asf1 does not affect the stability of the newly synthesized histones per se, but instead histone binding by Asf1 promotes the efficient acetylation of specific residues of newly synthesized histone H3.

The eukaryotic genome is packaged into a nucleoprotein structure known as chromatin. The basic repeating unit of chromatin, the nucleosome, is made up of 147 base pairs of DNA wrapped around a histone octamer (two molecules each of histone proteins H2A, H2B, H3, and H4) (1). Chromatin provides a formidable obstacle to the cellular machinery gaining access to the DNA. Indeed, it is becoming apparent that chromatin is a highly dynamic structure that tightly regulates all nuclear processes that use DNA as a substrate; including transcription, DNA replication, repair, and recombination (2–4). Thus, the mechanisms by which chromatin structures are made and modified are fundamental questions of broad interest.

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TABLE 1
Yeast strains used in this study

| Yeast Strain | Description |
|--------------|-------------|
| JCY0011      | W303a MAT a ade2-1; trpl-1; can1-100; leu2-3,112; his3-11,15; ura3; GAL + psi; GCN5::9myc::TRP1; asf1::his5 + (this study) |
| JKT0010      | W303a MAT a; his3-11; leu2-3,112; lys2; trpl-1; ura3-1; bar1::LEU2 (Ref. 35) |
| JKT0018      | W303a MAT a; his3-11; leu2-3,112; lys2; trpl-1; ura3-1; bar1::LEU2; asf1::his5 + (Ref. 35) |
| MAY0009      | W303a MAT a; ade2-1; can1-100; his3-11; leu2-3,112; trpl-1; ura3-1; hht1-hhf1::KAN; hht2-hhf2::NAT; asf1::his5 +; pH57 [HTA1 HTB1 hht2d(1-27) HHT2 LEU2 CEN]; (pASF1:ASF1 URA3) (this study) |
| MAY0010      | W303a MAT a; ade2-1; can1-100; his3-11; leu2-3,112; trpl-1; ura3-1; hht1-hhf1::KAN; hht2-hhf2::NAT; asf1::his5 +; pH57 [HTA1 HTB1 HHT2 LEU2 CEN]; (pASF1:ASF1 URA3) (this study) |
| MAY0022      | W303a MAT a; his3-11; leu2-3,112; trpl-1; ura3-1; asf1::his5 + (pASF1:6HIS URA3) (this study) |
| MAY0023      | W303 MAT a; his3-11; leu2-3,112; trpl-1; ura3-1; asf1::his5 + (pGAL:ASF1::6HIS:V5 URA3) (this study) |
| MAY0024      | W303a MAT a; ade2-1; can1-100; his3-11; leu2-3,112; trpl-1; ura3-1; hht1-hhf1::KAN; hht2-hhf2::NAT; asf1::his5 +; pH57 [HTA1 HTB1 HHT2 LEU2 CEN]; (pASF1:ASF1 URA3) (this study) |
| MAY0038      | W303a MAT a; his3-11; leu2-3,112; lys2; trpl-1; ura3-1; bar1::LEU2; gen5::KAN; asf1::his5 + (this study) |
| MAY0014      | W303a MAT a; his3-11; leu2-3,112; lys2; trpl-1; ura3-1; bar1::LEU2; gen5::KAN; asf1::his5 + (this study) |
| ROY1169      | W303 MAT a; ade2-1; lys2; can1-100; his3-11; trpl-1; ura3-1; caj1::LEU2; hht1-hhf1::KAN; hht2-hhf2::NAT; TELV5::URA3; HMRa::ADE2; can1-100 (Ref. 6) |
| Z1466        | W303a DNA a ade2-1; trpl-1; can1-100; leu2-3,112; his3-11,15; ura3; GAL + psi; GCN5::myc::TRP1(36) |
| JCY0025      | S288c MAT a; ura3::his3Δ his3Δ met15A (pGAL1Asf1 URA3) |
| JCY0026      | S288c MAT a; ura3::his3Δ his3Δ met15A (pGAL1Asf1 URA3) |
| JCY0027      | S288c MAT a; ura3::his3Δ his3Δ met15A gen5::KAN X6 (pGAL1Asf1 URA3) |
| JCY0028      | S288c MAT a; ura3::his3Δ his3Δ met15A sp10::KanMX6 (pGAL1Asf1 URA3) |

Recent evidence also indicates that lysine 56 in the first α helix of H3 is acetylated on newly synthesized histone H3 (15). This modification is predicted to allow a looser interaction of the histones with DNA, suggesting that it may contribute to chromatin assembly (15, 16). Interestingly, recent work has indicated that deletion of the gene encoding the histone chaperone Asf1 greatly reduces the levels of acetylation of H3 on lysine 56 (17, 18). Similarly, our previous work had found that yeast lacking the histone chaperone Asf1 has greatly reduced levels of acetylation on lysine 9 of histone H3 (19).

