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Steady-state Levels of Histone Acetylation in *Saccharomyces cerevisiae**

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The importance of control of the levels of histone acetylation for the control of gene expression in eukaryotic chromatin is being elucidated, and the yeast *Saccharomyces cerevisiae* has proven to be an important model system. The level of histone acetylation in yeast is the highest known. However, only acetylation of H4 has been quantified, and reports reveal loss of acetylation in histone preparations. A chaotropic guanidine-based method for histone isolation from intact wild-type cells or from a single-step nuclear preparation with butyrate preserves acetylation of all core histones. Histone H4 has an average of more than 2 acetylated lysines per molecule, distributed over 4 sites. Histones H2A, H3, and H2B have 0.2, ~2, and >2 acetylated lysines per molecule, respectively, distributed across 2, 5, and 6 sites. Thus, yeast nucleosomes carry, on average, 13 acetylated lysines per octamer, *i.e.* just above the threshold of 10–12 deduced for transcriptionally activated chromatin of animals, plants, and algae. Following M_r 100,000 ultrafiltration in 2.5% acetic acid, yeast histone H3 was purified to homogeneity by reversed-phase high pressure liquid chromatography. Other core histones were obtained at 80–95% purity.

The yeast *Saccharomyces cerevisiae* is a unicellular eukaryotic model system used in many studies that aim to define in molecular detail the components of chromatin and the regulatory machinery of gene transcription (1–3). It has been demonstrated in recent years that a localized high level of histone acetylation is required to allow gene transcription to start or continue (4–9). When histone deacetylases (HDACs)[1] are inhibited by *N*-butyrate or by specific inhibitors like trichostatin A (TSA), gene transcription tends to increase (10–13). Conversely, localized recruiting of HDAC activities to methylated DNA results in a repressed state of heterochromatin (9, 14–17). Currently, analysis of histone acetylation is frequently performed in Western blots of SDS gels using commercial α-acetyllysine antibodies (7, 18–24), but this methodology does not show whether histone acetylation is maintained at levels present *in vivo*. Commonly, histones, nuclei, or chromatin of yeast are prepared from spheroplasts without the use of HDAC inhibitors (20, 25–27). Acid-urea (AU) or acid-urea-Triton X-100 (AUT) gels reveal loss of histone H4 and H3 acetylation (8, 22, 27, 28). Proteolysis of histone H3 is observed (19, 27).

The highest levels of histone acetylation have been reported by Davie *et al.* (29) when 50–100 mM butyrate was present during spheroplasting and isolation of nuclei. Histone H4 contained 2.0 ± 0.3 acetylated lysines per molecule, *i.e.* 10% tetra- and 20% triacetylated H4 forms with less than 15% unmodified histone. High levels of acetylation were also shown in two-dimensional gels for other core histones, but these were not quantified.

Over the years a method of histone purification from nuclei of *Physarum* (30, 31), alfalfa (32), and *Chlamydomonas* (33) has been developed that is based on the use of guanidine as a chaotropic salt that prevents protease, deacetylase, and phosphatase action. Histones are obtained quantitatively and at reasonable to high purity. When applied to whole cells of multiple plant species (34, 35), the method gives consistently high histone yield with only limited reduction in histone purity. In this publication we present its use, including a novel ultrafiltration step that increases histone purity, for the quantitative preparation of fully acetylated histones from whole yeast cells. It allowed, for the first time, the determination of the steady-state acetylation levels of all four core histones of yeast. A simple method for isolating nuclei without loss of histone acetylation is also described. Histone solubilization from such crude nuclei followed by reversed-phase HPLC yields undegraded, purified core histones of yeast.

