Dynamically Acetylated Histone Association with Transcriptionally Active and Competent Genes in the Avian Adult β-Globin Gene Domain*

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In chicken immature erythrocytes, class 1 acetylated histones are rapidly tri- and tetra-acetylated and rapidly deacetylated. Class 2 acetylated H3 and H4 are rapidly acetylated to mono- and di-acetylated isoforms and slowly deacetylated. Our previous studies suggested that class 1 acetylated histones were primarily associated with transcriptionally active DNA (β-globin) but not competent DNA (β-globin). Chromatin salt solubility (chromatin fiber oligomerization) is directly influenced by hyperacetylation. In this study we investigated the association of class 1 histones with β-globin DNA by measuring their loss of solubility rates in 150 mM NaCl and 3 mM MgCl2 as a function of hyperacetylated histone deacetylation. Expressed and competent chromatin was associated with class 1 acetylated histones. As most active chromatin and hyperacetylated histones are associated with the low salt-insoluble residual nuclear material containing the nuclear matrix, we investigated whether hyperacetylated histones are bound to the β-globin DNA in this fraction. In chromatin immunoprecipitation assays, we found that the β-globin and e-globin coding regions are bound to hyperacetylated H3 and H4. Our observations are consistent with a model in which nuclear matrix-associated histone acetyltransferases and deacetylases mediate a dynamic attachment between active and competent chromatin and the nuclear matrix.

Histone acetylation is a dynamic process catalyzed by histone acetyltransferases and histone deacetylases. Transcriptionally active chromatin is thought to be associated with histones that are rapidly acetylated and deacetylated, whereas histones situated along transcriptionally inactive DNA are either unacetylated or statically mono- or di-acetylated (1). In chicken immature erythrocytes, 4% of the modifiable lysine residues located within the N-terminal tails of core histones become dynamically acetylated and deacetylated (2). The core histones within these cells display a similar rate of acetylation (t1/2 = 12 min for mono-acetylated H4) (3). However, these histones can be divided into two classes based on the extent of dynamic acetylation along their N-terminal tails and the rate at which the N-terminal acetylated lysine residues become deacetylated. Class 1 acetylated histones become tri- or tetra-acetylated when exposed to sodium butyrate, a histone deacetylase inhibitor. When the inhibitor is removed, these hyperacetylated histones are rapidly deacetylated (t1/2 = 5 min for tetra-acetylated H4) (4, 5). Class 2 acetylated H3 and H4 histones become mono- or di-acetylated in the presence of sodium butyrate at the same rate as class 1 histones and then are slowly deacetylated (t1/2 = 90 min for H4 when mono-acetylated) once this inhibitor is removed.

Chromatin fractionation studies have shown that chicken immature erythrocyte chromatin fragments soluble in 3 mM MgCl2 or 0.15 mM NaCl are enriched in transcriptionally active DNA sequences and class 1, dynamically hyperacetylated histones (5, 6). In reconstitution experiments, chromatin fragments containing transcriptionally active/competent DNA sequences are more resistant to 0.15 mM NaCl precipitation caused by the addition of exogenously added H1 histones (7). Transcriptionally competent chromatin is sensitive to DNase I digestion but transcriptionally silent. Further, the degree of salt solubility of the chromatin fragments containing the transcriptionally active/competent DNA sequences in 150 mM NaCl correlates with the level of histone hyperacetylation (7). In fact, the level of histone acetylation was shown to be the primary determinant for the resistance of transcriptionally active/competent DNA fragments to H1-induced salt precipitation. In support of these findings, the treatment of mouse fibroblast cells with trichostatin A, a histone deacetylase inhibitor, induces histone hyperacetylation and increases the rate of exchange of a mobile fraction of H1 (8). Histone acetylation also has a profound effect on higher order compaction of chromatin. Acetylating core histones past a threshold level of 12 acetates/octamer disrupts higher order folding and oligomerization of chromatin fibers (9). Thus, in addition to interfering with chromatin fiber-fiber interactions (9, 10), histone acetylation enhances the 0.15 mM NaCl solubility of chromatin fragments by altering H1-mediated condensation of transcriptionally active/competent DNA.

