Postsynthetic Methylation of Core Histones in K562 Cells Is Associated with Bulk Acetylation but Not with Transcriptional Activity†

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ABSTRACT: The relationship between the postsynthetic modification of core histones by methylation and transcriptionally active chromatin was investigated in K562 erythroleukemia cells. Cells were incubated with L-[*methyl*-^{3}H]methionine in the presence of cycloheximide. Under these conditions, only histones H3 and H4 were detectably methylated. Chromatin was fractionated by several methods, including low-salt elution, mononucleosome gel mobility shift using HPLC-purified HMG 17, and cesium chloride–guanidine equilibrium gradient centrifugation of formaldehyde–fixed chromatin. By these latter two methods, chromatin highly enriched for transcriptionally engaged or competent genes was isolated. A significant correlation was noted between postsynthetic modification of histones by methylation and by the slow-turnover form of acetylation. However, there was no enrichment of methylated histones in the transcriptionally competent fraction of chromatin isolated by HMG 17 binding. Moreover, only minor enrichment of methylated histone H3.1 and no enrichment of methylated histones H3.2 and H4 was detected in transcriptionally engaged chromatin isolated by gradient centrifugation. Chromatin soluble in low-salt buffer was found to be significantly enriched in methylated histones, but not in active genes. We conclude that histone methylation is associated with both transcriptionally active and transcriptionally inactive chromatin. The function of this modification, like that of bulk histone acetylation, remains to be determined.

It is well established that chromatin that contains actively transcribed gene domains is maintained in a state that is more sensitive to degradation by nucleases, such as DNase I (Cartwright et al., 1982). Our previous studies on HMG 17 binding to chromatin in vitro have shown that these proteins can preferentially bind to core mononucleosomes that contain genes from transcriptionally active domains and that binding preference is probably mediated by features intrinsic to the core histones (Brotherton & Ginder, 1986; Brotherton et al., 1990). Core histones undergo a wide variety of postsynthetic modifications, including acetylation, ubiquitination, phosphorylation, and methylation [for a review, see Wu et al. (1986)], and these modifications have all been implicated as possible mediators of the "open", DNase I sensitive structure of active chromatin.

All four core histones can undergo reversible methylation of internal lysines (Wu et al., 1986). Histone methylation is a relatively stable modification that has a slow turnover rate in some cells (Honda et al., 1975), but not others (Shepherd et al., 1971). Modification appears to take place after assembly into chromatin (Shepherd et al., 1971; Honda et al., 1975). The function of the methylation in chromatin is unknown, but is probably not random as it displays tissue-specific and cell cycle specific patterns (Shepherd et al., 1971; Lee & Loh, 1977). It has been proposed that this modification may play a role in chromatin condensation and mitosis (Honda et al., 1975), the nuclear response to heat shock (Arrigo, 1983; Desrosiers & Tanguay, 1988), the response to nucleosome structure disruption by agents such as ethium bromide, novobiocin, and VM-26 (teniposide) (Desrosiers & Tanguay, 1989), and maintenance of the transcriptionally active chromatin state (Lee & Loh, 1977; Camato & Tanguay, 1982; Desrosiers & Tanguay, 1988; Hendzel & Davie, 1989).

In this study, we used uninduced K562 cells to determine whether there is a relationship between histone methylation and the transcriptionally active chromatin state. K562 cells are a human erythroleukemia cell line derived from a patient with the *bcr-abl* translocation (Lozzio & Lozzio, 1975) that constitutively express embryonic and fetal globin genes (Groudine et al., 1983). Three distinct methods for the fractionation of chromatin were employed. Our results demonstrate that the acetylated species of the histones H3 and H4 are preferentially methylated. However, these modified histones are not enriched in chromatin containing transcriptionally competent genes. Methylated histone H3.1 is modestly enriched in chromatin containing transcriptionally engaged genes, but methylated histones H3.2 and H4 are not. These results suggest that core histone methylation does not play a significant role in the maintenance of transcriptionally active chromatin. Like the slow-turnover form of core histone acetylation with which it is associated, the role of histone methylation remains to be determined.

