Histone Acetylation Alters the Capacity of the H1 Histones to Condense Transcriptionally Active/Competent Chromatin*

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The relationship between histone acetylation and the capacity of H1 histones to cause the 0.15 M NaCl-induced aggregation/precipitation of transcriptionally active/competent gene chromatin fragments was investigated. Previous studies have shown that transcriptionally active/competent, but not repressed, gene chromatin polynucleosomes, which were isolated from chicken erythrocytes, remained soluble in 0.15 M NaCl after being reconstituted with H1 histones. This result suggested that some component of the active/competent gene nucleosome altered the capacity of the H1 histones to condense the chromatin fiber. Recently, Hebbes et al. (Hebbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988) EMBO J. 7, 1395-1402) demonstrated directly that active, but not repressed, gene chromatin of chicken erythrocytoid cells contains high levels of acetylated histones. Here, we show that the solubility of active/competent gene chromatin fragments in 0.15 M NaCl is dependent on the level of acetylated histone species, with induction of hyperacetylation increasing the solubility of this gene chromatin. Also, we show that lowering the levels of the acetylated histone forms reduces the ability of the active/competent gene chromatin fragments to resist exogenously added H1-histone-induced 0.15 M NaCl aggregation/precipitation. These results suggest that histone acetylation alters the capacity of the H1 histones to form compact higher order chromatin structures such that active/competent gene chromatin is maintained in a less folded state than the bulk of chromatin.

The H1 (or linker) histones, which play a key role in the folding of chromatin, are general repressors of gene expression (Weintraub, 1985). Mature chicken erythrocyte nuclei contain linker histones H1 and H5, an extreme variant of H1, in a stoichiometric ratio of 0.4 of H1 and 0.9 of H5 per nucleosome (Bates and Thomas, 1981). At the final stages of erythropoiesis, histone H5 accumulates in erythroid nuclei. Although there is more than enough H1 histones to cover the entire genome of immature cells, this high level of linker histones does not prevent the transcription of erythroid-specific histone H5 and β-globin genes (Affolter et al., 1987).

Transcriptionally active/competent chromatin is thought to be in an unfolded conformation (as reflected, for example, by an increased sensitivity to DNase I). Most active gene-enriched chromatin fractions contain increased levels of modified and variant histone species. Nucleosomes containing such histones may vary somewhat in structure from the bulk and thereby affect nucleosome-dependent transitions to higher order structures. Differences in nucleosomal structure that result in differences in higher-order structure may be of primary importance in determining the role of chromatin structure in the regulation of transcription.

One of the most universally observed features of active gene-enriched chromatin is the presence of hyperacetylated nucleosomal histones (Reeves, 1984; Vidali et al., 1988; Loidl, 1988). Hyperacetylation of the amino-terminal region of the nucleosomal histones (H2A, H2B, H3, and H4) may be linked to the chromatin solubility in the presence of Mg2+ and destabilization of chromatin higher order structures (Perry and Chalkley, 1981, 1982, Allau et al., 1982, Amunzio et al., 1988). Although not all data make a clear distinction, acetylation also seems to affect the structure of the nucleosome (a good discussion of this point is in Oliva et al., 1987). Numerous reports have suggested that histone acetylation functions in the maintenance or control of the transcriptional and/or replicative capacity of chromatin regions (see Loidl, 1988, and Vidali et al., 1988 for discussions).

In immature chicken erythrocytes, approximately 3.7% of modifiable histone lysine sites are undergoing acetylation and deacetylation (Zhang and Nelson, 1986, 1988, a and b). These sites are rapidly acetylated (half-life of 12 min) and deacetylated (Zhang and Nelson, 1986, a and b). The histones undergoing dynamic acetylation are associated with active/competent DNA (Nelson et al., 1986; Alonso et al., 1987; Zhang and Nelson, 1988a). Moreover, Hebbes et al. (1988) have demonstrated directly that active, but not repressed, genes of chicken erythroid cells are associated with significant amounts of acetylated histones.

