Histone Deacetylase Is a Component of the Internal Nuclear Matrix*

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In chicken immature erythrocytes, approximately 4% of the modifiable histone lysine sites participate in active acetylation. There are two categories of actively acetylated histone H4. Although both are acetylated at the same rate ($t_{1/2} = 12$ min), one is acetylated to the tetraacetylated form and is rapidly deacetylated (class 1), and the other is acetylated to mono- and diacetylated forms and is slowly deacetylated (class 2). We show that the chromatin distribution of the class 1 labeled tetraacetylated H4 species paralleled that of the transcriptionally active DNA sequences. For example, the chromatin fragments of the insoluble nuclear material contained 76% of the active DNA and 74% of the labeled tetraacetylated H4. Class 2 labeled acetylated H4 species were found in repressed chromatin and were enriched in active/competent gene-enriched chromatin fragments. The majority of the histone deacetylase activity (75–80%) was located with the insoluble residual nuclear material. Further, approximately 40–50% of the enzyme activity was associated with nuclear matrices prepared by two methods using high salt and intermediate/high salt extraction. Histone deacetylase was solubilized by extracting the nuclear matrices with high salt and 2-mercaptoethanol, a procedure that generates nuclear pore-lamina complexes. These results demonstrate that histone deacetylase is a component of the internal nuclear matrix.

Highly acetylated histones are complexed to transcriptionally active/competent DNA (Allegra et al., 1987; Zhang and Nelson, 1988a; Hebbes et al., 1988; Ip et al., 1988; Ridsdale et al., 1990; Boffa et al., 1990). Importantly, genetic analysis of histone H4 acetylation has shown that acetylation of H4 has an essential role in chromosome dynamics (Magee et al., 1990). Histone deacetylase has been shown to alter nucleosome structure and to alter the capacity of the H1 histones to condense the transcriptionally active/competent gene chromatin fiber which may in turn facilitate transcription (Norton et al., 1989, 1990; Walker et al., 1990; Oliva et al., 1990; Ridsdale et al., 1990).

Histone acetylation is a very dynamic process. This cannot be appreciated in studies that analyze steady-state levels of acetylated histones in chromatin preparations. In chicken immature erythrocytes approximately 4% of the modifiable histone lysine sites participate in active acetylation and deacetylation (Zhang and Nelson, 1986). In these cells there is only one rate of acetylation which has a $t_{1/2}$ of approximately 12 min (Zhang and Nelson, 1988a; Hendzel and Davie, 1991). However, there are two categories of metabolically active histone acetylation. One type of acetylated histone species becomes hyperacetylated (e.g. the tetraacetylated form of histone H4) in the presence of sodium butyrate, a histone deacetylase inhibitor. Upon removal of the inhibitor, the hyperacetylated histone species are rapidly deacetylated ($t_{1/2} = 5$ min; Zhang and Nelson, 1988b). We refer to this type of metabolically active acetylation as dynamic, class 1 acetylation.

Another population of the metabolically active acetylated histone species only achieve low levels of acetylation (e.g. mono- and diacetylated forms of histone H4) in the presence of sodium butyrate. In the absence of butyrate these histones are slowly deacetylated. We refer to this type of acetylation as class 2 acetylation.

Transcriptionally active gene chromatin has a soluble and/or insoluble nature; that is, active genes are located in two types of chromatin fragments: those that are soluble in 0.15 M NaCl and/or 2 mM MgCl₂ and those that co-fractionate with the low salt-insoluble residual nuclear material (Rose and Garrard, 1984; Cohen and Sheffrey, 1985; Stratling et al., 1986; Einck et al., 1986; Stratling, 1987; Delcuve and Davie, 1989). Interestingly, the partitioning of inducible genes with the low salt-insoluble residual nuclear material was related to their transcriptional activity (Stratling et al., 1986; Einck et al., 1986).

