The β -globin domain in immature chicken erythrocytes: enhanced solubility is coincident with histone hyperacetylation

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ABSTRACT

A 60 minute exposure of chicken immature erythrocytes to n-butyrate shifts actively acetylated and deacetylated histones to hypermodified forms. Micrococcal nuclease digestion of nuclei from n-butyrate treated cells and subsequent fractionation of the chromatin releases 40-45% of the adult β -globin (β^A) nucleohistone into a soluble fraction. This is an eleven fold enrichment over the soluble chromatin from untreated cells (Ferez and Nelson (1985) Nucleic Acids Res. 13, 1977-1995). The enhanced β^A chromatin solubility and induced histone hyperacetylation are coincident. Removal of n-butyrate from the cell incubation medium allows rapid histone deacetylation and a striking reduction in β^A chromatin solubility. Chromatin from cells incubated in the absence of n-butyrate, or in medium containing 10 mM NaCl or 2% dimethylsulfoxide, does not exhibit histone hyperacetylation, or the acquired solubility of β^A chromatin. We show that the H4 histone co-isolated with the β^A DNA is in a hyperacetylated state and present evidence that the n-butyrate incubation increases the solubility of both coding and noncoding chromatin regions in the β -globin domain.

INTRODUCTION

Histone acetylation or deacetylation plays a role in histone deposition (1), DNA replication (2,3) and spermatogenesis in some species (4-6). Although histone acetylation is thought to relax chromatin for transcription (7), little is known of the role of this modification in transcriptional events. There is correlative evidence that histone acetylation and deacetylation are germane to transcription. Active segments of the genome are sensitive to DNase I (8,9), as are regions associated with hyperacetylated histones (10-15). Histone acetylation precedes RNA synthesis in human lymphocytes stimulated by phytohemagglutinin (16). One of the earliest effects of estradiol-17 β on rat uterus (17) or of aldosterone on rat kidney (18) is the incorporation of acetate into histone. These hormones are known to directly stimulate transcription in target cells. However, histone acetylation and transcription per se are not tightly coupled. Inhibition of transcription prior to hormonal stimulation (17,18), or hydrocortisone

suppression of phytohemagglutinin stimulated RNA synthesis (19) does not alter the enhanced acetate incorporation into histone.

As adult chicken red blood cells mature, there is a concomitant reduction in transcriptional activity and rate of incorporation of ^3H -acetate into histone (20,21). However, again it should be noted that histone acetylation and transcription in avian erythrocytes are not directly coupled. The abolition of RNA polymerase II transcription by α -amanitin or actinomycin D has no effect on the rate of incorporation of acetate into histone, or the rate of release of acetate (21). Brotherton *et al.* (22), using the deacetylase inhibitor n-butyrate (23-27), demonstrated that only a small subset of the total histone is available for modification in the chicken mature red blood cells. We have verified this result and established that only 3.6% of the immature and 1.9% of the mature erythrocyte acetyltable lysine sites are actively acetylated and deacetylated (28). Based on these results, we postulate that in the terminally differentiated, nonreplicating red blood cell, acetylation and deacetylation are confined to a set of transcriptionally active or potentially active chromatin domains.

A short n-butyrate incubation of immature erythrocytes results in an eleven fold enhancement of β^A chromatin solubility (29). We demonstrate that this solubility increase is coincident with histone hyperacetylation and that the solubilities of both coding and noncoding regions of the β -globin domain are enhanced.

MATERIALS AND METHODS

Preparation of immature red blood cells: Chicken immature erythrocytes were prepared as described previously (29).

Labeling and treatment of immature erythrocytes: Routinely, immature red blood cells from two chickens were resuspended in 40 mls of Swim's S-77 medium (pH 7.2), equilibrated for 10 minutes at 37° , and ^3H -acetate labeled (20 mCi/40 ml; ICN 26 Ci/mmol) at 37° for the times indicated in the figure legends (15-60 minutes). Nuclei were either prepared directly, or cells were washed 2X in fresh Swim's S-77 medium containing 10 mM Na n-butyrate (or other appropriate chemicals) and incubated at 37° in 100-200 mls of medium for the times indicated in the figure legends. Histone specific activities were typically $5\text{--}20 \times 10^3$ dpm ^3H per μg of total histone.