We have demonstrated that the 0.15 M NaCl-soluble polynucleosomes from mature or immature chicken erythrocyte nuclei are highly enriched in transcriptionally active (e.g. histone Hb and β-globin) and competent (e.g. ε-globin) genes. These salt-soluble polynucleosomes are enriched in acetylated species of histones H2B, H2A.Z, and H4, poly- and monoubiquitinated species of H2A and H2B, and histone variants H3.5 and H2A.Z. Moreover, these active/competent gene-enriched chromatin fragments are complexed with linker histones H1 and H5 (Ridsdale and Davie, 1987; Nickel et al., 1986; Delcuve and Davie, 1989). Reconstitution experiments revealed that active/competent gene-enriched chromatin fragments are much more resistant than repressed gene chromatin fragments to exogenously added linker-histone-induced precipitation in 0.15 M NaCl (Ridsdale et al., 1988). These observations suggest that some feature of the active/competent gene chromatin fragments prevents the linker histones from folding the fiber into a compact higher order structure.
In this report, we investigated whether histone acetylation influences the capacity of H1 histones to aggregate/precipitate chromatin fragments in 0.15 M NaCl. Our results show that the degree of solubility of the active/competent gene chromatin fragments in 0.15 M NaCl is correlated with the level of acetylated histone species. Furthermore, the results suggest that the level of histone acetylation is the major determinant of the resistance of active/competent gene chromatin fragments to H1/H5-induced salt precipitation. These results suggest that histone acetylation alters the capacity of linker histones to form higher order chromatin structures such that transcriptionally active/competent gene chromatin is maintained in a less folded state than the bulk of chromatin.

MATERIALS AND METHODS

Isolation and Digestion of Nuclei with Micrococcal Nuclease—Ane mia was induced in adult white Leghorn hens by phenylhydrazine (acetyl cell injections) (Ferenz and Nelson, 1985). Blood was collected and washed with the buffy coat in ice cold 75 mM NaCl, 25 mM EDTA. For the incubation in the presence or absence of sodium butyrate, red blood cells were divided into two portions and washed once with NaCl, 1.87 medium, and then either with sodium butyrate added to 10 mM. The cells were incubated with gentle agitation for 60 min at 37 °C in the same media at a density of approximately 25 ml of cells (volume when packed by low speed centrifugation) in 500-ml final volume. After the incubation, the cells were collected by centrifugation, frozen, and stored at -70 °C.

Nuclei were isolated and digested with micrococcal nuclease as previously described, with all digestions being for 30 min unless otherwise specified (Ridsdale and Davie, 1987). The wash and digestion buffers for butyrate-treated cells contained 30 mM sodium butyrate.

Fractionation of Chromatin Fragments—Immature erythrocyte chromatin was fractionated as described (Delcuve and Davie, 1987). Briefly, digested nuclei were resuspended in 10 mM EDTA, pH 7.2, 1 mM phenylmethylsulfonyl fluoride. The soluble (S1) and insoluble (P1) fractions were collected. NaCl was added to fraction S2 to a final concentration of 0.15 mM NaCl and insoluble (P2) and soluble (S2) fractions were obtained. To size-resolve the chromatin fragments of fraction S30, this fraction was applied onto a Bio-Gel A-5m column. The DNA content in each chromatin fraction was determined either by diphenylamine assay (Giles and Myers, 1965) or measuring the absorbance at 260 nm.

Electrophoretic Analysis of Proteins—Proteins of lyophilized chromatin samples were analyzed by AUT-15% polyacrylamide gels, sodium dodecyl sulfate-15% polyacrylamide gels, and two-dimensional electrophoresis (AUT into sodium dodecyl sulfate) as described by Nickel et al. (1997).

Preparation and Reconstitution of Stripped Chromatin Fragments with Linker Histones—Dry CM-Sephadex (30 mg/ml) was added to the EDTA-released chromatin fragments, and then NaCl to 0.25 M from a 4 M stock (with stirring). The suspension was stirred at 4 °C for 1 h. The chromatin fragments stripped of the linker histones were collected by filtration. The CM-Sephadex was first washed with 0.6 M NaCl and then 1.0 M NaCl. The second wash contained the H1 and H5 histones, in proportions equivalent to what is found in nuclei, and which were essentially free from other proteins. The linker histone preparation was dialyzed against water, lyophilized, and stored as a concentrated solution at -20 °C. The stripped chromatin was dialyzed against 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, stored at 4 °C and used within a few days.

