The Yeast Histone Acetyltransferase A2 Complex, but Not Free Gcn5p, Binds Stably to Nucleosomal Arrays*

Received for publication, May 4, 2000, and in revised form, May 22, 2000
Published, JBC Papers in Press, May 23, 2000, DOI 10.1074/jbc.M003783200

Ramon Sendra‡, Christin Tse§, and Jeffrey C. Hansen§¶

From the ‡Departament de Bioquímica i Biologia Molecular, Universitat de València, E-46100 València, Spain and the §Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284

We have investigated the structural basis for the differential catalytic function of the yeast Gcn5p-containing histone acetyltransferase (HAT) A2 complex and free recombinant yeast Gcn5p (rGcn5p). HAT A2 is shown to be a unique complex that contains Gcn5p, Ada2p, and Ada3p, but not proteins specific to other related HAT A complexes, e.g. ADA, SAGA. Nevertheless, HAT A2 produces the same unique polyacetylation pattern of nucleosomal substrates reported previously for ADA and SAGA, demonstrating that proteins specific to the ADA and SAGA complexes do not influence the enzymatic activity of Gcn5p within the HAT A2 complex. To investigate the role of substrate interactions in the differential behavior of free and complexed Gcn5p, sucrose density gradient centrifugation was used to characterize the binding of HAT A2 and free rGcn5p to intact and trypsinized nucleosomal arrays, H3/H4 tetramer arrays, and nucleosome core particles. We find that HAT A2 forms stable complexes with all nucleosomal substrates tested. In distinct contrast, rGcn5p does not interact stably with nucleosomal arrays, despite being able to specifically monoacetylate the H3 N terminus of nucleosomal substrates. Our data suggest that the ability of the HAT A2 complex to bind stably to nucleosomal arrays is functionally related to both local and global acetylation by the complexed and free forms of Gcn5p.

Histone acetylation is a dynamic process in vivo involving multiple acetyltransferases and deacetylases enzymes (1–3). Acetylation occurs at specific lysine residues of each of the core histone N termini, and influences chromatin structure and function at all levels from the folded chromatin fiber to the nucleosome (4–6). Because of the key role of acetylation in transcription and replication, a great deal of attention has been focused on the properties and functions of recently identified histone acetyltransferase enzymes (HATs).¹ HAT A enzymes (e.g. Gcn5p, Esa1p) primarily are involved in transcriptional regulation, whereas HAT B enzymes (e.g. Hat1p) are thought to participate in replication coupled-chromatin assembly. The Tetrahymena equivalent of yeast Gcn5p (Gen5p) was the first specific HAT A enzyme to be cloned and overexpressed (7), and the Gcn5 family of HATs remains the focus of intense investigation (see Refs. 5, 8, and 9, and references therein).

The catalytic function of yeast Gcn5p in vitro is strongly dependent on whether the enzyme is free or is a component of large multiprotein complexes such as HAT A2, ADA, and SAGA (8, 9). Purified ADA and SAGA complexes both have been shown to contain the non-DNA-binding, transcriptional adaptor proteins Ada2p and Ada3p (8–10). Consistent with these in vitro results, genetic experiments in yeast have documented that mutations in Ada2p or Ada3p yield phenotypes identical to loss of Gcn5p in terms of both transcriptional regulation at specific gene loci (11, 12) and cell growth (11, 13). In addition, several specific proteins have recently been identified as unique components of ADA or SAGA. For example, Ahs1 is found only in the ADA complex (14), whereas certain members of the Spt and TAF proteins are unique components of the SAGA complex (10, 15, 16). There is compelling evidence that Gcn5p-containing HAT A complexes play key roles in transcriptional regulation by acetylating small regions of chromatin in the vicinity of specific genes (16–19). However, essentially nothing is known about either the solution properties of these complexes, or the extent to which they are involved in the maintenance of the high levels of global genome-wide acetylation in yeast, e.g. at steady-state there are ~13 acetyl per histone octamer throughout the entire Saccharomyces cerevisiae genome in log growth phase (20).

To address these issues, we have analyzed the subunit composition and enzymatic properties of the HAT A2 complex, and used sucrose density gradient centrifugation to determine the relative strength and stability of HAT A2 and free rGcn5p interactions with different nucleosomal substrates. Results indicate that the substrate acetylation pattern of the HAT A2 complex is very similar to that of the larger ADA and SAGA complexes, even though HAT A2 lacks many of the subunits found in ADA and SAGA. We also observe that HAT A2, but not free Gcn5p, binds stably to nucleosomal substrates. The relevance of these results to the mechanisms of both local and global acetylation by type A histone acetyltransferases is discussed in detail.