Nuclear isolation and digestion: Nuclei were isolated by two centrifugations at $2500 \times g$ in Nuclear Digestion Buffer (NDB) plus 0.5% Triton X-100. NDB: 0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 3 mM MgCl_2 , 1 mM CaCl_2 ,

10 mM Na n-butyrate, 15 mM MES (2-N-morpholinosulfonic acid), 0.1 mM PMSF (phenylmethanesulfonylfluoride), pH 6.6 (NaOH). The Na n-butyrate (Pfaltz and Bauer, solid) and PMSF (stock solution: 0.1 M in isopropanol) were added immediately prior to use of the solutions. Nuclei used for chromatin fractionations were resuspended in NDB at 70 A_{260nm} units/ml and digested for 5 minutes at 37° with micrococcal nuclease (1 unit/50 µg of DNA). The nuclease was inactivated by addition of 1/50 volume of 0.1 M EGTA (pH 7.4).

Chromatin fractionation following micrococcal nuclease digestion:

Samples were centrifuged at 10,000 x g to obtain supernatant (S) and pellet (P) components.

Sucrose density gradient centrifugation: To obtain the soluble mononucleosome and soluble oligonucleosome samples in figure 5, the supernatant fraction from a nuclear digest was loaded directly on 5-30% sucrose gradients (34 ml, SW 27 tubes) and centrifuged 24 hours at 24,000 rpm in a SW 27 rotor (Beckman). The gradients were buffered with NDB containing 1/50 volume 0.1 M EGTA (pH 7.4). 3.5 ml fractions were collected from the gradients and pooled as described in the text.

Histone isolation, electrophoresis and fluorography: Histones were prepared (30) and electrophoresed on Triton acid-urea gels (31). The 23 cm slab gels were 14% acrylamide, 0.2% bisacrylamide and contained 0.3% Triton X-100, 5% acetic acid and 8 M urea. Subsequent silver staining was according to Wray *et al.* (32). Preparation for fluorography was by the method of Bonner and Laskey (33) and Laskey and Mills (34). Each lane of the gel was typically loaded with 10⁵ dpm of tritium in the form of acetate labeled histone. The vacuum-heat dried gels were exposed to sensitized Kodak XAR-5 X-ray film for 3-7 days at -80°C in Kodak cassettes.

DNA preparation, electrophoresis and nitrocellulose filter

hybridization: DNA preparation and hybridization were as described previously (29). 30 µg of DNA were electrophoresed on each lane of the 1% agarose gels.

RESULTS

Coupling between histone acetylation and deacetylation and β^A chromatin solubility. We have previously demonstrated that chromatin containing β^A sequences is preferentially solubilized by a short n-butyrate incubation of immature chicken erythrocytes (29). The coupling between the solubility of adult β-globin chromatin and histone acetylation and deacetylation is demonstrated during a time course of incubation in the presence and after

Figure 1: Time course of histone acetylation and deacetylation in the presence and absence of n-butyrate in the cell incubation medium. Cells were labeled for 40 minutes with ^3H -acetate, the ^3H -acetate removed, and the cells incubated for 0, 15, 30 and 60 minutes with 10 mM n-butyrate. A portion of the 60 minute incubated cells were washed two times in Swim's S-77 medium without n-butyrate and further incubated 15, 30 and 60 minutes (15, 30, 60) in the absence of n-butyrate. Histones were isolated from whole nuclei, electrophoresed on a Triton acid-urea gel and a fluorogram prepared. The fluorogram is depicted. (1), (2), (3) and (4) at the right edge of the figure indicate the location of mono-, di-, tri- and tetra-acetylated forms of the histones. Assignment of the histone species is according to Urban, Franklin and Zweidler (64) and Urban and Zweidler (65).

removal of n-butyrate (figures 1 and 2). Cells labeled for 40 minutes with ^3H -acetate were incubated 0, 15, 30 and 60 minutes in Swim's medium with 10 mM n-butyrate, and a portion of the cells treated for 60 minutes further

Figure 2: Solubilization of β^A chromatin in response to the presence or absence of n-butyrate in the cell incubation medium. Nuclei from the samples used in figure 1 were mildly digested with micrococcal nuclease and the chromatin fractionated into supernatant (S) and pellet components. Isolated supernatant DNA was treated with alkali (0.3 N NaOH, 37°C, 18 hours), electrophoresed on a 1% agarose gel and stained with ethidium bromide (panel A). The DNA was transferred to nitrocellulose, hybridized to nick-translated pHB1001 and an autoradiogram prepared from the washed filter (panel B).

incubated in the absence of n-butyrate. Figure 1 documents the extent of acetylation of the radiolabeled histones in the presence and after the removal of the short chain fatty acid. In the presence of the deacetylase inhibitor, n-butyrate, acetate is incorporated into, but not removed from the histones, shifting labeled species to hypermodified forms. After 15-60 minutes of incubation in n-butyrate, bands corresponding to highly acetylated H2B and H4 are clearly visualized on the fluorogram. H3 also becomes more highly acetylated (as can be distinctly seen in figure 3, lane 3), but the pattern of modifications is often somewhat blurred. Removal of n-butyrate after the 60 minute incubation results in a dramatic and rapid (15 minutes or less) deacetylation of the hyperacetylated proteins.