EXPERIMENTAL PROCEDURES

Culture of Yeast S. cerevisiae—Strain SNY28, a gift from A. Cooper, was chosen as a wild-type haploid strain of yeast with few auxotrophic markers (*MATα leu2–3, 112 ura3–52 his4–519 ade6*). It was grown at 30 °C in YPD (1% yeast extract (Difco), 2% bactopeptone (Difco), 2% glucose, pH 7) with a doubling time of 90 min to late log phase ($5-7 \times 10^7$ cells/ml) or in supplemented SD medium (yeast nitrogen base (Difco) with ammonium sulfate and 120 mg of leucine, 20 mg of uracil, 20 mg of histidine, and 20 mg of adenine per liter, pH ~3.9) with a doubling time of 150 min to late log phase ($3-4 \times 10^7$ cells/ml). To facilitate acetate labeling in YPD the pH value of the medium was adjusted to 4 (36) by addition of 40 ml of 0.5 M sterile succinic acid per liter. A translation inhibitor, cycloheximide, was added to 10 μg/ml from fresh, non-sterile stock (2 mg/ml in ethanol) 10 min prior to addition of acetate. High specific activity [^{3}H]NaAc (9×10^{13} Bq/mol, NEN Life Science Products) was added at $2-10 \times 10^7$ Bq (0.5–3 mCi) per $1-1.5 \times 10^{10}$ cells in 200–500 ml. After continued incubation, as described, cells were harvested by centrifugation for 5 min at 800 × *g* and 4 °C, typically as $1-2 \times 10^{10}$ cell aliquots (1–2 ml of cell pellet) in 250-ml conical polypropylene centrifugation tubes. The cells were washed once with 10 ml of sterile water and collected in 50-ml conical polypropylene tubes. Cell pellets were flash-frozen in a bath of methanol with dry ice and stored at −80 °C for at least 1 h.

Preparation of Histones—Histones were extracted from whole cells by adding on ice to each frozen cell pellet 3 ml of 40% guanidine hydrochloride (practical grade (Sigma), filtered), 0.1 M potassium phosphate, adjusted by KOH to pH 6.8, with 0.1 ml of 2-mercaptoethanol per liter (named 40%G buffer). Glass beads (0.5-mm diameter) were added

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[1] The abbreviations used are: HDAC(s), histone deacetylase(s); AU, acid-urea; AUT, acid-urea-Triton X-100; HAT, histone acetyltransferase; HPLC, high pressure liquid chromatography; NIB, nuclear isolation buffer; SD, synthetic defined; TSA, trichostatin A; YPD, yeast extract/bactopeptone/dextrose.

until no free fluid was seen, typically to a total volume of 10 ml. Once cell pellets were defrosted, each tube was vortexed vigorously for 1 min, evolving free, foamy fluid. Additional glass beads were added until no free fluid was seen, typically to a total volume of 13 ml, and vortexed for 1 min. Cell homogenate was collected by pipette, and the glass beads were washed twice with 1 ml of 40%G. The combined 40%G cell extract was processed exactly as described below for the sonicated 40%G nuclear extract.

Nuclear isolation buffer (NIB) contained 0.25 M sucrose, 10 mM $MgCl_2$, 2.5 mM spermidine, 0.5 mM spermine, 20 mM HEPES, 100 mM *N*-butyric acid, 0.1% Triton X-100 (w/v), 5 mM 2-mercaptoethanol and was adjusted to pH 7 with KOH. Immediately before use, the protease inhibitor, phenylmethylsulfonyl fluoride, freshly dissolved to 50 mM in isopropanol, was added to 1 mM. Crude nuclei were released from cell pellets into 3 ml of ice-cold NIB by twice vortexing with glass beads exactly as described above. NIB was added to 30 ml, the turbid supernatant was filtered through 2 layers of Miracloth (Calbiochem), and the glass beads were washed once with 10 ml of NIB. NIB was designed for insolubility of chromatin and nuclei, with butyrate at a high concentration to prevent histone deacetylation (29). Based on DNA recovery, yeast chromatin was quantitatively obtained from the Miracloth filtrate by centrifugation for 10 min at 30,000 × *g* (4 °C). The crude nuclear pellet was resuspended by vortexing and repeated pipetting in 10 ml of NIB and collected again by centrifugation. To each pellet, 4 ml of 40%G buffer was added. Histones were solubilized, and DNA was fragmented by vigorous sonication on ice with a sonication microprobe twice for 30 s with a cooling break.