EXPERIMENTAL PROCEDURES

Materials—Whole chicken blood was purchased from Pel-Freez and used to obtain free histones (21), intact core histone octamers (22), and nucleosome core particles (23) as described. 208-12 DNA templates consisting of 12 tandem repeats of a 208-base pair sequence derived from the Lytechinus 5 S rRNA gene (24) were grown and purified from plasmid using pPOL208-12 as described (22). 12-mer nucleosomal arrays were obtained after salt dialysis reconstitution of a mixture of purified chicken erythrocyte histone octamers and 208-12 DNA as described (25). pUC18 supercoiled plasmid was isolated from Esherichia coli DH5α cells using the Maxi plasmid purification kit (Qiagen) according to the manufacturer’s instructions. Linearized pUC18 plasmid was obtained by BamHI endonuclease digestion of the supercoiled plasmid.

¹ The abbreviations used are: HAT, histone acetyltransferase; TAU, Triton X-100/acetic acid/urea; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; E64, trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane; TAF, TATA-associated factor.
mid for 3 h at 37 °C with 2 units/µg DNA. All chemicals were of reagent grade.

**Purification of HAT A2 and Recombinant Gcn5—**Gcn5 was expressed and purified from BL21 cells containing plasmid pBSET- 

gcn5 as described previously (26). *Saccaromyces cerevisiae* strain BQS2421 (MATα ura3-52 lys2-801 trp1-901 his3-200 leu2-3,112 met15 trp2-3 ura3-52; D301) lacking the histone HAT genes h2-TRPL1 and h1-TRPL3, which lacks cytoplasmic HAT B and nuclear HAT A3 enzymes (see Ref. 27), was used to purify the HAT A2 complex. Eight-liter batches of BQS2421 cells were grown to exponential phase at 25 °C in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose), harvested by centrifugation, and spheroplasted by Zymolase digestion as described (28). Crude extracts were obtained by ultracentrifugation of the S. cerevisiae histones or nucleosomal substrates and free DNAs without added enzyme, were prepared with DB buffer to the same final volume. All samples were incubated at 4 °C for 30 min, and then layered on the top of an 11-mL linear 10–30% (w/v) sucrose gradient (DB buffer lacking glycerol), and ultracentrifuged in a Beckman SW41 Ti rotor at 30,000 rpm for 14 h at 4 °C. Each centrifuge tube subsequently was fractionated (0.54 mL) from bottom to top, and the A200 (or the A200 in the case of HAT A2 sample alone) was measured. Portions of each fraction were mixed with free histones and assayed for HAT activity as described above. Results of the ultracentrifugation experiments were completely reproducible using different batches (n = 3) of purified HAT A2 complex preparations.

**Western Blotting and Immunoprecipitation—**Typically, 20–25 µL of the indicated chromatographic fractions were used for Western blotting after electrophoresis on a 10% SDS-polyacrylamide gel. Protein gels were transferred to nitrocellulose using a semi-dry transfer apparatus (Bio-Rad). The blots were blocked and probed using standard procedures (30), and visualized using an ECL chemiluminescent detection kit (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Anti-Ahcl antisera raised in rabbits (13) was a gift from J. L. Workman (Pennsylvania State University, State College, PA). Rabbit polyclonal antibodies against Gen5p and Ada2p (31) proteins were kindly provided by S. L. Berger (Wistar Institute, Philadelphia, PA). Anti-Spt7 and 20 antibodies (probes yE-19 and yC-20, respectively; Santa Cruz Biotech) also were used in Western blotting experiments, but in these cases secondary antibody-alkaline phosphatase conjugates were used for detection. Identical bands were visualized with rabbit and commercial goat anti- 

sers. Rabbit polyclonal antibodies against Spt7 and 20 proteins were generously provided by F. Winston (Harvard Medical School, Boston, MA). Antiis against TAP 60 and 68 proteins were kindly provided by C. Peterson (University of Massachusetts Medical Center, Worcester, MA). For immunoprecipitation experiments, rabbit anti-Ada2p, anti- 

H, and non-immune antisera were mixed with purified HAT A2 fractions (100 µL) and incubated for 2 h at 4 °C. 15 µL of pre-equilibrated Protein A-Sepharose (Amersham Pharmacia Biotech) were then added and allowed to rotate 4 h at 4 °C on a rotating wheel. After centrifugation, supernatants were collected, and the beads washed four times with 0.4 mg of immunoprecipitation buffer (50 mM HEPES, pH 7.8, 150 mM NaCl, 10% glycerol, 0.1% Tween 20, 1 mM PMSF, 2 mM EDTA, 2 µg/mL chymostatin). Input material, supernatants, and beads were directly assayed for HAT activity using free histones as a substrate.