In addition to being salt-soluble, transcriptionally active/competent DNA fractionates with the insoluble nuclear material that remains following low ionic extraction of chromatin fragments from micrococcal nuclease-digested nuclei (11). Approximately 76% of the transcriptionally active histone H5 and β-globin DNA sequences and 30.5% of the transcriptionally competent e-globin DNA sequences are located with the low salt-insoluble nuclear material, which includes chromatin fragments associated with the nuclear matrix of chicken immature erythrocytes (5). The low salt-insoluble nuclear material of butyrate-treated immature erythrocytes contains 74% of class 1, tetra-acetylated H4 and 26.5% of class 2, mono- and di-
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acetylated H4 along with 75–80% of the nuclear histone deacetylase and acetyltransferase activities (12). The co-enrichment of transcriptionally active DNA sequences and class 1 tetra-acetylated H4 in the low salt-insoluble nuclear material suggests that the histones associated with transcriptionally active DNA sequences bound to the nuclear matrix are class 1 dynamically and rapidly acetylated and deacetylated.

Crane-Robinson and co-workers mapped the distribution of proteins containing acetylated lysine residues along the entire β-globin chromatin domain and in regions adjacent to this domain (1). Their study showed that the core histones situated along the β-globin chromatin domain are acetylated, whereas the histones located in the DNase I-insensitive regions outside the domain are hypoacetylated. However, this study analyzed only the steady state levels of acetylated core histones along β-globin domain DNA sequences in soluble chromatin fragments; low salt-insoluble chromatin fragments, which contain most of the dynamically acetylated histones and transcriptionally active β-globin DNA sequences, were excluded from analysis. Further, the antibody used to map the distribution of acetylated histones recognized acetylated histone and acetylated non-histone chromosomal proteins (13).

Whether the dynamics of histone acetylation varies between transcriptionally active and competent DNA sequences within the β-globin domain remains to be determined. In addition, little is known about the distribution of acetylated histones along the sections of the β-globin domain that are associated with the nuclear matrix. In this study we determined whether class 1, dynamically acetylated histones are associated with the transcriptionally active adult β-globin and transcriptionally competent e-globin DNA sequences of salt-soluble chromatin fragments (chromatin fibers unable to oligomerize at the ionic conditions tested) and low salt-insoluble chromatin fragments.

MATERIALS AND METHODS

Isolation and Treatment of Immature Chicken Erythrocytes—Immature erythrocytes were isolated from anemic, young adult White Leghorn chickens as previously described (7). Immature erythrocytes were collected in an ice-cold buffer containing 75 mM NaCl, 25 mM EDTA, and 25 mM Tris-HCl (pH 7.5). Cells were resuspended in an equal volume of Swims S-77 medium (Sigma) and then incubated in the presence or absence of 10 mM sodium butyrate for 60 min at 37°C. The erythrocytes were then washed three times in ice-cold Swims’s media, resuspended in fresh Swims media prewarmed to 37°C, and incubated for 0, 5, 10, 15, and 30 min at 37°C. Following treatment, the erythrocytes were immediately resuspended in ice-cold Swims’s media, collected by centrifugation, and stored at −80°C. Three different preparations were analyzed in this study.

Fractionation of Erythrocyte Chromatin Fractionation—Chromatin fragments soluble in 150 mM NaCl because of their inability to oligomerize were isolated from chicken immature erythrocytes as previously described (7). All buffers contained 1 mM phenylmethylsulfonyl fluoride (PMSF). In brief, nuclei from immature erythrocytes were suspended to 70 A260 units/ml in a digestion buffer (0.25 mM sucrose, 60 mM KCl, 15 mM NaCl, 10 mM sodium butyrate, 15 mM Pipes, pH 6.6) containing 3 mM MgCl2 and 1 mM CaCl2. The nuclei were then digested with 1 unit of micrococcal nuclease/50 µl of total DNA for 5 min at 37°C and centrifuged at 9000 × g for 10 min at 4°C. The addition of EGTA to the supernatant terminated the reaction. The supernatant containing the salt-soluble chromatin fragments was isolated.