Histone purification was based on the method developed for nuclear preparations of *Physarum* (30) and *Chlamydomonas* (33) and whole cell preparations of alfalfa (32, 37). Insoluble debris was removed by centrifugation for 10 min at 30,000 × *g* (4 °C). The supernatant was acidified to 0.25 N HCl, incubated on ice for at least 15 min, and clarified by centrifugation for 30 min at 30,000 × *g* (4 °C). It was diluted with 0.1 M potassium phosphate, pH 6.8, to the refractive index of a solution of 5% guanidine hydrochloride, 0.1 M potassium phosphate, pH 6.8, with 0.1 ml of 2-mercaptoethanol per liter (named 5%G buffer). The pH value was adjusted to 6.8 with 5 N KOH, and 0.4 μl of 2-mercaptoethanol was added per ml of final volume. In case the extract had become cloudy, typically observed for extracts from whole cells and (near-)stationary yeast cultures, it was clarified by centrifugation for 20 min at 7,000 × *g*.

A suspension with 0.2 ml of settled Bio-Rex 70 resin (Bio-Rad, 200–400 mesh, equilibrated in 5%G buffer and free of fines) was added to extract from 10^{10} SNY28 cells, *i.e.* at an approximate ratio of 1 ml of settled resin for histones extracted from cells with 1 mg of DNA (30). Histone yield remained unaffected if twice as much resin was used, was almost unchanged with half the amount of resin, but decreased sharply if even less resin was added. The suspension was incubated overnight at room temperature under continuous gentle mixing. The resin was allowed to settle, and the turbid supernatant was removed by aspiration. The resin was repeatedly resuspended into 40 ml of 5%G buffer, allowed to settle, and the supernatant was aspirated until it remained completely clear. Allowed to pack into a conical 2–10-ml polypropylene Poly-Prep column (Bio-Rad), the resin was washed with aliquots of 5%G buffer, totaling at least 8 column volumes. The rate of free buffer flow was reduced by a short 25-gauge needle, and the resin was eluted with 10 column volumes of 40%G buffer. The eluate was dialyzed in a Spectrapor3 dialysis membrane (M_r 3500 nominal cut-off) twice for 1 h and once overnight at 4 °C against at least 100 volumes of 2.5% acetic acid with 0.1 ml of 2-mercaptoethanol per liter.

Histone purity was increased by ultrafiltration through Amicon YM100 membranes (Millipore, M_r 100,000 nominal cut-off) in Centricon-100 or CentriPlus-100 centrifugation units or by stirred-cell ultrafiltration. In 2.5% acetic acid, hydrophobic non-histone proteins with widely differing molecular sizes were effectively retained by YM100 membranes. Histone yield was quantitative if the retentate was diluted 1 time with 10 volumes of 2.5% acetic acid and filtered.

Salt-free histones were recovered by lyophilization and dissolved in 0.1–0.4 ml of 8 M urea, 1 M acetic acid, 50 mM NH_4OH, including full reduction by dithiothreitol, as described (38). They were routinely fractionated for 54 min by reversed-phase HPLC on a Zorbax Protein-Plus column (4.6 × 250 mm) between 35 and 53% acetonitrile (v/v) in 0.1% trifluoroacetic acid at 1 ml/min and monitored by absorbance at 214 nm. Radioactivity in 0.5-ml fractions was determined by liquid scintillation counting. Selected fractions were pooled, lyophilized, and analyzed by SDS, AU, or AUT gels with 8 M urea and 9 mM Triton X-100, exactly as described before (37–40). Procedures for gel staining, densitometry, and fluorography have been described previously (33).