In chicken immature erythrocytes, transcriptionally active DNA sequences are located in chromatin fragments that are soluble in 0.15 M NaCl and in chromatin fragments that are associated with the low salt-insoluble residual nuclear material (Delcuve and Davie, 1989). The latter chromatin fraction was found to be enriched in histones H3 and H4 which were undergoing methylation and in newly synthesized histones, both of which paralleled active gene content (Hendzel and Davie, 1989, 1990). The association of the class 1, dynamically acetylated histones with the 0.15 M NaCl or 2 mM MgCl₂-soluble, active gene chromatin fragments has been demonstrated (Zhang and Nelson, 1988a; Ridsdale et al., 1990). However, whether the histones associated with the active gene chromatin fragments bound to the low salt-insoluble residual nuclear material partake in dynamic acetylation remains to be determined. Furthermore, little is known about the chromatin distribution of the class 2 acetylated histones.

Dynamic acetylation is a consequence of the activities of two histone-modifying enzymes, the histone acetyltransferase(s) and the histone deacetylase(s). Because dynamic acetylation is a nonrandom phenomenon restricted to specific regions of the eucaryotic genome, to aid in the understanding

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of this process it is necessary that the nuclear distribution of these two enzymes be determined. In chicken erythrocytes the histone acetyltransferase(s) is highly enriched in the transcriptionally active/competent gene-enriched, 0.15 M NaCl-soluble polyconosomes (Chan et al., 1988). In contrast, little is known about the distribution of histone deacetylase(s) within the nucleus. Hay and Candido (1983a, 1983b) have reported the presence in HeLa cells of histone deacetylase activity in high molecular weight material after mild micrococcal nuclease digestion. This fraction resembles the nuclear scaffold in that it is partially dissociated with 2-mercaptoethanol or neocuproine (Hay and Candido, 1983a). Mold and McCarty (1987) reported that histone deacetylase is associated with a unique class of mononucleosomes which can be resolved by non-denaturing polyacrylamide gel electrophoresis.

In this study we determined the chromatin distribution of the metabolically active class 1 and class 2 acetylated histone H4 species and the histone deacetylase activity in chicken immature and mature erythrocytes. We provide evidence that dynamic class 1 histone H4 acetylation was limited to the transcriptionally active chromatin regions. Dynamically acetylated H4 histone species were associated with the active gene and the NaCl-soluble chromatin fragments and the active chromatin fragments bind to the residual nuclear material. Class 2 acetylated histone H4 species were found in repressed, active and competent chromatin, with a preference for the latter two chromatin regions. The majority of the histone deacetylase activity was located with the residual nuclear material. We demonstrate that the histone deacetylase is a component of the internal nuclear matrix.

MATERIALS AND METHODS

Isolation and Incubation of Chicken Erythrocytes

Chicken mature and immature erythrocytes were isolated from adult White Leghorn chickens as described (Hendzel and Davie, 1989). Cells were washed once with an isotonic buffer (130 mM NaCl, 5.2 mM KCl, 7.5 mM MgCl2, 10 mM Hepes, pH 7.2, and once in Swim's S-77 medium (Sigma), pH 7.2). Cells resuspended at one-third volume packed erythrocytes and two-thirds volume medium were preincubated for 30 min with 20 μM cycloheximide. Cells were then labeled with [3H]acetic acid, sodium salt (27 Ci/mmol; ICN Radiochemicals) at a final concentration of 0.1 μCi/ml. Labelling proceeded for either 15 or 60 min as indicated. Cells were collected by centrifugation and washed with Swim's S-77 medium containing 0.1 mM sodium acetate and 10 mM sodium butyrate. Subsequently, cells resuspended in the same medium with sodium acetate and sodium butyrate were incubated a further 60 min essentially as described by Zhang and Nelson (1988a).

Nuclei Isolation, Digestion, and Chromatin Fractionation

Nuclei were digested and the chromatin fractionated as described previously (Delucave and Davie, 1989) except that the micrococcal nuclease incubations (Pharmacia LKB Biotechnology Inc.) were for 20 min. Under these digestion conditions 2.0-2.4% of the nuclear DNA was rendered acid soluble. The 0.15 M NaCl-soluble chromatin fragments of fraction S10 were size fractionated on either a 2.8 or 90-cm Bio-Gel A-5m column at a flow rate of 25 ml/h or a 2.8 × 20-cm Bio-Gel A-5m column at a flow rate of 1.2 ml/min. Protein concentration of the column fractions was determined by the Bio-Rad protein microassay. Fraction P1 was extracted with 0.6 M NaCl. The suspension was then kept on ice for 10 min and then centrifuged at 12,000 × g for 10 min to obtain a soluble (S0.4) and an insoluble fraction (P1).

