Gen5p, a Transcription-related Histone Acetyltransferase, Acetylates Nucleosomes and Folded Nucleosomal Arrays in the Absence of Other Protein Subunits*

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Histone acetylation is a reversible dynamic process that occurs at specific lysine residues in the N termini of all the core histone proteins and has been correlated with several key biological processes, including nucleosome assembly and modulation of gene expression (1–4). The recent discoveries of the specific histone acetyltransferases (HATs), Hat1p (5, 6) and Gcn5p (7), have directly linked histone acetylation with nucleosome assembly and transcriptional activation, respectively. Hat1p has recently been localized in the nucleus as well (8). Gcn5p is the catalytic subunit of several type A histone acetyltransferases (HATs). Previous studies performed under a limited range of solution conditions have found that nucleosome core particles and nucleosomal arrays can be acetylated by Gcn5p only when it is complexed with other proteins, e.g. Gcn5-Ada, HAT-A2, and SAGA. Here we demonstrate that when assayed in buffer containing optimum concentrations of either NaCl or MgCl2, purified yeast recombinant Gcn5p (rGcn5p) efficiently acetylates both nucleosome core particles and nucleosomal arrays. Furthermore, under conditions where nucleosomal arrays are extensively folded, rGcn5p acetylates folded arrays ~40% faster than nucleosome core particles. Finally, rGcn5p poly-acetylates the N termini of free histone H3 but only monoacetylates H3 in nucleosomes and nucleosomal arrays. These results demonstrate both that rGcn5p in and of itself is catalytically active when assayed under optimal solution conditions and that this enzyme prefers folded nucleosomal arrays as a substrate. They further suggest that the structure of the histone H3 N terminus, and concomitantly the accessibility of the H3 acetylation sites, changes upon assembly into nucleosomes and nucleosomal arrays.

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nucleosomes and stored as described previously (18). Core histones used in histone acetyltransferase assays were concentrated to ~1.0 mg/ml and dialyzed into 15 mM Tris-Cl, 0.25 mM EDTA, 0.05% Tween 20, 2.5 mM dithiothreitol, 5.0% glycerol, pH 7.8 (DB buffer). Saturated 12-mer nucleosomal arrays (12 octamers/DNA) were assembled by combining the reconstituted core histones with core histone octamers and performing the salt dialysis method previously as described (18). Reconstitutes were then concentrated to ~1.0 mg/ml and dialyzed into DB buffer. Nucleosomal core particles were isolated by digestion of native chicken oligonucleosomes with micrococcal nuclease as described (19). Core particles were concentrated to ~1.0 mg/ml and dialyzed in DB buffer. All samples were concentrated using an Amicon Centricron concentrator (molecular weight cutoff 10,000).

Expression and Purification of Recombinant GCN5—A 50-ml culture of Escherichia coli BL21 cells containing pRSET-yGCN5 was grown in LB broth containing 0.1 mg/ml ampicillin to an A600 of ~0.3–0.5. Cells were then induced with 0.4 mM isopropyl-1-thio-D-galactopyranoside for 3 h. Recombinant Gcn5p was purified using a Ni2+ -agarose affinity column as described (20), concentrated to ~70 µg/ml, and dialyzed into DB buffer. The purity of the isolated rGcn5p was determined by silver staining of overloaded SDS-polyacrylamide gels.

Analytical Ultracentrifugation—Sedimentation velocity experiments were performed using a Beckman XL-A analytical ultracentrifuge equipped with absorption optics as described (20). The A280 of the solute was adjusted to 0.6 and 0.8. The sedimentation distribution coefficient was determined by the method of van Holde and Weischat (21) using Ultrascan data analysis software version 2.95 (Borries Demeler; University of Texas Health Science Center).

HAT Activity Assays—HAT activity assays were performed with 15 µg of either free histone proteins, nucleosome core particles, or 12-mer nucleosomal arrays as substrates using a slightly modified version of the procedure described previously (22). In a final reaction volume of 80 µl, 5 µl (0.35 µg) of rGcn5p was mixed with 0.2 µCi of [3H]acetyl-CoA (7.2 Ci/mmol; ICN) and DB buffer containing the indicated salt concentration (shown on Fig. 1). Reactions were initiated by the addition of either histone or nucleosomal substrates, incubated for 20 min at 30 °C, and quenched by spotting the sample onto GF/F glass filters (Whatman). Filters were washed 4 times with 5 ml of 25% trichloroacetic acid and then 4 times with 5 ml of 100% ethanol using a Millipore vacuum manifold. The amount of [3H]acetate incorporated into the substrate was determined by scintillation counting. In some cases, 0.1 µCi of [3H]acetyl-CoA (63 mCi/mmol; ICN) was used in the above reaction, and samples were loaded onto either an 18% SDS-polyacrylamide gel (23) or acetic acid/urea/Triton (AUT) (24) gel for gel analyses.