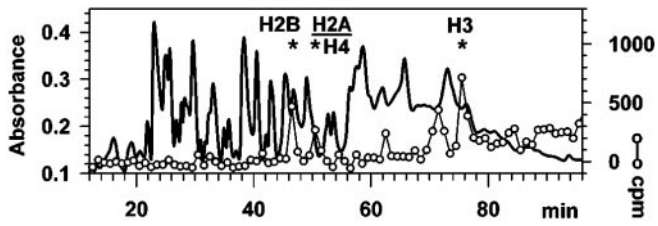

FIG. 1. **Fractionation of crude histones extracted from yeast cells.** Histones were extracted from late-log SNY28 cells in YPD medium, pH 7, with cycloheximide after labeling for 10 min with tritiated acetate. Proteins were fractionated by reversed-phase HPLC at 1 ml/min by a gradient of 30–60% acetonitrile in 0.1% trifluoroacetic acid. Protein elution was monitored by absorbance at 214 nm (*continuous thick line*). Radioactivity was monitored by liquid scintillation counting (cpm per fraction) (*thin line with open circles*). Elution peaks containing histones detected by gel electrophoresis (Fig. 2) are marked by *asterisks* and histone names.

RESULTS

Histone Preparation from Whole Cells—Histones were extracted from intact, flash-frozen wild-type haploid yeast cells using glass beads directly in a chaotropic solution with guanidine at a final homogenate concentration exceeding 20% (see "Experimental Procedures"). This has been shown to prevent proteolysis and histone deacetylation and to solubilize histones from chromatin, isolated nuclei, or sonicated plant cells (30–35). Following the established procedure of clarification by centrifugation, acidification, dilution of the guanidine concentration to 5%, and adsorption of histones to a limiting amount of Bio-Rex 70 resin, histones were eluted in a single step into 40%G buffer, dialyzed into 2.5% acetic acid, and lyophilized. Proteins were fractionated by reversed-phase HPLC (Fig. 1). Core histones were identified in fractions by SDS (Fig. 2*A*), AU (Fig. 3), AUT (Fig. 2*B*), and gradient Triton Acid-Urea (38) (results not shown) gel electrophoresis, based on known characteristics of size, charge modifications, and affinity for the non-ionic detergent Triton X-100. Pulse-labeling with tritiated acetate in the presence of the translation inhibitor, cycloheximide, assisted in identification of post-synthetically modified histones (Fig. 1) and enabled unambiguous identification of the number of acetylated lysines in each band of each core histone in the Coomassie and fluorography gel patterns (Fig. 3). Histones H2B, H3, H4, and H2A are modified maximally at 6, 5, 4, and 2 sites, respectively. The steady-state level of acetylation of the core histones of yeast in growing cultures is presented in Table I. In all preparations the level of histone H4 acetylation was at least as high as the level measured by Davie *et al.* (29) in nuclei prepared in 100 mM butyrate. In some preparations, histone H4 acetylation was as much as 20% higher.

Histone Preparation from Nuclei— Despite Bio-Rex 70 selectivity, histone extracts from whole yeast cells contain more non-histone proteins (Figs. 1 and 2) than seen in preparations from whole plant cells (34, 35). A NIB was designed with butyrate as an essential HDAC inhibitor (29), polyamines and divalent cations for chromatin insolubility, and Triton X-100 to minimize inclusion of cytoplasmic and organellar proteins. Yeast cells were homogenized with glass beads in NIB, and a crude nuclear-chromatin preparation was collected by centrifugation. No attempt was made to keep nuclei intact or to purify them. Histones were solubilized immediately by sonication in 40%G buffer and collected by the standard Bio-Rex 70 adsorption and elution procedure. After fractionation by HPLC and pooling based on the acetate labeling profile (Fig. 4*A*), histone yield and acetylation levels were quantitated following AU gel electrophoresis (Fig. 5). Without affecting histone yield, the number and level of discrete non-histone proteins in the HPLC elution profile (Fig. 4*A*) and in histone pools (Fig. 5) were