Experimental Procedures

Cell Culture and Labeling. A line of K562 cells that constitutively express γ-globin were obtained from Dr. G. Ginder and have been previously described (Chen et al., 1988). For labeling, cells were resuspended to 2.5×10^8 cells/mL in RPMI with 10% bovine calf serum and preincubated for 30 min at 37 °C with 2×10^{-4} M cycloheximide (Sigma). In some experiments, 25 mM sodium butyrate, pH 7.0, was added to the preincubation and all subsequent buffers. L-[*methyl*-^{3}H]Methionine (70–85 Ci/mmol; Amersham Corp.) was added to the cell suspension (final concentration between 50 and 75 μCi/mL) which was incubated for 60 min at 37 °C with constant mixing. Cells were collected by centrifugation and processed immediately. Methylation turnover studies were performed as follows. Cells were labeled under standard conditions in the presence of 25 mM butyrate. After two

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washes of ice-cold media without label, cells were resuspended at 2.5 × 10^8 cells/mL in RPMI with 10% bovine calf serum and 5 mM butyrate at 37 °C. Aliquots were removed at 0, 5, 10, 30, 60, and 120 min, and histones were prepared as previously described (Brotherton et al., 1981). These histones were submitted to Triton-urea–acetic acid gel [12.5% (w/v) acrylamide, 0.08% (w/v) bis(acrylamide)] electrophoresis followed by fluorography (Brotherton et al., 1981).

Nuclei Isolation, Digestion, and Fractionation. Nuclei were isolated as described previously (Brotherton & Ginder, 1986). For salt elution fractionation of chromatin, washed nuclei were resuspended at 50 A_{260}/mL in RSB [10 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl),[1] pH 7.4, and 5 mM $MgCl_2$] and 0.25 M sucrose with 1 mM PMSF. $CaCl_2$ was added to 1 mM and staphylococcal nuclease (Boehringer) at 25 units/mL. The preparation was incubated to 37 °C with constant mixing to allow 20–50% of the chromatin to be digested to mononucleosomes; this was typically 10 min. The reaction was stopped by adding EGTA to 2 mM, and the nuclei were pelleted by centrifugation. Low-salt elution was then carried out according to the method described previously (Ridsdale & Davie, 1987; Hendzel & Davie, 1989). Briefly, nuclei were resuspended in 10 mM EDTA (pH 7.5) and 1 mM PMSF and incubated on ice for 30–90 min. An aliquot was removed (fraction T), and EDTA-insoluble nuclear material was pelleted (fraction P_E) by centrifugation at 12000*g* for 10 min. After removal of a sample of the supernatent fraction (fraction S_E), the remainder was made 150 mM with NaCl. After centrifugation, a soluble fraction (fraction S_{150}) and insoluble fraction (fraction P_{150}) were obtained. Histones were recovered by acid elution (Brotherton et al., 1989).

For fractionation of mononucleosomes by HMG 17 gel mobility shift, trimmed core mononucleosomes were prepared as described previously (Brotherton et al., 1986). HMG 17 was prepared from 5% perchloric acid extracted calf thymus nuclei by reverse-phase HPLC (C4, Vydac) (Brotherton et al., 1990). Gel mobility shift fractionation of HMG 17 bound and HMG 17 unbound mononucleosomes was carried out in 0.15 × 16 cm 5% acrylamide–0.2% bis(acrylamide) gels in 1 × TBE (0.089 M Tris, 0.089 M borate, and 2.5 mM EDTA, pH 8.4). After first-dimension electrophoresis, gels were stained in ethidium bromide in water. Lanes were excised, soaked in 0.9 N acetic acid–8 M urea for 30 min, and then placed over a Triton–urea–acetic acid–polyacrylamide gel (Brotherton et al., 1981). Histones were released by the method of protamine displacement (Richards & Shaw, 1982). After this second-dimension electrophoresis, gels were stained with Coomassie blue, photographed, and submitted to fluorography (Bonner & Laskey, 1974; Laskey & Mills, 1975).

Chromatin was also fractionated, after formaldehyde cross-linking, by a previously described method (Ip et al., 1988), with minor modifications. Briefly, labeled cells were resuspended in cold RPMI media containing 1% fresh formaldehyde for 4–24 h at 4 °C with constant shaking. The cells were then washed and resuspended in fresh media and incubated overnight at 4 °C, or washed with several changes of media for 2–4 h at room temperature. The fixed cells were washed 3 times with 0.25 M sucrose–RSB and 1% Triton X-100 and then washed once in the same buffer without detergent. Nuclei from cells fixed for 4 h with formaldehyde were also incubated with dithiobis(succinimidyl propionate)