To understand how the histone chaperone Asf1 is leading to normal levels of acetylation on lysines 9 and 56 of histone H3, we further investigated the relationship between Asf1 and histone H3 acetylation. We show that Asf1 is not required for stabilization of the newly synthesized histones per se, but instead Asf1 promotes the Gcn5-mediated S-phase peak of acetylation of lysine 9 on newly synthesized histone H3. Consistent with this result, overexpression of Asf1 results in greatly increased acetylation of lysines 9 and 56. The role of Asf1 in modulating acetylation of nascent H3 is dependent on its ability to bind to histones H3/H4. As such, we propose that Asf1 properly presents the newly synthesized histone H3 for acetylation by Gcn5 and the histone acetyltransferase (HAT) mediating Lys-56 acetylation prior to chromatin assembly.

MATERIALS AND METHODS

Yeast Strains and Growth—All strains were made using standard yeast molecular genetics techniques and are listed in Table 1. All yeast growth was in yeast extract peptone supplemented with 2% dextrose or galactose if stated as such in the figure legend. All analyses were performed in the logarithmic phase of growth. URA3 plasmids were maintained by growth in minimal medium lacking uracil. Cell cycle arrest of BARI-deleted strains was achieved by addition of α-factor to a final concentration of 15 nm for multiple hours. Arrest was assessed by visual inspection at the time of the experiment and confirmed by flow cytometry analysis of propidium iodide stained cells. Release from arrest was achieved by washing the cells and resuspending in fresh medium containing Pronase.

Western Analysis—Equivalent numbers of cells (1 × 10^7) grown in log phase were centrifuged and washed in 1-volume ddH2O. The pellets were resuspended in 75 μl of sample buffer (0.06 M Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (v/v) SDS, 5% (v/v) 2-mercaptoethanol, and 0.0025% (w/v) bromphenol blue). 10 μl of each whole cell extract was electrophoresed on a 15% SDS-polyacrylamide gel and transferred to nitrocellulose for immunoblotting. The following antibodies were used for Western blot analysis: anti-His (Qiagen, 34660), H3 (Abcam, ab-1791), H4 (Cell Signaling, 2592), AcH3K9 (Cell Signaling, 9671), AcH3K9K14 (Upstate, 15-599), AcH4 (Upstate, 06-933), AcH4K12 (Cell Signaling, 2591), and AcH3K56 (Upstate, 07-677). Secondary antibodies conjugated to horseradish peroxidase were detected using enhanced chemiluminescence (Amersham Biosciences) followed by exposure to autoradiography film. The linearity of the signal was verified by taking a range of exposures for all blots shown to ensure that the presented images were not subjected to saturation.