Preparation of Nuclear Matrices

High Salt Nuclear Matrix Isolation—NaCl matrices from chicken erythrocytes were prepared according to the procedure of Cockerill and Garrard (1986). The 2 M NaCl-soluble material (fraction 2 M NaCl) was saved and it was dialyzed against 10 mM Tris-HCl, 10 mM EDTA, 150 mM NaCl (TEN), pH 8.0, at 4 °C. The nuclear matrix (fraction 2 M NaCl) was washed once in RSB (10 mM Tris-HCl, 10 mM NaCl, 3 mM MgCl2, 10 mM sodium butyrate), pH 7.5, containing 1 mM phenylmethylsulfonyl fluoride and resuspended in 10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride.

Intermediate-High Salt Nuclear Matrix Isolation—Nuclear matrices from chicken erythrocytes were prepared as described by Robarge et al. (1988). Nuclei were resuspended at a concentration of 40 A260/ml in RSB, 0.25 M sucrose, pH 7.5, and digested for 1 h at room temperature with 200 μg/ml DNase I. Nuclei were collected by centrifugation and the supernatant (fraction S), which contained very low histone deacetylase activity, was discarded. The nuclear pellet was resuspended in 0.4 M KCl, 0.2 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 0.25 M sucrose, and 10 mM Tris-HCl, pH 7.4. After a 15-min incubation on ice the matrices were collected by centrifugation, and both the pellet (fraction 0.4 M KCl) and supernatant (fraction 0.4 M KCl S) were saved. The pellet was resuspended in the same buffer with 2.0 M KCl and treated as before. The supernatant (fraction 2.0 M KCl S) and pellet (fraction 2.0 M KCl) fractions were dialyzed against TEN, pH 8.0. Often a precipitate would form after dialysis, and this was resuspended in the supernatant, and the samples were assayed as suspensions.

Nuclear Pore-Lamina Complex Isolation—Nuclear pore-lamina complexes were prepared from chicken erythrocytes as described by Kaufmann et al. (1983). Nuclei were resuspended at a concentration of 49 A260/ml in RSB, 0.25 M sucrose, pH 7.5, and digested for 1 h on ice with 500 μg/ml DNase I and 500 μg/ml RNase A (boiled). The nuclear pellet was saved after centrifugation. The supernatant (fraction S), which contained very low histone deacetylase activity, was discarded. The nuclear pellet was resuspended in a low salt buffer (10 mM Tris-HCl, pH 7.4, and 0.2 mM MgSO4). High salt buffer (10 mM Tris-HCl, pH 7.4, 0.2 mM MgSO4, 2 M NaCl) was then added to a final NaCl concentration of 1.6 M. 2-Mercaptoethanol was added subsequently with gentle agitation to a final concentration of 1% (v/v), and the suspension was incubated on ice for 15 min. The nuclear pore-lamina complexes (pellet) and supernatant were collected by centrifugation. The pellet was resuspended in the same manner in the absence of 2-mercaptoethanol. After a 15-min incubation on ice, the pellet (fraction 1.6 M NaCl P) and supernatant were collected. The supernatants from the salt-extracted nuclear pellets were pooled, yielding fraction 1.6 M NaCl S. The pellet was resuspended in low salt buffer, and all fractions were dialyzed overnight against TEN, pH 8.0.

Assay for Deacetylase Activity

Chromatin fractions were brought to equivalent A260 concentrations (typically 4 absorbance units in a final volume of 300 μl) in TEN buffer. For the suspension of nuclear matrices or nuclear envelopes and the accompanying supernatant fractions, a portion (300 μl) of the fraction was assayed. Approximately 100 μg of acid-soluble histones isolated from [3H]acetyl-labeled erythrocytes was added to each reaction. Cells were then incubated for 60 min at 37 °C. Nonenzymatic release of label was typically below 100 dpm. Incubations were typically done in triplicate. Incubation was terminated by adding acetic acid/HCl to a final concentration of 0.12 N/0.72 N, and 2 volumes of ethyl acetate were added to the reaction mix. The samples were centrifuged for 2 min in a microcentrifuge. Half of the added volume of ethyl acetate was then counted in a scintillation counter to determine the amount of liberated label. The enzyme activity of fraction P0, which was the chromatin fraction with the highest level of activity, was linear up to 16 A260 units in a 1-h incubation, and the enzyme activity was linear with time up to 2 h at 4 A260 units of fraction P0.