[1] Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

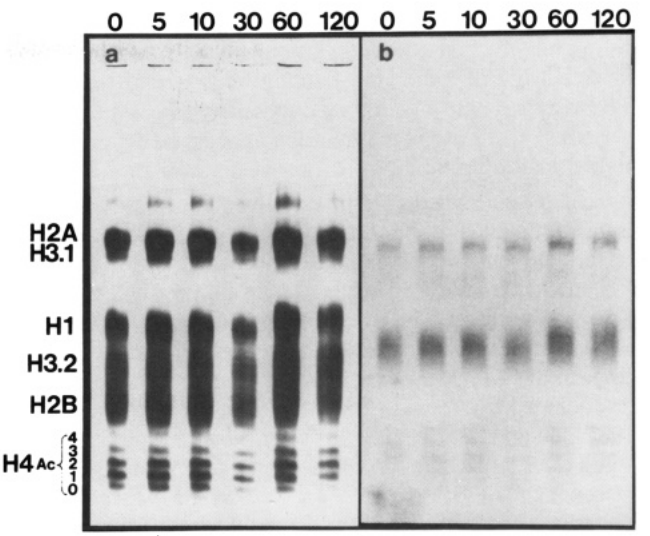

FIGURE 1: Postsynthetic methylation of histones H3 and H4 is stable in K562 cells. K562 cells were labeled with [*methyl*-^{3}H]methionine, after preincubation with cyclohexamide (see Experimental Procedures). Cells were washed and then incubated for the indicated times in media without radiolabel or cyclohexamide. Nuclei were made, and histones were extracted with 0.4% H_2SO_4. (a) Coomassie blue stained Triton–urea–acetic acid. (b) Fluorogram of gel.

(Pierce) at 7.5 μg/mL in 0.25 M sucrose, 50 mM acetic acid–triethanolamine, pH 7.2, 5 mM $MgCl_2$, 50 mM KCl, and 0.2 mM PMSF for 90 s with constant mixing. Cross-linked nuclei were washed 3 times in 2 M NaCl, 5 M urea, and 10 mM EDTA; nuclei were then resuspended at 1–2 mg/mL in this same buffer and sonicated 3 times for 15 s at 350 W (medium tip, Braun-Sonic 1510). The resultant solution was centrifuged at 25000*g* for 10 min, and the supernatant was then dialyzed against 50 mM Tris, pH 8, and 1 mM EDTA overnight at 4 °C. Equilibrium centrifugation was then performed on 4.6-mL sales, after adding 1.7 g of guanidine hydrochloride and 1.2 g of cesium chloride, at 35000 rpm for 72 h at 4 °C in a Beckman SW56 rotors. Fractions of 0.2 mL were collected from the bottom of each tube. DNA and proteins were recovered according to the method described elsewhere (Ip et al., 1988). Gradient performance was monitored by the addition to each gradient tube of (10–20) × 10^3 cpm of [^{3}H]thymidine labeled, cross-linked K562 chromatin as an internal control (Ip et al., 1988).

DNA Blotting and Hybridization Analysis. DNA in agarose gels was transferred to nylon (Zeta-Probe, Bio-Rad) by alkaline Southern blot (Reed & Mann, 1985). DNA in nucleohistone gels was electroblotted by previously described methods (Brotherton & Ginder, 1986). Cloned DNA probes for the human γ-globin gene and avian malic enzyme genes have been described elsewhere (Groudine et al., 1983; Brotherton et al., 1990). The avian malic enzyme probe used (C4 malic) hybridizes to unique restriction bands in human genomic DNA when hybridized and washed under low-stringency conditions (data not shown). Malic enzyme activity is not detected in K562 cell extracts. Random primer labeling and hybridization procedures are as previously described (Brotherton et al., 1990).

Results

K562 cells were labeled with [*methyl*-^{3}H]methionine in the presence of cyclohexamide after preincubation in cyclohexamide. Cells were washed in media without radiolabel or cyclohexamide and then incubated in fresh media for up to 2 h. Aliquots were removed at various times, and total nuclear histones were isolated (Brotherton et al., 1981). Shown in

Figure 1 is the gel and fluorogram obtained. When this labeling method is used, methylation is chiefly present on histones H3.1, H3.2, and H4. In addition, no significant loss of label is apparent in any histone fraction over the 2-h cold chase period. Thus, histone methylation appears to be a relatively stable postsynthetic modification of histones in K562 cells.