DNA Preparation and Hybridization—DNA from S150, MgCl2-soluble and MgCl2-insoluble chromatin fractions was extracted with an equal volume of phenol/chloroform/isomyl alcohol (25:24:1). The resulting DNA fragments were precipitated with sodium acetate and ethanol and suspended in 10 mM EDTA buffer (pH 8), quantified by UV spectrophotometry, and then either slot blotted using a Schleicher and Schuell slot blotting manifold or Southern blotted onto Hybond N+-charged nylon membrane as previously described (11). For the slot blot analysis, an amount of DNA was applied to each slot such that the relationship between the signal and the amount of DNA slotted was linear. Thus, the signal intensity from each slot was directly proportional to the amount of hybridizable DNA sequence. The slot or Southern blot was then hybridized overnight at 42°C to 6 × 106 cpm of 32P-labeled DNA with a specific activity of ~1 × 109 cpm/µg DNA. Following hybridization, the slot or Southern blot was washed to remove nonspecifically bound probe. In the Slot blot analysis, the amount of probe hybridized to each slot was quantified by a phosphorimager (Bio-Rad, CA). DNA probes recognizing the β-globin, e-globin and vitellogenin gene regions were used (11). The β- and e-globin DNA sequences of salt-soluble chromatin and the second intronic sequence of the β3- and e-globin genes, respectively. Both probes were 500 base pairs in length, with the β3- and e-globin introns being ~800 and 600 base pairs, respectively, from the transcription start site (14, 15). The vitellogenin DNA probe is 3.6 kilo base pairs in length and recognizes the 5′ region of the vitellogenin gene.

Protein Electrophoresis and Western Blotting—Histones were isolated from nuclei and chromatin preparations by extraction with 0.4 N H2SO4 as previously described (5). Protein concentrations were determined using the Bio-Rad protein microassay. Acid-Urea-Triton 15% polyacrylamide gel electrophoresis and transfer of the proteins to nitrocellulose were performed as previously described (11). Acetylated isoforms of H3 and H4 were detected by immunostaining the membrane with polyclonal antibodies to di-acetylated H3 and penta-acetylated H4 (Upstate Biotech).

Chromatin Immunoprecipitation (ChIP) Assay—PE was isolated as previously described (7) with the exception that the nuclei were digested with 15 units of micrococcal nuclease/ml of total DNA for 10 min at 37°C. The PE fraction was resuspended in CSK buffer (10 mM Pipes, pH 6.9; 150 mM sodium succinate, 150 mM KCl, 3 mM EDTA, 0.5% (v/v) thiodiglycol) to ~10 A260 units/ml. Formaldehyde was added to the PE suspension to a final concentration of 1% for 10 min on ice, and the cross-linking reaction was quenched by the addition of Tris-HCl (pH 8) to a final concentration of 125 mM. The suspension was dialyzed overnight at 4°C against double-distilled water and 0.5 mM PMSF and then concentrated to ~4–5 ml using PEG 6000–9000 Carbowax. The final concentration was NaCl, Tris-HCl, EDTA, Trxon-100, and SDS to 250 mM, 25 mM (pH 7.5), 5 mM, 1%, and 0.1%, respectively (SB250 buffer). The DNA within the suspension was reduced to 500 base pair fragments by sonication on ice for a total time of 4 min at 30% output (Sonifier Cell Disruptor 350, Branson Sonic Power Company). The 4-min period of sonication was divided up into 16 15-s pulses with 15-s resting intervals on ice in between each pulse. The sonicated PE suspension was then diluted to ~9 A260 units/ml and centrifuged for 10 min at 9000 × g to remove insoluble material. The resulting suspension was made up to 1 mM PMSF and 50 µg/ml leupeptin. A volume of 2.5 µl of antibody to di-acetylated H3 or penta-acetylated H4 was added to 500 µl of the suspension, and the mixture was incubated overnight at 4°C. The suspension was then incubated for 3 h at 4°C on an orbital with 20 µl of a 90/50 protein A-Sepharose slurry (Zymed Laboratories Inc., Ontario, Canada) that had been pretreated overnight at 4°C with 0.1 µg/ml of sonicated salmon sperm DNA and 1 mg/ml of bovine serum albumin. To control for nonspecific binding of DNA to protein A-Sepharose, 500 µl of the suspension was incubated for 3 h at 4°C with 20 µl of the 50:50 protein A-Sepharose slurry in the absence of primary antibody. The protein A-Sepharose of both samples was then centrifuged at 2000 × g for 10 min, washed twice with 1 ml of RIPA (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.1% SDS, 0.5% sodium deoxycholate, 1.0% Nonidet P-40), 1 ml of high salt buffer (500 mM NaCl, 1.0% Nonidet P-40, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA), 1 ml of LiCl wash buffer (250 mM LiCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM EDTA, 50 mM Tris-HCl, pH 8.0), and two times with 1 ml of TE buffer (pH 8.0). A volume of 100 µl of TE buffer was then hybridized overnight at 4°C to 6 × 106 cpm of 32P-labeled DNA with a specific activity of ~1 × 109 cpm/µg DNA. Following hybridization, the slot or Southern blot was washed to remove nonspecifically bound probe. In the Slot blot analysis, the amount of probe hybridized to each slot was quantified by a phosphorimager (Bio-Rad, CA). DNA probes recognizing the β-globin, e-globin and vitellogenin gene regions were used (11). The β- and e-globin DNA sequences of salt-soluble chromatin and the second intronic sequence of the β3- and e-globin genes, respectively. Both probes were 500 base pairs in length, with the β3- and e-globin introns being ~800 and 600 base pairs, respectively, from the transcription start site (14, 15). The vitellogenin DNA probe is 3.6 kilo base pairs in length and recognizes the 5′ region of the vitellogenin gene.

