Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6364–6368, July 1995
Cell Biology

An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei

(acetylation/chromatin)

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Communicated by Elizabeth H. Blackburn, University of California, San Francisco, CA, March 21, 1995

ABSTRACT **Macronuclei of the ciliated protozoan *Tetrahymena thermophila* possess a histone acetyltransferase activity closely associated with transcription-related histone acetylation. Nothing definitive is known concerning the polypeptide composition of this activity in *Tetrahymena* or any comparable activity from any cellular source. An acetyltransferase activity gel assay was developed which identifies a catalytically active subunit of this enzyme in *Tetrahymena*. This activity gel assay detects a single polypeptide of 55 kDa (p55) in crude macronuclear extracts, as well as in column-purified fractions, which incorporates [^{3}H]acetate from [^{3}H]acetyl-CoA into core histone substrates polymerized directly into SDS polyacrylamide gels. p55 copurifies precisely with acetyltransferase activity through all chromatographic steps examined, including reverse-phase HPLC. Gel-filtration chromatography of this activity indicates a molecular mass of 220 kDa, suggesting that the native enzyme may consist of four identical subunits of 55 kDa. Furthermore, p55 is tightly associated with di-, and greater polynucleosomes and therefore may be defined as a component of histone acetyltransferase type A—i.e., chromatin associated.**

Posttranslational core histone acetylation is a well-studied, dynamic process, long considered a hallmark of transcriptionally active chromatin (1–5). Although core histones are among the most conserved proteins in eukaryotes and the specific lysine residues which undergo acetylation are in some cases absolutely conserved (6–8), the functional role of acetylation in the formation of either newly synthesized or transcriptionally competent chromatin remains poorly understood.

We and others have approached this problem by attempting to isolate and characterize the major enzyme systems involved in establishing the steady state balance of histone acetylation in different biological situations. Using a variety of conventional methods and activity assays, numerous groups have partially purified histone acetyltransferase from yeast (9), calf thymus (10), rat liver (11), and porcine liver (12, 13). While several reports have suggested polypeptides which copurify with activity, in no case has the enzyme been purified to homogeneity nor has a polypeptide been directly linked to histone acetyltransferase activity.

A major problem encountered in the purification of histone acetyltransferase A is that it is present in nuclei in extremely low quantity, with estimates suggesting a single enzyme molecule for every 300–30,000 nucleosomes (9, 10, 14). Thus, due to the low abundance of the enzyme, as well as its reported instability in increasingly purified preparations, conventional purification attempts have not been successful.

Avoiding some of these difficulties led us to develop a powerful assay to detect histone acetyltransferase-related polypeptides in standard SDS/polyacrylamide gels. In the ciliated protozoan *Tetrahymena*, macronuclei provide an enriched source of a histone acetyltransferase that is closely associated with transcription-related histone acetylation (15, 16). By exploiting the ability of macronuclear histone acetyltransferase to incorporate [^{3}H]acetate from [^{3}H]acetyl-CoA into core histone substrates polymerized in SDS/polyacrylamide gels, a single polypeptide of 55 kDa (p55) has been detected. This assay has allowed us to avoid exhaustive steps in identifying p55 as a catalytically active subunit of macronuclear histone acetyltransferase A. To our knowledge, this is the first report which directly links histone acetyltransferase activity to a single polypeptide in any biological system.

MATERIALS AND METHODS

Cell Culture and Isolation of Nuclei. *Tetrahymena thermophila* was grown axenically as described (17). Logarithmic-phase cells were harvested and macronuclei isolated according to Gorovsky *et al.* (17) and were used immediately or stored in isolation buffer at −80°C.

Histone Acetyltransferase Extraction. Purified macronuclei were extracted in buffer A [50 mM Tris·HCl, pH 8.0/10% (vol/vol) glycerol/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/0.1 mM EDTA] containing 0.5 M NaCl essentially as described (16).

Standard Liquid Assay of Histone Acetyltransferase Activity. Enzyme samples were incubated at 30°C in a total volume of 30 μl of buffer A containing 10 mM butyric acid and 25 mg of calf thymus histones (Sigma, type IIA). The reaction was started by the addition of [^{3}H]acetyl-CoA (50 nCi; 3.2 Ci/mmol; 1 Ci = 37 GBq; Amersham) to a final concentration of 0.5 μM and terminated after 10 min by spotting the entire mixture onto Whatman P 81 filters (2 × 2 cm) according to Horiuchi and Fujimoto (18). Inhibitors were prepared by dilution in dimethyl sulfoxide and were preincubated with the enzyme for 10 min on ice, then brought to 30°C prior to the start of the assay. [^{3}H]Acetate incorporation was determined by liquid scintillation and nonspecific counts were subtracted. One unit of activity is defined as the amount of enzyme required to incorporate 1 pmol of acetate into calf thymus histones in 10 min at 30°C.