Solubility of the β^A chromatin was monitored for the same samples used for figure 1. Nuclei were digested, fractionated, the DNA from the soluble chromatin fraction purified, alkali treated and electrophoresed on a 1%

Figure 3: Extent of histone acetylation in response to acetate, n-butyrate, NaCl and DMSO incubation of immature erythrocytes. Cells were labeled for 15 minutes with ^3H -acetate, the ^3H -acetate removed, and the cells incubated 1 hour in Swim's medium with either no additional chemicals (lane 1), or 10 mM Na-acetate (lane 2), 10 mM Na n-butyrate (lane 3), 10 mM NaCl (lane 4) or 2% DMSO (lane 5). Histones were isolated, electrophoresed on a Triton acid urea gel and the resultant fluorogram depicted in the figure. The band just above H2A.1 may be di-acetylated H2A.1 (66). The band above H3.2Ac4 may be an acetylated, or acetylated and phosphorylated form of the histone.

agarose gel (figure 2, panel A). Most of the DNA isolated from the supernatant fractions is nucleosome core size (29), with some dimer and trimer size DNA in the soluble fraction from the 30-60 minute n-butyrate incubated samples. The slightly greater amount of dimer and trimer size DNA in the

supernatant after n-butyrate incubation (lanes 30 and 60) is presumably the result of histone hyperacetylation (15,35). The DNA in panel A was transferred to nitrocellulose and the filter samples hybridized to pHb1001, a plasmid containing a chicken adult β -globin cDNA sequence (36). The autoradiogram shows the effects of the presence and removal of n-butyrate from the cell incubation medium. The adult β -globin chromatin solubility clearly correlates with the pattern of histone acetylation and deacetylation depicted in figure 1. After a 1 hour n-butyrate incubation, the supernatant chromatin is 11 fold enriched for β^A sequences (29). Removal of the n-butyrate results in a rapid and dramatic disappearance of the β^A DNA (figure 2, panel B, lane 15).

The soluble β^A chromatin in figure 2, lanes 15-60 is "oligomer" size, spanning the dimer to pentamer region of the stained gel (29). This is a result of the very mild digestion conditions used for these experiments (1-2% of the DNA rendered acid soluble); more extensive hydrolysis reduces the soluble oligomer chromatin to monomer size.

Only n-butyrate fully promotes histone hyperacetylation and β^A chromatin solubility. Both elevated salt concentrations (37) and chemical inducers (38,39) can effect gene activation or transcription in numerous cell types. Since n-butyrate is an inducer of globin transcription in Friend erythroleukemic cells (38,39), we have investigated the specificity of β^A chromatin solubilization in relation to inhibition of histone deacetylation.

^3H -acetate labeled immature red blood cells were incubated 1 hour in Swim's medium, or medium plus 10 mM Na acetate, 10 mM Na n-butyrate, 10 mM NaCl or 2% DMSO. The fluorogram prepared after electrophoresis of the histones on a Triton acid-urea gel (figure 3) shows that only n-butyrate completely inhibits histone deacetylation. This is evidenced by the higher levels of labeled histone-acetate in lane 3 compared to the other lanes. The short chain fatty acid, acetate, has a small inhibitory effect on deacetylation in hepatoma tissue culture cells (25), and a small inhibitory effect on deacetylation in the chicken immature red blood cell (lane 2, note the small amounts of tetra-acetylated radiolabeled H2B and H4). Neither 10 mM NaCl (lane 4), nor 2% DMSO (lane 5), the potent globin gene inducer in Friend Erythroleukemic cells, affects deacetylation in the immature erythrocyte. The levels of acetylation for NaCl and DMSO treated cells resemble those found for cells incubated for one hour in Swim's alone (lane 1).

