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Modulation in Proportions of H1 Histone Subfractions by Differential Changes in Synthesis and Turnover during Butyrate Treatment of Neuroblastoma Cells†

Jeffrey M. Hall and R. David Cole*
Department of Biochemistry, University of California, Berkeley, California 94720
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ABSTRACT: Mouse neuroblastoma cells treated with millimolar concentrations of butyrate adjusted their recipe of histone H1 subfractions over the course of several days, eventually attaining an enrichment of about 5-fold in $H1^0$. This adjustment in the proportions of the H1's, which was essentially complete by 4 days, was brought about by changes in synthesis and turnover that were different for each of the three H1 subfractions. As the cells stopped dividing, the synthesis of all histones slowed substantially, but core histones were affected more than the H1's. Transiently, therefore, there was an overproduction of H1's relative to core histones, but the excess H1 was eventually removed by turnover. The very slow turnover of $H1^0$ and the rapid turnover of H1c were not substantially affected by butyrate treatment, but the turnover of H1ab was greatly accelerated by butyrate. Acetylation of the core histones was not necessary for maintenance of elevated $H1^0$ levels in the nondividing cells, although we did not rule out its involvement in the initial accumulation of $H1^0$.

The relative proportions of histone H1 subtypes vary according to the state of differentiation of the tissue from which the histones are derived [for review, see Cole (1984)]. In earlier work aimed at understanding how the recipe for H1 subtypes is regulated, we showed that the H1 subtypes differed among themselves in the responsiveness of their synthesis to hormones (Stellwagen & Cole, 1969; Hohmann & Cole, 1971), and there were analogous studies on shifting patterns of synthesis of H1 variants in developing sea urchins (Ruderman et al., 1974). The former experiments, by relating H1 synthesis to core histone synthesis, indicated that H1 synthesis was not strictly coordinated with DNA replication [see also Gurley & Hardin (1969)], and the latter study, by including measurements of mRNA (Ruderman et al., 1974), showed that at least some regulation of the recipe of H1 subtypes occurs pretranslationally. More recently (Pehrson & Cole,

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1982) we used cultured neuroblastoma cells to demonstrate that H1 subtypes are synthesized and turn over in the absence of DNA replication, and this extended the earlier studies by revealing differences among the H1 subfractions in turnover as well as in synthesis. Furthermore, the patterns of synthesis and turnover were different when rapidly growing neuroblastoma cells were compared to cells that had been deprived of serum for 4 days in order to stop cell division and induce differentiation; the differences in patterns of synthesis and turnover were reflected in the change of the proportions of H1 subfractions accumulated in the chromatin. The sum of these observations is that the recipe of H1 variants is regulated according to the state of differentiation, during DNA replication and in the absence of replication, and the regulation involves differential responses in mRNA production and in both synthesis and turnover of the H1's.

Although our recent studies showed a difference in the pattern of synthesis and turnover for each H1 subfraction, comparing rapidly dividing cells to nondividing ones, the sequence of events was not clear in the transition from one state to the other. Neither was it established that neuroblastoma cells deprived of serum for 4 days had adjusted their H1 histone patterns to the ultimate steady state. The present work was undertaken to learn such details. Among other things, the picture of transitional adjustments that emerged provided a better appreciation of the relative importance of synthesis and turnover in the regulation of H1 subtype recipes. During the transition a temporary overburden of H1 was deposited on the chromatin before a steady state was reached, and the role of turnover in the control of H1 subfraction recipes was given considerable emphasis.

Experimental Procedures

Cell Culture. Mouse neuroblastoma cells N-155 (from Marc Kirschner's laboratory, University of California, San Francisco) were grown in 100 × 20 mm tissue culture dishes at 37 °C with 10% CO_2 in DMEM[1] supplemented with glutamine (2 mM) and 10% fetal calf serum. For the inhibitor studies reported in this paper this medium was further supplemented with 500 mg/mL (5 mM) sodium butyrate or 2 mL/100 mL (250 mM) Me_2SO.

Labeling of Histones. For the metabolic studies reported in this paper, the medium was removed from the plate and the cells were washed once with phosphate-buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4). Three milliliters of lysine-free DMEM supplemented with either 10 μCi of L-[^{14}C]lysine (ICN) or 100 μCi of L-[^{3}H]lysine (ICN), plus 10% fetal calf serum and 2 mM glutamine, was added, and the cells were incubated at 37 °C for the pulse time period.

