Yeast Contains Multiple Forms of Histone Acetyltransferase*

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We have assayed several methods to quantitatively recover yeast histone acetyltransferases in an attempt to study the multiplicity of enzymatic activities. Two methods, namely (NH₄)₂SO₄ precipitation and salt dissociation of chromatin in 0.5 M NaCl, yielded convenient preparations of total histone acetyltransferases. DEAE-Sepharose chromatography of the crude extracts resulted in the separation of three peaks of activity when total yeast histones were used as substrate. However, the scanning of the enzymatic activity toward individual histones along the chromatography, achieved by determining the specific activity of the individual histones after incubating whole histones and [¹⁴C]acetyl-CoA with the chromatographic fractions, yielded four peaks. The first two peaks showed specificity toward H2B and H3, respectively. Although they partially overlapped, rechromatography on cation exchangers allowed us to resolve the two activities, and several criteria were used to prove that they correspond to different enzyme molecules. The last two peaks were H4-specific, but the present data suggest that one of the activities is chromatin-bound, whereas the other surely corresponds to the cytoplasmic B-form of the enzyme. The enzyme specific for yeast H2B acetylates chicken erythrocyte H2A, rather than H2B. The detected multiplicity of yeast histone acetyltransferases may correspond to the multiplicity of roles proposed for histone acetylation.

Acetylation of the ε-amino groups of lysyl residues is the most widely studied reversible modification of histones, but only in the last few years are its functional roles being disclosed. These include the deposition and assembly of histones into nucleosomes during DNA replication (1–3), the substitution of histones during differentiation (for instance, the replacement of histones by protamines during spermatogenesis) (4–7), together with the already classical, proposed role in transcription (8–10). It has recently been emphasized that there exist 26 lysyl residues/nucleosome that can be acetylated; and therefore, a notable heterogeneity of nucleosome acetylation modes exists (11). It is possible that acetylation of a certain histone at a given site may serve for some particular function, whereas the acetylation of a different histone, or even the same histone at a different site, may play a different role.

Some examples in this line have been described. Nonrandom acetylation of the four potential lysines of H4 has been reported in Tetrahymena (3, 12, 13), Physarum (14), and cuttlefish (15). It has been concluded that acetylation of a single lysyl residue is related to histone deposition, whereas the turnover of acetyl groups at other lysines may be connected with other chromatin functions. On the other hand, whereas all four core histones become acetylated in connection with chromatin assembly during replication, only H2A and H2B are acetylated during the G₂ period of the Physarum cell cycle (16), in which active transcription occurs. Although nonspecific hyperacetylation of histones causes little, if any, nucleosome destabilization, specifically acetylated Physarum histones are bound to DNA with a cell cycle-dependent tightness, as revealed by protamine competition assay (16). Acetylation of histones may thus play a variety of roles depending on the specific lysines modified (11). All these roles may imply a displacement of particular histones, which will, in turn, depend on the specific modification achieved (11, 16).

The multiplicity of target histones and/or acetylation sites may require a multiplicity of histone acetyltransferases and/or histone deacetylases, the enzymes responsible for the turnover of acetyl groups. In most eukaryotes, two main types of histone acetyltransferase have been characterized by the criteria of chromatographic behavior, histone specificity, and subcellular localization (17–21). Histone acetyltransferases A are nuclear enzymes that catalyze the transfer of acetyl groups from acetyl-CoA to all core histones (18, 19, 22–24). Acetyltransferases B are cytoplasmic enzymes, and they seem to be responsible for the acetylation of H4 before chromatin assembly (18, 20, 24, 25).

It is not yet clear whether histone acetyltransferases actually are single enzyme molecules; but in view of the multiplicity of roles proposed for histone acetylation (11), it may be possible that activities A and B are composed of several isoenzymic forms.

The yeast Saccharomyces cerevisiae is an organism adequate to address the question of the multiplicity of histone acetyltransferases (26). Its histones rank among the most heavily acetylated (27, 28), and the existence of histone acetyltransferases A (21, 29) and B (21) has been described. In this paper, we describe the presence of multiple isoenzymes of yeast histone acetyltransferase A by using a novel and simple experimental approach.