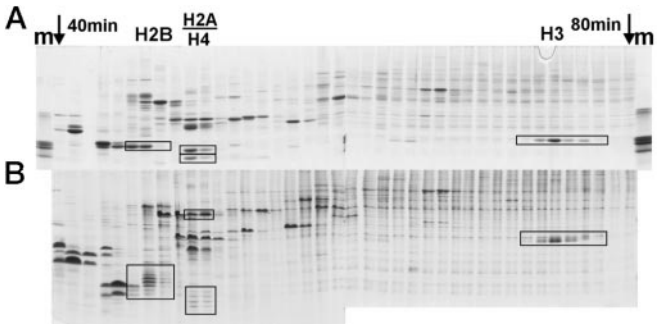

FIG. 2. **Gel electrophoresis of HPLC-fractionated histones extracted from yeast cells.** Fractions from parallel HPLC runs between 40 and 80 min (Fig. 1) were lyophilized and analyzed by SDS (*A*) and AUT (*B*) gel electrophoresis. Calf thymus histones (*m*) were used as gel markers. Identified core histones are *boxed* in both gel systems and named *above* the SDS gel pattern.

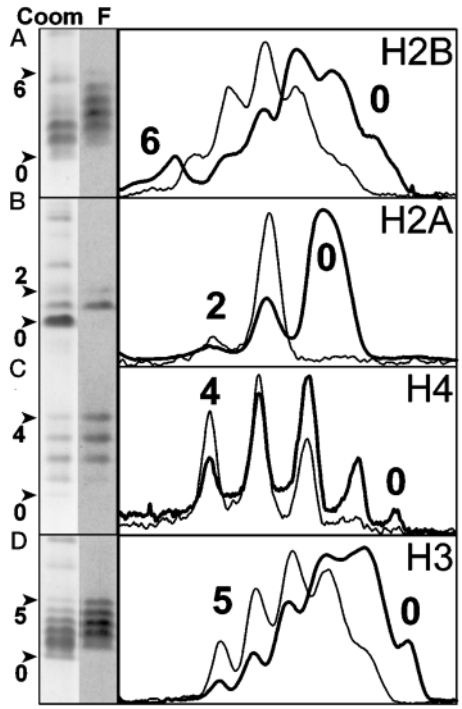

FIG. 3. **Acetylation analysis of HPLC-purified histones extracted from yeast cells.** Histones were extracted from yeast cells, labeled with tritiated acetate for 30 min in YPD, pH 7, with cycloheximide, fractionated by HPLC (Fig. 1), pooled, lyophilized, and analyzed in 30-cm AU gels. Coomassie-stained gel lanes (*Coom*) (electrophoresis from top to bottom) are shown aligned with 48-day exposure fluorographs of the same gel lanes (*F*) to the *left* of densitometric tracings (electrophoresis from left to right) of Coomassie dye (*thick line*) and fluorography (*thin line*) for histones H2B (*A*), H2A (*B*), H4 (*C*), and H3 (*D*). The non-acetylated and maximally acetylated forms are marked with *arrowheads*. In these preparations, penta- and hexa-acetylated H2B forms are only visible in the fluorograph. They are obscured by non-histone proteins in the Coomassie pattern.

sharply reduced. Histone H3 was consistently detected as a distinct HPLC absorbance peak, and histone H2B and H4 peaks could be identified. In general, histone yield was increased (Fig. 5, *lane c*), because the cell homogenate could be collected more completely in this procedure. Importantly, the nuclear isolation method met the critical requirement that histone acetylation was completely preserved, both in the quantity (Table I) and in the distribution of steady-state and dynamically acetylated histone species (compare whole cell histones in *lanes a* and *b* with histones isolated from crude nuclei in *lane c* of Fig. 5).

It was noted that acetate labeling of histones in yeast cells

TABLE I
Average number of acetylated lysines per core histone and histone octamer in yeast

Histone	YPD7 Cells[a]	YPD4 Cells[b]	YPD4 Nuclei[c]
H2A	0.3 ± 0.1	0.22 ± 0.01	0.24 ± 0.01
H2B	1.8 ± 0.1	2.06 ± 0.20	2.30 ± 0.10[d]
H3	1.8 ± 0.1	2.04 ± 0.14	2.09 ± 0.10
H4	2.4 ± 0.1	2.06 ± 0.04	2.03 ± 0.02
Octamer	12.8 ± 0.4	12.8 ± 0.7	13.3 ± 0.5

[a] The acetylation level of histones isolated from yeast cells grown in YPD, pH 7, was calculated from the percentage of charge-modified bands of Coomassie-stained AU gels, as determined by densitometry. The standard error includes variations observed between parallel gel lanes but is primarily determined by variations between five independent cell cultures and histone preparations.