Stripped chromatin was reconstituted with varying quantities of the mixed H1 and H5 preparation as described (Ridsdale et al., 1988). Both chromatin and histones were dialyzed against 10 mM Tris-Cl, pH 8.0, or our Copos 5 acetate buffer, and then dialyzed against varying concentrations, were added to a constant amount of chromatin, and dialysis was continued for 2 h, then for 1 h against 0.25 M NaCl, 1 mM EDTA and then against 1 mM EDTA overnight, both buffered with 10 mM Tris-Cl, pH 8.0. The reconstituted material was fractionated by the addition of NaCl to 0.15 M as described above.

Electrophoresis and Blotting of DNA—For Southern blot analysis, DNA samples were purified as described (Ridsdale and Davie, 1987). Agarose gel electrophoresis and transfer of DNA to membranes were carried out by standard procedures. For slot blot analysis, purified DNA was made to 0.3 M NaOH and incubated at 65 °C for 30 min, cooled to room temperature, neutralized by the addition of an equal volume of 2 M ammonium acetate, pH 7.0, and applied to nitrocellulose using a Schleicher and Schuell slot blotting manifold.

Hybridization with nick-translated probes was carried out as described by Thomas (1989). Autoradiograms of hybridized slot blots were scanned with a densitometer, and the data were analyzed as described (Ridsdale et al., 1988; Delcuve and Davie, 1989). The intensity of the hybridization signal per µg of DNA was determined for DNA which was isolated from fraction S2 and the chromatin fractions. The values obtained for the DNA of each chromatin fraction were divided by the percent S2 DNA, determining the partitioning ratio of DNA sequences among the different chromatin fractions. If the partitioning ratio was >1, the analyzed DNA sequence was enriched in that chromatin fraction, but if the partitioning ratio was <1, the analyzed DNA sequence was depleted. The percentage of a DNA sequence in a chromatin fraction was obtained by multiplying the partitioning ratio of a sequence by the percentage of S2 DNA in that fraction. For example, the percent gene chromatin soluble for a given S100 fraction = fraction S100 hybridization signal × fraction S2 hybridization signal × percent DNA soluble in fraction S100.

The cloned DNA probes used were: pCBG 18.7, a unique intronic sequence of the embryonic e-globin gene, and pCBG 14.4, a unique intronic sequence of the adult b-globin gene (Villepont et al., 1982) which were acquired from H. Martinson (University of California, San Diego, California). pVIT25B/H from A. Ruiz-Carrillo (Centre de Recherche en Cancerologie, Quebec) is a 2.5-kilobase pair fragment of the histone H5 gene (Ruiz-Carrillo et al., 1983). pVIT, a genomic clone of the vimentin gene (Zehner and Paterson, 1983), was a gift from B. M. Paterson (National Cancer Institute, Bethesda, MD).

RESULTS

Effect of Histone Acetylation on Solubility of Transcriptionally Active/ Competent Gene Chromatin Fragments in 0.15 M NaCl—Immature chicken erythrocytes were incubated in the presence or absence of sodium butyrate, an inhibitor of the histone deacetylase (Candido et al., 1978). In the presence of sodium butyrate, nuclease-soluble histones complexed to active/competent genes become hyperacetylated, while in the absence of sodium butyrate, these histones have reduced levels of acetylated species (Alonso et al., 1987; Zhang and Nelson, 1988a). Chromatin isolated from these cells was fractionated. The distribution of DNA among chromatin fractions P1, S100, and S150 was 25.9 ± 0.4%, 54.4 ± 5.9, 66.3 ± 6.0, and 7.8 ± 0.8 (butyrate; n = 3) and 20.2 ± 2.6, 79.9 ± 2.6, 70.5 ± 3.0, and 9.5 ± 0.1 (±butyrate; n = 3), respectively. Thus, incubation of the cells with or without butyrate did not influence the fractionation of the bulk of the chromatin fragments. The partitioning of active and competent DNA with fraction P2 which is not shown in this report, was not affected by these incubations (Delcuve and Davie, 1989).