To determine whether histone methylation is associated with transcriptional activity, three methods for fractionating transcriptionally active or engaged chromatin were applied to [^{3}H]methyl-labeled K562 cells. In this first method employed, K562 cells were again labeled with [*methyl*-^{3}H]methionine in the presence of cycloheximide, after preincubation in cycloheximide. Nuclei were then prepared, and chromatin was fractionated by selective low-salt elution (Ridsdale & Davie, 1987; Hendzel & Davie, 1989). In this method, nuclei are digested briefly with micrococcal nuclease. Pelleted nuclei are resuspended in 10 mM EDTA–1 mM PMSF; chromatin solubilized (fraction S_E) in this low salt is further fractionated by the addition of 150 mM NaCl into soluble (fraction S_{150}) and precipitated (fraction P_{150}) chromatin. On the basis of studies in adult avian reticulocytes, it has been reported that transcriptionally active gene sequences (e.g., β-globin in adult reticulocytes) partition into both the S_{150} fraction and the EDTA-insoluble residual nuclear material (called P_E). Competent genes, which are sensitive to DNase I but transcriptionally inactive (e.g., embryonic ϵ-globin), are enriched in the S_{150} fraction (Hendzel & Davie, 1989). The reported distribution of adult avian reticulocyte DNA in fractions S_E, S_{150}, P_{150}, and P_E is 83%, 6%, 77%, and 17%, respectively (Hendzel & Davie, 1989). When this method was applied to K562 cells, the distribution of DNA among chromatin fractions S_E, S_{150}, P_{150}, and P_E was 38%, 33%, 6%, and 62%, respectively. The extent of core histone methylation in each chromatin fraction is shown in Figure 2a. Since the mass of the histones is about equal to that of DNA, the ratio of ^{3}H-cpm/mg of DNA reflects the relative activity of histone labeling in each fraction. As shown, the most extensive labeling was present in the S_{150} fraction. However, as this fraction contained only 33% of the total DNA, only 42% of the total ^{3}H-methylation was present in this fraction; 32% of the total label was in the P_E fraction.

The distribution of K562 DNA in the various fractions was different in our studies than those reported previously (Hendzel & Davie, 1989). In the study reported here, the proportion of DNA in the S_{150} and P_E fractions was significantly increased relative to the P_{150} fraction compared to the previously published report. To determine if this altered distribution of DNA also altered the distribution of active genes, we did the following. First, DNA was recovered from each fraction of K562 chromatin, subjected to electrophoresis, Southern-blotted, and examined for the distribution of the active γ-globin gene (Groudine et al., 1983) and inactive malic enzyme gene (Brotherton et al., 1990). No significant enrichment of the active γ-globin gene in DNA recovered from the S_{150} or P_E chromatin fractions was detected. In particular, the ratio of detected active γ-globin gene sequences to inactive malic gene sequences in fraction S_{150} is 0.73. For fraction P_E, this ratio is 1.5. Similar ratios are obtained if the gene content is compared to total DNA, rather than other genes. Second, these studies were repeated in adult chicken reticulocytes. Results using these cells were more similar to those reported previously (Hendzel & Davie, 1989). In particular, the distribution of DNA among chromatin fractions S_E, S_{150}, P_{150}, and P_E was 57%, 15%, 42%, and 43%, respectively. There was an over 3-fold increase in the [^{3}H]methyl cpm in the S_{150} fraction relative to both the S_E and P_{150} fractions (Figure 2b).

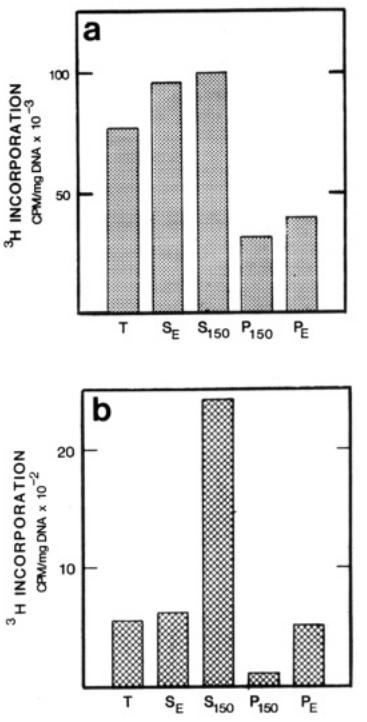

FIGURE 2: Distribution of methylated histones among fractions of K562 and adult avian reticulocyte chromatin. (a) K562 cells were incubated in the presence of cycloheximide and L-[*methyl*-^{3}H]-methionine as described under Experimental Procedures. The chromatin was fractionated, and the acid-soluble proteins of each fraction were isolated. Proteins in these extracts were almost exclusively histones by gel electrophoresis. Radioactivity was determined by mixing 200 μg of protein with 10 mL of Budget-Solve (RPI) scintillation fluid. (b) Reticulocyte nuclei were incubated in the presence of cycloheximide and L-[*methyl*-^{3}H]methionine as described under Experimental Procedures. The chromatin was fractionated, and the acid-soluble proteins of each fraction were isolated. Radioactivity was determined by mixing 200 μg of protein with 10 μL of scintillation fluid.