[b] Acetylation level of histones in yeast cells grown in YPD, pH 4 (two independent preparations) (Fig. 5, *sample b*).

[c] Acetylation level of histones isolated from NIB nuclei prepared from yeast cells grown in YPD, pH 4 (two independent preparations) (Fig. 5, *sample c*).

[d] The acetylation level of histone H2B in nuclear preparations is equal to that of cell preparations. The removal of non-histone bands in the nuclear preparation (Fig. 5*A*, *sample c*), which overlap with multi-acetylated H2B bands in whole cell preparations (Fig. 5*A*, *sample b*), allows increased accuracy in measuring acetylation levels.

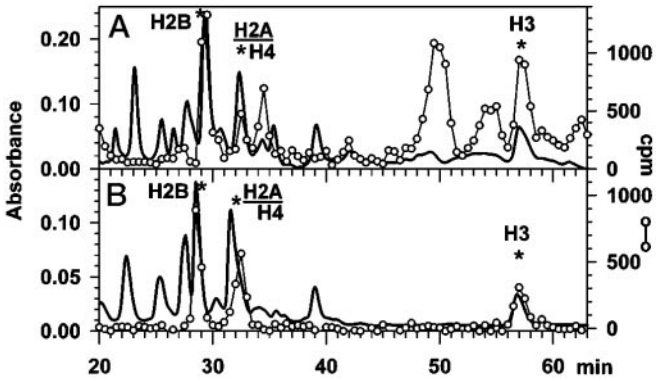

FIG. 4. **Fractionation of histones extracted from yeast nuclei.** *A*, histones were extracted from the nuclear pellet prepared from yeast cells, labeled with tritiated acetate for 23 min in YPD, pH 4, with cycloheximide. Histones were fractionated by HPLC between 35 and 53% acetonitrile and are marked by *asterisks* and histone names (*H2B*, *H2A*, *H4*, and *H3*). All other details are as in Fig. 1. B, histone preparation from nuclei prepared from yeast cells, labeled with tritiated acetate for 31 min in SD, pH 4, with cycloheximide. Prior to lyophilization, the dialysate in 2.5% acetic acid was filtered through an YM100 ultrafiltration membrane by Centricon-100 centrifugation.

grown in YPD medium was slow (25). In YPD medium, glucose represses acetate uptake. Thus, free diffusion of the undissociated acetic acid limits uptake to less than 1% of the total tritiated acetate supplied (36). However, decreasing the pH value of the YPD medium by succinic acid to 4, below the 4.76 pK_a of acetate, or using SD medium at pH 4, increased the level of undissociated acetic acid to 70% of total acetate added and, consequently, the rate of acetate entry into the cells and the rate of acetate incorporation into histones (Fig. 5). The steady-state level of acetylation labeling of histones appeared unaffected (results not shown).

Histone Purification by Ultrafiltration—Histones extracted from yeast cells or nuclei and fractionated by reversed-phase HPLC are not pure. In addition to discrete protein species, which co-elute with histone H2B or with H2A/H4, histone H3 preparations are contaminated by multiple non-histone proteins (Fig. 5), which elute broadly above 45% acetonitrile (Figs. 1, 2, and 4*A*) and which cause HPLC column fouling. Selective solubilization of histones from the lyophilizate into 0.1 or 0.4 N H_2SO_4 or HCl (common solvents to extract histones from nu-