Analogous to the results in figure 3, the n-butyrate incubation specifically enhanced β^A chromatin solubility (figure 4). The nuclei isolated for

Figure 4: Solubility of β^A chromatin following acetate, n-butyrate, NaCl and DMSO incubation of immature red blood cells. Nuclei from the samples used for figure 3 were mildly digested with micrococcal nuclease and the chromatin fractionated into supernatant (S) and pellet. Isolated supernatant DNA was treated with alkali, electrophoresed on a 1% agarose gel (panel A), transferred to nitrocellulose, hybridized to nick-translated pHb1001 and an autoradiogram prepared (panel B). Lanes 1-5 correspond to 1-5 in figure 3.

figure 3 were used to prepare soluble chromatin, the DNA from the soluble fraction isolated and electrophoresed as shown in figure 4, panel A. The autoradiogram obtained after hybridization to pHb1001 (panel B), reveals that neither incubation in the absence of added chemicals (lane 1), nor in the presence of DMSO or NaCl (lanes 4 and 5) results in the solubilization of adult β -globin chromatin. The 10 mM acetate lane 2 contains a small amount of soluble adult β -globin chromatin compared to the control lane (lane 1), corresponding with the small amount of higher acetylated histone forms observed in lane 2, figure 3. A comparison of the control lane with the 10 mM n-butyrate lane 3, illustrates the maximally enhanced β^A chromatin solubility as a result of the n-butyrate exposure. We conclude that n-butyrate incubation promotes β^A chromatin solubility coincident with the inhibition of histone deacetylation. The increased solubility of this chromatin as a result of the n-butyrate incubation is exactly the effect predicted if histone acetylation and deacetylation are preferentially occurring on the histones associated with the adult β -globin gene.

H4 associated with soluble β^A chromatin is hyperacetylated. The soluble chromatin isolated from n-butyrate incubated cells was separated into oligo-nucleosomes and monosomes by 5-30% sucrose density gradient centrifugation. As described previously (29), the soluble oligomers, not the mononucleosomes, are highly enriched for β^A DNA after mild digestions. We verified that our soluble oligomer chromatin was 30 fold enriched for β^A sequences, and then visualized the protein in the various fractions directly on a silver stained Triton acid urea gel as depicted in figure 5. Most of the nonhistone proteins in the soluble chromatin fraction (lane S) remain at the top of the sucrose density gradient (data not shown). A complete analysis of the protein bands in the soluble oligomer, soluble monomer, supernatant and pellet fractions requires two dimensional gel electrophoresis. However, an analysis is possible for the H4 species on the Triton acid urea gel since they are well resolved and free from other protein bands. As seen in the silver stained gel (Ag stain) and fluorogram (FL), the bulk of H4 is present as H4Ac4 in the oligomer lanes (O). An overexposure of the same lane (O') displays the other H4 bands. Soluble monosomes (lane M) from the sucrose gradient contain all levels of H4-acetate, with the nonacetylated and monoacetylated forms of H4 predominating. It is evident that when the soluble chromatin (lane S) is fractionated into monomer and oligomer components, the higher acetylated forms of H4 dominate in the soluble oligomer fraction. These results demonstrate that after a short n-butyrate incubation of the immature erythrocytes, the H4 associated with the soluble β^A DNA is hyperacetylated.

N-butyrate exposure enhances the solubility of coding and noncoding regions of the β -globin domain. We have assayed for regions of the immature red blood cell genome that are solubilized by the n-butyrate incubation. The results of such an analysis are presented in figure 6. Immature red blood cells were incubated for 1 hour in 10 mM n-butyrate, nuclei isolated, mildly cleaved with micrococcal nuclease and the chromatin fractionated by differential centrifugation. In figure 6, lanes S contain DNA from the soluble chromatin and lanes P from the insoluble chromatin fractions. When the DNA in lanes 2S and 2P are hybridized to pHb1001 (β^A cDNA probe), the typical enrichment for β^A sequences is seen in the supernatant. No enrichment is evident in the soluble fractions (lane 3S and 4S) for the transcriptionally inactive ovalbumin (pOV230; ref. 40) or lysozyme (pls-1; ref. 41) chromatin. We also confirm the β^A chromatin solubility (lane 5S) using a probe (pC8G18.7; ref. 42) containing a unique sequence in the second intron of β^A .