Fig. 1 shows the protein content of fractions S2 and S150 isolated from cells incubated in the presence or absence of butyrate. Acetylation levels can be easily judged by examining the different acetylated forms of H4. The levels of acetylated histones do not change dramatically in the S2 fractions which contain the bulk of erythrocyte chromatin. This is consistent with the results of Zhang and Nelson (1986) who show that approximately 1–2% of the chicken erythoid genome is composed of actively acetylated and deacetylated histones. The level of acetylated histones in the salt-soluble chromatin (fraction S150) isolated from butyrate-treated cells is significantly different from those of chromatin fraction S150 obtained from cells incubated in the absence of butyrate. Separation of the proteins of the salt-soluble chromatin fractions by two-dimensional PAGE demonstrates that fraction...
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S150 (+butyrate) has greater amounts of the acetylated species of histones H4, H2B, and H3 than fraction S150 (−butyrate) (see Fig. 1). The amount of ubiquitinated histones in the salt-soluble chromatin fractions is similar.

The chromatin fragments of fraction S150 were size-resolved by gel exclusion chromatography. Fig. 2 demonstrates that incubation of cells in the absence of butyrate results in a major decline in the 260 nm absorbing material in the polyacrylamide gel. Ac indicates acetylated forms of the nucleosomal histones. The ubiquitin adducts of histones H2A and H2B are depicted as uH2A and uH2B, respectively. Note that on SDS gels the ubiquitinated histone species migrate as doublets (Nickel et al., 1989).

The right-hand panel shows a Coomassie Blue-stained AUT PAGE gel pattern containing proteins (10 μg) of chromatin fractions SEDTA and S150 which were isolated from immature erythrocytes incubated in the presence (+) and absence (−) of butyrate. The Aα, Aγ, and Aε indicate the non-, di-, and tetraacetylated forms of histone H4, respectively. The left-hand panels show the same SEDTA fractions further resolved by electrophoresis into a second dimension SDS, 15% polyacrylamide gel. Ac indicates acetylated forms of the nucleosomal histones. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B, respectively. Note that in SDS gels the ubiquitinated histone species migrate as doublets (Nickel et al., 1989).

In this study, we determined whether modulating the level of acetylated histone species would alter the ability of active/competent gene chromatin fragments to added linker histone-induced NaCl precipitability. The salt-soluble, active/competent gene-enriched chromatin fragments, which contained histones H1 and H5, were enriched in acetylated and ubiquitinated histone species. These observations suggested that some component(s) of the active/competent gene nucleosome altered the capacity of the H1 histones to condense the active/competent gene chromatin fiber in 0.15 M NaCl.

The percentage of DNA sequences in the salt-soluble and -insoluble chromatin fractions was quantified (Table I). The percentage of active/competent DNA sequences in fraction S150 (+butyrate) is greater than that in fraction S150 (−butyrate). The enrichment of competent and active DNA in the salt-soluble chromatin fraction decreased 2.0 ± 0.7-fold (n = 4; combined average for ε-globin and vimentin) and 1.0 ± 0.7-fold (n = 3; combined average for β-globin and histone H5), respectively, when cells were incubated in the absence of butyrate.

The partitioning of the repressed DNA (vitellogenin) among the salt-soluble and -insoluble chromatin fractions is not affected by incubation in the presence or absence of butyrate, and repressed DNA is salt-soluble primarily as mononucleosomes in 150 mM NaCl.

In contrast, the salt-soluble chromatin fragments from cells incubated in the absence of butyrate are depleted in competent DNA sequences (approximately 0.6-fold). In addition, there is an increase in the content of competent DNA found in the aggregation-prone, salt-insoluble chromatin fraction P150 as a result of cells being incubated in the absence of butyrate. These observations suggested that some component(s) of the active/competent gene nucleosome altered the capacity of the H1 histones to condense the active/competent gene chromatin fiber in 0.15 M NaCl.

In this study, we determined whether modulating the level of acetylated histone species would alter the ability of active/competent gene chromatin fragments to resist exogenously added linker histone-induced NaCl precipitation. Histone H1/H5-stripped chromatin fragments of mature chicken erythrocytes were reconstituted with varying linker nucleosomal histone ratios (Ridsdale et al., 1988). Active/competent gene chromatin fragments were more resistant than bulk chromatin fragments to added linker histone-induced NaCl precipitability.