Preparation and Analysis of Samples

DNA was isolated and quantitated as described by Delucave and Davie (1989). Histones were isolated from the various chromatin fractions by extraction with 0.4 N H2SO4, as described (Nickel et al., 1987). Polyacrylamide gel electrophoresis was performed as described (Nickel et al., 1987). Fluorography was performed as described by Hendzel and Davie (1989).
RESULTS

Dynamically Acetylated Histones Are Preferentially Associated with Transcriptionally Active Gene Chromatin—To label the dynamically acetylated (class 1) histones, chicken immature erythrocytes were pulse labeled with \(^3\)Hlacetate for 15 min followed by a 60-min chase period in Swinn's S-77 medium containing 10 mM sodium butyrate. Fig. 1B, lane T, shows that during this 60-min chase period a portion of the metabolically active histone H4 population became hyperacetylated, with approximately 33.7% of the labeled histone H4 being tetraacetylated. In addition to histone H4, the acetylated species of histones H2A, H2A.Z, H3.2, H3.3, and H2B were also labeled (Fig. 1B, lane T). We observed that of the two histone H3 variants, H3.2 and H3.3, the latter variant preferentially participated in dynamic acetylation. Densitometric analysis of electrophoretic patterns of histones resolved on long AUT (acetic acid/6.7 M urea, 0.375% (w/v) Triton X-100)-polyacrylamide gels (not shown) demonstrated that histone variant H3.3 was labeled to a specific activity 3.2-fold greater than that of histone H3.2, the major histone H3 variant in chicken erythrocytes.

We have described previously a chromatin fractionation protocol that separates chromatin fragments differing in their content of transcriptionally active, competent, and repressed DNA sequences (Fig. 2; Table I; Delucove and Davie, 1989). Transcriptionally active DNA sequences (e.g. β-globin, histone H5, and histone H2A.F) were enriched in 0.15 M NaCl-soluble chromatin fragments (fraction S\(_{150}\)). 0.15 M NaCl-soluble oligonucleosomes (fractions F\(_{II}\) and F\(_{III}\), and chromatin fragments associated with the residual nuclear material (fraction P\(_E\)). Transcriptionally competent DNA sequences were enriched in 0.15 M NaCl-soluble chromatin fragments (fraction S\(_{150}\)) and 0.15 M NaCl-soluble polynucleosomes (fraction F\(_I\)) whereas the majority of the repressed DNA was localized with 0.15 M NaCl-insoluble chromatin fragments (fraction P\(_{III}\)). Transcriptionally competent DNA sequences (e.g. c-globin, c-myc, thymidine kinase), which are no longer expressed in immature or mature erythrocytes of adult birds, retain a DNase I-sensitive structure.

Fig. 1 demonstrates that relative to total histones, the levels of the labeled acetylated histones were elevated in the 0.15 M NaCl-soluble chromatin fragments (fractions S\(_{150}\), F\(_I\), F\(_{II}\), F\(_{III}\), and F\(_{VII-V}\)) which contained salt-soluble mononucleosomes) and fraction P\(_E\). The 0.15 M NaCl-insoluble chromatin fragments (fraction P\(_{150}\)) had low amounts of the labeled histones, with mono- and diacetylated histone H4 forms being labeled. Labeled tetraacetylated histone H4 was not detectable in this fraction. The highest concentration of labeled histones was present in the 0.15 M NaCl-soluble oligonucleosomes (F\(_{II}\) and F\(_{III}\)) and the content of labeled histones H2A.Z and ubiquitinated H2B in these fractions was significant. Labeled histone H2A.Z, but not ubiquitinated H2B, was detected in fraction P\(_E\).