Figure 5: Extent of H4 acetylation of soluble mononucleosomes, soluble oligonucleosomes and supernatant and pellet chromatin fractions as visualized on a silver stained Triton acid urea gel. Nuclei from cells incubated 1 hour in 10 mM n-butyrate were mildly digested with micrococcal nuclease and fractionated into soluble (S) and insoluble (P) components. Soluble nucleohistone was fractionated into monosome (M) and oligonucleosome (O) com-

ponents on a sucrose density gradient as described in the Materials and Methods. Histones were isolated, electrophoresed on a Triton acid urea gel and silver stained (Ag stain). The five species of H4 are indicated as: p - parental (non-acetylated); 1, mono-; 2, di-; 3, tri-; and 4, tetra-acetylated H4. (W) = histone from whole nuclei. The (O) and (O') lanes are lighter and darker prints of the same lane. The fluorogram (FL) of the oligomer (O) lane is shown on the right hand side of the figure. Assignment of H4Ac4 in the fluorogram is based on the location of radiolabeled H4 species in other lanes of the same fluorogram (not shown).

The results of interest are in the last two pairs of S and P lanes. In lanes 6S and 6P, the DNA has been reannealed to pC8G21.6, a noncoding sequence approximately 3 kb 3' of ϵ , but within the β -globin domain (42). The chicken DNA in this sequence is of moderate repetition frequency as deduced from an analysis of hybridization to whole Bam HI digested chicken DNA (data not shown). It is evident that portions of the chromatin containing this sequence are soluble (lane 6(S)) and insoluble (lane 6(P)). We ascribe the hybridization of the probe to the pellet DNA to homology with DNA sequences in inactive domains. In lanes 7, S and P, we have reannealed the DNA to pC8G3.9, a subcloned fragment isolated from Hpa II digested pC8G4 (43). The

Figure 6: Both coding and noncoding regions of the β -globin domain are solubilized by the n-butyrate incubation. Immature erythrocytes were incubated 1 hour in the presence of 10 mM n-butyrate, nuclei isolated, mildly digested with micrococcal nuclease and the chromatin fractionated into soluble and insoluble components. DNA was isolated, treated with alkali and six identical sets of supernatant (S) and pellet (P) DNA samples electrophoresed on a 1% agarose gel (lane 1, ethidium bromide stained S and P DNA). DNA was transferred to nitrocellulose, the filter cut into six S and P pairs and the samples hybridized to nick-translated (2) pHB1001, (3) pOV230, (4) pls-1, (5) pC8G18.7, (6) pC8G21.6 and (7) pC8G3.9. Autoradiograms prepared from the washed filters are shown in 2-7. Plasmids pC8G21.6 and pC8G3.9 contain non-coding DNA fragments from within the β -globin domain.

subclone contains approximately 500 base pairs of unique DNA located 2 kb 5' of the ρ gene. The sequence is also within the β -globin domain but outside the coding regions (43). This chromatin is solubilized by the n-butyrate incubation (lane 7(S)) and appears to be more rapidly cleaved to mononucleosome size compared to the other samples. In control experiments, the soluble chromatin isolated from non-butyrate treated cells is markedly depleted in this DNA sequence (data not shown).

The results shown in lanes 6 and 7 contrast with those obtained with the probes pOV230 and pls-1 (figure 6, lanes 3(S) and 4(S)) which demonstrate no solubilization of the transcriptionally inactive ovalbumin or lysozyme chromatin. Based on the enhanced solubility of both the transcribed and nontranscribed β -globin nucleohistone, we postulate that histone acetylation and deacetylation are preferentially occurring on both coding and noncoding regions of the β -globin domain, possibly over the entire β -globin region.

DISCUSSION

The behavior of β -globin chromatin from n-butyrate incubated chicken immature erythrocytes is fully coincident with the preferential occurrence of active histone acetylation and deacetylation on this region of the genome. A short exposure of cells to 10 mM n-butyrate inhibits histone deacetylation and preferentially solubilizes oligomer size chromatin enriched in β -globin DNA and hyperacetylated H4. The n-butyrate solubilization is specific for active or potentially active chromatin. Ovalbumin and lysozyme nucleohistone, inactive in chicken immature red blood cells, remain insoluble during the n-butyrate incubation.

Enhanced β^A chromatin solubility does not appear to be related to gene induction by n-butyrate. Dimethylsulfoxide, used at a concentration which induces β -globin synthesis in Friend cells (39), has no detectable effect on nucleohistone solubility during a one hour incubation period. The addition of 10 mM n-butyrate to the Swim's incubation medium slightly increases the ionic strength. We therefore also tested for a "salt induction" effect (37) in the presence of 10 mM NaCl and found that NaCl incubation has no effect on β -globin chromatin solubility. Neither DMSO nor NaCl inhibits histone deacetylation.