The salt-soluble, active/competent gene-enriched chromatin fragments, which contained histones H1 and H5, were enriched in acetylated and ubiquitinated histone species. These observations suggested that some component(s) of the active/competent gene nucleosome altered the capacity of the H1 histones to condense the active/competent gene chromatin fiber in 0.15 M NaCl.
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FIG. 3. Southern blot analysis of DNA from chromatin fractions. Immature chicken erythrocyte nuclei, which were isolated from cells incubated in the presence (+) and absence (−) of butyrate, were digested with micrococcal nuclease for 10 min as described under "Materials and Methods." DNA (10 μg) of the chromatin fractions was electrophoretically resolved on a 1% agarose gel, stained with ethidium bromide (DNA), or transferred onto nitrocellulose and hybridized to 32P-labeled probes as indicated. c-globin is shown as Globin.

TABLE I
The effect of butyrate treatment on the 0.15 M NaCl solubility of various gene chromatin fragments

The salt solubility of gene chromatin fragments isolated from immature chicken erythrocytes incubated in the presence (+Bu) or absence (−Bu) of sodium butyrate was determined. The percentage of DNA in the EDTA-released chromatin fragments (SE) that is soluble in 0.15 M NaCl was determined by absorbance at 260 nm, and the percentage of each sequence in S150 that fractionates into Ss was determined by hybridization of slot blots and densitometric analysis of the autoradiograms (see "Materials and Methods").

| Fraction | % DNA | % H5 | % c-globin | % vimentin | % vitellogenin |
|----------|-------|------|------------|------------|---------------|
| S150 (+Bu) | 11.4  | 65   | 41         | 42         | 2             |
| S150 (−Bu) | 9.7   | 25   | 6          | 7          | 2             |
| Ratio (+/−) | 1.2   | 2.6  | 6.8        | 6.0        | 1.0           |

density = 1.2), more polynucleosomes of +butyrate-treated cells remain soluble in 0.15 M NaCl than those of −butyrate-treated cells.

The histones, which were isolated from the salt-soluble chromatin fragments, were electrophoretically resolved on AUT 15% polyacrylamide gels. Fig. 5 shows that as the amount of linker histones added increases to native levels (linker histone density = 1), there is an increase in the content of hyperacetylated histone H4 and histone H2B species associated with the salt-soluble chromatin fragments of +butyrate-treated cells. This increase in the level of acetylated histone species is not observed for salt-soluble chromatin fragments of −butyrate-treated cells. Note that the salt-soluble chromatin fractions contain linker histones H1 and H5. We have previously demonstrated that these linker histones are associated with the salt-soluble polynucleosomes (Ridsdale et al., 1988).

Fig. 6 shows the solubility of reconstituted chromatin as a function of the amount of linker histone added. Butyrate incubation does not significantly alter the solubility of bulk chromatin at any given amount of added linker histone. The sigmoidal shape of the curve defined by this relation suggests a cooperative interaction among linker histones in giving rise to salt-precipitable chromatin structures.

Fig. 7 shows a quantitative assessment of the amount of competent c-globin and repressed vitellogenin gene chromatin soluble in 0.15 M NaCl as a function of the amount of linker histone added. The same relationship is shown for total chromatin. Note that the total chromatin and vitellogenin curves are distinctly sigmoidal in shape. The c-globin gene chromatin fragments isolated from cells incubated in the presence or absence of butyrate show markedly different degrees of solubility as a function of linker histone density. c-Globin gene chromatin fragments from butyrate-incubated cells remain completely soluble until the amount of linker histones added is equivalent to the levels of these histones found in native chromatin fragments. Further increases in the amount of linker histones reconstituted onto the chromatin fragments result in a decline in the solubility of the globin gene chromatin fragments. However, c-globin chromatin fragments, which were isolated from cells incubated in

FIG. 4. Size distribution of DNA fragments isolated from salt-soluble reconstituted chromatin. Chromatin fragments isolated from cells incubated in the presence (+) or absence (−) of butyrate were used for reconstitution with linker histones. Linker histone was added to 0 or 1.2 times the levels of fraction S0. The percentage of H1 histone-reconstituted chromatin fragments that were soluble in 0.15 M NaCl was 8.4 and 6.3% for + and −butyrate, respectively. DNA from 0.15 M NaCl-soluble, linker histone-reconstituted chromatin fragments was electrophoresed on 1% agarose gels.
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FIG. 5. Proteins associated with the 0.15 M NaCl-soluble fractions of H1/H5-reconstituted chromatin fragments. H1/H5-stripped chromatin fragments of fraction SE, which were isolated from cells incubated in the presence (+) and absence (−) of butyrate, were reconstituted with linker histones at various stoichiometric ratios. Proteins (10 μg) of these fractions were analyzed by AUT PAGE, and Coomassie Blue-stained gel is shown. Panels A and B show gel patterns obtained in two separate reconstitution experiments. A, A, and A, indicate the non-, di-, and tetra-acetylated forms of H4, respectively. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B, respectively. In the last four lanes of panel A, histone H5 migrates partially with H2B.