RESULTS

We have previously shown that the steady-state acetylation level on histone H3 lysine 9, but not on the other residues in the N-terminal tails of H3 and H4, was greatly reduced in asynchronously growing yeast deleted for the gene encoding the histone chaperone Asf1 (19) (Fig. 1A). This reduced acetylation on histone H3 Lys-9 upon deletion of ASF1 was observed in multiple independently derived strains, indicating that it was not because of a second site mutation in the strain background (data not shown). Histone acetylation on lysine 9 can be divided into two types: (a) deposition-specific acetylation that occurs on nascent newly synthesized histones prior to chromatin assembly; and (b) acetylation on chromatin, such as during the processes of transcriptional regulation. To understand whether one or both types of lysine 9 acetylation were reduced in the absence of Asf1, we examined H3 lysine 9 acetylation through the cell cycle. We arrested yeast deleted for ASF1 (asf1Δ) and wild type yeast in G1 phase of the cell cycle with α-factor, then released them from the arrest, and took samples through the subsequent cell cycle (Fig. 1A). We assigned the approximate phase in the cell cycle from flow cytometry analysis of DNA content (Fig.
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FIGURE 1. Asf1 is required for the S-phase peak of H3 lysine 9 acetylation. A, acetylation of H3 Lys-9 was examined in WT (JKT0010) and asf1Δ (JKT0018) yeast. Asy refers to asynchronous cells, whereas mins refers to the number of minutes following release from α-factor. Equal amounts of whole cell extracts in the specified part of the cell cycle, determined by the flow cytometry analysis of DNA content on the right, were electrophoresed on 15% SDS gels and transferred to nitrocellulose. Wild type asynchronous samples were loaded onto each gel as a comparison. The membranes were probed with antibodies specific to H3 and acetylated H3 Lys-9. B, schematic shown on the left indicates the locations on plates of strains included in the analysis. H4TailΔpASF1(MAY0023), H4TailΔasf1ΔΔpASF1(MAY0009), H3TailΔpASF1(MAY0024), and H3TailΔasf1ΔΔpASF1(MAY0010) strains were streaked on medium lacking uracil (−Ura) as a control for growth and on 5'-fluoroorotic acid (5'-FOA) to test for viability. All strains contain a plasmid that has a copy of the ASF1 gene on a URA3-based plasmid. 5'-FOA was used to counter-select against the URA3 plasmid.

1A). Upon Western blotting total cell extracts for acetylated H3 lysine 9 through the cell cycle, we observed a striking peak of acetylation correlating with S-phase in the wild type cells (Fig. 1A), which presumably reflected the deposition-specific acetylation of H3 Lys-9. This result shows that the vast majority of H3 Lys-9 acetylation in the cell occurs in an S-phase-specific peak (Fig. 1). This pattern closely resembles that of acetylation of lysine 56 on newly synthesized histones. The H4 N-terminal tail and on 5'-fluoroorotic acid (5'-FOA), which presumably reflected the deposition-specific acetylation of H3 Lys-9. This result shows that the vast majority of H3 Lys-9 acetylation in the cell occurs in an S-phase-specific peak (Fig. 1). This pattern closely resembles that of acetylation of lysine 56 on newly synthesized histone H3 was viable (Fig. 1A). Although it is possible that the inviability is because of other roles of Asf1 in the cell, this result suggests that loss of H3 lysine 9 and lysine 56 acetylation together with loss of the acetylatable lysines on histone H4 may result in a level of acetylation on newly synthesized histones that falls below the threshold required for chromatin assembly in yeast (14).

There are two possibilities that could explain the reduced level of the deposition-specific acetylations on histone H3 (lysines 9 and 56) in asf1 mutants. First, the ability of Asf1 to act as a histone chaperone may stabilize the soluble pool of histones H3/H4, by either reducing its turn over via degradation or by reducing its rate of assembly onto chromatin. Second, Asf1 may promote the ability of the HATs to perform acetylation on newly synthesized H3. To differentiate between these two possibilities, we examined the deposition-specific acetylation on histone H4. If Asf1 is stabilizing H3 it would also be expected to stabilize H4, as H3 and H4 always exist as heterodimers. We had previously shown that the levels of acetylation of H4 lysines 5 and 12 are not altered in yeast lacking ASF1 (19). However, these analyses examined histone acetylation in asynchronous
cultures of cells, and it was unknown whether the transcription-specific acetylation may be masking a defect in the deposition-specific acetylation of histone H4. To investigate this possibility, we examined H4 Lys-12 acetylation through the cell cycle to determine whether there is an S-phase-specific peak (Fig. 2), as seen for H3 Lys-9 acetylation (Fig. 1A). We observed a clear S-phase-specific peak of H4 Lys-12 acetylation in wild type cultures of cells, indicating that the deposition-specific acetylation of H4 Lys-12 is the most abundant form of this modification in the cell. Importantly, the S-phase-specific peak of H4 Lys-12 acetylation was also present in asf1 mutant cells, albeit slightly delayed because asf1 mutants had an elongated S-phase (supplemental Fig. 2). Because the deposition-specific acetylation of H3 but not H4 is affected in asf1 mutant cells, and because H3 and H4 are always tightly associated as heterodimers in the cell, this result indicates that Asf1 does not affect histone acetylation indirectly by stabilizing soluble histones H3/H4.

