Multiple Histone Acetyltransferases Are Associated with a Chicken Erythrocyte Chromatin Fraction Enriched in Active Genes*

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We have examined salt-soluble chromatin released by micrococcal nuclease from a 15-day-old chicken embryo erythrocyte nuclei for histone acetyltransferase (HAT) activities. This chromatin is enriched in transcriptionally active sequences from within the active β-globin locus and contains elevated levels of acetylated core histones. HAT activities present in this fraction target histones H4, H3, and H2A when the chromatin itself is used as the substrate. In gel HAT activity assay demonstrates that the salt-soluble chromatin fraction contains four acetyltransferase molecules distinguished by their different molecular masses (47, 33, 32, and 28 kDa). Further separation of the chromatin by centrifugation through sucrose gradients shows that the acetyltransferases segregate into chromatin-bound and chromatin-free populations. The 32- and 28-kDa HATs are associated with chromatin, whereas the 47- and 33-kDa HAT molecules are not. The chromatin-bound HAT activities predominantly target H4 to give the diacetyl and triacetyl species; some acetylation of H2A can also be seen. Our results suggest that the chromatin-associated acetyltransferases have a role in gene regulation.

The N-terminal tails of the core histone proteins are subject to a number of different posttranslational modifications that can confer specific modification states of chromatin involved in a number of different biological processes (as reviewed in Ref. 1). Of these processes, the reversible acetylation of specific lysine residues is a key modification linked to transcriptional activation (2). Since the original proposal by Allfrey and coworkers (3) that acetylation could facilitate the passage of polymerase through chromatin, acetylation and its relationship with gene regulation have been intensively studied. Immunoprecipitation studies using antibodies capable of selecting hyperacetylated chromatin have established a direct link between the modification and the actively transcribing αD-globin chromatin of embryonic chicken erythrocytes (4). The identification of the Tetrahymena p55 acetyltransferase as the yeast Gcn5 homologue underlined the importance of acetylation in transcriptional regulation (5, 6). Afterward, a number of transcription factors and coactivators had been identified as acetyltransferases (7–10). The majority of these enzymes function in multiprotein complexes and provide a mechanism of specifically targeting acetylation to active promoters to confer a localized acetylation state of the chromatin (10, 11). Recently, the elongation factor Elp3 has been shown to possess histone acetyltransferase (HAT)¹ activity (12) and could acetylate the core histones as the transcription complex passes along the template.

A more widespread role for acetylation was indicated by our studies with the chicken β-globin domain. In these experiments, we found that high levels of the modification were not restricted to the transcribed genes but were spread throughout the domain spanning some 33 kilobases of DNA. This hyperacetylation was found to correlate closely with the boundaries of the domain as defined by generalized DNase I sensitivity (13) and the 5'-insulator element at the boundary (14). At the human β-globin locus, acetylation of histones H3 and H4 marks a broad open chromatin region with a peak of H3 acetylation at the upstream locus control region and at the active β-globin gene (15). Similarly, widespread H3/H4 acetylation peaking at the locus control region has also been reported in the human growth hormone locus (16). Histone H4 hyperacetylation over a chromatin region spanning 120 kilobases upstream of the Xist somatic promoter was also found in female mouse embryonic stem cells (17). These experiments imply that acetylation, in addition to a role in transcription, may also be involved in establishing or maintaining the transcriptionally competent chromatin conformation.

It is possible that the domain-wide acetylation we had previously observed in the chicken β-globin domain may not be achieved through the same enzyme mechanisms that were used for the specific targeting of acetylation to the promoters. We, therefore, investigated whether chromatin preparations enriched in active gene sequences contained novel acetyltransferases, which could have a role in this type of acetylation. We report that chromatin, released from embryonic chicken erythrocyte nuclei by micrococcal nuclease and subsequently salt fractionated, contains enriched levels of both active DNA sequences and elevated levels of acetylated core histones. Significantly, this chromatin also contains at least four different HAT molecules, three of which are much smaller than previously described acetyltransferases. These acetyltransferases have been further separated by sucrose gradient centrifugation into free and chromatin-bound populations. Our data suggest that these molecules have a role in active chromatin. In particular, it is possible that one or more of the chromatin-bound molecules could have a role in domain-wide acetylation.