FIG. 6. The solubility of chromatin fragments as a function of linker histone density. Chromatin fragments of fraction SE (SEDTA) were isolated from cells incubated in the presence (□) or absence (△) of butyrate. The linker histone-stripped chromatin fragments were reconstituted with linker histones at various stoichiometric ratios. The percentage of chromatin soluble in 0.15 M NaCl at any given amount of H1 histones is shown as the mean value of several determinations. As these values are similar for both + and −butyrate-treated cells, combined means are also shown (△). Standard deviations (error bars) are indicated. The number of different experiments (+ and −butyrate) for each linker density are: 0, n = 3; 0.2, n = 4; 0.4, n = 4; 0.5, n = 10; 0.7, n = 10; 0.8, n = 11; 0.9, n = 13; 1.0, n = 10; 1.1, n = 4.

the absence of butyrate, lost most of their ability to resist linker histone-induced NaCl precipitation. Thus, when about 11% of the bulk chromatin fragments are soluble in 0.15 M NaCl, -globin gene chromatin fragments from cells incubated in the presence or absence of butyrate are 100% and 18% soluble, respectively. This represents a 5.6-fold difference in the solubility of competent gene chromatin fragments. Active gene chromatin fragments, which are considerably shorter than competent gene chromatin fragments (Delcuve and Davie, 1989), also had this difference in salt solubility, with the ratio of histone H5 gene chromatin solubility (+butyrate:−butyrate) at a nominal 1-fold linker histone density being 2.1 ± 0.2 (n = 4). (The solubility of bulk chromatin fragments at this linker histone density was 11.3 ± 1.5 for chromatin isolated from butyrate-treated cells and 11.0 ± 1.0 for chromatin isolated from cells incubated in the absence of butyrate (n = 4).) Within each experiment with reconstituted chromatin (six separate experiments), active/competent gene chromatin was always more enriched in the NaCl-soluble chromatin fragments from cells incubated in the presence of butyrate than those from cells incubated in its absence.

FIG. 7. The solubility of gene chromatin fragments as a function of linker histone density and butyrate incubation. Chromatin fragments of fraction SE (SEDTA) were isolated from cells incubated in the presence (filled symbols with +) and absence (empty symbols with −) of butyrate. DNA prepared from the 0.15 M NaCl-soluble fraction of chromatin reconstituted with varying quantities of linker histone was applied to nitrocellulose by slot blotting and hybridized against labeled gene probes for -globin (GLOBIN) and vitellogenin (VITELW). Hybridization was quantified by densitometric scanning of the autoradiograms as described under “Materials and Methods.” The results are expressed as the percentage of gene chromatin fragments in fraction SE that are soluble in 0.15 M NaCl.

DISCUSSION

Linker histones are known to act in the salt-dependent formation of higher order chromatin structures which are observable by electron microscopy (Thoma et al., 1979). The formation of aggregation-prone, NaCl-precipitable structures shows a similar histone dependency (this study; Allan et al., 1981). The results of Widom’s (1986) study indicate that aggregation is a built-in property of the 30 nm fiber. Thus,
the aggregation/precipitation property of a chromatin fragment in 0.15 M NaCl in vitro may correlate with the capacity of the chromatin fiber to form higher order structures in vitro.

The results of this study provide evidence that histone acetylation has a role in altering the capacity of the linker histones to fold the chromatin fiber. The bulk of erythrocyte chromatin fragments aggregate and precipitate in 0.15 M NaCl. In contrast, active/competent gene-enriched chromatin fragments, which are associated with linker histones, do not precipitate in 0.15 M NaCl and are in an unfolded conformation (Ridsdale et al., 1988). Elevated levels of hyperacetylated nucleosomal histones increase the 0.15 M NaCl solubility of active/competent gene chromatin fragments, while lowered levels of acetylated histones decrease the 0.15 M NaCl solubility of these fragments. Reconstitution experiments with linker histones demonstrate that lowered levels of the acetylated histones reduce the ability of active/competent gene chromatin fragments to resist linker histone-induced salt precipitation. These observations suggest that the H1 histones associated with highly acetylated nucleosomes are not able to condense the active/competent gene chromatin fiber.