**RESULTS**

**Properties of the Yeast HAT A2 Complex—**We have previously isolated from *S. cerevisiae* a Gen5p-containing histone acetyltransferase complex termed HAT A2 (28). In these studies the molecular mass of HAT A2 was found to be ~170 kDa when determined by sucrose density gradient ultracentrifugation (28), consistent with our observation that HAT A2 sediments more slowly than the globular ~200-kDa nucleosome core particle (see Fig. 5C). However, analysis by either standard exclusion chromatography on Sephacryl S-300 HR (Fig. 1A), or by fast protein liquid chromatography on a Superdex 200 HR 10/30 column (data not shown), reproducibly yielded an apparent molecular mass for HAT A2 of ~480 kDa. Note that the position of the HAT A2 complex was monitored by both liquid HAT assays (Fig. 1A) and by its distinct acetylation pattern of exogenously added free histones as determined by fluorography (Fig. 1B). Since gel filtration and sedimentation should give the same results for globular macromolecules, these findings strongly suggest that the shape of HAT A2 is asymmetric. Consequently, the actual molecular mass of HAT A2 probably lies somewhere between 200 and 500 kDa. Most importantly for the interpretation of the results shown below, these data demonstrate that the HAT A2 is significantly smaller than the ~800-kDa ADA and ~1.8-MDa SAGA complexes.

The subunit composition of the HAT A2 complex, which had not been determined in earlier studies (28), is shown in Fig. 1. Western blots of the column fractions from Fig. 1A indicate that Ada2p and Gen5p (Fig. 1C) are present in the HAT A2 complex (fractions 25–27 from the gel filtration column). The

**Histone Acetyltransferase Assays—**Liquid HAT activity assays were performed with chicken core histones or nucleosomal substrates and [1-14C]acetyl-CoA (54 mCi/mmol, ICN) as described (26). Briefly, an aliquot from the indicated chromatographic or sucrose gradient fractions was used for HAT A2. The dialyzed supernatant was made 25% with trichloroacetic acid and then four times in 5 ml with 100% ethanol using a Millipore vacuum manifold. Scintillation counting was performed with chicken core histones or nucleosomal substrates and free DNAs without added enzyme, were prepared with DB buffer to the same final volume. All samples were incubated at 4 °C for 30 min, and then layered on the top of an 11-mL linear 10–30% (w/v) sucrose gradient (DB buffer lacking glycerol), and ultracentrifuged in a Beckman SW41 Ti rotor at 30,000 rpm for 14 h at 4 °C. Each centrifuge tube subsequently was fractionated (0.54 mL) from bottom to top, and the A200 (or the A200 in the case of HAT A2 sample alone) was measured. Portions of each fraction were mixed with free histones and assayed for HAT activity as described above. Results of the ultracentrifugation experiments were completely reproducible using different batches (n = 3) of purified HAT A2 complex preparations.

**RESULTS**

**Properties of the Yeast HAT A2 Complex—**We have previously isolated from *S. cerevisiae* a Gen5p-containing histone acetyltransferase complex termed HAT A2 (28). In these studies the molecular mass of HAT A2 was found to be ~170 kDa when determined by sucrose density gradient ultracentrifugation (28), consistent with our observation that HAT A2 sediments more slowly than the globular ~200-kDa nucleosome core particle (see Fig. 5C). However, analysis by either standard exclusion chromatography on Sephacryl S-300 HR (Fig. 1A), or by fast protein liquid chromatography on a Superdex 200 HR 10/30 column (data not shown), reproducibly yielded an apparent molecular mass for HAT A2 of ~480 kDa. Note that the position of the HAT A2 complex was monitored by both liquid HAT assays (Fig. 1A) and by its distinct acetylation pattern of exogenously added free histones as determined by fluorography (Fig. 1B). Since gel filtration and sedimentation should give the same results for globular macromolecules, these findings strongly suggest that the shape of HAT A2 is asymmetric. Consequently, the actual molecular mass of HAT A2 probably lies somewhere between 200 and 500 kDa. Most importantly for the interpretation of the results shown below, these data demonstrate that the HAT A2 is significantly smaller than the ~800-kDa ADA and ~1.8-MDa SAGA complexes.