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The mono- and di-acetylated H4 isoforms accumulated at 15 min, pronounced as that observed for the tetra-acetylated H4 isoform. Levels of tri-acetylated H4 also decreased at 10 min, although this decrease was not as pronounced as that observed for the tetra-acetylated H4 isoform. Levels of tri-acetylated H3 did not change initially, but at 10 min a decrease in the levels of this acetylated form became apparent. Levels of penta-acetylated H3 dropped further, plateauing at a very low level. Levels of tri-acetylated H3 did not change initially, but at 10 min a decrease in the levels of this acetylated H3 isoform was observed. At 5, 10, and 15 min following butyrate removal, the levels of mono- and di-acetylated H3 decreased but at a much slower rate than the highly acetylated H3 isoforms.

Immunostaining the blots with anti-penta-acetylated H4 antibodies revealed that the levels of tetra-acetylated H4 rapidly declined at 10 min (Fig. 1). These levels declined further over the next 20 min. Similarly, the levels of tri-acetylated H4 also decreased at 10 min, although this decrease was not as pronounced as that observed for the tetra-acetylated H4 isoform. The mono- and di-acetylated H4 isoforms accumulated at 15 and 30 min post-butyrate removal. In summary the immunoblot analyses show that class 1 highly acetylated H3 and H4 isoforms declined to low levels by 10 min following incubation of cells in media lacking butyrate.

**RESULTS**

**Rate of Deacetylation of Hyperacetylated H3 and H4**—Immature erythrocytes were incubated with 10 mM sodium butyrate for 60 min to induce a state of histone hyperacetylation. The erythrocytes were then incubated in the absence of sodium butyrate for 0–30 min to deacetylate the hyperacetylated histones. Histones were extracted from the nuclei of cells collected at various time points following butyrate removal and subjected to acid/urea/Triton X-100 gel electrophoresis and immunoblotting. The resulting membrane was immunostained with antibodies to hyperacetylated H3 or H4 (Fig. 1). Western blot analysis of the total nuclear histone extracts showed a large drop in the levels of penta-acetylated H3 or H4 (Fig. 1). The proteins were transferred to nitrocellulose and immunostained with antibodies to hyperacetylated H3 or H4 (B). 1, 2, 3, 4, and 5 designate the mono-, di-, tri-, tetra-, and penta-acetylated histone isoforms, respectively.