Nelson and colleagues have shown that altering the level of acetylated histones has a profound effect on the solubility of active/competent gene chromatin fragments in buffers containing 3 mM MgCl₂, with increased levels of acetylated histones resulting in enhanced solubility (Alonso et al., 1987). It should be noted that H1/H5-stripped chromatin fragments are insoluble in 3 mM Mg²⁺, but they are soluble in 0.15 M NaCl (Ausio et al., 1986). To be precipitated in 0.15 M NaCl, chromatin fragments must be complexed with H1 histones.

Removal of the basic amino terminal “tails” of the nucleosomal histones, which contain the sites of acetylation, does not prevent the H1 histones from binding to the chromatin fiber (Allan et al., 1982). But removal of the tails does interfere with the capacity of the linker histones to condense the chromatin fiber (Allan et al., 1982). Thus, acetylation of lysyl residues located within the amino-terminal basic domains of the nucleosomal histones may have the same effect on altering H1 histone action as does the removal of this basic domain.

The EDTA-soluble competent gene chromatin fragments isolated from micrococcal nuclease-digested immature erythrocyte nuclei are considerably longer than the active gene chromatin (Allan et al., 1982). Thus, acetylation of lysyl residues may alter the capacity of linker histones to fold the chromatin fiber (Allan et al., 1982). The EDTA-soluble competent gene chromatin fragments isolated from micrococcal nuclease-digested immature erythrocyte nuclei are considerably longer than the active gene chromatin (Allan et al., 1982). This disparity in chromatin fragment sizes may account for the more noticeable transition of competent (6-fold) versus active (2-3-fold) gene chromatin to an aggregation-prone state (native or linker histone reconstituted) as a consequence of deacetylation. In studies of exchange of linker histones between chromatin fragments, Thomas and Rees (1983) demonstrated that at an ionic strength of 0.75 M, histone H5 of short chromatin fragments preferred to associate with long chromatin fragments that had formed higher order structures. Short active gene chromatin fragments may be more susceptible than long competent gene chromatin fragments to losing their linker histones. The loss of linker histones and the reduced ability to form higher order structures would tend to decrease the salt-induced aggregation and precipitation of active gene chromatin fragments.

Recently, Norton et al. (1989) reported that histone acetylation reduces the amount of negative DNA supercoils constrained by the nucleosome. Removal of the nucleosomal histone tails also leads to the loss of DNA normally constrained by the nucleosome (Allan et al., 1982). Conversely, Morse and Cantor (1985, 1986) demonstrated that plasmid DNA reconstituted with acid-extracted histones was constrained from thermal untwisting, and that trypsin treatment of the reconstituted chromatin did not diminish this constraint. However, yeast minichromosomes, which contain highly acetylated histones (Davie et al., 1981), do allow thermal untwisting of the DNA (Morse et al., 1987). The liberation of DNA from the nucleosome and/or change in nucleosome shape as a consequence of acetylation may alter the path of the DNA entering and leaving the nucleosome which in turn may alter the interaction between H1 histones and nucleosomal/linker DNA (Allan et al., 1980). These alterations in DNA path and linker histone-nucleosome interaction may prevent (or alter) the formation of compact higher order structures.

In conclusion, our observations suggest that, in immature chicken erythrocytes, dynamic acetylation of nucleosomal histones complexed with transcriptionally active/competent DNA prevents histone H1 and H5 interactions that allow for normal condensation of the chromatin fiber. Chan et al. (1988) presented evidence indicating that histone acetyltransferase is preferentially associated with the active/competent gene chromatin domains. This would ensure that the transcriptionally active gene chromatin is maintained in a less folded state than the bulk of chromatin.

Acknowledgments—We wish to thank Darcy Salo and Jon Olsen for excellent technical assistance.

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J. Biol. Chem. 1990, 265:5150-5156.

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