To ascertain the extent to which the histones in each chromatin fraction were participating in dynamic class 1 acetylation, we determined the percentage of the labeled histone H4 forms of each fraction which were tetraacetylated (Fig. 3). The small amount of H4 of fraction P\(_{150}\) which was metabolically active was of the class 2 acetylation type as indicated by the label being localized in the mono- and diacetylated species (Fig. 3, P\(_{150}\))

| Fraction | % DNA | % Competent | % Active | % H4A4 | % H4A1 + A2 |
|----------|-------|-------------|----------|--------|-------------|
| S\(_{150}\) | 7.1   | 41.7        | 29.4     | 33.4   | 26.8        |
| F\(_I\) | 0.5   | 14.4        | 2.0      | 2.4    | 2.8         |
| F\(_{II}\) | 1.3   | 18.0        | 11.0     | 13.1   | 9.7         |
| F\(_{III}\) | 1.6   | 4.2         | 7.4      | 19.5   | 7.3         |
| P\(_E\) | 21.0  | 30.5        | 76.3     | 73.5   | 26.5        |
| S\(_{150}\) | 16.3  | 25.8        | 44.2     | 39.3   | 14.2        |
| P\(_E\) | 4.7   | 6.7         | 32.1     | 35.6   | 8.7         |
| P\(_{150}\) | 71.9  | 29.0        | 9.6      | ND     | 52.8        |

Fig. 2. Chromatin fractionation procedure. For details of the procedure see "Materials and Methods."
either 15 min fluorography and densitometry after labeling with [3H]acetate for both 15 min or 2 h, as indicated. The relative distribution of label among the mono- (1), di- (2), tri- (3), and tetra- (4) acetylated species of histone H4 was then plotted with the value for monoaacetylated (1) histone H4 set arbitrarily at 1.0 for each chromatin fraction.

chromatin fibers associated with the residual nuclear material (P1, 0.15) were mainly of the dynamic, class 1 acetylation type, with 43.4 and 44.9% of the labeled H4 being tetraacetylated, respectively. Of the various chromatin fractions the metabolically active acetylated H4 species of fraction P1 showed the strongest bias toward the dynamic, class 1 acetylation type.

To determine the chromatin distribution of the dynamically acetylated class 1 histones, the percentage of labeled tetraacetylated histone H4 located in each chromatin fraction was ascertained. Table I demonstrates that the distribution of the labeled tetraacetylated H4 species (class 1 acetylation) paralleled that of active DNA among the chromatin fractions. A similar match in the partitioning of competent DNA was not so obvious. The chromatin fragments associated with the residual nuclear material (fraction P1) contained the majority of the active DNA and labeled tetraacetylated histone H4 species. The remainder of the labeled tetraacetylated histone H4 was located with the active and competent gene-enriched 0.15 M NaCl-soluble chromatin fragments (fraction S15N). Size fractionation of these fragments demonstrated that the 0.15 M NaCl-soluble oligonucleosomes (F1 and FII) had a greater proportion of the labeled tetraacetylated H4 than the 0.15 M NaCl-soluble polynucleosomes, illustrating the parallel partitioning of class 1 acetylated histones and active DNA. Similar results were obtained when we repeated these experiments with mature erythrocytes.

Metabolically Active Class 1 Acetylated Histone Species in Fraction P1 Co-purify with Active Gene Chromatin—The chromatin fragments associated with the residual nuclear material contained competent and repressed DNA as well as an enrichment in active DNA. Fig. 4A shows that the salt elution characteristics of bulk DNA and active DNA sequences from the P1 fraction differ. Bulk chromatin was eluted more readily than the transcriptionally active histone H5 gene chromatin. We used this differential extractability of bulk versus active gene chromatin to obtain a greater level of enrichment of active gene chromatin. Fig. 4C shows that after the treatment of the fraction P1 with 0.6 M NaCl, the amount of labeled histones associated with the insoluble chromatin (fraction P1, 0.6M) was elevated. A striking 51.1% of the labeled H4 of fraction P1, 0.6M was tetraacetylated. Moreover, the percentage of total labeled tetraacetylated H4 (dynamic, class 1 acetylation type) in fraction P1, 0.6M correlated with the enrichment in active gene chromatin fragments (Table I).