Incubation of immature erythrocytes in Swim's medium plus 10 mM Na acetate produces results intermediate between those for cells incubated in the presence or absence of 10 mM n-butyrate. Acetate has a slight inhibitory

effect on histone deacetylation, and a small portion of the adult β -globin chromatin becomes soluble during the one hour incubation. Densitometer scans of the autoradiogram shown in figure 4 indicate that the supernatant fraction from the acetate treated cells contains about 4-5 fold less adult β -globin chromatin than from the n-butyrate incubated cells. We do not know if 10 mM acetate produces a mass action effect, reducing the rate of hydrolysis of histone-acetate, or whether the Na acetate preparation contains small amounts of contaminating short-chain fatty acids (i.e., n-butyrate or propionate). Either of these possibilities would cause the partial inhibition of histone deacetylation. The intermediate results obtained with acetate support the notion that active histone acetylation and deacetylation are preferentially occurring on the β -globin chromatin. The partial inhibition of histone deacetylation slightly increases the amount of soluble β -globin nucleohistone.

To observe the enhanced solubility of active or potentially active chromatin, the presence of a divalent cation (ie, Mg^{++}) in the fractionation buffer is essential (29). The presence of 3 mM $MgCl_2$ during centrifugation following micrococcal nuclease digestion is also required to obtain a soluble oligomer chromatin fraction enriched in highly acetylated histones (data not shown). This observation is consistent with experiments demonstrating that histone hyperacetylation enhances polynucleosome solubility in the presence of $MgCl_2$ (15,35), providing further evidence that the n-butyrate induced solubility of the β -globin chromatin is a result of histone hyperacetylation. The pleiotropic effects of n-butyrate *in vivo* (44,45), however, preclude a final conclusion that the solubilization is solely due to histone hyperacetylation. Alterations in protein composition or other histone modifications as a result of the n-butyrate incubation may be relevant to the enhanced chromatin solubility.

Our results suggest that the β -globin histones in immature erythrocytes are not preserved in a permanent, highly acetylated state. Permanently hyperacetylated chromatin would be soluble without n-butyrate incubation of the cells (15,35). The potentially active chromatin conformation, as delineated by moderate DNase I sensitivity (8,9) is therefore likely to be maintained by factors (46-48) other than histone hyperacetylation.

We favor two potential mechanisms for transcription related action of the histone acetyltransferase and deacetylase enzymes. The enzymes may be loosely bound to chromatin, free to move and randomly distribute within the

nucleoplasm. Histones are preferentially modified in the active chromatin domains because these regions are more open and the histone substrate more accessible to the enzymes. A second, more likely mode of action, is that these enzymes are bound to chromatin, either localized or activated within specific domains. In fact, based on the data of Libby (17,18), Yamamoto and Alberts (49) proposed that "waves" of histone acetylation precede steroid activated transcription within specific target cell chromatin regions. The latter hypothesis is supported by evidence that the acetyltransferase (50-58) and often the deacetylase (59,60) enzymes are tightly bound to chromatin, requiring 0.4-0.5 M NaCl or higher salt concentrations for their removal. Further support for chromatin bound enzymes comes from the observation that in isolated male mealy bug nuclei, the maternal active chromosomes incorporate seven times more labeled acetate than the inactive paternal chromosomes (61). The acetyltransferases are clearly not dissociated during nuclear or chromatin preparation and are preferentially active in transcriptionally active chromosomal domains. It is not unreasonable to propose that chromatin bound histone acetyltransferases and deacetylases move linearly along the chromatin fiber, sequentially acetylating and deacetylating histones on specific nucleohistone domains (49,59,60). An intriguing possibility is that histone acetylation and deacetylation contribute to modulating domain structure, thereby functioning to regulate nonhistone protein and RNA polymerase II entry onto the DNA. The effect of inhibiting histone acetylation would be to reduce the frequency of RNA polymerase II transcription. Conversely, inhibition of RNA polymerase II transcription in the immature chicken erythrocyte need not inhibit acetate incorporation or removal from the histones (21).

Enhanced chromatin solubility (15,35) and DNase I sensitivity (10-15) are related to histone hyperacetylation, but reports are both consistent (62) and inconsistent (10,63) with the perception that histone acetylation "loosens" nucleosome, or nucleosome higher order structure. Clearly, further work is required to understand the contributions of histone acetylation in permitting or regulating cellular events such as transcription.

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