Asf1 itself does not have HAT activity (data not shown) indicating that a distinct protein mediates H3 Lys-9 and Lys-56 acetylation with HAT activity. To identify which HAT is responsible for the S-phase peak of acetylation on lysine 9 on newly synthesized H3, we examined yeast deleted for GCN5, HAT1, SPT10, or SAS3 encoding HATs and the YNG2 component of the NuA4 HAT complex (data not shown). When we examined yeast deleted for GCN5 (gcn5Δ), we observed a complete absence of the S-phase peak of acetylation of H3 Lys-9 but not H4 Lys-12 (Fig. 3A). This result indicates that Gcn5 is the HAT for the deposition-specific acetylation of soluble histone H3 in vivo. To rule out an indirect affect of ASFI deletion on Gcn5 expression or stability we compared Gcn5 protein and transcript levels in asf1Δ and wild type yeast. We found no significant changes in the transcript levels of any known HAT or histone deacetylase upon the deletion of ASFI (21). Furthermore, the steady-state levels of Gcn5 protein are indistinguishable between wild type and asf1 mutant yeast (Fig. 3B). Gcn5 however is not the HAT responsible for the S-phase peak of acetylation of histone H3 Lys-56 (supplemental Fig. 3). As such, our data indicate that Asf1 may present the newly synthesized histone H3 to Gcn5 or promote the activity of Gcn5 toward lysine 9.

We were interested to investigate whether any of the mutant phenotypes of yeast deleted for ASFI (6) may be a consequence of the absence of Gcn5-mediated deposition-specific acetylation of lysine 9 of H3. We found that yeast deleted for GCN5 or ASFI were equally sensitive to the radiomimetic agent bleomycin than either single mutation (Fig. 3C). The growth defect of the gcn5Δasf1Δ strain was even more sensitive to bleomycin than either single mutation (Fig. 3C). These results indicate that Asf1 and Gcn5 have non-overlapping roles in the cell that are not related to the requirement of both proteins for acetylation of H3 Lys-9.

To further investigate the relationship between Asf1 and H3 acetylation, we asked whether excess Asf1 could promote H3 acetylation.
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A

![Image of Western blot analysis](image)

B

![Image of Western blot analysis](image)

C

![Image of Western blot analysis](image)

**FIGURE 4.** Overexpression of Asf1 promotes Lys-9 and Lys-56 acetylation of H3. A, overexpression of Asf1 increases H3 Lys-9 acetylation. A schematic of constructs used for analysis is shown at the top. Strain O/X Asf1 (MAY0022) has the Asf1 gene under the pGAL1 promoter with a V5:His6 epitope tag. WT (MAY0025) has the Asf1 gene under the endogenous promoter and a His6 epitope. Strains described were grown in 2% galactose, and total cell extracts were Western blotted to detect the His epitopes on Asf1. The slight increase in size of the O/X Asf1 is because of the V5 epitope not found on WT. At the bottom is shown a lighter exposure of the same blot with the Asf1 in the O/X Asf1 in the linear range of detection. Right panel, Western blot analysis of the same protein extracts used above, probing for histone H4, total H4 acetylation, H3, and histone H3 Lys-9 and Lys-14 acetylation levels. B, overexpression of Asf1 leads to increased levels of H3 Lys-56 acetylation. Total extracts from the strains used in A, with the addition of an asf1Δ (JKT0018) strain, were Western blotted for histone H3 and for H3 acetylated on Lys-56. C, Gcn5 is not required for the increased H3 Lys-9 acetylation that results from overexpression of Asf1. WT (JCY0025), sas3Δ (JCY0026), gcn5Δ (JCY0027), and spt10Δ (JCY0028) strains containing the pGAL1Asf1 plasmid were grown in 2% glucose or 2% galactose to induce the overexpression of Asf1 O/X Asf1. Total protein extracts were Western blotted to determine whether any of these HATs were required for the increased acetylation on Lys-9 of H3 that resulted from the overexpression of Asf1. Western blotting for H3 serves as a normalization control for loading differences.