**Chromatin Distribution of Class 2 Acetylated Histone Species**—To label the class 2 acetylated histones, chicken immature erythrocytes were pulse labeled with [3H]acetate for 2 h followed by a 60-min chase in Swim's S-77 medium containing 10 mM sodium butyrate. Incubation of cells in the absence of sodium butyrate resulted in the loss of the 0.15 M NaCl solubility of the active and competent gene polynucleosomes. Incubation of the cells with sodium butyrate was necessary to restore this solubility (Ridsdale et al., 1990). The slow rate of deacetylation of the class 2 acetylated histones and the rapid deacetylation of the class 1 acetylated histones result in the preferential labeling of the class 2 acetylated histones during this 2-h labeling period. Fig. 3 shows that the predominating labeled H4 species of unfractionated chromatin (T2h) were the mono- and diacetylated forms. For all of the chromatin fractions except the 0.15 M NaCl-soluble oligonucleosomes (fraction F1, 2h), the labeled mono- or diacetylated H4 species were at a higher level than the labeled tetraacetylated H4 (Fig. 3).

The content of the labeled histones of each chromatin fraction was similar to those shown in Fig. 1, with 0.15 M NaCl-soluble oligonucleosomes (fractions F1 and FII) having the greatest concentration of labeled histones and the 0.15 M NaCl-insoluble chromatin fragments having the lowest levels (not shown). The chromatin distribution of the labeled mono- and diacetylated histones (class 2 acetylated histone forms) was different from that of class 1 acetylated (tetraacetylated H4) histone (Table I). Although the 0.15 M NaCl-soluble poly- and oligonucleosomes (fractions F1, FII, and FIII) were enriched in class 2 acetylated histones (5.6-, 7.5- and 4.6-fold, respectively), the 0.15 M NaCl-insoluble chromatin fragments contained a substantial amount of the labeled mono- and diacetylated histone H4 species (Table I). Further, these labeled histone forms were only slightly enriched (1.3-fold) in the chromatin fragments complexed to the insoluble nuclear material (fraction P1). These observations suggest that class 2 acetylated histones were associated with repressed, competent and active gene chromatin fragments, with a preference toward competent gene chromatin fragments. Obviously, the chromatin distribution of the class 2 acetylated histones did not follow the chromatin distribution of the class 1 acetylated histones.
not parallel that of the active DNA.

**Distribution of Histone Deacetylase Activity in Chicken Immature and Mature Erythrocyte Chromatin**—The analysis of the immature and mature erythroid chromatin distribution of the dynamically acetylated histones demonstrated that the majority of the rapidly deacetylated, class 1 histone H4 was localized with the chromatin fragments complexed to the insoluble nuclear material. This observation suggested that the majority of the histone deacetylase activity would also be localized in this fraction. Table II shows the activity of the histone deacetylase of the chromatin fractions. Fraction PE was found to possess the majority of the enzyme activity. Of three fractionations, the proportion of the total histone deacetylase activity located in the EDTA-insoluble residual nuclear material (fraction PE) was highly reproducible, with this fraction containing 77.6–78.9% of the enzyme activity.

Zhang and Nelson (1988b) reported that the deacetylation rate of labeled histone H4 of mature erythrocytes was lower than that of immature cells. Consistent with this observation, we found that the histone deacetylase activity of mature erythroid nuclei was approximately 35% that of the enzyme activity of immature erythrocyd nuclei. However, the distribution of the histone deacetylase activity among the mature erythroid chromatin fractions was similar (Table II).

Whereas treatment of the residual nuclear material (fraction PE) with 2 M NaCl extracted approximately 90% of the associated chromatin (see Fig. 4), the histone deacetylase activity was not solubilized, suggesting that the enzyme was not bound to chromatin. Also, the enzyme activity remained associated with the residual nuclear material that was treated with 10 mM 2-mercaptoethanol, indicating that the enzyme was not retained by the residual nuclear material (nuclear matrix) solely via disulfide bonds.

**Histone Deacetylase Is a Component of the Nuclear Matrix**—The observation that histone deacetylase activity was primarily located in fraction PE suggested that the enzyme may be a component of the nuclear matrix. Nuclear matrices of immature and mature erythrocytes were prepared by extensively digesting nuclei with DNase I and subsequently extracting the residual nuclear material with 2 M NaCl (Fig. 5, high salt matrix). Table II shows that 50–57% of the histone deacetylase activity was associated with the nuclear matrix of mature and immature erythrocytes.