EXPERIMENTAL PROCEDURES

Preparation of Chromatin—Nuclei were prepared from 15-day-old chicken embryos as described previously (4) but by using 10 mM NaCl in cell lysis and nuclear wash buffers. Nuclei were washed three times in a digest buffer (10 mM NaCl, 10 mM sodium butyrate, 10 mM Tris-HCl, pH 7.5, 3 mM MgCl₂, 1 mM CaCl₂, 0.1 mM PMSF, 0.1 mM benzamidine) and resuspended at a concentration of 5 mg/ml DNA. The nuclei were

¹ The abbreviations used are: HAT, histone acetyltransferase; PAGE, polyacrylamide gel electrophoresis; AUT, acetic acid/urea/Triton; PMSF, phenylmethylsulfonyl fluoride.
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preincubated at 37 °C for 3 min and then digested with 200 units/ml micrococcal nuclease ( Worthington) for 4 min at 37 °C. The digestion was terminated with 10 mM Na2EDTA and the suspension was chilled on ice. Nuclei were centrifuged at 4000 × g for 2 min, and the supernatant was retained. The pellet was resuspended in a lysine buffer (10 mM Na2EDTA, 10 mM Tris-HCl, pH 7.5, 10 mM sodium butyrate, 0.1 mM PMSF, 0.1 mM benzamide), incubated on ice for 10 min, and centrifuged as above retaining supernatant S2. The pellet was then resuspended in 0.25 mM Na2EDTA, 10 mM sodium butyrate, 10 mM Tris-HCl, pH 7.5, 0.1 mM PMSF, 0.1 mM benzamide, and incubated for 90 min at 4 °C before centrifuging and retaining the supernatant S3. For salt fractionation, the chromatin-containing supernatants were centrifuged. 1 mM NaCl was added to the released chromatin to a final concentration of 100 mM, and the sample was incubated in ice for 10 min to allow H1-containing chromatin to precipitate. The precipitate was removed by centrifuging at 10,000 × g for 10 min, and the salt-soluble chromatin in the supernatant was retained. Salt-soluble chromatin was then either dialyzed overnight against 10 mM Tris-HCl, pH 7.5, 10 mM sodium butyrate, 0.25 mM Na2EDTA, 0.1 mM PMSF, 0.1 mM benzamide or was loaded directly onto a 5–25% linear sucrose gradient in the same buffer and centrifuged at 36,000 rpm in a Beckman SW 41ti rotor for 14 h at 4 °C.

HAT Assays—To assay for chromatin-bound acetyltransferase activity in chromatin samples or across sucrose gradients, 50 μl of each sample were taken and made into a sucrose gradient of 50% 1 M NaCl, pH 7.9, 10 mM sodium butyrate. To this buffer, 0.1 μCi of [3H]acetate-CoA (2–10 Ci/mmol, Amersham Pharmacia Biotech) was added, and the sample was incubated at 37 °C for 60 min. The samples were spotted onto glass fiber filters that were incubated in 20% trichloroacetic acid for 20 min followed by two 15-min washes in 5% trichloroacetic acid. Filters were then incubated in acetic acid and ethanol (1:1) for 10 min and air-dried. Scintillant was added to the filters before counting. To test for acetyltransferases in the subnucleosomal regions of sucrose gradients, HAT assays were performed as above except that 20 μg of calf thymus total histone ( Worthington) was added.

In Gel HAT Activity Assays—In gel HAT activity assays were performed essentially as described by Brownell and Allis (5). Samples from salt-soluble chromatin, subnucleosomes, and mono-dinucleosomes were concentrated and digested with 10 units of DNase I ( Worthington) at 3 mM MgCl2 for 30 min at 37 °C. Samples were made to 1× SDS-loading buffer and electrophoresed through 13% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, with either the calf thymus total histone at 0.1 mg/ml or no substrate (autoacetylation) polymerized into the resolving gels.

Immunodepletion—For immunodepletion assays, 5 μg of sucrose gradient purified mononucleosomes from salt-soluble chromatin were used. Either 20 μl of pan acetyl-lysine or 10 μl of H4 antisem was preincubated with 100 μl of 50% protein A-Sepharose and washed three times in 1 ml of 10 mM Tris-HCl, pH 7.5, 10 mM sodium butyrate, 0.25 mM Na2EDTA, 0.1 mM PMSF, 0.1 mM benzamide. The chromatin and protein A-antibody were combined in a volume of 200 μl and incubated for 2 h on ice with gentle agitation. After incubation, the immune complex was pelleted at 4000 × g for 2 min, the supernatant was retained, and the samples were taken and assayed for HAT activity. DNA was recovered from the immunoprecipitates after washing the resin three times in the above buffer and eluting in 1.5% SDS. DNA was recovered from equal proportions of the supernatant and immunoprecipitate fractions by phenol/chloroform extraction before hybridization analysis.