(pH 8.0) was added to the protein A-Sepharose along with 0.5 mg/ml proteinase K, 0.5% SDS, and 100 mM NaCl. The mixture was incubated overnight at 37 °C, and then at 68 °C for 6 h. The mixture was centrifuged at 2200 × g for 30 s, and the supernatant was extracted once with an equal volume of phenol/chloroform/isoamyl (25:24:1). The DNA in the supernatant was precipitated with 20 µg/ml of glycojen carrier, one-tenth the volume of 3 M sodium acetate, pH 5.3, and 3 volumes of absolute ethanol. The DNA was then resuspended in double-distilled water, quantified by fluorometry, slotted on to a Hybond N charged nylon membrane, and hybridized to the previously mentioned β-globin, ε-globin, and vitellogenin gene probes.

**Effect of Histone Deacetylation on Globin Chromatin Fragments Oligomerization in 0.15 M NaCl**—Our previous studies showed that the solubility of active/competent gene chromatin fragments in 0.15 M NaCl is dependent on the level of acetylated histone species (7). Furthermore, histone hyperacylation interferes with the ability of chromatin fibers to form high molecular weight oligomers (9). Because the inability of chromatin fragments to oligomerize in 0.15 M NaCl is dependent upon histone acetylation status, the rate of deacetylation of histones associated with transcriptionally active and competent chromatin fragments can be determined by studying their rate of loss of 0.15 M NaCl solubility and gain of ability to oligomerize.

Soluble chromatin fragments (fraction S.E.) were isolated from nuclei of cells incubated for various times (0, 5, 10, 15, and 30 min) following the removal of butyrate. Typically 60% of the A_{260} absorbing material was released into this fraction. The SE chromatin fraction was made 0.15 M in NaCl, and the salt-soluble chromatin fragments (fraction S150) were isolated. The DNA fragments were analyzed by slot blot hybridization with probes to the β-globin, ε-globin, and vitellogenin DNA sequences. Fig. 2 shows that incubation of cells in the absence of butyrate results in a decline in the content of β-globin and ε-globin DNA sequences in the 0.15 M NaCl-soluble chromatin fragments. The content of vitellogenin DNA sequences in the salt-soluble chromatin fraction was not altered throughout the 30-min incubation. The parallel drop in salt solubility and gain in ability of the β-globin and ε-globin chromatin fragments to oligomerize suggests that the deacetylation rates of the histones associated with these chromatin fragments are similar.

**Effect of Histone Deacetylation on MgCl₂ Solubility of Globin Chromatin Fragments**—Mononucleosomes released from micrococcal nuclease-digested erythrocyte nuclei into buffers containing 3 mM MgCl₂ are enriched in the transcriptionally active β-globin DNA sequences and largely depleted in inactive DNA sequences. Inhibiting histone deacetylation increases the enrichment of active mononucleosomes released from the nucle-
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The content of not shown). These observations are identical to the results of isolated from cells incubated in the absence of butyrate (data ever, this enrichment was not observed with mononucleosomes the mononucleosomal fraction released from the nuclease-di-

To test whether the status of dynamically acetylated histones affected the release of competent β-globin mononucleosomes from nuclease-digested nuclei, nuclei isolated from cells incubated in the absence or presence of butyrate for 60 min were digested with micrococcal nuclease, and the chromatin fragments released during digestion and those remaining with the nuclei were collected. The percentage of chromatin released from the nuclease-digested nuclei was similar for each preparation (4.4 to 5%). β-globin DNA sequences were enriched in the mononucleosomal fraction released from the nuclease-di-
gested nuclei of butyrate-treated immature erythrocytes. However, this enrichment was not observed with mononucleosomes isolated from cells incubated in the absence of butyrate (data not shown). These observations are identical to the results of Zhang and Nelson (4). The content of ε-globin DNA sequences in the mononucleosome fraction was greater from nuclei iso-
lated from cells incubated from butyrate compared with that from nuclei of cells incubated in the absence of butyrate (Fig. 3).

Because hyperacetylation directly influences the MgCl₂ sol-

hybridization signal intensity versus time following removal of butyrate showed that the release of βA-globin and ε-globin mononucleosomes was markedly reduced by 5 min followed by a more gradual decline (Fig. 4). In contrast to the βA-globin and ε-globin mononucleosomes, the deacetylation of hyperacety-
ated histones did not alter the release of vitellogenin mono-
nucleosomes from the nuclease-digested nuclei. The sudden decrease in the release of βA-globin and ε-globin mononucleosomes within the first 5 min of incubation in the absence of butyrate closely follows the timing of class 1 hyperacetylated histone deacetylation, particularly that of H3 (see Fig. 1). In summary the rapid decline in the release of βA-globin and ε-globin mononucleosomes from nuclease-digested nuclei parallels the rapid deacetylation of the hyperacetylated class 1 histones.