There is concern that the direct high salt extraction of nuclei may induce precipitation of enzymes in the nuclear matrix. For example, Roberge et al. (1988) demonstrated that RNA polymerases I and II were associated with the high salt matrix but not with the intermediate/high salt matrix (see Fig. 5). Thus, we prepared nuclear matrices by the intermediate/high salt matrix isolation procedure. Table III shows that 42% of the histone deacetylase remained bound to the intermediate/high salt prepared nuclear matrices of immature erythrocytes. These results provided strong evidence that approximately half of the histone deacetylase activity of mature and immature erythrocytes was complexed to the nuclear matrix.

We attempted to determine the histone deacetylase activity associated with the nuclear scaffold prepared by the lithium diiodosalicylate low ionic strength procedure (Mirkovich et al., 1984). However, we found that lithium diiodosalicylate irreversibly denatured the enzyme, and we were unable to detect enzyme activity in either soluble or scaffold fractions (data not shown).

To determine whether the histone deacetylase was a component of the internal nuclear matrix or of the nuclear pore-lamina fraction, nuclear pore-lamina complexes were isolated according to the protocol of Kaufmann et al. (1983) (see Fig. 5, nuclear pore-lamina complex). Treating nuclear matrices with 1% 2-mercaptoethanol, 1.6 M NaCl solubilized the internal matrix, leaving the nuclear pore-lamina complexes. This procedure was recently used to solubilize and purify a protein (ARBP) which is responsible for binding DNA to the nuclear matrix (von Kries et al., 1991). Table III shows that approximately 95% of the histone deacetylase activity was found in the 1% 2-mercaptoethanol, 1.6 M NaCl extract. The nuclear pore-lamina complex contained less than 5% of the histone deacetylase activity. These results demonstrated that like...
enriched chromatin fractions. The enrichment of the class 2 acetylated histones in the 0.15 M NaCl-soluble poly- and oligonucleosomes. Approximately 3 mg of fraction S150 in a volume of 1.0 ml was applied to a Bio-Gel A-5m (2.5 x 20 cm) column. The column was run at a flow rate of 1.2 ml/min in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 150 mM NaCl, and 1-mi fractions were collected. A portion (290 µl) of the column fraction was then assayed for deacetylase activity as described under “Materials and Methods.”

ARBP the histone deacetylase is a specific component of the internal nuclear matrix.

Distribution of Histone Deacetylase Activity among the Salt-soluble Poly- and Mononucleosomes—Fig. 6 shows the elution of enzyme activity versus the elution of the 0.15 M NaCl-soluble chromatin fragments from a Bio-Gel A-5m column. Four peaks of enzyme activity were detected. Peaks 1 and 2 were associated with the poly- and oligonucleosomes, respectively. The third peak of enzyme activity eluted with the mononucleosomes, and the fourth peak eluted from the column in a position consistent with the elution of free enzyme.2 Approximately 28, 15, and 57% of the histone deacetylase activity in fraction S150 was present in the 0.15 M NaCl-soluble poly- and oligonucleosomes (peaks 1 and 2), mononucleosomes (peak 3), and free enzyme (peak 4), respectively. The specific enzyme activity of the oligonucleosomes (fraction 30; 14.7 dpm/µg of protein) was greater than that of the polyribonucleosomes (fraction 18; 12.2 dpm/µg of protein) or mononucleosomes (fraction 40; 10.7 dpm/µg of protein).

DISCUSSION

Chicken immature erythrocytes contain two categories of metabolically active acetylated histones. Both groups of histones are acetylated at the same rapid rate, but they differ in both the extent of acetylation and the rate of deacetylation. We provide evidence that the dynamically, class 1 acetylated histones, which attain high acetylation levels and are rapidly deacetylated, are complexed principally to active DNA. Parallel chromatin distributions of the active DNA sequences and labeled tetraacetylated histone H4 species were observed, with the 0.15 M NaCl-soluble oligonucleosomes and the chromatin fragments bound to the insoluble nuclear material containing the majority of the active DNA and labeled tetraacetylated histone H4. The strong bias of the dynamically acetylated class 1 histones to active gene-enriched chromatin regions was not apparent with the class 2 acetylated histones. These metabolically active acetylated histones, which are acetylated to low levels and are slowly deacetylated, were located in repressed, competent and active chromatin regions. However, it is important to note that the class 2 acetylated histones were more abundant in active and competent gene-enriched chromatin fractions. The enrichment of the class 2 acetylated histones in the 0.15 M NaCl-soluble poly- and oligonucleosomes was 8.0-fold greater than the 0.15 M NaCl-insoluble, repressed gene chromatin fraction. This implies that the majority of the histones associated with the 0.15 M NaCl-insoluble chromatin fragments do not participate in metabolically active acetylation, and their presence in the acetylation levels observed on Coomassie Blue-stained AUT gels.