Protein Extraction and Electrophoresis—Proteins from all chromatin fractions were isolated by phenol/chloroform extraction as previously described (4) and electrophoresed on 15% polyacrylamide acetic acid/urea/Triton gels as described by Bonner et al. (18) or electrophoresed on 15% SDS-PAGE.

Southern Blots and Hybridizations—DNA extracted from released, salt-insoluble, and salt-soluble chromatin fractions was recovered by phenol/chloroform extraction and loaded directly onto 1.2% agarose gels. After electrophoresis, gels were incubated in 0.5 M NaOH, 1.5 M NaCl for 30 min and the DNA transferred to Hybond N+ membranes (Amersham Pharmacia Biotech) in 20× SSC. After transfer, the membranes were rinsed in 2× SSC, blotted dry, and baked at 80 °C for 30 min. DNA hybridizations were performed by use of 50 ng of genomic probe for D, B, β-globin, and α-globin probes (13), labeled by random priming to specific activities of 4 – 8 × 107 dpm/μg. Filters were prehybridized for 60 min and hybridized for 2 h using QuickHyb solution (Stratagene, La Jolla, CA) at 65 °C. After hybridization, filters were washed as follows: twice in 2× SSC, 0.1% SDS for 5 min at 65 °C, once with 2× SSC, 0.1% SDS for 30 min at 65 °C, once with 0.2× SSC, 0.1% SDS for 20 min at 65 °C, and finally twice with 2× SSC, 0.1% SDS for 5 min at 65 °C. All hybridization and washing procedures were performed in bottles in a hybridization oven (Hyblot, Franklin, MA). Filters were blotted dry and exposed to PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Salt-soluble Chromatin Contains HAT Activity—Nuclei were prepared from 15-day-old chicken embryo erythrocytes and subsequently digested with micrococcal nuclease and lysed with EDTA to release approximately 50% of the DNA content into the supernatant. This chromatin-containing supernatant was further fractionated into salt-soluble (~30% of released) and salt-insoluble (~70% of released) fractions by the addition of 1 mM NaCl to 100 mM as described previously (19). This technique, which fractionates chromatin on the basis of H1-mediated aggregation and differential acetylation, has been extensively used to prepare chromatin enriched in both active DNA sequences and in acetylated core histones (20, 21). To determine whether both the released and the aggregation-resistant, salt-soluble chromatin contained HAT activities, the two samples were dialyzed, and equal proportions were assayed for HAT activities by incubating in the presence of [3H]acetate-CoA using the chromatin itself as a substrate. Fig. 1a gives the initial characterization of the chromatin. The ethidium bromide-stained gel shows that the salt-soluble chromatin comprises mainly mononucleosomes and dinucleosomes, but larger oligonucleosomes are also present in lower amounts. This contrasts with the distribution of chromatin released from the nuclei, which contains a broad distribution of fragments. When tested for HAT activities, similar counts were recorded for both the released and salt-soluble chromatins, despite the higher quantity of material in the released fraction. Chromatin released by micrococcal nuclease, therefore, contains significant levels of HAT activity, which is largely retained in the salt-soluble fraction. To show the principal targets of the acetyltransferase activity, proteins from the released and salt-soluble chromatin were extracted, and equal proportions were resolved by SDS-PAGE and fluorography was performed. The Coomassie Blue-stained gel shows that the salt-soluble chromatin is depleted in the linker histones H1/H5 but retains a full complement of core histones. In both released and salt-soluble chromatin, the fluorograph shows that histones H4, H3, and to a lesser extent H2A are the principal targets for acetylation. No acetylation of the linker histones is observed in the released chromatin.