Acetylation. Although deletion of **ASF1** reduced H3 Lys-9 acetylation, we found that the overexpression of Asf1 in yeast greatly increased the levels of acetylated H3 Lys-9 but had no effect on H4 acetylation (Fig. 4A). Notably, overexpression of Asf1 did not increase levels of H3 Lys-9 acetylation on chromatin (supplemental Fig. 1). Similarly, although deletion of **ASF1** reduced H3 Lys-56 acetylation, the overexpression of Asf1 greatly increased the levels of acetylated H3 Lys-56 in the cell (Fig. 4B). The increased Lys-9 acetylation was not dependent on Gcn5, suggesting that overexpression of Asf1 caused additional HATs to acetylate this residue (Fig. 4C). Because acetylation on H4 did not increase upon overexpression of Asf1, these results suggest that the increased H3 acetylation was not because of the excess Asf1 stabilizing the pool of soluble acetylated histones H3/H4. Instead, these data are consistent with wild type levels of Asf1 normally being limited in the cell for the optimal presentation of soluble histone H3 to the HATs, such that overexpression of Asf1 results in more H3 Lys-9 and Lys-56 acetylation.

**DISCUSSION**

Asf1 Is Likely to Present the Newly Synthesized Free Histone H3 to the HATs—Given that Asf1 only affects acetylation of soluble H3, not soluble H4 or chromatin-bound histones (Fig. 2 and supplemental Fig. 1), we propose that Asf1 delivers or presents the histones in an appropriate manner to the HATs for H3 Lys-9 and Lys-56 acetylation. Previous evidence has shown that a complex containing Gcn5 purifies with newly synthesized histones, and this was proposed to be the HAT for S-phase-specific acetylation of H3 (23). Consistent with this prediction, we show that the S-phase peak of histone H3 Lys-9 acetylation does not occur in gcn5 mutants (Fig. 3), demonstrating that Gcn5 is the HAT for acetylation of Lys-9 on newly synthesized histone H3 in the cell. It is possible that Asf1 regulates the activity of Gcn5 via a direct interaction. However, no direct interaction between Asf1 and Gcn5 has been reported so far. Asf1 has been reported to bind to the bromodomain-containing proteins Brd1 and Brd2 in yeast and Brahma in Drosophila (24, 25). As Gcn5 does contain a bromodomain (26), it may be a potential binding partner of Asf1 that merits further investigation in the future. Notably, no detectable HAT activities toward full-length H3 co-purified with Asf1 from yeast cells, although an H4-specific
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HAT activity (presumably the SAS complex) did co-purify with Asf1 (data not shown). As such, if there is an interaction between Asf1 and Gcn5, it must be very transient. Although Spt10 is the HAT required for acetylation of H3 Lys-56 on chromatin (16), the identity of the HAT required for acetylation of soluble H3 is unknown, the SAS complex is an interesting candidate because inactivation of this complex does not cause sensitivity to DNA damaging agents (27) as is the case for yeast unable to acetylate H3 Lys-56 (15).

It is possible that the binding of histones H3/H4 to Asf1 may induce a conformational change in histone H3 that makes it a more favorable target for acetylation by Gcn5 and the Lys-56 HAT. Because H3 Lys-9 and Lys-56 are not included in the structure of the Asf1-H3/H4 complex (22), we do not yet know whether binding to Asf1 induces a conformational change toward the N-terminal region of H3 that may improve the fit into the active site of Gcn5 and Lys-56 HAT. However, this is a possibility, as we observed numerous conformational changes throughout histones H3 and H4 upon binding to Asf1 as compared with their conformation in the nucleosome structure (29). Furthermore, we do not think that the influence of Asf1 on H3 acetylation levels could be because of any potential role for Asf1 in shielding histone H3 from deacetylation following its deposition onto the DNA because the crystal structure of Asf1 bound to H3/H4 (22) indicates that Asf1 must be removed from histones H3/H4 to enable interaction with histones H2A/H2B and the DNA within the nucleosome. Our biophysical and structural analyses have clearly shown that the binding of Asf1 to the histone H3/H4 heterodimer envelops the surface of H3 that would otherwise mediate formation of the H3/H4 heterotetramer (30). Upon deletion of ASF1, this H3-H3 interaction interface of the H3/H4 heterodimer is no longer shielded, and it is likely that the soluble H3/H4 in the cell would associate into H3/H4 heterotetramers prior to chromatin assembly. H3/H4 heterotetramerization in the absence of Asf1 may itself induce conformational changes in the soluble histone H3 that may in turn reduce its ability to be acetylated. This will be difficult to determine until a molecular structure of the free H3/H4 heterotetramer is solved. Alternatively, the proposed heterotetramerization of H3/H4 in the absence of Asf1 may present new binding sites for proteins that block access to the HATs of soluble H3.