In addition, the enrichment of active adult β-globin in the S_{150} and P_E fractions was similar to that described previously (Hendzel & Davie, 1989). The ratio of detected β-globin gene sequences to total DNA in fraction S_{150} DNA is 5.9. For fraction P_E, this ratio is 1.8. These results confirm the reported association of active histone methylation with avian reticulocyte chromatin that is soluble in low salt and demonstrate that this association is also present when chromatin from K562 cells is examined. However, an association between active transcription and histone methylation cannot be considered established, as the S_{150} fraction of K562 chromatin is neither significantly enriched nor depleted of transcribed genes.

To determine if core histone methylation correlated with transcriptional competence (i.e., genes sensitive to DNase I, but transcriptionally inactive) or with being transcriptionally engaged, K562 chromatin labeled with [*methyl*-^{3}H]methionine was fractionated by two additional methods. The first of these methods utilizes HPLC-purified HMG 17 in a gel mobility shift assay that separates mononucleosomes containing transcriptionally competent genes from bulk chromatin (Brotherton et al., 1990). The utility of this assay using mononucleosomes prepared from K562 chromatin is documented in Table I. As shown, when 25–30% of the total mononucleosomes are HMG 17 bound, there is a 3–4-fold enrichment of an active gene marker, γ-globin, in the bound fraction relative to a repressed gene marker, malic enzyme. The specificity of binding for mononucleosomes containing competent genes is lost when 50% or more of the total mononucleosomes are bound. When core

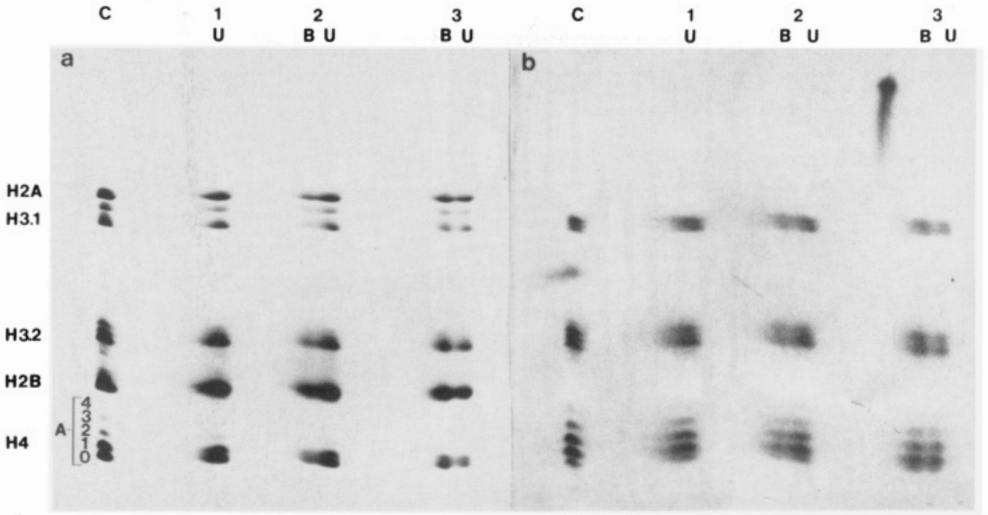

FIGURE 3: Triton–urea–acetic acid gel analysis of K562 cell histone methylation in HMG 17 bound and unbound mononucleosomes. Mononucleosomes were isolated from K562 cells incubated with [*methyl*-^{3}H]methionine in the presences of cycloheximide. Trimmed, core mononucleosomes were titrated with HMG 17 and subjected to nondenaturing gel electrophoresis. After brief ethidium bromide staining, gel lanes were excised, and second-dimension Triton–urea–acetic acid gel electrophoresis was performed (see Experimental Procedures). (a) Coomassie blue stained gel; (b) fluorogram of gel. (c) Total mononucleosome histone control. U, unbound mononucleosomes. B, HMG 17 bound mononucleosomes. Lane 1, no added HMG 17; lane 2, 30% HMG 17 bound; lane 3, 55% HMG 17 bound. Acetylated forms of histone H4 are indicated, with A0 indicating the unacetylated state. Note that methylation is confined to histones H4, H3.2, and H3.1 and that methylation is present chiefly on acetylated histones.