To test the distribution of active and inactive DNA sequences in the different fractions, DNA was recovered from the released, salt-insoluble pellet and salt-soluble supernatant. Equal proportions were electrophoresed and Southern blots were performed. Filters were then hybridized with probes from inside and outside the β-globin domain. The results of the hybridizations are shown in Fig. 1b. Quantitation of the blots shows that for the inactive chromatin, probe D, approximately 75% of the sequences present in the released chromatin are found in the salt-insoluble chromatin fraction and 25% in the soluble fraction. This fragment is located immediately outside the globin domain and is contained within DNase I-resistant chromatin, which is not highly acetylated (13). In contrast, the majority of the sequences from within the globin domain B, β-globin, and β′-globin are present in the salt-soluble fraction (65–80% of released). The blots also show the relative size distribution of the different sequences in each fraction. Sequences from within the domain are relatively evenly distributed from the monomer through to higher oligonucleosomes, despite the bias of the DNA toward the shorter fragments that are evident in the soluble fraction. The majority of the inactive sequences, however, are found in the higher oligonucleosomes.
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Salt-soluble Chromatin Contains Multiple HAT Molecules—To determine the number and size of any acetyltransferase molecules present in the salt-soluble chromatin, we used the in gel HAT activity assay essentially as described by Brownell and Allis (5). Salt-soluble chromatin was prepared, concentrated, and digested with DNase I to remove the DNA that interferes with the assay. Proteins were then separated through denaturing SDS gels containing either histone or no substrate polymerized into the resolving gel. After electrophoresis, SDS was removed from the gel, and the proteins were treated with 8 M urea before renaturing. The gel was then incubated in the presence of [3H]acetetyl-CoA to allow any renatured HAT molecules present in the sample to transfer [3H]acetetyl groups to the histones polymerized into the gel matrix. After washing, the gel was dried and fluorography was performed. The results of the assay are given in Fig. 3. For this assay, approximately 30 μg of chromatin were used per track. Gels were electrophoresed such that the core histones were run off the bottom. The Coomassie Blue-stained gel shows a number of different proteins present in the salt-soluble chromatin not seen on a normal loading of 2–3 μg (see Fig. 1a). The fluorograph shows the presence of four bands that have acetyltransferase activity classified by approximate molecular mass; there are a 47-kDa HAT, two similarly sized acetyltransferases 33- and 32-kDa HAT, and a smaller 28-kDa HAT. In the case of the small 33-, 32-, and 28-kDa HAT molecules, the individual bands cannot readily be identified because of the abundance of residual linker histones running in the same region of the gel. It is important to note that the in gel HAT activity assay only reveals the size of HAT molecules and not the native size of any complex of which they might be a component.

In addition to the in gel HAT activity assay with histones H4, H3, and H2A are all labeled. For H4, the Ac-2 and Ac-3 are the most heavily labeled species, which is confirmed by the densitometry traces. Some labeling of Ac-1 and Ac-4 is also evident at a lower level. This labeling pattern may well be influenced by the high base-line level of acetylation of the chromatin. However, it could not be altered by pulse-chase labeling experiments or by preparing chromatin in the absence of sodium butyrate (data not shown). This pattern of acetylation suggests a specific targeting of acetylation to H4 resulting in the diacetyl and triacetyl species.
Fig. 2. Acetylation levels of histones from released and salt-soluble chromatin. a, samples of released (r) and salt-soluble (ss) chromatin before and after labeling with [3H]acetyl-CoA. Equal quantities of the histones from released and salt-soluble chromatin were separated by AUT-PAGE and fluorography was performed. b, densitometry traces of the H4 region from the AUT-PAGE gel and fluorograph of the released and salt-soluble chromatin.

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Fig. 3. In gel HAT activity assay of proteins from salt-soluble chromatin. 30 μg of salt-soluble chromatin (ss) was loaded onto three 13% SDS-PAGE gels. One gel was stained with Coomassie Brilliant Blue, whereas the two remaining gels were subjected to an in gel activity assay, with (histone) or without (auto) the incorporation of calf total histone polymerized into the gel matrix. Acetyltransferases are indicated by approximate molecular masses (kDa).

In these experiments it has not been possible to assign the histone acetyltransferases to specific bands in the Coomassie Blue-stained gels. For the chromatin sample, labeled histones were extracted and resolved by both SDS and AUT-PAGE, and fluorography was performed. The fluorograph of the SDS gel shows that histone H4 and to a lesser extent H2A are labeled, but the H3 activity seen in the sample before separation on sucrose gradient is no longer present (Fig. 2). The Coomassie Blue-stained acetic acid urea gel shows that the gradient-purified chromatin contains a similar level of acetylated histone to the bulk salt-soluble chromatin, notably the reduced level of H4 Ac-0. The nonhistone proteins HMG14 and HMG17 are also evident. The fluorograph of this gel shows that for H4, Ac-2 and Ac-3 are the major species labeled histones were extracted and resolved by both SDS and AUT-PAGE, and fluorography was performed. The fluorograph of the SDS gel shows that histone H4 and to a lesser extent H2A are labeled, but the H3 activity seen in the sample before separation on sucrose gradient is no longer present (Fig. 1). To test for free HAT activities in the subnucleosomal region of the gradient, HAT assays were performed but with the addition of exogenous calf thymus histones. This process shows a broad peak of HAT activity that can be attributed to HAT molecules which are not intimately associated with the chromatin fragments.