The subunit composition of the HAT A2 complex, which had not been determined in earlier studies (28), is shown in Fig. 1. Western blots of the column fractions from Fig. 1A indicate that Ada2p and Gen5p (Fig. 1C) are present in the HAT A2 complex (fractions 25–27 from the gel filtration column). The
finding that extracts from a yeast ADA3 deletion strain lacked the HAT A2 complex (data not shown) suggests that Ada3p also is a component of HAT A2, and is required for structural integrity of the complex. These observations are consistent with the strong genetic, physical, and functional association of Ada2p and Ada3p observed by others (11, 31–33), and the findings that Ada2p and Ada3p are present in all yeast Gcn5p-containing HAT complexes isolated to date (8, 9). Certain TAFs (10, 14), whereas the latter observations are unique to these studies. The pattern of acetylation of free and nucleosomal substrates by HAT A2 (Fig. 2A) is distinctly different from that observed previously for rGcn5p (Ref. 26; see Fig. 2B). Particularly, although rGcn5p can strongly polyacetylate free histones, HAT A2 moderately acetylated nucleosomal H2B, only very weakly acetylated nucleosomal H4, and did not acetylate nucleosomal H2A (Fig. 2A). The former observation is consistent with recent results obtained with the ADA and SAGA complexes (10, 14), whereas the latter observations are unique to these studies. The pattern of acetylation of free and nucleosomal histones by HAT A2 in 1–2 mM MgCl2 was the molecular basis for the different acetylation patterns of nucleosomal substrates by HAT A2 obtained over a 80-min reaction period is shown in Fig. 2A. HAT A2 polyacetylated free histone H3 and to a lesser extent free H4 and H2A (Fig. 2B). In terms of nucleosomal substrates, HAT A2 polyacetylated the H3 N termini of nucleosomal arrays and nucleosome core particles extensively and to an equal extent (Fig. 2A). However, unlike the case for free histones, HAT A2 moderately acetylated nucleosomal H2B, and did not acetylate nucleosomal H2A (Fig. 2A). The former observation is consistent with recent results obtained with the ADA and SAGA complexes (10, 14), whereas the latter observations are unique to these studies. The pattern of acetylation of free and nucleosomal histones by HAT A2 in 1–2 mM MgCl2 was the same as that observed in low salt DB buffer (data not shown), indicating that HAT A2 acetylation of nucleosomal arrays occurs equally well under conditions that promote extensive array folding (22). Importantly, the pattern of acetylation of nucleosomal substrates by HAT A2 (Fig. 2A) is distinctly different than that observed previously for rGcn5p (Ref. 26; see Fig. 2B). Particularly, although rGcn5p can strongly polyacetylate free histone H3 and acetylate free H4 to a lesser extent (Fig. 2B, lane 2), the enzyme in its free form only is able to monoacetylate the H3 N termini nucleosomal substrates (Fig. 2B, lane 3).

Interaction of HAT A2 with Nucleosomal Arrays—The molecular basis for the different acetylation patterns of nucleosomal substrates and free histones by HAT A2 and free Gcn5p (Fig. 2) is unknown. To determine whether HAT A2 and free Gcn5p interact differently with nucleosomal arrays, which is consistent with the proposed functions of HAT A complexes (8, 9, 34, 35), HAT A2 and rGcn5p were incubated with 12-mer nucleosomal arrays for 30 min, followed by ultracentrifugation.
for 14 h in linear 10–30% sucrose density gradients. The same buffer DB used to obtain the substrate specificity results (Fig. 2A) was also used for the HAT A2-nucleosomal array incubations and the sucrose density gradients. The A_{260} of each gradient fraction was measured to determine the position of nucleosomal arrays, whereas the HAT A2 position was identified by adding exogenous free histones to each fraction and performing liquid HAT assays. The position of HAT A2 in all cases was verified by fluorography of SDS gels of portions of each gradient fraction. Isolated HAT A2 complexes were sedimented on linear 10–30% sucrose density gradients as described in part 3A. The position of the nucleosomal arrays in the gradient was determined from the absorption of each column fraction at 260 nm (solid line). The position of HAT A2 was determined by standard HAT assays (dashed line). D, sedimentation profile of a mixture of HAT A2 and 12-mer nucleosomal arrays in the absence of acetyl-CoA. Isolated HAT A2 was mixed with 208-12 nucleosomal arrays in the absence of acetyl-CoA, followed by ultracentrifugation and gradient fractionation as described in part 3A. The position of the nucleosomal arrays in the gradient was determined from the absorption of each column fraction at 260 nm (solid line). The position of HAT A2 was determined by standard HAT assays (dashed line). D, sedimentation profile of a mixture of HAT A2 and 12-mer nucleosomal arrays in the absence of acetyl-CoA. The same experiment as in C was performed, except that the incubations occurred in the presence of acetyl-CoA (40 μM).

Fig. 3 shows the representative elution profiles obtained after sedimentation of 12-mer nucleosomal arrays alone, HAT A2 alone, and HAT A2/nucleosomal array mixtures. The 12-mer nucleosomal array peak eluted in fraction 10 (Fig. 3A), whereas the HAT A2 peak eluted in fraction 18 (Fig. 3B). After mixing nucleosomal arrays and HAT A2 for 30 min in the absence of acetyl-CoA, the nucleosomal array peak and the major peak of HAT A2 activity co-eluted in fraction 9 (Fig. 3C).