Microsequencing Analysis—The N termini of histone H3 were sequenced with a Perkin-Elmer Applied Biosystems 477A gas/liquid protein sequencer using the N-terminal Edman degradation procedure. Briefly, free core histones were incubated with rGcn5p, 2.5 µCi of [3H]acetyl-CoA, and 2.25 nmol of unlabeled acetyl-CoA for 90 min and then resolved on an 18% SDS-polyacrylamide gel. The histones were transferred to PdV-polyvinylidene difluoride membrane (Millipore) at 1.5 mA/cm2 in a Millipore semi-dry electrophoresis unit. The band corresponding to histone H3 subsequently was excised and sequenced as described.

RESULTS

Recombinant Gcn5p Acetylates Nucleosomes and Nucleosomal Arrays in the Presence of Optimal Concentrations of Monovalent or Divalent Cations—Previous assays of rGcn5p activity have been performed exclusively in buffer containing 50 mM Tris-HCl and either no salt (7, 10, 13, 25) or a mixture of 50 mM KCl plus 50 mM NaCl (12). To determine whether rGcn5p possesses the ability to acetylating nucleosomal substrates when studied over a wider range of salt conditions, HAT assays were conducted in the presence of 15 mM Tris-HCl and either 0–300 mM NaCl or 0–100 mM MgCl2, using free core histones, nucleosome core particles, and defined 12-mer nucleosomal arrays as substrates. The ability of rGcn5p to acetylate free histones remained the same in all the NaCl or MgCl2 concentrations tested indicating that these salts do not affect Gcn5p activity per se (Fig. 1A). In contrast, both nucleosome core particles and nucleosomal arrays were acetylated by rGcn5p only in the presence of a relatively narrow range of NaCl or MgCl2, with maximum acetylation observed in 50 mM NaCl or 2 mM MgCl2 (Fig. 1, B and C, respectively). Under conditions utilized previously by other laboratories, i.e. 0 or 100 mM monovalent cations, we also detected essentially no HAT activity using nucleosomal substrates (Fig. 1B). In addition, rGcn5p acetylated nucleosomal arrays in 50 mM KCl but not 50 mM KCl/50 mM NaCl (data not shown), indicating that the previously reported inability of rGcn5p to acetylate nucleosomal substrates was because of the use of non-optimal salt concentrations rather than an inhibitory effect of K+. Finally, it is important to note that despite the lack of rGcn5p activity in ≥100 mM NaCl alone (Fig. 1B), acetylation of nucleosomes and nucleosomal arrays was observed in the presence of the physiologically relevant mixture of 150 mM NaCl and 2 mM MgCl2 (data not shown).

Acetylation of nucleosome core particles and nucleosomal arrays by rGcn5p in 50 mM NaCl was very similar, although nucleosomal arrays appeared to be slightly preferred under these conditions (Fig. 1B). In contrast, rGcn5p acetylated nucleosomal arrays ~40% faster than nucleosome core particles in 2 mM MgCl2 (Fig. 1C). The enhanced rates of acetylation of nucleosomal arrays relative to nucleosome core particles was strongly correlated with the degree of array folding; nucleosomal arrays were only partially folded in 50 mM NaCl and extensively folded in 2 mM MgCl2 (Fig. 1D; see Refs. 18 and 20), whereas nucleosome core particles sedimented at the expected value of 11 S (26) in both salt conditions (Fig. 1D, inset). Importantly, no salt-dependent histone dissociation occurred under the conditions used for the HAT assays as indicated by both the sedimentation data (Fig. 1D) and native agarose gel electrophoresis (data not shown). In addition, neither nucleosomes nor nucleosomal arrays were acetylated by rHat1p under comparable ionic conditions, indicating that the ability to acetylate nucleosomal substrates was rGcn5p-specific (data not shown). Taken together, the data in Fig. 1 demonstrate both that rGcn5p alone is capable of significantly acetylating nucleosomal substrates under optimal salt and substrate conditions and that rGcn5p more efficiently acetylates extensively folded nucleosomal arrays compared with partially folded arrays and nucleosome core particles.