MATERIALS AND METHODS AND RESULTS

Histone Specificity of Acetyltransferases—Fig. 3 shows the results of electrophoretic separation of yeast histones after acetylation with [¹⁴C]acetyl-CoA catalyzed by several fractions from the chromatogram in Fig. 2. It is obvious that a

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1 Portions of this paper (including "Materials and Methods," part of "Results," and Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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* This work was supported in part by Grant PB85-233 from Comisión Interministerial de Ciencia y Tecnologia (Spain). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
histone hydrolase activity must elute in peak I, and it causes the degradation of histones, as clearly seen in the Coomassie Blue-stained gel and, better still, in the corresponding fluorogram. Attempts to inhibit proteolytic activity without affecting histone acetyltransferase activity have been unsuccessful. The experiment of Fig. 3 and a parallel incubation with the fractions from Fig. 1 allowed us to construct elution profiles of the H3-, H2B-, and H4-acetylating activities by the activity scanning method (Fig. 4, A and B). The accuracy of the method is evidenced by the low relative standard errors of 10 independent measurements of both the fluorogram and the stained gel that, as an example, are given in Fig. 4A. The appearance of two peaks with H4-acetylating activity, for instance, cannot be attributed to lack of statistical significance of the valley between them. It has to be noted that, with the method used, the presence of proteolysis does not invalidate the results because the labeling intensity of the intact histone band was normalized to the protein content (staining) of the same band. It may be possible that a specific histone hydrolase would exist with substrate specificity toward acetylated histones. In this instance, the results of Fig. 4 would not be valid from a quantitative point of view (SLD² values of the peaks), but the qualitative features (position of the individual histone peaks) would remain unchanged. Keeping this in mind, it is obvious that peak I contains an H2B-acetylating activity, that peaks II and III exhibit specificity toward H4, and that the activity capable of acetylating H3 elutes between peaks I and II. The enzyme in peak III seems to be absolutely specific for H4. Finally, it is noteworthy that the elution profile of histone acetyltransferase activity, as determined with total histones (Figs. 1 and 2), does not coincide with the individual profiles given in Fig. 4, i.e. not all the individual peaks coincide with peaks obtained with whole histones. Actually, the latter result from the summation of the individual curves given in Fig. 4, plus the curve corresponding to the acetylating activity toward polypeptides other than core histones, minus the curve corresponding to the deacetylase activity.

The following experiments were carried out to determine whether the existence of these four peaks of activity means that four different enzymes exist in yeast.

The H2B- and H3-acetylating Activities Correspond to Different Enzymes—Several lines of evidence demonstrate that the resolution of H2B- and H3-acetylating activities in Fig. 4 (A and B) is actually due to the existence of two different enzymes with distinct molecular properties.

As pointed out before, fractionation with PEG to obtain a crude histone acetyltransferase preparation was unsuccessful because the enzymatic activity was partitioned between PEG10 and PEG20. However, it was found that, in the analysis of the histone specificity of the enzymatic activities in PEG10 and PEG20, the H3-acetylating activity was exclusively present in PEG10, whereas the activities capable of acetylating H2B and H4 were partitioned between both fractions. This result indicates that we are actually dealing with at least two different enzymes.

The experiment shown in Fig. 5 is still clearer in that respect. Peak I from an experiment like that of Fig. 2 was rechromatographed on CM-Accell at pH 7.5. Most of the activity appeared in the retained fraction; and as shown in the fluorogram in Fig. 6, it exclusively acetylates H2B. A small peak of activity also appeared in the nonretained fraction (Fig. 5), and it is H3-specific (Fig. 6). The low recovery of H3-acetylating activity is due to the fact that it elutes from DEAE-Sepharose between peaks I and II (Fig. 4), and it was...
FIG. 5. Cation-exchange chromatography of peak I from experiment like that of Fig. 2. The appropriate fractions containing H2B- and H3-acetylating activity were pooled and submitted to preparative high performance liquid chromatography on CM-Accell at pH 7.5 as described under "Materials and Methods." \(A_{280}(\cdots\cdots)\) was continuously monitored, and the histone acetyltransferase activity toward total chicken erythrocyte histones was determined in the even-numbered protein-containing fractions and expressed as incorporated disintegrations/minute under standard assay conditions (●). The NaCl gradient is represented (— — —).

FIG. 6. Histone specificity of acetyltransferases resolved by CM-Accell chromatography. The assay was carried out as described under "Materials and Methods" by incubating yeast histones with \([{}^{14}C]\)acetyl-CoA in the presence of the fractions (identified by their numbers) from the chromatogram in Fig. 5. Both the Coomassie Blue-stained gel (A) and the fluorogram (B) are shown.

soley peak I which was re-chromatographed on CM-Accell. In fact, the larger the number of fractions taken from the tail of DEAE-Sepharose peak I, the higher the H3-acetylating activity recovered in the nonretained peak from ion-exchange chromatography. The use of other cation exchangers gave results similar to those of CM-Accell. Re-chromatography of DEAE-Sepharose peak I on SP-Sephadex, for instance, yielded a retained fraction containing the H2B-acetylating enzyme and a nonretained fraction that acetylates H3.