The spectrum of labeled acetylated histones of the active gene-enriched chromatin fractions, the 0.15 M NaCl-soluble oligonucleosomes, and the insoluble chromatin associated with the residual nuclear material were similar, but some differences were noted. Labeled histones H2A, H2AZ, H3.2, H3.3, H2B, and H4 were present in both chromatin fractions. Labeled ubiquitinated H2B was found only in the salt-soluble oligonucleosomes. Although the 0.15 M NaCl-soluble polyribonucleosomes contained H2AZ, it was labeled to a much lower specific activity than the H2AZ of the 0.15 M NaCl-soluble oligonucleosomes. It is of interest to note that although the amount of histone H3.2 is considerably lower than that of histone H3.3, the level of labeled histone H3.3 was similar to that of H3.2. Waterborg (1990) reported a similar observation that the alfalfa minor histone H3 variant H3.2 was also preferentially acetylated.

The observation that rapid acetyl group turnover occurs primarily with histones of active gene chromatin (Boffa et al., 1990; Ip et al., 1988; Zhang and Nelson, 1988a, 1988b; this study) predicts that the modifying enzymes, histone acetyltransferase and histone deacetylase, would also be localized in the active gene chromatin domains. Chan et al. (1988) demonstrated that in chicken erythrocytes the histone acetyltransferase was associated with the nuclease-sensitive regions of the active gene-enriched, 0.15 M NaCl-soluble poly/ oligonucleosomes. Our results show that the histone deacetylase was also found in the active gene-enriched oligonucleosomes as well as active gene-enriched insoluble chromatin fragments associated with the residual nuclear material. These observations are in agreement with other reports showing that the deacetylase of HeLa cells was located in a high molecular weight fraction with some of the characteristics of the nuclear scaffold (Hay and Candido, 1983a, 1983b) and with a subpopulation of mononucleosomes (Mold and McCarty, 1987).

The nuclear matrix is operationally defined as the residual nuclear structure that remains after the high salt extraction of nuclease-digested nuclei. It may represent an underlying structural framework that is the putative site of many nuclear metabolic processes (Cook, 1988; Verheijen et al., 1988). The histone deacetylase is a component of this internal nuclear matrix. The following observations support this conclusion: the enzyme activity was associated with the low salt-insoluble residual nuclear material after a micrococcal nuclease digest, the high salt nuclear matrix, and the intermediate salt/high salt nuclear matrix. We found that more than 40% of the histone deacetylase activity was located in the intermediate salt/high salt nuclear matrix. This result is in marked contrast to the < 10% of the RNA polymerase I and II which remained associated with the intermediate salt/high salt nuclear matrix (Roberge et al., 1988) and argues that the histone deacetylase is associated with such a nuclear structure. Histone deacetylase was solubilized by extracting the nuclear matrices with high salt and 2-mercaptoethanol, a procedure that generates empty shells of nuclear pore-lamina complexes. This result demonstrates that as with the matrix/scaffold attachment region-binding protein (von Kries et al., 1991), histone deacetylase is a component of the internal nuclear matrix.

The dual locations of the histone deacetylase with active gene chromatin regions and the internal nuclear matrix raises

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2 M. J. Hendzel and J. R. Davie, unpublished results.
intriguing questions as to the enzyme’s function. In addition to its enzymatic role in modulating the levels of acetylated histones complexed to transcriptionally active DNA, the histone deacetylase may also have a structural role similar to that of topoisomerase II (Adachi et al., 1991). It is conceivable that histone deacetylase complexed to transcriptionally active chromatin may mediate the dynamic interaction of active gene chromatin with the internal nuclear matrix.

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