The location and pattern of residues on histone H3 that are acetylated in a deposition-specific manner vary largely between different species (10, 13). Toward differentiating between some of the possible models above, it would be interesting to determine whether the acetylation of the different deposition-related H3 residues is reduced in other organisms lacking Asf1 function. This may provide insight into whether Asf1 is inducing a conformational change in the newly synthesized histones upon binding to them, or whether the presentation of H3/H4 as a heterodimer by Asf1 makes them more favorable for acetylation.

A final possibility is that Asf1 may influence the cellular or sub-cellular localization of the soluble histones, which may affect their delivery to the HATs. It is interesting to note that HAT1, which acetylates Lys-5 and Lys-12 on newly synthesized H4; although somewhat surprisingly, the SAS complex cannot acetylate histones bound to Asf1 (28). Although the HAT for acetylation of soluble Lys-56 is unknown, the SAS complex is an unlikely candidate because inactivation of this complex does not cause sensitivity to DNA damaging agents (27) as is the case for yeast unable to acetylate H3 Lys-56 (15).

It is possible that the binding of histones H3/H4 to Asf1 may induce a conformational change in histone H3 that makes it a more favorable target for acetylation by Gcn5 and the Lys-56 HAT. Because H3 Lys-9 and Lys-56 are not included in the
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the intense nuclear staining provided by the chromatinized histones.

Whatever the details of how the histone chaperone Asf1 affects acetylation of lysines 9 and 56 residues on soluble H3, it is clear that the histone binding ability of Asf1 is absolutely required for these acetylation events (Fig. 5). It is important to note that the histone H3/H4 molecules that are bound to Asf1 are seemingly 100% acetylated at every deposition-specific acetylatable lysine in the N-terminal tails (6). By contrast, analysis of all newly synthesized histones H3/H4 shows them to be less than 100% acetylated (10). This saturated acetylation on the newly synthesized H3/H4 bound to Asf1 is fully consistent with our proposal that the binding of H3/H4 to Asf1 potentiates their acetylation. Furthermore, the fact that overexpression of Asf1 greatly increases the levels of Lys-9 and Lys-56 acetylation in the cell (Fig. 4) suggests that the amounts of Asf1 are limiting in the cell, as far as its role in presenting newly synthesized histone H3 to Gcn5 and the Lys-56 HAT.

Finally, having found that acetylation of the soluble histones at H4 Lys-12 and H3 Lys-9 acetylation is apparent by an S-phase peak (Figs. 1–3), we now have a simple assay for the identification of enzymes involved in the acetylation and deacetylation of newly synthesized histones. The HAT for Lys-12 acetylation on newly synthesized H4 is known to be HAT1 (32), and we have now shown the HAT for acetylation of newly synthesized H3 Lys-9 is Gcn5. Similarly, this approach could be applied to identify the histone deacetylases that deacetylate H4 Lys-12 and H3 Lys-9 rapidly after chromatin assembly.

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REFERENCES
1. Van Holde, K. E. (1989) Chromatin, Springer-Verlag, New York
2. Donaldson, A. D. (2005) Trends Genet. 21, 444–449
3. Melior, J. (2005) Mol. Cell 19, 147–157
4. Peterson, C. L., and Cote, J. (2004) Mol. Cell. Biol. 24, 10180–10192
5. Sobel, R. E., Cook, R. G., Perry, C. A., Annunziato, A. T., and Allis, C. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1237–1241
6. Jackson, V., Shires, A., Tanphaichitr, N., and Chalkley, R. (1976) J. Mol. Biol. 104, 471–483
7. Annunziato, A. T., and Seale, R. I. (1983) J. Biol. Chem. 258, 12675–12684
8. Kuo, M. H., Brownell, J. E., Sobel, R. E., Ranalli, T. A., Cook, R. G., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Nature 383, 269–272
9. Ma, X. J., Wu, J., Altheim, B. A., Schultz, M. C., and Grunstein, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6693–6698
10. Sobel, R. E., Cook, R. G., Perry, C. A., Annunziato, A. T., and Allis, C. D. (1994) Cell 116, 51–61
11. Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. (2004) Cell 116, 471–483
12. Peterson, C. L., and Cote, J. (2004) Mol. Cell. Biol. 24, 10180–10192
13. Adkins, M. W., Howar, S. R., and Tyler, J. K. (2004) Mol. Cell. Biol. 24, 10180–10192