Table I: Proportion of Total DNA and Active and Inactive Genes in the HMG 17 Bound Fraction of K562 Mononucleosomes[a]

% total bound		
DNA	γ-globin	malic enzyme
25	39	11
30	43	17
53	50	45

[a] Trimmed core mononucleosimes were prepared from K562 nuclei and titrated with HMG 17 as described under Experimental Procedures. The proportion of total DNA and of DNA sequences that hybridize to probes for the active γ-globin gene and inactive malic enzyme gene in the HMG 17 bound fraction of mononucleosomes was determined and is expressed as a percentage of the total DNA.

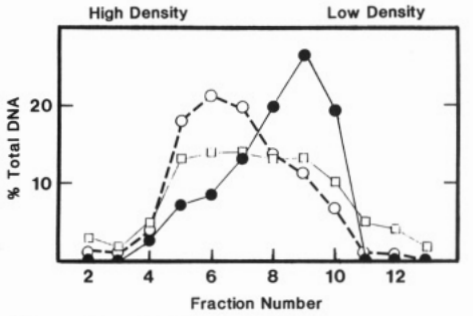

FIGURE 4: Separation of active chromatin from inactive chromatin by a CsCl density gradient. K562 cells were fixed for 24 h and sonicated as described. Chromatin was fractionated on CsCl density gradients and DNA purified from fractions around the DNA peak. Total DNA in each fraction was directly submitted to agarose gel electrophoresis and gene content analyzed by blot hybridization. Open squares, distribution of DNA in the gradient. Open circles, distribution of DNA hybridizing to the probe for the malic enzyme gene. Closed circles, distribution of DNA hybridizing to the probe for the γ-globin gene.

histones contained in the HMG 17 bound and unbound mononucleosomes are examined by second-dimension triton–urea–acetic acid gel electrophoresis, no enrichment of ^{3}H-methylated histones is detected in the bound fraction (Figure 3). Methylation is confined to histones H3 and H4. Similar to earlier observations, an association between methylation and bulk acetylation is detected (Hendzel & Davie, 1989), and no association between bulk acetylation and HMG 17 binding is present (Brotherton & Ginder, 1986).

The second method employed allows the isolation of transcriptionally engaged chromatin (Ip et al., 1988). In this method, labeled cells are cross-linked with formaldehyde. Nuclei are then extracted and sonicated in 2 M NaCl, 5 M urea, and 10 mM EDTA. Chromatin solubilized by sonication (>80% of total, using K562 nuclei) is then fractionated by cesium chloride–guanidine hydrochloride equilibrium centrifugation. Chromatin fractions that are cross-linked to RNA polymerase have a lighter density than bulk chromatin (Ip et al., 1988). This method was validated for K562 cells, as shown in Figure 4. DNA recovered from lighter density fractions was typically 2.5–5-fold enriched in transcriptionally active genes. The study in Figure 4 was generated by using cells incubated for 24 h with formaldehyde, but similar results were also obtained with cells incubated for only 4 h (data not shown). It is reported that greater enrichment for a given gene can be obtained by increasing its rate of transcription (Ip et al., 1988). However, although hemin induction is reported to up-regulate γ-globin transcription in the K562 cell line used (G. Ginder, personal communication), no significant effect on the distribution of DNA containing the γ-globin gene in the CsCl–GuHCl gradient was detected after either 1 h or 24 h of hemin induction (data not shown). Histones recovered from gradient fractions were subjected to electrophoresis after reversal of cross-links. As shown, the distribution of total methylation for histones H3.2 and H4 is similar to the content of total histone protein in these fractions (Figure 5). In particular, no enrichment of methylation was detected in fractions 8–14, the fractions enriched in transcriptionally engaged chromatin. In contrast, there is an apparent enrichment of methylated histone H3.1, relative to total H3.1 protein, in fractions 8–14. This enrichment has been quantitated by scanning densitometry (Figure 6), and is approximately 2-fold in fractions 8–10. In addition, several other methylated protein bands are enriched in the region of the gradient that is enriched in lighter density gradient fractions (Figure 5). These protein bands are probably not histones as they are not detected in similar gels of methylated histones recovered from non-cross-linked K562 nuclei (Figure 1) or from nucleosomal core