Gcn5p Polyacetylates Free Histone H3 but Only Monoacetylates Nucleosomal H3—Acetylated free histones, nucleosome core particles, and nucleosomal arrays next were electrophoresed on an 18% SDS-polyacrylamide gel to determine which specific core histones had been modified by rGcn5p (Fig. 2). When free histones were incubated with rGcn5p, both H3 and H4 were acetylated in 50 mM NaCl (lane 1) and 2 mM MgCl2 (lane 2), consistent with previous results (7, 10, 12, 13). In contrast, under these conditions rGcn5p acetylated essentially only H3 in both nucleosomal arrays (lanes 5 and 6) and nucleosome core particles (lanes 9 and 10). In agreement with the liquid HAT assays (Fig. 1), the extent of H3 acetylation of nucleosomal arrays in 2 mM MgCl2 was significantly greater than that observed for nucleosome core particles (Fig. 2; lanes 6 and 10, respectively). No acetylation incorporation was observed in the absence of rGcn5p. These results demonstrate that rGcn5p primarily modifies H3 in nucleosomal core particles and nucleosomal arrays.

Given that the assembly of histones into nucleosomes decreases the overall accessibility of the H3 N termini to rGcn5p (see Fig. 1, A–C), we investigated whether the acetylation target sites in the H3 N termini also were affected. Free histones, nucleosome core particles, and nucleosomal arrays were incubated with an excess of rGcn5p and acetyl-CoA in either 2 mM MgCl2 or 50 mM NaCl for 0–90 min and analyzed by AUT gel electrophoresis (24). The results obtained in 2 mM MgCl2 are shown in Fig. 3. Under these conditions, the N termini of free histone H3 were polyacetylated. In contrast, only monoacety-
lated H3 was observed in both core particles and nucleosomal arrays. No unacetylated H3 molecules remained after 90 min of incubation, indicating that all of the H3 N termini were either polyacetylated or monoacetylated in free histones or nucleosomal substrates, respectively (data not shown). Hence, when assembled into nucleosomes, only the total number of acetylatable sites within the H3 N termini were limited to acetylation by rGcn5p. Results obtained in 50 mM NaCl were similar to those in MgCl2 (data not shown).

To determine the specific H3 residues acetylated in the three different substrates, microsequence analyses of the first 30 amino acid residues were performed (Fig. 3, C–E). The amount of radioactivity present in each cycle was determined by scintillation counting. Results indicated that polyacetylation of free H3 occurred at lysines 9, 14, and 18 (Fig. 3C). In contrast, H3 was acetylated only at lysine 14 in both nucleosomal core particles and nucleosomal arrays (Fig. 3D and E). These data indicate that the structure and accessibility of the H3 N termini change upon assembly of free histones into nucleosomes and nucleosomal arrays. Recently, it has been reported that Gcn5p is required either directly or indirectly for the acetylation of residues 9, 14, and 18 of histone H3 in vivo (13, 27). Our results are both consistent with these observations and extend them by showing that rGcn5p can directly acetylate all three residues of free H3 in the absence of other proteins in vitro.

**DISCUSSION**

The recruitment of the histone acetyltransferase, Gcn5p, to specific genes (16, 27) and the ability of acetylation to decondense repressive higher order chromatin structures (28, 29) have provided important clues into the molecular basis of how acetylation facilitates gene expression. Our results indicate that Gcn5p action and chromatin decondensation may be directly linked. Although it has been proposed that Gcn5p must interact with other protein subunits to acetylate nucleosomal arrays (10, 12–14, 30), our studies have demonstrated unequivocally that rGcn5p per se is capable of acetylating both nucleosomes and nucleosomal arrays under appropriate ionic conditions, including those that approximate the ionic environment present in the nucleus. Furthermore, rGcn5p exhibits a marked preference for extensively folded nucleosomal arrays as...
a substrate. Cumulatively, these observations necessitate several fundamental changes in the present model of how Gcn5p functions to regulate gene expression. Gcn5p is currently viewed as an inactive enzyme that is recruited to specific promoters through interaction(s) with transcriptional coactivators and when complexed subsequently leads to local acetylation-dependent disruption of the structure of specifically targeted nucleosomes (16, 27). The available data now indicate that Gcn5p both is capable of acetylating chromatin in the absence of coactivators and likely functions at the level of global chromosomal domains (i.e. $\geq 2–3$ nucleosomes) by facilitating targeted chromatin decondensation. In support of the latter view, it is well established that extensively folded nucleosomal arrays are repressive to transcription by eukaryotic RNA polymerases (28, 31, 32), and it has recently been shown that an unexpectedly low threshold level of acetylation dramatically enhances transcription initiation and elongation by causing array decondensation (28). Importantly, studies performed with antibodies that specifically recognize histone H3 diacetylated at Lys-91 and Lys-14 (27) do not contradict a more global function for Gcn5p, because these experiments would not have detected any nucleosomes acetylated only at Lys-14 of H3 (Fig. 3) that were located away from the promoter.