The K_m for acetyl-CoA ranged from 0.5 to 1.5 μM when using the standard assay and varying acetyl-CoA concentration while maintaining histones at 5 mg/ml. The K_m for histones was determined at constant acetyl-CoA (5.0 μM) by varying the concentration of calf thymus histones and ranged from 200 to 300 mg/ml.

Acetyltransferase Activity Gel Assay. Crude macronuclear extracts or column-purified fractions were analyzed for histone acetyltransferase activity following electrophoresis in SDS/polyacrylamide gels containing calf thymus histones or bovine serum albumin by using a procedure modified from Kameshita and Fujisawa (19). Samples were dissolved in SDS/PAGE sample buffer, but not boiled, and then loaded onto standard

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Abbreviation: RP-HPLC, reverse-phase HPLC.

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SDS/polyacrylamide gels modified such that protein substrates were dissolved in the resolving gel at 1 mg/ml prior to polymerization.

Following electrophoresis, gels were washed for 1 h at room temperature in buffer B (50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol/0.1 mM EDTA) containing 20% (vol/vol) isopropanol and then for 30 min in buffer B. Gels were then incubated in buffer B containing 8 M urea for 1 h and then overnight at 4°C in buffer B containing 0.04% Tween 40 (Sigma). The gels were then washed in buffer A prior to incubation with [^{3}H]acetyl-CoA (10 μCi) for 30 min at 30°C. Finally, the labeled gels were washed with 5% trichloroacetic acid to remove unbound radiolabel and fluorographed.

Chromatin Preparation and Characterization. Macronuclei were digested with micrococcal nuclease and the resulting solubilized chromatin was resolved on a 5–20% sucrose gradient exactly as described (16). Forty 0.3-ml fractions were collected and analyzed for DNA and histone acetyltransferase activity.

Gel Permeation Chromatography. A 200-ml aliquot of macronuclear extract was applied to a Superose 12 10/30 gel-permeation column (1.0 cm × 30 cm; packed volume 24 ml) equilibrated in buffer A containing 0.3 M NaCl and operated at 15°C with a flow rate of 0.5 ml/min. Fractions of 0.75 ml were collected and assayed for protein content and acetyltransferase activity.

Hydroxyapatite Chromatography. A Bio-Gel HTP column (0.5 cm × 5 cm; Bio-Rad) was equilibrated at 15°C in buffer C [25 mM Na_2HPO_4, pH 8.0/1 M urea/10% (vol/vol) glycerol/0.01% Brij 35/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride] at a flow rate of 1 ml/min, loaded with macronuclear extract, and washed with five column volumes of buffer C before eluting bound proteins with a 50-ml gradient of 25 to 250 mM Na_2HPO_4. Acetyltransferase activity, detected by using the standard liquid assay, eluted as a single peak from 80 to 160 mM Na_2HPO_4.

Cation-Exchange Chromatography. The active fractions recovered from hydroxyapatite chromatography were loaded onto a Mono S 5/5 column (Pharmacia) equilibrated in buffer D [50 mM Hepes, pH 8.0/1 M urea/10% (vol/vol) glycerol/0.01% Brij 35/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride] and operated at a flow rate of 1.0 ml/min at 15°C. The column was washed with buffer D before elution with a 30-ml gradient of 0 to 500 mM NaCl; histone acetyltransferase activity eluted in a single peak from 130 to 300 mM NaCl.

Reverse-Phase (RP) HPLC. RP-HPLC was performed by using a Smart System (Pharmacia) equipped with a C_8 column (Aquapore RP-300; 2.1 mm × 22 cm; Brownlee Labs) operated at 20°C. The column was equilibrated with 0.1% trifluoroacetic acid and eluted with a linear gradient of 0 to 70% acetonitrile containing 0.1% trifluoroacetic acid over 35 min at a flow rate of 0.25 ml/min.