The extremely distinct chromatographic behavior of H3- and H2B-acetylating activities on ion exchangers indicates that they actually reside in different enzyme molecules. These two histone acetyltransferases not only differ in their ionic properties, but also in their thermal stability. The experiment depicted in Fig. 7 shows that preincubation at 45 °C resulted in the complete abolition of the H3-acetylating activity of a nonretained SP-Sephadex fraction, whereas the retained H2B-specific fraction was still 75% active after preincubation. A similar strategy has been used to demonstrate that the histone acetyltransferases from Tetrahymena macro- and micronuclei are different (13). Again, these results clearly show that both activities are associated with different enzymes.

The Two H4-acetylating Activities Probably Reside in Different Enzyme Molecules—Two peaks of H4-acetylating activity are apparent in the experiments of Fig. 4. Apart from their different behavior on DEAE-Sepharose chromatography, an additional experiment showed that both activities
the chromatin-associated material is expected to be present in DEAE-Sepharose chromatography. The Coomassie Blue-stained gel (A) and the fluorogram (B) are shown. The bands running above core histones in lanes C correspond to H1 and H5.

reside in different cellular environments. Spheroplasts lysed in buffer A were centrifuged at 100,000 × g for 1 h without adding NaCl. Under these conditions of low ionic strength, the chromatin-associated material is expected to be present mainly in the sediment, whereas the cytoplasmic histone acetyltransferases (B enzymes) would appear mainly in the supernatant. Fig. 8 shows that the H4-acetylating activity in peak III is a soluble enzyme, whereas most of the activity associated with peak II sediments at low ionic strength and can be extracted with 0.5 M NaCl. Thus, this activity seems to be chromatin-bound and probably has to be considered different from the cytoplasmic histone acetyltransferase B specific for H4. The lack of unequivocal evidence for a clear-cut uneven distribution of the activity prevents a more definite assessment of the existence of two different molecular species of H4-specific yeast histone acetyltransferases.

The Enzyme Specific for H2B in Yeast Acetyltransferases Chicken erythrocyte H2A. Incorporation of labeled acetate into yeast (lanes Y) or chicken erythrocyte (lanes C) whole histones was carried out as described for Fig. 3. The source of enzyme was a fraction from peak I from DEAE-Sepharose chromatography. The Coomassie Blue-stained gel (A) and the fluorogram (B) are shown. The bands running above core histones in lanes C correspond to H1 and H5.

FIG. 9. H2B-specific yeast enzyme acetylates chicken erythrocyte H2A. Incorporation of labeled acetate into yeast (lanes Y) or chicken erythrocyte (lanes C) whole histones was carried out as described for Fig. 3. The source of enzyme was a fraction from peak I from DEAE-Sepharose chromatography. The Coomassie Blue-stained gel (A) and the fluorogram (B) are shown. The bands running above core histones in lanes C correspond to H1 and H5.

DISCUSSION

To quantitatively recover enzymatic activities, we have tested the possibility of applying, to yeast cells, three of the methods currently used to prepare histone acetyltransferases. This objective was essential to our purpose of determining the number of molecular species of yeast histone acetyltransferases. Two of the three methods, namely the (NH₄)₂SO₄ and ultracentrifugation procedures, were effective in recovering whole histone acetyltransferase activity, and they were alternatively used.

When assaying the enzymatic activity with whole histones, three peaks of activity were resolved under our DEAE-Sepharose chromatographic conditions. To study histone specificity in the chromatographic fractions, the common practice consists of assaying the acetylating activity of pooled fractions from chromatographic peaks toward isolated histones (19, 33), histone mixtures (17, 19, 23–25), or nucleosomes (17, 24, 29); and so we did in our previous work (21). Here, we describe a novel and simple approach, the activity scanning method, that consists of scanning the acetylating activity of individual fractions along the chromatographic peaks toward the different histone classes. The particular procedure we chose to determine the SLD values was a convenient one, giving high reproducibility and accuracy; and it has already been used by other workers (34), although in different contexts. Alternatively, a densitometric procedure could have been used together with an adequate curve-resolving program (29). However, we should emphasize that the novelty of the activity scanning method is based upon the idea that a peak of enzymatic activity toward total histone does not necessarily coincide with a peak of activity toward individual histones.

The activity scanning method gave the first clue to multiplicity of yeast histone acetyltransferases, as we were able to resolve four activities specific for H2B, H3, and H4 (two activities). The different behavior of the two H4-acetylating activities (Fig. 8) rules out the possibility that the appearance of peaks II and III (Figs. 1 and 2) was an artifact due to the presence of histone deacetylase activity. Rather, it strongly supports the idea that there exist two H4-specific enzymes, one chromatin-bound and another cytoplasmatic (B form).