To determine the acetylation patterns for the different histones, proteins were extracted from the chromatin sample (monomer-dimer) and from the subnucleosomal region, and they were electrophoresed and fluorography was performed. In addition, samples from both the chromatin and subnucleosomal regions of the gradient were taken and analyzed by in gel HAT activity assays. The results of the analyses are given in Fig. 4b. For the subnucleosomal region of the gradient, the fluorograph of the labeled histones shows H3 and H4 acetylation. The in gel HAT activity assay shows the presence of the 47- and 33-kDa HAT molecules. For the chromatin sample, labeled histones were extracted and resolved by both SDS and AUT-PAGE, and fluorography was performed. The fluorograph of the SDS gel shows that histone H4 and to a lesser extent H2A are labeled, but the H3 activity seen in the sample before separation on sucrose gradient is no longer present (Figs. 1 and 2). The Coomasie Blue-stained acetic acid urea gel shows that the gradient-purified chromatin contains a similar level of acetylated histone to the bulk salt-soluble chromatin, notably the reduced level of H4 Ac-0. The nonhistone proteins HMG14 and HMG17 are also evident. The fluorograph of this gel shows that for H4, Ac-2 and Ac-3 are the major species labeled at ratios similar to those in the bulk salt-soluble chromatin (see Fig. 2). The in gel HAT activity assay of this chromatin reveals the presence of the 32- and 28-kDa HAT molecules. There is also some 33-kDa HAT present in the chromatin sample, although the majority of this acetyltransferase is found in the subnucleosomal region of the gradient.

To further test the stability of the interaction of the acetyltransferases with the chromatin, salt-soluble chromatin was prepared and treated with buffers containing NaCl up to 400 mM before separation by sucrose gradient. This treatment did not remove the HAT activity from the chromatin, indicating that chromatin/HAT interaction is stable (data not shown).

In these experiments it has not been possible to assign the histone acetyltransferase to specific bands in the Coomassie Blue-stained gels. For example, the majority of the 33-kDa HAT, which runs in the same region as the linker histone H1 in the salt-soluble chromatin (Fig. 3), is found in the subnucleosomal region of the sucrose gradient (Fig. 4a). Although this region does not contain any linker histones, the band cannot be clearly identified because of the number of proteins with similar molecular masses (Fig. 4b). Similarly the 28-kDa acetyl-
transferase cannot be identified because of the presence of H5 in either the salt-soluble or the sucrose gradient-purified chromatin (Figs. 3 and 4b). An in gel HAT activity assay of purified monomers using a higher percentage gel is able to partially separate a number of bands from the H5, but the HAT molecule cannot unambiguously be identified (data not shown).

The separation of the salt-soluble chromatin by centrifugation through sucrose gradients allows a distinction between free and chromatin-bound HAT molecules to be made. This experiment indicates that the 28- and 32-kDa HAT molecules are primarily associated with chromatin whereas 47- and 33-kDa HAT molecules are not. Given that the interactions between the HAT molecules and the chromatin survive salt treatments and the forces of centrifugation, it is a possibility that the molecules are specifically associated with chromatin derived from active chromosomal locations.

Chromatin-bound HAT Activities Are Associated with Active Chromatin—To test whether the HAT activity in the salt-soluble monomer is associated with the acetylated, active chromatin, monomers from the first half of the peak containing the highest HAT activity were taken and incubated with either the pan acetyl-lysine antibodies, which are highly specific for acetylated active chromatin (4, 13, 25), or with H4 antibodies as control. After the removal of antibody-chromatin complexes, the resulting supernatants were assayed for residual HAT activity. The results of the immunodepletion are given in Fig. 5. The HAT activity in the chromatin incubated with H4 antibodies is similar to that of the input sample incubated with the buffer alone, and it shows little or no depletion of activity. In contrast, the pan acetyl-lysine antibodies almost completely deplete the HAT activity from the chromatin, strongly indicating that chromatin-bound HAT molecules are associated with the active fraction. In these experiments, although we were unable to detect HAT activities in the antibody-chromatin complex, probably because of antibody binding to the histone tails and thus preventing acetylation, we were able to recover the DNA from the immunoprecipitates for hybridization analysis. This DNA along with the DNA from the chromatin supernatants was hybridized to the active βA-globin and inactive D probes, shown in Fig. 5. The Southern blots show that the chromatin incubated with the pan acetyl-lysine antibodies is severely depleted in the βA-globin sequences, whereas the chromatin incubated with H4 antibodies has a similar level to untreated chromatin. In contrast, the inactive probe D sequences are found largely in the supernatants of both chromatin samples. The DNA recovered from the pan acetyl-lysine immunoprecipitate contains the majority of the βA-globin and little or no inactive D sequences, confirming the selectivity of the antibodies for active chromatin. The H4 immunoprecipitate shows a small hybridization signal for both βA-globin and D. This level of hybridization would be expected because the antibodies only precipitate a small proportion of the chromatin, as revealed by the ethidium bromide gel. The immunodepletion of the HAT activity with the pan acetyl-lysine antibodies strongly implies a role for the chromatin-bound acetyltransferases in active chromatin.
DISCUSSION