RESULTS

Histone Acetyltransferase Activity Gel Assay. We chose highly purified macronuclei as an enriched source of histone acetyltransferase A because macronuclear chromatin is characterized by a high steady state level of transcription-related histone acetylation (15) and we can be reasonably confident that our crude enzyme preparation is free of cytoplasmic acetyltransferase B contamination. In pilot experiments, we observed that histone acetyltransferase activity was recovered following exposure to (and subsequent removal of) 8 M urea and 1 M NaCl, suggesting that denaturing the enzyme did not result in an irreversible loss of activity. This property of the enzyme was thus exploited in developing an acetyltransferase assay. A modified "activity gel" assay was pursued so that we might directly link polypeptides with acetyltransferase activity, even from complex protein mixtures. If successful, the advantage of such an assay is obvious, combining the excellent resolution of polypeptides by SDS/PAGE with the ability of histone acetyltransferase (or a catalytic subunit) to incorporate [^{3}H]acetate into core histone substrates.

Fig. 1 shows that when crude macronuclear extract was subjected to activity gel analysis, a single band migrating with a molecular mass of 55 kDa was detected exclusively in the fluorograph of the histone-containing gel. When identical enzyme samples were electrophoresed in gels containing either bovine serum albumin, a nonsubstrate protein (Fig. 1, lane 5) or no protein substrate (Fig. 1, lane 6) and then processed in the assay, no [^{3}H]acetate incorporation was detected. Furthermore, inactivating the enzyme by boiling samples prior to loading the gel (Fig. 1, lane 3), or by preincubating samples with *N*-ethylmaleimide (Fig. 1, lane 4 and Table 1) also resulted in failure to incorporate [^{3}H]acetate.

The incorporation of [^{3}H]acetate at the position of 55 kDa is not due to autoacetylation of a polypeptide in the enzyme sample. An activity gel assay identical to that shown in Fig. 1, lane 2, was analyzed as follows: immediately after incubation with [^{3}H]acetyl-CoA, the region of the gel corresponding to 55 kDa was excised, equilibrated in SDS sample buffer, and reelectrophoresed in a standard SDS/12% polyacrylamide gel. After the gel was processed for fluorography and exposed to film, [^{3}H]acetate was detected exclusively in histones liberated from the activity gel and resolved in the second gel; no radiolabel was detected in the 55-kDa region (data not shown). By comparison to the positions of histone markers, it appeared that H3 was the most heavily labeled product under these assay conditions.

Thus, the activity gel assay detects the transfer of [^{3}H]acetate into histones in a manner dependent on the presence of a polypeptide of 55 kDa possessing histone acetyltransferase activity. Furthermore, the acetylation reaction is heat labile and sensitive to *N*-ethylmaleimide, suggesting that [^{3}H]acetate incorporation requires the presence of an enzyme activity which has not been irreversibly inactivated. These data demonstrate that, despite the exceedingly complex protein complement of the macronuclear extract, as demonstrated by silver staining (Fig. 1, lane 1), only one labeled product band is

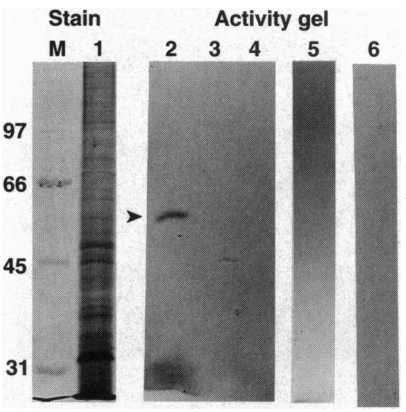

FIG. 1. A 55-kDa polypeptide specifically labels histones in an acetyltransferase activity gel assay. Crude macronuclear histone acetyltransferase activity was subjected to electrophoresis in SDS/8% polyacrylamide gels in which histones (lanes 2, 3, and 4), bovine serum albumin (lane 5), or no protein substrates (lanes 1 and 6) were incorporated prior to polymerization. Following electrophoresis, the gels were prepared for the activity gel assay and processed for fluorography (lanes 2–6) or silver stained (lane 1); M, molecular weight markers. In some cases, the enzyme was inactivated prior to loading the gel either by boiling for 5 min in sample buffer (lane 3) or by incubation with 10 mM *N*-ethylmaleimide (lane 4). [^{3}H]Acetate was incorporated into histones in a single region of the gel corresponding to a molecular mass of 55 kDa (arrowhead, lane 2). The gel was exposed for 1 week.