These results demonstrate that HAT A2 binds to nucleosomal arrays. Identical elution profiles were obtained when HAT A2 and nucleosomal arrays were incubated in the presence of 40 μM acetyl-CoA (Fig. 3D), indicating that HAT A2-nucleosomal array interactions remained stable after acetylation of endogenous nucleosomal histones by HAT A2 during the 30-min incubation period. The very small but reproducible peak present in fraction 18 (Fig. 3, C and D) represents the amount of unbound HAT A2 present at the start of centrifugation, and in all cases consisted of <3% of the total HAT A2 activity detected under these binding conditions. Finally, overexposure of the fluorograms of the HAT assays revealed that only a very small amount of “trailing” HAT A2 activity (<5–10% of the total) could be detected in fractions 12–18 (data not shown), indicating that very little HAT A2 dissociated from the nucleosomal arrays during the course of the sucrose density gradient experiments.

To determine the extent to which free rGcn5p was able to interact with nucleosomal arrays, we sedimented a mixture of rGcn5p and 12-mer nucleosomal arrays in DB buffer + 2 mM MgCl₂ and 40 μM acetyl-CoA, the optimal conditions for monoaacetylation of the H3 N termini of nucleosomal arrays by rGcn5p (25). The broader nucleosomal array gradient profile and the faster sedimenting peak seen in 2 mM MgCl₂ (Fig. 4A) compared with DB (Fig. 3A) reflect the extensive folding of the nucleosomal arrays in 2 mM MgCl₂ (22, 37, 38). Importantly, in distinct contrast to the HAT A2 complex, mixtures of rGcn5p and nucleosomal arrays (Fig. 4C) resolved into separate HAT activity and A_{260} peaks characteristic of free enzyme (Fig. 4B) and nucleosomal arrays (Fig. 4A), respectively. Thus, rGcn5p was not able to form a stable complex with 12-mer nucleosomal arrays, despite being able to utilize these arrays as a substrate
for acetylation. Note that the nucleosomal array binding properties of both free rGcn5p and HAT A2 were independent of the folded state of the array; rGcn5p was not able to bind to nucleosomal arrays in DB buffer (where the arrays are unfolded), whereas HAT A2 bound strongly to extensively folded nucleosomal arrays in 2 mM MgCl₂ (data not shown). Detailed investigations of the effects of monovalent cations and salt concentration on the stability of HAT A2-nucleosomal array interactions are in progress.

**Macromolecular Determinants of HAT A2-Nucleosomal Array Interactions**—To better define which constituents of the nucleosomal array (e.g., core histone N termini, linker DNA) were responsible for stable interactions with HAT A2, sucrose density gradients initially were used to analyze mixtures of HAT A2 and 12-mer rDNA templates assembled with either trypsinized histone octamers (Fig. 5A) or H3/H4 tetramers (Fig. 5B). The $A_{260}$ and HAT activity profiles in both cases overlapped precisely and closely resembled the profiles observed for intact nucleosomal arrays (Fig. 3, C and D). The level of trailing HAT activity in peaks 12–18 in all cases remained very low (data not shown), and were indistinguishable from the amounts seen with intact nucleosomal arrays. Thus, neither the core histone N termini nor H2A/H2B dimers were required for stable HAT A2-nucleosomal array interactions. Note that the relative difference in the gradient fraction peaks of the intact (peak 10), trypsinized (peak 12), and H3/H4 tetramer arrays (peak 13) in DB (Figs. 3 and 4) mimicked previously observed differences in the actual sedimentation coefficients of these same arrays in low salt as measured by analytical ultracentrifugation (22, 37, 39).

To determine whether nucleosomal subunits and(or) linker DNA were involved in stabilizing HAT A2-nucleosomal array interactions, we next characterized the ability of HAT A2 to interact with nucleosome core particles. Despite lacking any extranucleosomal DNA, the $A_{260}$ and HAT activity profiles of mixtures of nucleosome core particles and HAT A2 (Fig. 5C) overlapped precisely, and the peak was shifted to a lower fraction number relative to the peak migration of the free constituents. The same result also was observed in linear 5–18% gradients (data not shown). These data suggest that HAT A2 binds to the nucleosomal subunits of nucleosomal arrays rather than linker DNA. Further support for this conclusion comes from the observation that HAT A2 acetylates nucleosome core particles (which lack linker DNA) and 12-mer nucleosomal arrays (which contain 12 stretches of linker DNA) identically (Fig. 2A). We also used the sucrose density gradient approach to analyze HAT A2 interactions with linear 208-12 DNA, linear pUC18 DNA, and supercoiled pUC18 DNA. Incubations with linear 208-12 DNA (Fig. 5D) and both types of pUC DNAs (data not shown) yielded overlapping $A_{260}$ and HAT activity profiles similar to those obtained for nucleosomal arrays. However, more HAT A2 activity appeared to be present in the trailing fractions than was observed for any of the nucleosomal templates (data not shown). Thus, HAT A2 interacts with naked DNA, albeit with a reduced relative affinity compared with nucleosomal arrays.