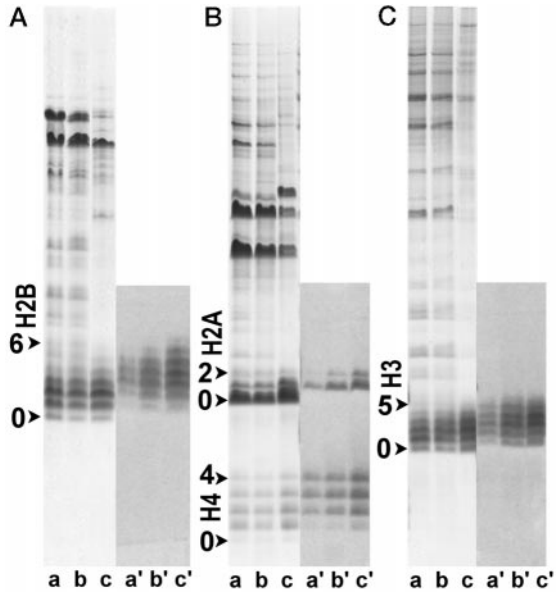

FIG. 5. **AU gel analysis of histones extracted from yeast cells and nuclei.** Yeast cells were labeled with tritiated acetate for 23 min in YPD medium with cycloheximide at pH 7 (*a*) or pH 4 (*b* and *c*). Histones were extracted from whole cells (*a* and *b*) or from nuclei collected from NIB cell homogenates (*c*), fractionated by HPLC (Fig. 4) into histone H2B (*A*), H2A with H4 (*B*), and H3 (*C*) pools and analyzed by densitometry in AU gels, Coomassie stained (*lanes a*, *b*, and *c*), and fluorographed for 82 days (lanes *a′*, *b′*, and *c′*). Sections *A*, *B*, and *C* are shown aligned, allowing comparison of the relative mobilities of all histone bands in AU gel electrophoresis. *Numbered arrowheads* mark non- and maximally acetylated histone forms with 6, 2, 4, and 5 acetylated lysines in histone H2B (*A*), H2A and H4 (*B*), and H3 (*C*), respectively.

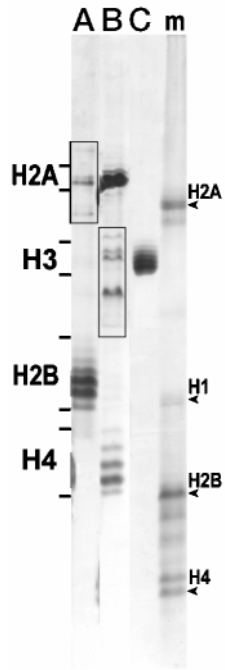

FIG. 6. **Assessment of yeast histone purification by gel electrophoresis.** Histones were extracted from NIB-produced yeast nuclei. The histone dialysate was ultrafiltered through YM100 membranes, lyophilized, and fractionated by HPLC as in the legend to Fig. 4*B*. The Coomassie-stained AUT gel pattern of pooled histone H2B (*lane A*), of pooled histone H2A with H4 (*lane B*), and of pooled histone H3 (*lane C*) is marked on the *left* with *marks* at the levels of non- and maximally acetylated histone bands. Discrete non-histone proteins in histone pools are marked by *boxes*. The identity of major calf thymus histone markers (*m*) is shown along the *right*.

clei) failed (results not shown). However, ultrafiltration of the histone extract in 2.5% acetic acid dialysis solvent through YM100 membranes (M_r 100,000 nominal cut-off, Amicon) removed all column-fouling protein species from the filtrate, irrespective of molecular weight as judged by SDS gel analysis, as well as a number of discrete non-histone proteins. Moreover, the majority of acetate label, incorporated *in vivo* in the presence of cycloheximide (Fig. 4*A*), was also removed. The only labeled protein species collected in the Centricon-100 ultrafiltrate were histones, which were quantitatively recovered (Fig. 4*B*). Centricon-30 (M_r 30,000 nominal cut-off) ultrafiltration was equally effective in histone purification but reduced histone yield. Reversed-phase HPLC fractionation produced homogeneous, pure histone H3, based on SDS, AU, and AUT gel analysis (Fig. 6). A small number of discrete non-histone protein species with M_r 20,000–50,000 represented 5–10 and 10–20% of total protein in the H2B and H2A/H4 histone pools, respectively (Fig. 6). These contaminants could be separated from the histones by Bio-Gel P60 (Bio-Rad) conventional column gel filtration in 10 mM HCl with 50 mM NaCl, a method that separates histone H2A from H4 (41) (results not shown).