Table 1. Inhibition of histone acetyltransferase activity

Inhibitor	IC_{50}, mM
p-Hydroxymercuribenzoate	0.01
N-Ethylmaleimide	0.1
Cerulenin	0.1
Iodoacetamide	1

The concentration of dimethyl sulfoxide in all reactions was 3.3% (vol/vol).

detected in this assay. We will refer to this 55-kDa polypeptide as p55.

p55 Is Bound to Chromatin. Previous studies on macronuclear histone acetyltransferase suggested that this activity is bound to chromatin, cosedimenting with polynucleosomes containing two or more core particles on sucrose gradients, but is not tightly associated with mononucleosomes (16). To determine if p55 is chromatin associated and exhibits similar cosedimenting properties, macronuclei were digested with micrococcal nuclease and fractions containing mono-, di-, and polynucleosomes were identified by using standard procedures (Fig. 2*A* and ref. 20). To examine whether histone acetyltransferase activity cosedimented with these purified chromatin fragments, aliquots of each fraction were incubated with [^{3}H]-acetyl-CoA prior to SDS/PAGE and fluorography. By design, this assay measures chromatin-associated acetyltransferase activity by using endogenous nucleosomal histones as substrate. In agreement with published results (16), no evidence of label incorporation was found with mononucleosomes (Fig. 2*B*, lane 1). In contrast, [^{3}H]acetate incorporation was observed when examining the fluorograph of lanes 2, 3, and 4 of Fig. 2*B*. This incorporation increases and correlates positively with the presence of di- and polynucleosomes. Furthermore, this effect is likely due to increasing enzyme concentration since the amount of histone substrate was approximately equal in each sample. These results suggest that *in vivo* histone acetyltransferase may preferentially bind near the linker region of chromatin, although other subnuclear localizations have also been reported (21).

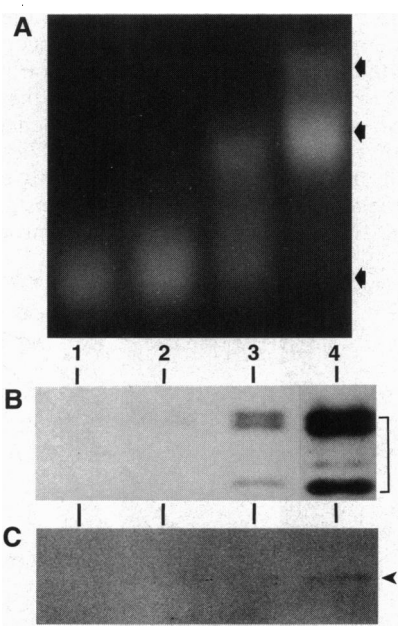

FIG. 2. p55 is associated with chromatin. Chromatin was isolated from macronuclei digested with micrococcal nuclease, subjected to sucrose-gradient centrifugation, and fractionated. (*A*) Fractions containing mono- (lane 1) as well as di- and polynucleosomes (lanes 2, 3, and 4) were identified by comparison with standard DNA markers after electrophoresis in a 1.5% agarose gel stained with ethidium bromide (see arrows). (*B*) Aliquots of each fraction were incubated with [^{3}H]acetyl-CoA prior to SDS/12% PAGE and fluorography. Despite approximately equal loading of core histones, [^{3}H]acetate incorporation was limited to fractions containing di- or polynucleosomes (lanes 2, 3, and 4); the mononucleosome fraction shows no significant incorporation (lane 1). Only the core histone portion of the fluorograph is shown. (*C*) Fractions were further analyzed in the activity gel assay. Note that p55 is also detected exclusively in fractions of di- and polynucleosomes (lanes 3 and 4; see arrowhead), and not in fractions containing primarily mononucleosomes (lanes 1 and 2).

The chromatin fractions shown in Fig. 2*A* were further analyzed in the activity gel assay. Fig. 2*C* shows that p55 is detected exclusively in fractions containing di- and polynucleosomes (Fig. 2*C*, lanes 3 and 4) and is not observed in fractions consisting primarily of mononucleosomes (Fig. 2*C*, lanes 1 and 2). Together, these data demonstrate the coincidence of p55 with histone acetyltransferase activity detected by conventional assay and suggest that p55 is also tightly associated with di- and greater chromatin subunits (compare lanes 3 and 4 in Fig. 2 *B* and *C*). On the basis of these results, we operationally define p55 as a component of nuclear/chromatin-associated, histone acetyltransferase A.

p55 Is a Catalytic Subunit of Native Histone Acetyltransferase. The molecular weight of native macronuclear histone acetyltransferase was estimated by gel-permeation chromatography (Fig. 3*A*) and glycerol-gradient sedimentation (data not shown). From these analyses, a Stokes radius of 51.0 Å and a sedimentation coefficient of 10.7S were estimated, corresponding to a globular protein with a molecular mass of 220 kDa (22).