As chromatin folds into progressively higher order structures with increasing salt concentrations, the N termini undergo significant changes in their location and accessibility (33, 34). Hence, the most likely explanation for the ability of Gcn5p-transcription factor complexes to acetylate nucleosomal substrates in the absence of salt in vitro (7, 10, 13, 25, 30) is that the H3 N termini simply become more accessible under these conditions because of the presence of the Gcn5p-associated proteins. Although there is clearly a genetic relationship between Gcn5p and Ada proteins in yeast in vivo (35, 36), the mechanistic roles of these accessory proteins remain to be fully defined. One function may be to potentiate the activity of Gcn5p (30). However, actions unrelated to inducement of Gcn5p catalytic activity, such as targeting to specific chromo-

FIG. 2. Acetylated histones resolved on an 18% SDS-polyacrylamide gel. A, HAT activity assays were conducted essentially as described under “Experimental Procedures” with the exception that 0.1 $\mu$Ci of [14C]acetyl-CoA was used. Ten micrograms of each sample was loaded and electrophoresed. Bands were visualized by Coomassie Blue staining. Free core histones were incubated with rGcn5p in the presence of 50 mM NaCl (lane 1) or 2 mM MgCl$_2$ (lane 2). Free histones incubated without Gcn5p in the presence of NaCl or MgCl$_2$ are in lanes 3 and 4, respectively. Nucleosomal arrays were incubated with rGcn5p in the presence of 50 mM NaCl (lane 5) or 2 mM MgCl$_2$ (lane 6). Nucleosomal arrays incubated without Gcn5p in NaCl or MgCl$_2$ are in lanes 7 and 8, respectively. Nucleosome core particles were incubated with rGcn5p in the presence of 50 mM NaCl (lane 9) or 2 mM MgCl$_2$ (lane 10). Nucleosomal core particles incubated without Gcn5p in NaCl or MgCl$_2$ are in lanes 11 and 12, respectively. B, fluorogram of the gel in A. The lanes correspond to the lanes indicated in A.

FIG. 3. Determination of the degree of acetylation of free core histones, nucleosomal core particles, and nucleosomal arrays. A, HAT activity assays were carried out by incubation of different substrates with rGcn5p and 2.25 nmol of unlabeled acetyl-CoA plus 1.59 nmol of [14C]acetyl-CoA (63 mCi/mmol). Reactions were stopped at $t = 0, 30, \text{and } 60$ min and electrophoresed on an AUT gel. Bands were visualized by Coomassie Blue staining. B, fluorography of the same gel in A. C, microsequencing analysis of free histones incubated with rGcn5p for 90 min as described under “Experimental Procedures.” Shown are the sequencing cycles versus disintegrations/min. Each amino acid residue from the N terminus of H3 corresponds to 1 cycle. Acetylatable lysine residues are indicated as 9, 14, 18, 23, and 27. D, microsequencing analysis of nucleosomal arrays as described in C. E, microsequencing analysis of nucleosomal core particles as described in C.
nucleosomal loci or regulation of multiple acetylation site usage, also must be considered. In this regard, it should be noted that the mammalian Gcn5p has an extra ~350-amino acid N-terminal extension not found in the yeast homologue (37, 38), which may serve to replace some or all of the functions contributed by the yeast accessory proteins.