**DISCUSSION**

Our studies have characterized in detail the interaction of purified yeast HAT A2 complex with nucleosomal arrays. The primary technical approach used, sucrose density gradient ultracentrifugation, provides rigorous information about the relative strength and stability of macromolecular interactions (36). A major finding of this work is that only the HAT A2 complex binds stably to nucleosomal arrays under conditions where nucleosomal arrays efficiently are acetylated by both free Gcn5p and HAT A2. The evidence for stable HAT A2-nucleosomal array interactions is as follows. First, only 1–3% of the HAT A2 is not bound after a 30-min incubation with length-defined 12-mer nucleosomal arrays. These incubations were performed in the presence of a significant molar excess of nucleosomal arrays, mimicking the situation encountered by HAT A2 in vivo. Second, ~90% of the HAT A2 precisely coelutes with the nucleosomal arrays after 14 h of sedimentation. Finally, very little (~5–10%) of the bound HAT A2 dissociates from the nucleosomal arrays during the course of the long sucrose density gradient experiment. Functionally important ramifications of the stable HAT A2-nucleosomal array interactions observed in these studies are discussed below.

Insight into the determinants of HAT A2-nucleosomal array interactions also has been obtained. Stable binding is not dependent on whether HAT A2 is catalytically active during incubation with nucleosomal arrays (Fig. 3, compare C and D), or whether the nucleosomal arrays are folded or unfolded (data not shown). In addition, the stability of the interactions is not substantially influenced when either the core histone N termini or H2A/H2B dimers are removed from the nucleosomal array (Fig. 5, A and B). The former observation is of particular importance in that it indicates that interaction of the N termini with the Gcn5p component of HAT A2 provides no detectable stabilization of HAT A2-nucleosomal array interactions, either prior to or during catalysis. HAT A2 appears to interact as stably with nucleosome core particles (Fig. 5C) as with nucleosomal arrays (Fig. 3), and also acetylates core particles and arrays identically (Fig. 2). Collectively, these observations suggest that HAT A2 interacts with the nucleosomal subunits of nucleosomal arrays. Further, the finding that HAT A2 binds to linear (Fig. 5D) and supercoiled naked DNAs with reduced affinity relative to nucleosomal arrays suggests that both nu-
nucleosomal DNA and structured domain(s) of the histone octamer may mediate interactions with HAT A2.

Several Gcn5p-containing HAT A complexes have been isolated to date, including HAT A2, ADA, and SAGA (8, 9, 34, 35). Our compositional analyses and substrate specificity data suggest that HAT A2 may be the “core” enzymatic complex for this family of histone acetyltransferases. The physically smaller HAT A2 complex (Fig. 1A) contains Gcn5p, Ada2p, and Ada3p, but does possess proteins specific to SAGA (e.g. Spts 7 and 20, TAFs 60 and 68), or ADA (e.g. Aci1). Nevertheless, HAT A2 exhibits the same distinctive enzymatic properties of the SAGA and ADA complexes (10, 14, 40, 41). Specifically, HAT A2 strongly polyacetylates histone H3 and, to a lesser extent, the H2B and H4 N termini of nucleosomal substrates. Thus, the functions of proteins specific to ADA and SAGA clearly are not related to modification of the catalytic activity of Gcn5p within the HAT complexes. Instead, they most likely involve targeting of HAT complexes to specific locations in the genome, and physical linkage of the complexes with components of the transcriptional machinery at these genomic sites (discussed further below). The steric accessibility of the various core histone N termini to HAT A2 will be altered when HAT A2 is stably bound to nucleosomal substrates compared with when histones are free in solution (see Ref. 5, and references therein). This likely explains why HAT A2 can acetylate nucleosomal but not free H2B, and why HAT A2 only very weakly acetylates nucleosomal H4 and cannot acetylate nucleosomal H2A despite acetylating these histones when they are free (Fig. 2A). It is worth noting that none of the known individual components of HAT A2 (i.e. Gcn5p, Ada2p, Ada3p) are chromatin- or DNA-binding proteins. Thus, the ability of HAT A2 to interact with nucleosomal arrays either originates from undefined HAT A2 subunits, or from the establishment of a complex-specific nucleosomal array binding domain during HAT A2 assembly. Finally, although it has yet to be tested, we suspect from their biological functions that ADA and SAGA also will bind nucleosomal arrays stably (see below).