To determine if p55 codistributes with native (220-kDa) acetyltransferase, aliquots of the fractions recovered from the gel-filtration experiment (Fig. 3*A*) were analyzed by SDS/PAGE and the activity gel assay or silver staining. Close comparison of the activity gel fluorograph (Fig. 3*C*) with the corresponding stained gel (Fig. 3*B*) demonstrates that p55 cannot be positively identified by stain. However, p55 is easily detected in the activity gel and is limited to only those fractions in which acetyltransferase activity is also detected when using the standard liquid assay (Fig. 3*A*). Upon direct comparison, the distribution of activity shown in Fig. 3*A* and the distribution of p55 shown in Fig. 3*B* are in close agreement—i.e., both assays detect acetyltransferase activity in fractions 6–11. Thus, p55 appears to be an integral component of the native 220-kDa enzyme.

Enrichment of p55. With the goal of obtaining a fraction highly enriched for p55, several conventional protein purification methods were evaluated for the recovery and enrichment of acetyltransferase activity by using both the standard liquid and activity gel assays. A single peak of enzyme activity was recovered following both hydroxyapatite and cation-exchange chromatography. Without exception, p55 copurified precisely with acetyltransferase activity (data not shown).

Fractions enriched for acetyltransferase activity following ion-exchange chromatography were then applied to an RP-HPLC C_8 column (Fig. 4). As might be expected, acetyltransferase activity could not be detected immediately following resolution by RP-HPLC. However, when fractions were dried and renatured prior to being tested in the standard liquid assay, partial activity was recovered. Despite exposure to acetonitrile/trifluoroacetic acid and the denaturing properties inherent to RP-HPLC, approximately 10% of the loaded activity was recovered eluting from 50 to 52% acetonitrile (Fig. 4 *Inset*, fraction 6).

In an attempt to identify a polypeptide correlating directly with activity, aliquots of each RP-HPLC fraction were again analyzed by SDS/PAGE and the activity gel assay (Fig. 5*B*) or silver stain (Fig. 5*A*). As shown in Fig. 5*B*, p55 was detected exclusively in the lanes corresponding to the input sample loaded onto the C_8 column (lane L) and fraction 6 (see Fig. 4). In the parallel stained gel (Fig. 5*A*), no polypeptides in lane L