Transcriptionally Active βA-Globin and Transcriptionally Competent ε-Globin Genes Associated with Fraction PE Are Bound to Hyperacetylated Histones H3 and H4—Previous studies have demonstrated directly that acetylated histones are associated with the transcriptionally active βA-globin and transcriptionally competent ε-globin genes in avian erythrocytes. However, these ChIP assays used soluble chromatin frag-

ments. Most highly acetylated histones and transcriptionally active βA-globin DNA sequences are associated with fraction PE, the low salt-insoluble residual nuclear material harboring chromatin associated with the nuclear matrix (5). To date, no studies have determined if transcriptionally active chromatin bound to the nuclear matrix is associated with highly acety-
ated histones. To address this question, chromatin fragments associated with the low salt-insoluble nuclear material of butyrate-treated immature chicken erythrocytes were briefly in-
cubated with formaldehyde. In addition to cross-linking his-
tones to DNA, formaldehyde incubation releases chromatin fragments from the nuclear matrix (17). The chromatin frag-

ments bound to hyperacetylated H3 and H4 were isolated by ChIPs. Previously in immunoblot experiments we showed that the antibodies used in the ChIP assays preferentially recog-
nized highly acetylated isoforms of H3 or H4 (18). However, the anti-di-acetylated H3 antibody (acetylated Lys-9 and Lys-14) was more discriminating for the highly acetylated H3 isoforms than was the anti-acetylated H4 antibody for the highly acety-
lated H4 isoforms. The DNA sequences bound to hyperacety-
lated H3 and H4 were isolated and analyzed by slot blot anal-
ysis using DNA probes to the intronic regions of the βA-globin and ε-globin genes and to the 5′ region of the vitellogenin gene (Fig. 5). A comparison of the hybridization signal intensities of the three probes in the input and acetylated H3-immunopre-

![Fig. 2](image1.jpg)

**FIG. 2.** β-Globin and ε-globin chromatin fragments gain ability to oligomerize in 150 mM NaCl at similar rates following removal of sodium butyrate. Avian immature cells were treated as described in the legend for Fig. 1. Chromatin fraction S150 was isolated from nuclease-digested nuclei as described under "Materials and Methods." Three µg of DNA isolated from NaCl-soluble chromatin fragments were blotted onto nylon membrane and hybridized to DNA probes recognizing intronic regions of the β-globin and ε-globin genes and the 5′ region of the vitellogenin gene. The intensity of hybridization to each slot was measured by a phosphorimager and plotted against the time of incubation in the absence of butyrate.

![Fig. 3](image2.jpg)

**FIG. 3.** Histone hyperacetylation influences the MgCl₂ solubility of transcriptionally competent ε-globin mononucleosomes. Eight µg of DNA from MgCl₂-soluble and -insoluble chromatin fractions of avian immature erythrocytes treated with or without sodium butyrate (But) for 60 min at 37 °C were electrophoresed on to a 0.8% agarose gel. The DNA was transferred to nylon membrane and hybridized to a probe containing the intronic sequence from the ε-globin gene. S and P designate lanes containing MgCl₂-soluble and -insoluble DNA, respectively. Mono* designates mononucleosomal-sized DNA fragments.
DNA probes recognizing an intronic region of the β-globin fragments were slotted onto nylon membrane and hybridized to a ChIP assay. Input represents the initial total pool of DNA fragments used in the pulse-chase studies. We conclude that cycloheximide does not significantly disturb the balance between histone acetyltransferase and histone deacetylase activity in chicken immature erythrocytes.