There is strong genetic (11, 17) and biochemical (19) evidence that the function of Gcn5p-containing HAT A complexes involves localized acetylation of a relatively small number of nucleosomes (i.e. –6 or less) at specific gene loci. The fact that the HAT A2 complex can bind strongly to the nucleosomal subunits of nucleosomal arrays is consistent with a localized function for Gcn5p-containing multisubunit complexes during gene expression. This observation also provides a mechanistic basis for understanding how such HAT complexes are able to acetylate only a relatively small region of chromatin, i.e. proteins that are not components of HAT A2 are likely to play a key role in targeting of HAT complexes to specific genetic loci as discussed above. Once targeted to the desired genomic region, the strong nucleosomal array binding capabilities should favor maintenance of a localized specific steady-state acetylation pattern, even under dynamic acetylation and deacetylation conditions. As has been proposed recently, the resulting pattern of acetylation (and other concurrent post-translational modifications) either may alter the structure of the N termini (5) and/or provide a specific recognition domain (6), in either case establishing a histone “code” that serves to attract other functional macromolecules to the specific region of chromatin (6).

Although our findings are consistent with a role of HAT complexes in localized acetylation, they argue against the involvement of these HAT complexes in global, genome-wide acetylation. It recently has been demonstrated that non-synchronized S. cerevisiae cells in log growth phase have an average steady-state level of acetylation throughout the entire genome of ~13 acetytes/nucleosome (20). Although there will be microheterogeneity in these levels that fluctuate with the cell cycle (18), the studies of Waterborg (20) ultimately indicate that much of the S. cerevisiae genome contains at least 13 acetytes/nucleosome throughout most of the cell cycle at steady-state. The function of this high level of global acetylation, as well as analogous global modifications in vertebrates (e.g. Refs. 42–44), presumably is linked in part to acetylation-induced nucleosomal array decondensation (45–47) and subsequent enhancement of the biological activity of the array (46). However, in contrast to their role in localized gene expression, the function of the HAT A enzymes in maintaining global acetylation is unknown. The finding that the HAT A2 binds stably to nucleosomal arrays suggests that HAT A complexes may be incapable of maintaining the high levels of global acetylation present in vivo, i.e. there are not enough HAT complexes in the cell to acetylate the entire genome presuming that they remain locally bound. However, free Gcn5p can acetylate the H3 N termini of nucleosomal arrays (Fig. 2B; Ref. 26) in the absence of stable nucleosomal array interactions (Fig. 4). Thus, it seems likely that either the free HAT enzymes themselves, or some fundamentally different type of non-genome binding HAT complex, must be involved in global acetylation events. In this regard, Krebs et al. (18) have shown that global monoacetylation of H3 by Gcn5p increases dramatically during S phase. Additionally, there is now evidence that some HAT complexes may function locally whereas others function more globally (19). Finally, it should be emphasized that, despite much recent progress, many key questions remain unanswered regarding the molecular and structural basis for the actions of HATs and their complexes in those situations where dynamic global or local core histone acetylation is linked to alteration of biological function.

Acknowledgments—We are indebted to Drs. S. Berger, J. Workman, C. Peterson, and F. Winston for supplying us with antibodies and to C. Peterson for critically evaluating the manuscript.