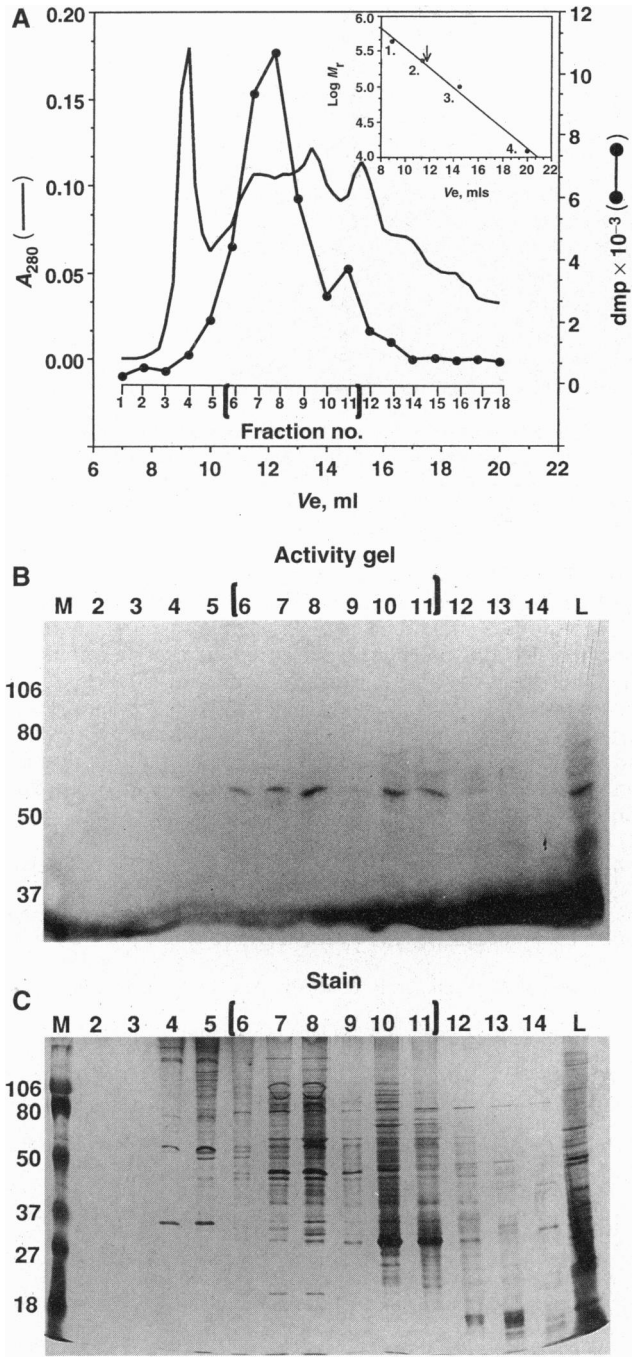

FIG. 3. p55 codistributes with native histone acetyltransferase. (*A*) Macronuclear extract was fractionated by gel-permeation chromatography; the absorbance profile at 280 nm (solid line) and enzyme activity determined by using the standard liquid assay (●) are shown. Total recovery of activity was 70%. *Ve*, elution volume. (*Inset*) Column was calibrated under identical conditions with a set of protein standards. The arrow indicates the elution volume of histone acetyltransferase and corresponds to a globular protein with an estimated molecular weight of approximately 220,000. Fractions recovered in *A* were precipitated, dried, and prepared for SDS/PAGE. Aliquots of each sample were then electrophoresed and analyzed by activity gel assay and fluorography (*B*) or by silver stain (*C*). Lane numbers correspond to the fractions collected in *A*. Note the precise agreement between fractions possessing acetyltransferase activity recovered in *A* with the occurrence of p55 (*B*, lanes 6–11). The sample in lane 9 was not completely recovered and appears underloaded in *B* and *C*. L, aliquot of sample loaded onto the column; M, molecular weight markers.

can be distinguished in the region corresponding to 55 kDa. However, a single polypeptide migrating at the position of 55

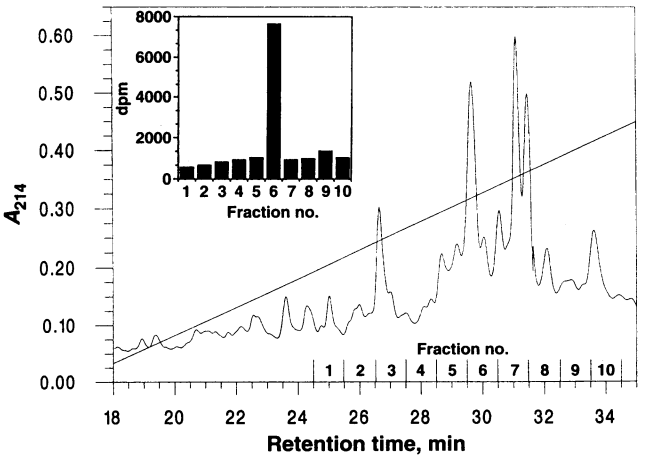

FIG. 4. Enrichment of histone acetyltransferase. Partially purified histone acetyltransferase obtained from hydroxyapatite followed by cation-exchange chromatography was subjected to RP-HPLC; the absorbance profile at 214 nm is shown. Fractions were collected as indicated, dried, and redissolved in buffer A containing 2 M urea and incubated overnight at 4°C before analysis using the standard liquid assay. At least partial activity was recovered in a single fraction eluting at 50–52% acetonitrile (*Inset*, fraction 6).

kDa is easily visualized by silver staining in the lane corresponding to fraction 6. Two-dimensional gel analysis of fraction 6 followed by silver staining and activity gel analysis revealed no other polypeptides migrating with a molecular mass of 55 kDa and indicates a relative pI for p55 of 6.7 (data not shown). Thus, following extraction from purified macronuclei, chromatography on hydroxyapatite, cation exchange, RP-HPLC, and SDS/PAGE, p55 appears as a distinct, homogeneous polypeptide.

DISCUSSION

In this report we have identified a catalytic subunit of histone acetyltransferase A from *Tetrahymena* macronuclei. To our knowledge, this is the first evidence directly linking this important activity to a homogeneous, SDS/polyacrylamide gel-purified polypeptide in any organism. By exploiting an activity gel assay, a single 55-kDa polypeptide is detected in SDS/polyacrylamide gels from either crude macronuclear extracts or column-purified material. Following purification on several conventional and one unconventional (RP-HPLC) chromatographic steps, p55 can be visualized as a discrete polypeptide on silver-stained SDS/polyacrylamide gels and possesses at least partial histone acetyltransferase activity.