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REFERENCES

1. Allfrey, V., Faulkner, R. M., and Mirsky, A. E. (1964) Proc. Natl. Acad. Sci. U. S. A. 51, 786–784
2. Tordera, V., Sendra, R., and Pérez-Ortíz, J. E. (1993) experientia 49, 786–788
3. Brownell, J. E., and Allis, C. D. (1996) Curr. Opin. Genet. Dev. 6, 176–184
4. Davie, J. R. (1997) Mol. Biol. Rep. 24, 197–207
5. Kleff, S., Andruulis, E. D., Anderson, C. W., and Sternglanz, R. (1995) J. Biol. Chem. 270, 24674–24677
6. Parthun, M., Widom, J., and Gottesring, D. (1996) Cell 87, 85–94
7. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmundson, D. G., Roth, S. Y., and Allis, C. D. (1996) Cell 84, 843–851
8. Ruiz-García, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortíz, J. E., and Tordera, V. (1996) J. Biol. Chem. 271, 12599–12605
9. Verreault, A., Kaufman, P. D., Kobayashi, R., and Stillman, B. (1998) Curr. Biol. 8, 96–108
10. Kuo, M. H., Brownell, J. E., Sobel, R. E., Ranalli, T. A., Cook, R. G., Edmundson, D. G., Roth, S. Y., and Allis, C. D. (1996) Nature 383, 269–272
11. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
12. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Cauda, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
13. Zhang, W.,Bone, J. R., Edmundson, D. G., Turner, B. M., and Roth, S. Y. (1998) EMBO J. 17, 3155–3167
14. Ruiz-García, A. B., Sendra, R., Pamblanco, M., and Tordera, V. (1997) FEBS 403, 186–190
15. Pollard, K. J., and Peterson, C. L. (1997) Mol. Cell. Biol. 17, 6212–6222
16. Utley, R. T., Ikeda, K., Grant, P. A., Cote, J., Steger, D. J., Eberharder, A., John, S., and Workman, J. L. (1998) Nature 394, 498–502
17. George, P., Demeler, B., Terpening, C., Paule, M. R., and van Holde, K. E. (1993) J. Biol. Chem. 268, 1947–1954
18. Hansen, J. C., Audo, J., Stanik, V. H., and van Holde, K. E. (1989) Biochemistry 28, 9129–9136
19. Ballester, E., Abad, C., and Franco, L. (1996) J. Biol. Chem. 271, 18817–18824
20. Schwarz, P. M., and Hansen, J. C. (1994) J. Biol. Chem. 269, 16284–16289
21. van Holde, K. E., and Weisheit, W. O. (1976) Biopolymers 17, 1387–1403
22. López-Rodas, G., Pérez-Ortíz, J. E., Tordera, V., Salvador, M. L., and Franco, L. (1996) Arch. Biochem. Biophys. 239, 184–190
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Bonner, W. M., West, M. H., and Stedman, J. D. (1980) Eur. J. Biochem. 109, 17–23
25. Wang, L., Mixzen, C., Ying, C., Candau, R., Barlev, N., Brownell, J., Allis, C. D., and Berger, S. L. (1997) Mol. Cell. Biol. 17, 519–527
26. van Holde, K. E. (1988) Chromatin, Springer-Verlag, New York
27. Kuo, M. H., Zhou, J., Jambeck, P., Churchill, M. E. A., and Allis, C. D. (1998) Genes Dev. 12, 627–639
28. Tse, C., Sera, T., Wolfe, A. P., and Hansen, J. C. (1998) Mol. Cell. Biol. 18, 4629–4638
29. Walia, H., Chen, H. Y., Sun, J. M., Holth, L. T., and Davie, J. R. (1998) J. Biol. Chem. 273, 14516–14522
30. Syntichaki, P., and Thireos, G. (1998) J. Biol. Chem. 273, 24414–24419
31. Hansen, J. C., and Wolfe, A. P. (1992) Biochemistry 31, 7977–7988
32. Hansen, J. C., and Wolfe, A. P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2339–2343
33. Usachenko, S. I., Ruvkin, S. G., Gavin, I. M., and Bradbury, E. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6845–6849
34. Fletcher, T. M., and Hansen, J. C. (1995) J. Biol. Chem. 270, 25359–25362
35. Georgakopoulos, T., Gounalaki, N., and Thireos, G. (1995) Mol. Gen. Genet. 246, 723–728
36. Candau, R., Zhou, J. X., Allis, C. D., and Berger, S. L. (1997) EMBO J. 16, 555–565
37. Smith, E. R., Belote, J. M., Schütz, R. L., Yang, X. J., Moore, P. A., Berger, S. L., Nakatani, Y., and Allis, C. D. (1998) Nucleic Acids Res. 26, 2948–2954
38. Xu, W., Edmondson, D. G., and Roth, S. Y. (1998) Mol. Cell. Biol. 18, 5659–5669
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