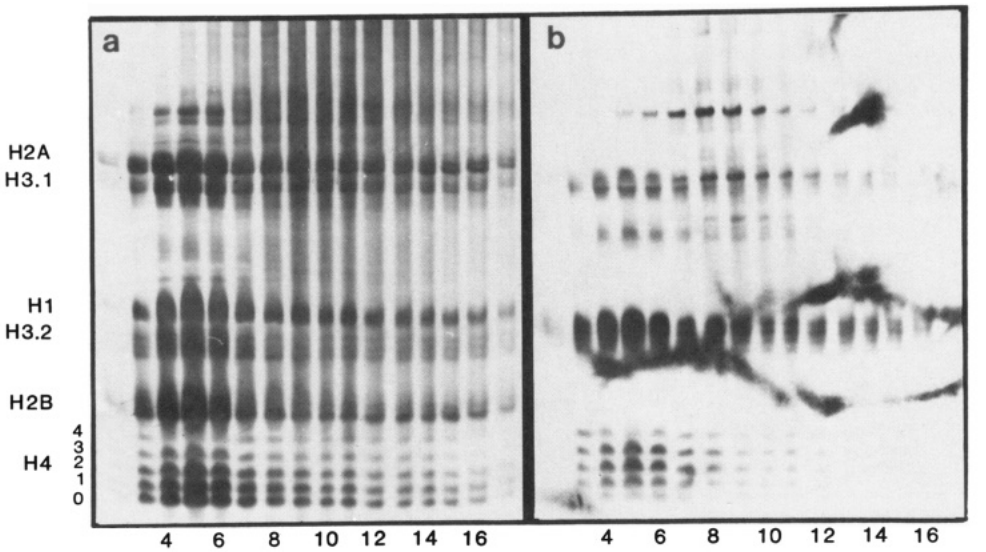

FIGURE 5: Distribution of methylated histones in the density gradient. K562 cells were labeled with [*methyl*]-^{3}H]methionine in the presence of cycloheximide and butyrate. Cells were cross-linked for 4 h with formaldehyde. Chromatin was subjected to cesium chloride density gradient fractionation. Histones were recovered by acid-extraction after reversal of cross-links, and subjected to Triton–urea–acetic acid gel electrophoresis. (a) Coomassie blue stained gel; (b) fluorogram of gel. The transcriptionally engaged γ-globin gene was enriched in fractions 8–14 in this experiment. Histones and acetylated variants of histone H4 are identified as in the legend to Figure 3.

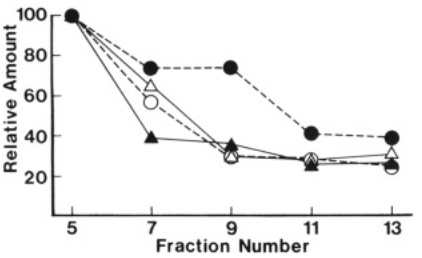

FIGURE 6: Quantitation of methylation of histones H3.1 and H3.2 isolated by density gradient centrifugation. The stained gel and fluorogram in Figure 5 were submitted to scanning densitometry. The relative absorbancy for individual bands was determined, and is expressed as a percentage of that in lane 5, which was arbitrarily set at 100%. Circles, histone H3.1; triangles, H3.2. Open symbols, total protein; closed symbols [^{3}H]methyl label. Note that although there is a progressive decrease in total methylated H3.1 and H3.2 across the gradient, the specific activity of methylated histone H3.1 is increased in fractions 7–11.

histones (Figure 2). It has been previously shown that nonhistone proteins can contaminate histones prepared from chromatin cross-linked in the presence of Lomant's reagent [dithiobis(succinimidyl propionate)] (Jackson, 1987). As noted previously, an association between methylation and bulk acetylation was present. In the experiment shown in Figure 5, 25 mM sodium butyrate was added during the [*methyl*-^{3}H]methionine preincubation and labeling incubations. This treatment leads to an increase in bulk histone acetylation of the slow-turnover variety (Covault & Chalkley, 1980; Brotherton et al., 1981). Repetition of these studies in the absence of butyrate resulted in a decrease in higher acetylated forms, but did not alter the conclusions drawn from experiments done in the presence of butyrate.