Our results show that class 1 histones, which are rapidly highly acetylated and deacetylated, are bound to transcriptionally active β-globin and transcriptionally competent ε-globin genes. In parallel β-globin and ε-globin chromatin fragments gained the ability to oligomerize in 150 mM NaCl as deacetylation of the hyperacetylated H3 and H4 isoforms progressed. Further, the rapid deacetylation of hyperacetylated H3 isoforms corresponded to a rapidly reduced solubility in 3 mM MgCl$_2$ of β-globin and ε-globin mononucleosomes from nuclease-digested nuclei. The loss of the hyperacetylated H3 histones may reverse the disruption of higher order globin chromatin structure, obstructing the release of mononucleosomes from the globin chromatin domain (9, 19). However, the extent of MgCl$_2$ solubility loss of the β-globin mononucleosomes was more acute than that of the ε-globin chromatin fragments. These and other studies show that a greater percentage of β-globin compared with ε-globin chromatin is soluble in 150 mM NaCl or 3 mM MgCl$_2$ (11). We interpret these studies to demonstrate that active coding regions of the β-globin gene are extensively associated with class 1 acetylated histones, whereas the competent ε-globin gene is a mosaic of class 1 and class 2 acetylated histones. This would explain why in our previous study the partitioning of β-globin DNA sequences precisely matched that of the hyperacetylated H4 isoforms, whereas competent ε-globin DNA sequences did not (5).

Crane-Robinson and co-workers have shown that the entire β-globin loop domain is associated with acetylated histones in soluble chromatin fragments (1). The low salt-insoluble chromatin fraction, which contains the bulk of the highly acetylated histones and transcriptionally active DNA, was excluded from their analyses. Our ChIP assays show for the first time that β-globin and ε-globin intron DNA sequences associated with the residual insoluble nuclear material are bound to highly acetylated H3 and H4. Fraction PE harbors most of the histone acetyltransferase and deacetylase activities. Further, histone acetyltransferase and histone deacetylase activities are associated with the nuclear matrix (12). Our observations are consistent with a model in which nuclear matrix-associated histone acetyltransferases and deacetylases mediate a dynamic attachment between transcriptionally active chromatin domains and the nuclear matrix. In the case of the β-globin domain, these dynamic interactions are not confined to the promoter region but also include the coding regions of expressed and competent genes. Our studies provide evidence that both β-globin and ε-globin intron DNA sequences are associated with class 1 dynamically acetylated histones, with β-globin intron DNA sequences having a higher concentration of this class of acetylated histones than that associated with the ε-globin intron DNA sequences. The rapid acetylation and deacetylation of the class 1 histones bound to the β-globin intron DNA sequences suggests that the core histone tails bound to the β-globin gene will be in frequent contact with nuclear matrix-bound histone acetyltransferases and deacetylases. The contacts between these enzymes and the competent ε-globin chromatin will be less frequent. Hence, these multiple dynamic interactions with the transcribed β-globin gene selectively retain this gene at nuclear matrix sites that are engaged in transcription.

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**FIG. 4. β-Globin and ε-globin chromatin fragments lose solubility in 3 mM MgCl$_2$ at similar rates following removal of sodium butyrate.** Three μg of DNA isolated from MgCl$_2$-soluble chromatin fragments were slotted onto nylon membrane and hybridized to DNA probes recognizing an intronic region of the β-globin and ε-globin genes and the 5’ region of the vitellogenin gene. The intensity of hybridization to each slot was measured by a phosphorimager and plotted against the time of incubation in the absence of butyrate.

**FIG. 5. β-Globin and ε-globin chromatin fragments associated with the low salt-insoluble fraction are bound to hyperacetylated H3 and H4.** Input, anti-hyperacetylated H3 (Ach3)-immunoprecipitated and anti-hyperacetylated H4 (Ach4) immunoprecipitated DNA was isolated and quantified by fluorometry. Two hundred ng of immunoprecipitated and input DNA were slotted into their respective slots and hybridized to probes recognizing an intronic region of the β-globin and ε-globin genes and the 5’ region of the vitellogenin gene. A volume of DNA nonspecifically bound to protein A-Sepharose was slotted that was equivalent to the volume of immunoprecipitated DNA. Input represents the initial total pool of DNA fragments used in the ChIP assay. IP represents DNA immunoprecipitated with anti-acetylated histone antibody. Non-specific (NS) represents DNA bound to protein A-Sepharose in the absence of primary antibody.
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