REFERENCES
1. Davie, J. R., and Hendzel, M. J. (1994) J. Cell. Biochem. 55, 98–105
2. Davie, J. R. (1998) Curr. Opin. Genet. Dev. 8, 153–178
3. Kuo, M. H., and Allis, C. D. (1994) BioEssays 20, 615–626
4. Grunstein, M. (1997) Nature 389, 349–352
5. Hansen, J. C., Tse, C., and Wolfe, A. P. (1998) Biochemistry 37, 17637–17641
6. Strahl, B. D., and Allis, C. D. (2000) Nature 404, 41–45
7. Brownell, J. E., Zhou, J., Ranalali, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Cell 84, 845–851
8. Grant, P. A., Sterner, D. E., Duggan, L. J., Workman, J. L., and Berger, S. L. (1996) Trends Cell Biol. 6, 183–203
9. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Obha, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
10. Pollard, K. J., and Peterson, C. L. (1997) Mol. Cell. Biol. 17, 6212–6222
11. Candau, R., Zhou, J. X., Allis, C. D., and Berger, S. L. (1997) EMBO J. 16, 555–565
12. Marcus, G. A., Silverman, N., Berger, S. L., Horuchi, J., and Guarente, L. (1994) EMBO J. 13, 4877–4885
13. Eberhart, A., Sterner, D. E., Schieltz, D., Hassan, A., Yates, J. R., III, Berger, S. L., and Workman, J. L. (1999) Mol. Cell. Biol. 19, 6621–6631
14. Roberts, S. M., and Winston, F. (1997) Genetics 147, 451–465
15. Grant, P. A., Schieltz, D., and Berger, S. L. (2000) Nature 404, 41–45
16. Reed, D. J., Reese, J. C., Yates, J. R., III, and Workman, J. L. (1998) Cell 94, 45–53
17. Kuo, M. H., Zhou, J., Jambeck, P., Churchill, M. E., and Allis, C. D. (1998) Genes Dev. 12, 627–635
18. Krebs, J. E., Kuo, M. H., Allis, C. D., and Peterson, C. L. (1999) Genes Dev. 13, 1412–1421
19. Vignali, M., Steger, D. J., Neely, K. E., and Workman, J. L. (2000) EMBO J. 19, 2629–2640
20. Waterborg, J. H. (2000) J. Biol. Chem. 275, 13007–13011
21. Johns, E. W. (1977) Methods Cell Biol. 16, 183–203
22. Schwarz, P. M., and Hansen, J. C. (1994) J. Biol. Chem. 269, 16284–16289
23. Yager, T. D., and van Holde, K. E. (1984) J. Cell. Biol. 99, 173–178
24. Ruiz-Garcı´a, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortí´n, J. E., and Tordera, V. (1998) J. Biol. Chem. 273, 12599–12605
25. Ruiz-Garcı´a, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortí´n, J. E., and Tordera, V. (1998) J. Biol. Chem. 273, 12599–12605
26. Tse, C., Ruiz-Garcı´a, A. B., Georgieva, E. I., Sendra, R., and Hansen, J. C. (1998) J. Biol. Chem. 273, 32388–32392
27. Ruiz-Garcı´a, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortí´n, J. E., and Tordera, V. (1998) J. Biol. Chem. 273, 12599–12605
Chromatin Binding by the HAT A2 Complex

28. Ruiz-Garcia, A. B., Sendra, R., Pamblanco, M., and Tordera, V. (1997) FEBS Lett. 403, 186–190
29. Bonner, W. M., West, M. H., and Stedman, J. D. (1980) Eur. J. Biochem. 109, 17–23
30. Harlow, E., and Lane, D. (eds) (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
31. Candau, R., and Berger, S. L. (1996) J. Biol. Chem. 271, 5237–5245
32. Horiuchi, J., Silverman, N., Marcus, G. A., and Guarente, L. (1995) Mol. Cell. Biol. 15, 1203–1209
33. Saleh, A., Lang, V., Cook, R., and Brandl, C. J. (1997) J. Biol. Chem. 272, 5571–5578
34. Davie, J. R., and Spencer, V. A. (1999) J. Cell. Biochem. 32/33, 141–148
35. Berger, S. L. (1999) Curr. Opin. Cell Biol. 11, 336–341
36. Kegeles, G., and Cann, J. R. (1978) Methods Enzymol. 48, 248–270
37. Tse, C., and Hansen, J. C. (1997) Biochemistry 36, 11381–11388
38. Carruthers, L. C., Bednar, J., Woodcock, C., and Hansen, J. C. (1998) Biochemistry 37, 14776–14787
39. Tse, C., Fletcher, T. M., and Hansen, J. C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12169–12173
40. Syntichaki, P., and Thireos, G. (1998) J. Biol. Chem. 273, 24414–24419
41. Grant, P. A., Eberharter, A., John, S., Cook, R. G., Turner, B. M., and Workman, J. L. (1999) J. Biol. Chem. 274, 5895–5900
42. Hebbes, T. R., Thorne, A. W., Clayton, A. L., and Crane-Robinson, C. (1992) Nucleic Acids Res. 20, 1017–1022
43. Hebbes, T. R., Clayton, A. L., Thorne, A. W., and Crane-Robinson, C. (1994) EMBO J. 13, 1823–1830
44. Keeshane, A. M., Lavender, J. S., O’Neill, L. P., and Turner, B. M. (1998) Dev. Genet. 22, 65–73
45. Garcia-Ramirez, M., Rochini, C., and Ausio, J. (1995) J. Biol. Chem. 270, 17923–17928
46. Tse, C., Sera, T., Wolffe, A. P., and Hansen, J. C. (1998) Mol. Cell. Biol. 18, 4629–4638
47. Pollard, K., Samuels, M. L., Crowley, K. A., Hansen, J. C., and Peterson, C. P. (1999) EMBO J. 18, 5622–5633