That p55 detected by the activity gel assay is indeed a component of histone acetyltransferase is supported by several observations. First, incorporation of [^{3}H]acetate is dependent on the appropriate histone substrates in the gel. No incorporation of label is detected in the presence of nonsubstrate proteins or in the absence of potential protein substrates or when macronuclear extracts are irreversibly inactivated. Second, fractions from a variety of chromatographic steps possessing acetyltransferase activity measured by using the standard liquid assay are in precise agreement with fractions in which p55 is detected by using the activity gel assay. Collectively, these data support the premise that the activity detected by using either assay is in fact due to the same enzyme.

In all of our experiments, only a single histone acetyltransferase activity was detected in macronuclear extracts. Because our salt-extraction method has been shown to recover greater than 95% of the histone acetyltransferase activity from macronuclei (16) and because we failed to resolve more than one peak of activity on any separation method, we conclude that we

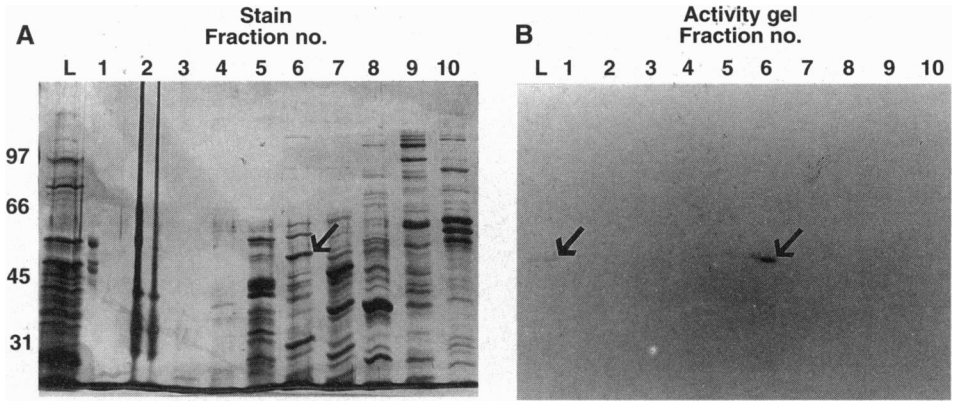

FIG. 5. Homogeneous p55 catalyzes histone acetyltransferase activity. Aliquots of the fractions shown in Fig. 4 were resolved by SDS/8% PAGE and visualized by either silver staining (*A*), or the activity gel assay and fluorography (*B*). The lane numbers are identical to the fractions shown in Fig. 4; the lane designated "L" is an aliquot of the enzyme sample injected onto the C_8 column. Note that in *B*, p55 is detected exclusively in lanes "L" and 6 (see arrows). Comparing the position of p55 with the corresponding silver stained gel, shows that a single polypeptide band migrates at the appropriate position (lane 6, *A* and *B*). The position of molecular mass marker proteins is indicated to the left *A*.

are working with a single activity. This finding is in agreement with earlier studies on histone acetyltransferase A from mammalian sources (10, 12) and yeast (9) for which evidence of only single chromatin-bound activities were reported. Similarly, macronuclear histone acetyltransferase A shares additional properties with these activities including the K_m values for histones and acetyl-CoA, as well as its sensitivity to sulfhydryl blocking agents (Table 1).

In principle, it should be possible to use the activity gel assay to identify catalytically active histone acetyltransferase subunits in other systems where extensive purification of the enzyme has proven difficult. It is unclear at this time whether any similarities exist between chromatin-bound histone acetyltransferases of the A class and type B cytosolic activities or whether p55 exists in other cellular compartments.

Relationship Between p55 and the Native 220-kDa Enzyme. The appearance of p55 in gel-filtration fractions eluting in the molecular mass range of 220 kDa suggests the intriguing possibility that the native enzyme contains at least one, and perhaps as many as four, catalytically active subunits of 55 kDa. Consistent with this hypothesis, exposure of macronuclear acetyltransferase to agents known to promote protein dissociation (for example, dimethylmaleic anhydride), followed by gel-filtration chromatography and subsequent renaturation, produces new peaks of activity eluting at the molecular weights of 110 and 55 kDa. As expected, p55 is detected in these new active fractions (unpublished observations).