Discussion

The chief findings in this paper are the following. First, core histones H3 and H4 are postsynthetically modified by methylation in K562 cells. Second, methylation of core histones appears to be relatively stable in K562 cells. Previous studies have suggested that methylation is a stable postsynthetic modification in developing trout spermatocytes (Honda et al., 1975) and avian reticulocytes (Hendzel & Davie, 1989) but displays cell cycle associated turnover in cultured mammalian cells (Shepherd et al., 1971). In our study, cells were incubated at high density when contact-inhibition of cell growth would be expected to occur. Therefore, we cannot determine whether progression through a normal growth cycle would have altered the apparent stability of the postsynthetic histone methylation in these cells. Third, modification by methylation is associated with histones modified by acetylation. Fourth, these modifications are in general not enriched in chromatin that contains either transcriptionally compentent or engaged genes. However, methylated histone H3.1, in addition to several other as yet unidentified methylated proteins, may be enriched in transcriptionally engaged chromatin. The significance of this finding is at present unclear as HMG 17 bound chromatin documented to 3–4-fold enriched in transcriptionally competent genes was not enriched in any methylated histone species, including methylated histone H3.1. In addition, a marked association between core histone methylation and acetylation was noted. The form of acetylation detected in these experiments was the type characterized by slow turnover. This form of acetylation occurs on up to 80% of the total nuclear core histones (Covault & Chalkley, 1980) and is not associated exclusively with transcriptionally active chromatin (Perry & Chalkley, 1982; Brotherton & Ginder, 1986; Ip et al., 1988).

The observation contained in this paper of a lack of association between methylation of histones H3.2 and H4 and transcirptional activity is at odds with studies on histone methylation in avian reticulocytes reported previously (Hendzel & Davie, 1989). What is the cause for this difference? The most likely explanation lies in the fact that avian adult reticulocytes are, in many respects, terminally differentiated cells despite retaining some low-level capacity for transcription of adult globin genes (and a few other genes as well). This problem is well illustrated by studies on histone acetylation in these cells, which have revealed that the bulk of histones are frozen in a state of modification and do not undergo the dynamic, steady-state modification typical of other cells (Brotherton et al., 1981). Alterations in bulk histone modification, easily detected in most cell lines cultured in the presence of butyrate, cannot be detected in avian reticulocytes. Although acetate label can be incorporated into histones in these cells, only a very minor fraction of total histones is able

to be so labeled, and detection is possible chiefly because endogenous pools of acetyl-CoA are so low (Brotherton et al., 1981). Although an association between histone acetylation and transcription has been repeatedly shown using avian reticulocytes (Ferenz & Nelson, 1985; Brotherton et al., 1986; Hebbes et al., 1988), this association may be an anomaly isolated to reticulocytes and due to the terminally differentiated state of these cells (Brotherton et al., 1986). Therefore, any report of an association between histone methylation and active transcription in avian reticulocyte nuclei may be difficult to interpret, and may be especially so when an association between methylation and bulk (i.e., slow-turnover) acetylation is also found (Hendzel & Davie, 1989). A further problem with some studies that have reported an association between transcription and histone methylation is that salt elution was used as the sole method of chromatin fractionation. It has been well documented that nucleosomes that contain hyperacetylated histones are more soluble in low-salt buffers than nucleosomes with histones that have less acetate modification and that this selective solubility is independent of the transcriptional activity of the DNA contained in the nucleosomes (Perry & Chalkley, 1982). Thus, chromatin isolated on the basis of increased solubility in the absence of salt may be enriched for both transcriptionally active chromatin and hyperacetylated chromatin, but these may be distinct populations within the solubilized chromatin. This possibility is perhaps established by our observation that the S_{150} fraction of reticulocyte chromatin is enriched in active genes but the same fraction of K562 chromatin is not; yet, the most actively methylated histones are found in this fraction of chromatin from either cell type.

What is the function of core histone modification by methylation? Unfortunately, a satisfactory answer cannot yet be given to this question. As a strong correlation exists between the slow-turnover form of histone acetylation and methylation, it may be that these processes share some common purpose. It has been proposed that the slow-turnover form of dynamic acetylation functions in the scanning of DNA interactions (Perry & Chalkley, 1982). In support of this hypothesis, it has recently been shown that DNA repair synthesis is enhanced in chromatin containing hyperacetylated nucleosomes (Ramanathan & Smerdon, 1989). Like acetylation, methylation decreases the ionic attractions between DNA and core histones by reducing the relative positive charge on the ϵ-nitrogen of the modified lysines (Shepherd et al., 1971). Therefore, it is entirely possible that these two modifications occur in concert to relax nucleosomes, and allow greater access to DNA by other proteins, such as enzymes involved in repair. However, at odds with this hypothesis is the observation of cell cycle specific patterns of methylation in synchronized mammalian cells (Shepherd et al., 1971). These patterns have been interpreted to suggest that methylation may somehow function in chromatin condensation during mitosis (Shepherd et al., 1971; Honda et al., 1975). Clearly, further work will be necessary to define the function of core histone modification by methylation. The results in this paper suggest that K562 cells are a suitable model system for this work.

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