The different properties of the H2B- and H3-acetylating activities (Figs. 5–7) also support the idea that they reside in different molecules, and it can be concluded that the presence of proteolysis does not cause a single histone acetyltransferase to appear as two activities.

Nevertheless, a notable histone hydrolase activity seems to exist, and this is especially prominent in fraction 38 from the DEAE chromatogram in Fig. 2, as shown by the fluorogram in Fig. 3. Although further work would be required for an unambiguous identification, most of the labeled high mobility peptides in Fig. 3B move like the products of histone proteolysis (35, 36). It is obvious from Fig. 3 that their specific activity is higher than that of the histones themselves. This finding would be explained if the proteolytic activity was specific for acetylated histones. It is interesting to note in this respect that H2A-specific proteolytic activity from HL-60 cell chromatin is unable to act on octamer-DNA complexes unless the histones are acetylated (36), although this effect may be mediated by the acetylation of the H3/H4 tetramer. According to Loidl’s hypothesis (11), acetylation of H2A/H2B may be a specific signal for transcription that precedes the removal of an H2A/H2B dimer. It would be tempting to speculate that this removal is produced by acetylation-dependent proteolysis. Experiments to test this hypothesis are being carried out in our laboratory.

If a protease specific for acetylated histones actually exists, the profiles shown in Fig. 4 may depart from reality if the elution maxima of histone protease and acetyltransferase do not coincide. At any rate, the remaining pieces of evidence...
concerning the multiplicity of histone acetyltransferases confirm the above established conclusions.

Travers at al. (29) have reported a 15 000-fold purification of a histone acetyltransferase A from yeast. These authors claimed that the enzymatic activity acetylates all four core histones. The apparent disagreement between their results and ours may be caused by the fact that they assay histone specificity by autoacetylation with acetyl-CoA of a chromatin fraction containing crude histone acetyltransferase, rather than with the purified enzyme. Probably, the enzyme they purified corresponds to our H2B- or H3-specific histone acetyltransferase.

Finally, we wish to comment that the substrate specificity of histone acetyltransferases toward free histones may change when nucleosomes are used as substrate (18, 22, 24, 32). The experiments with free histones are valid to demonstrate the multiplicity of histone acetyltransferases, and preliminary experiments with nucleosomes confirm the existence of at least three enzymes apart from the cytoplasmatic B-form. This multiplicity of histone acetyltransferases may be related to the multiplicity of roles proposed for histone acetylation (11).

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Preparation of crude histone acetyltransferase. This research has been planned in order to determine how many different histone acetyltransferases are present in yeast. A quantitative recovery of the enzymes is essential to our aim than an extensive purification of the crude enzyme extracts. The efficiency of the DEAE-Sepharose method used to recover yeast histone acetyltransferase activities has been assessed previously (12). We have found that the resolution has been improved under the present experimental conditions.

RESULTS

Preparation of crude histone acetyltransferase. This research was performed in the following way: yeast histone acetyltransferase activities could be found in the yeast histones as described elsewhere (32). Nac1 as described under Materials and methods it led to the recovery of the enzymatic activity (21, 22 for 1.0 M while the solubilization of nucleic acids was not improved by raising the salt concentration to 1.5 M. Therefore, this method was used to obtain crude preparations of whole histone acetyltransferase activities. As nucleic acids exhibit an inhibitory effect on the enzymatic activity (21, 22, 24) they were quantitatively removed from DEAE-Sepharose chromatography of crude histone acetyltransferase activities. By comparing Fig. 1 with Fig. 1 from ref. 21, it is obvious that the resolution has been improved under the present experimental conditions. The activity of histone deacetylase elutes as a single peak centered between peaks I and III. Therefore, it can be deduced that the question of whether peaks II and III actually correspond to two different activities and not to a single activity whose peak is artificially split by the presence of histone deacetylase. We have consistently found that the histone deacetylase obviously reduces the activity profile of the histone acetyltransferases activity, it does not cause any artificial splitting of activity peaks.

Histone deacetylase assay. The histone deacetylase activity was determined with biologically labeled chicken erythrocyte histones as described elsewhere (23).

**Fig. 1.** Elution of yeast histone acetyltransferases and deacetylases from DEAE-Sepharose CL-6B. A column (1.8 x 11 cm) was loaded with a crude preparation from 1 g of Saccharomyces cerevisiae. Chromatography was carried out as described by Lassen and Valne (17). The elution profile was monitored by measuring the absorbance of the eluate at 280 nm; fraction volume, 8.5 ml; flow rate, 35 ml/h. All the symbols are as in Fig. 1.