Purification studies on histone acetyltransferase from systems as diverse as yeast (9) and mammals (12) have reported the native molecular mass of these enzymes to be in the range of 100–110 kDa. Our demonstration that the *Tetrahymena* enzyme has a native size of approximately 220 kDa is, to our knowledge, the largest size estimate of native histone acetyltransferase from any source. Whether association or dissociation of enzyme subunits is occurring during extraction and purification of the activity in any of these systems remains to be determined.

Conservation of p55 in Other Systems. Our identification of p55 as a histone acetyltransferase A component polypeptide is similar to Grunstein and coworkers (9) who described a 55-kDa polypeptide that copurified extensively with a chromatin-bound histone acetyltransferase activity from yeast. Because this activity had a native size of 110 kDa, they proposed that the native structure may consist of two 55-kDa subunits. Unfortunately, the presence of numerous polypeptide contaminants in even their most purified fractions made them unable to directly establish that this 55-kDa polypeptide was related to the acetyltransferase. On the basis of the data reported in this study, it seems likely that the yeast 55-kDa polypeptide is a subunit of histone acetyltransferase A from yeast and that this polypeptide, like histone acetylation itself, is conserved across a wide range of eukaryotes. To that end, our finding that the *Tetrahymena* enzyme can regain at least partial activity after two relatively harsh treatments, RP-HPLC and SDS/PAGE, is encouraging, permitting the addition of these highly resolving fractionation methods in any purification sequence.

We acknowledge the efforts of Dr. Maria Davis and Ron Richman in some of the initial characterization of macronuclear histone acetyltransferase. This research was supported by a research grant from the National Institutes of Health to C.D.A. (HD16259).

1. Allfrey, V. G., Faulkner, R. & Mirsky, A. E. (1964) *J. Mol. Biol.* **196,** 379–388.
2. Hebbes, T. R., Thorne, A. W. & Crane-Robinson, C. (1988) *EMBO J.* **7,** 1395–1403.
3. Csordas, A. (1990) *Biochem. J.* **265,** 23–28.
4. Turner, B. M. (1991) *J. Cell Sci.* **99,** 13–20.
5. Loidl, P. (1994) *Chromosoma* **103,** 441–449.
6. Chicione, L. G., Schulman, I. G., Richman, R., Cook, R. G. & Allis, C. D. (1986) *J. Biol. Chem.* **261,** 1071–1076.
7. Turner, B. M. & Fellows, G. (1989) *Eur. J. Biochem.* **179,** 131–139.
8. Sobel, R. E., Cook, R. G. & Allis, C. D. (1994) *J. Biol. Chem.* **269,** 18576–18582.
9. Travis, G. H., Colavito-Shepanski, M. & Grunstein, M. (1984) *J. Biol. Chem.* **259,** 14406–14412.
10. Belikoff, E., Wong, L.-J. & Alberts, B. M. (1980) *J. Biol. Chem.* **255,** 11448–11453.
11. Wiktorowicz, J. E. & Bonner, J. (1982) *J. Biol. Chem.* **257,** 12893–12900.
12. Kelner, D. N. & MaCarty, K. S. (1984) *J. Biol. Chem.* **259,** 3414–3418.
13. Attisano, L. & Lewis, P. N. (1990) *J. Biol. Chem.* **265,** 3949–3955.
14. van Holde, K. E. (1989) *Chromatin* (Springer, New York), pp. 185–212.
15. Vavra, K. J., Allis, C. D. & Gorovsky, M. A. (1982) *J. Biol. Chem.* **257,** 2591–2598.
16. Chicione, L. G., Richman, R., Cook, R. G., Gorovsky, M. A. & Allis, C. D. (1987) *J. Cell Biol.* **105,** 127–135.
17. Gorovsky, M. A., Yao, M.-C., Keevert, J. B. & Pleger, G. L. (1975) *Methods Cell Biol.* **9,** 311–327.
18. Horiuchi, K. & Fujimoto, D. (1975) *Anal. Biochem.* **69,** 491–496.
19. Kameshita, I. & Fujisawa, H. (1989) *Anal. Biochem.* **183,** 139–143.
20. Gorovsky, M. A., Glover, C., Johmann, C. A., Keevert, J. B., Mathis, D. J. & Samuelson, M. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 493–503.
21. Hendzel, M. J., Sun, J.-M., Chen, H. Y., Rattner, J. B. & Davie, J. R. (1994) *J. Biol. Chem.* **269,** 22894–22901.
22. Siegel, I. H. & Monty, K. J. (1966) *Biochim. Biophys. Acta* **112,** 346–362.