We have examined chromatin released from 15-day-old chicken embryo erythrocytes by micrococcal nuclease digestion for acetyltransferase activities. This chromatin was found to contain significant acetyltransferase activities directed toward histones H4, H3, and H2A. These activities were retained in the salt-soluble chromatin, which is enriched in DNA sequences from the transcriptionally active β-globin domain. We have previously shown that the chromatin within the domain carries high levels of acetylated core histones and is preferentially sensitive to general DNase I digestion (13). This soluble chromatin fraction also contains elevated levels of acetylated core histones and is enriched in the nonhistone proteins HMG14 and HMG17, in line with previously described preparations (19, 26). It has been reported that HMG17 can be acetylated by PCAF (23), although we do not detect HMG17 acetylation in these experiments.

The in gel HAT activity assay revealed that the salt-soluble chromatin contains four different HAT molecules classified by approximate molecular masses (47-, 33-, 32-, and 28-kDa HAT molecules). The 47-kDa HAT is similar in size to a number of previously identified HATs, e.g. Tetrahymena p55, the yeast Gcn5 homologue (6), the short form of Gcn5 at 60 kDa (27), Esa-1 at 53 kDa (28), and the elongation factor Elp3 at 60 kDa (12). The remaining acetyltransferases are significantly smaller at 33-, 32-, and 28-kDa HATs and represent uncharacterized HAT molecules, the identity of which remains to be determined.

Centrifugation of the salt-soluble chromatin through sucrose gradients revealed that two of the acetyltransferases, the 32- and 28-kDa HATs, were chromatin-associated, whereas the 33- and 47-kDa HATs were not. To date, the majority of HATs have been found in multiprotein complexes (7, 10). We have considered the possibility that the chromatin-associated acetyltransferase molecules could simply be co-sedimenting within the gradients. If this were the case, then we would expect to see discrete peaks of HAT activity. Our finding that the activity is distributed throughout the chromatin fragments suggests that the activities are chromatin-associated (Fig. 4). However, because the in gel HAT activity assay disrupts multiprotein interactions, we do not know whether the HAT molecules bind chromatin individually or are part of a small complex, which only slightly affects the sedimentation characteristics of the chromatin.

Because of the overlap of different HAT activities it has not been possible to assign a particular histone target to an individual HAT molecule. We note, however, that the H3 activity present in the salt-soluble chromatin before centrifugation (see Figs. 1 and 2) is not present on the purified mono-dinucleosomes. This finding indicates that the activity could reside on either the 47- or 33-kDa HAT molecules. The predominant HAT activity associated with the chromatin targets H4 to give diacetyl and triacetyl species. Although we cannot assign the activity to either the 32- or 28-kDa molecules, these data indicate a specific role for the diacetyl and triacetyl species of H4 in active chromatin. These data are further supported by the ability of the pan acetyl-lysine antibodies to immunodeplete the salt-soluble mononucleosomes of HAT activity (Fig. 5). These antibodies are highly specific for acetylated, active chromatin (4). Our previous experiments have shown that chromatin precipitated by these antibodies are highly enriched in sequences within the β-globin domain and also contain all acetylated isoforms of H4 (4, 13, 25).

The experiments described here reveal the presence of a number of different acetyltransferases in a chromatin fraction enriched in active sequences including those of the β-globin domain. The data do not show whether the different HAT molecules actually operate within the globin domain because the chromatin preparation contains more than one specific subset of active genes. Future experiments will identify these HAT molecules and test whether any of them play roles in the domain-wide acetylation we have previously observed.

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