DISCUSSION

By avoiding extensive incubation of yeast for spheroplasting and isolation of nuclei, the steady-state acetylation levels of all core histones could be determined for the first time in the yeast *S. cerevisiae* (Table I). Davie *et al.* (29) had shown that 100 mM butyrate during spheroplasting of yeast cells could prevent histone deacetylation. However, common histone isolation procedures from yeast do not include the use of butyrate during spheroplasting and use only up to 30 mM butyrate during extensive procedures to isolate nuclei (20, 25–27). Consequently, AU gel electrophoresis (if shown) reveals variable loss of acetylation, most easily seen in the distribution of acetylated histone H4 species (8, 22, 27, 28). Even proteolysis of histone H3 is seen in SDS gel patterns (19, 27). The method described here is fast and can be applied directly to flash-frozen yeast cells. Extensive histone purification is achieved when histones are extracted from nuclei produced in a single step from frozen cells using a buffer that prevents loss of histone acetylation and maximizes histone recovery.

The steady-state acetylation level of whole-cell histone H4 (Table I) and its distribution across acetylated forms (Fig. 3*C*) mirrors the pattern described by Davie *et al.* (29) produced at 100 mM butyrate. We obtained the same result when crude nuclei were rapidly isolated from yeast in 50 mM butyrate (Table I). Our common observations enable the redesign of current methods of yeast chromatin preparation to preserve *in vivo* levels of histone acetylation for Western analysis of acetylation sites and levels.

The effective prevention of histone deacetylation *in vivo* under conditions of spheroplasting (25, 29) is remarkable as neither butyrate nor TSA will induce histone hyperacetylation in growing yeast cells (42). The latter observation was confirmed during culture of yeast cells for up to 3 h at 100 ng of TSA per ml (results not shown). This suggests that in growing yeast cells the steady-state pattern of histone acetylation (Table I) is defined by histone acetyltransferase (HAT) levels. Inhibition of HDAC activities under these conditions has no effect. The balance between HAT and HDAC activities appears delicate as histone deacetylation is readily observed during spheroplasting unless the then-dominant HDAC activity is inhibited by butyrate. This conclusion implies that in growing yeast cells all potential sites for histone acetylation, within reach of acetylating enzymes, are fully modified and are kept that way. This is in sharp contrast to animals (43, 44), algae (39), and plants (45, 46), where HDAC inhibitors like butyrate and TSA rapidly

induce histone hyperacetylation. Apparently, repressive HDAC activities dominate activating HAT action in these organisms.

This general conclusion agrees with the overall state of chromatin transcription. In most species, a limited fraction of the genome exists in a transcriptionally potentiated, competent, or transcribing state. Comparing the fraction of genome expressed with the steady-state level and distribution of histone acetylation, we have deduced that transcriptionally active or competent chromatin in plants and algae may carry 10−12 acetylated lysines per nucleosome, predominantly in histone H3 (33, 39, 47). Similar conclusions have been reached for animal cells where histone H4 is most highly acetylated. Biophysical studies of the transition between the folded and unfolded states of chromatin fibers also indicate a transition at 10−12 acetylated lysines per nucleosome (48, 49). It has been well established that the complete genome of *S. cerevisiae* exists as euchromatin with the exception of chromosome telomers and a few internal sites that are repressed by interaction with SIR proteins (8, 50). The overall steady-state acetylation of yeast nucleosomes with approximately 13 acetylated lysines per nucleosome (Table I) fits this model rather well. The average steady-state level of histone acetylation (Table I) is the highest ever reported. However, compared with the maximal level of 34 acetylated lysines in a yeast nucleosome, based on 6 lysines that can be acetylated in H2B, 2 in H2A, 5 in H3, and 4 in H4 (Fig. 3), the average level of nucleosome acetylation in yeast is rather moderate. Combining the methodologies described here with chromatin fractionation procedures, it should be possible to determine whether individual yeast nucleosomes have acetylation levels within a narrow range around 10−12 or vary between the extremes of 0 and 34 acetylated lysines per nucleosome.

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