Histone Extraction. The cell membranes were lysed on each plate of cells by a 3-min incubation at room temperature in 10 mL of buffer A (0.25 M sucrose, 50 mM Tris, 25 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.5) plus 0.1% Triton X-100. The nuclei were pelleted by a 5-min centrifugation at 1000*g* and then suspended in 1 mL of either 0.4 N H_2SO_4 (for preparation of total histone) or 0.25 mL of 5% PCA (for preparation of H1's and $H1^0$). Following an overnight incubation at 4 °C, cell debris was pelleted by a 10-min centrifugation at 12000*g*, and the supernatant was precipitated by 20% TCA. The precipitate was washed once with 100% ethanol and air-dried.

[1] Abbreviations: DMEM, Dulbecco's modified Eagle's medium; Me_2SO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; TCA, trichloroacetic acid; PCA, perchloric acid.

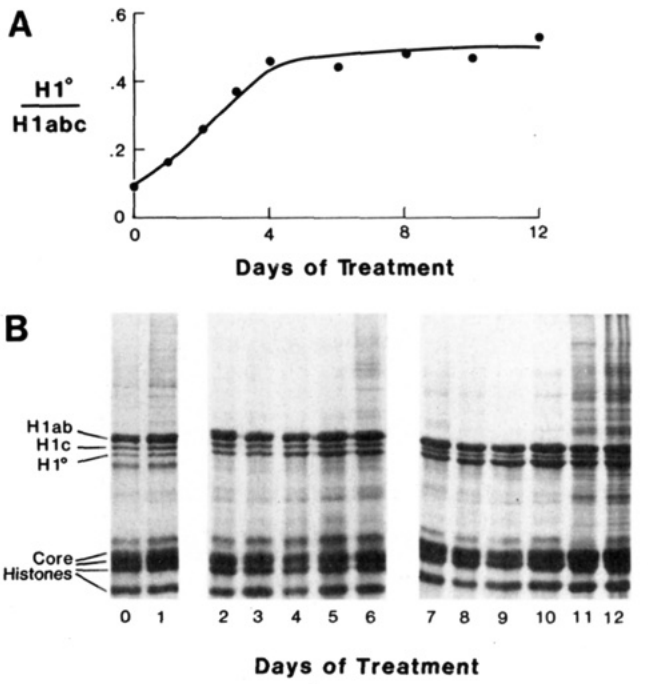

FIGURE 1: Accumulation of $H1^0$ relative to the other H1 histones during treatment with butyrate. Mouse neuroblastoma cells were grown in media containing 5 mM butyrate for up to 12 days. On each day of the treatment one 100-mm plate of cells was analyzed for the ratio of $H1^0$ relative to the other H1 histones (H1a, H1b, and H1c). (A) Graphical analysis of the $H1^0$/H1abc ratio during 12 days of butyrate treatment. (B) 12.5% SDS gel of histone proteins extracted from cells on each day of the 12-day treatment.

Gel Electrophoresis and Analysis. SDS–polyacrylamide gels were run according to the method of Laemmli (1970). These gels had either a 12.5% or 15% acrylamide separating gel. The gels were stained and destained as previously described (Pehrson & Cole, 1982) and scanned on a Kratos SD3000 xenon lamp spectrodensitometer with a Hewlett-Packard 3380A integrator attached. Care was taken to load gels so that the stain intensity was in the linear response range, which was determined in preliminary experiments. Autoradiograms were made by drying these gels and exposing them to Kodak X-Omat XAR-5 film. Alternatively, the gel bands were excised from the gel, dissolved in 1 mL of 30% H_2O_2 by heating at 75 °C for 4 h, mixed with 10 mL of Aquasol scintillation fluid (Amersham), and then counted. Since all H1 histones have about the same lysine content (30 mol %), no correction was made in comparing variants. Specific activities were then calculated from the radioactivity (CPM) of the gel band divided by its stain value (arbitrary units) from the densitometer.

Acetic acid/urea gels used to resolve the core histone acetylation were run according to the method of Hurley (1977), with the following modifications: 11 × 14 cm gels were poured with 7.5 g of urea, 7.5 mL of H_2O, 1.1 mL of acetic acid, 15 mg of thiourea, 100 μL of H_2O_2 (30%), and 5 mL of a stock acrylamide/bis(acrylamide) (64%/4%) solution. Gels were loaded and run in 5% acetic acid for 5 h at 20 mA.

Results

Butyrate Increases $H1^0$ Levels. Butyrate treatment results in a transient overaccumulation of H1 histone and an eventual large increase in $H1^0$. When NIE-115 cells were grown for 12 days in media containing 5 mM butyrate, the amount of $H1^0$ relative to the other three H1 histones increased with time (Figure 1). There was a period of rapid increase in the $H1^0$/H1abc ratio during the first 4 days of treatment to a level

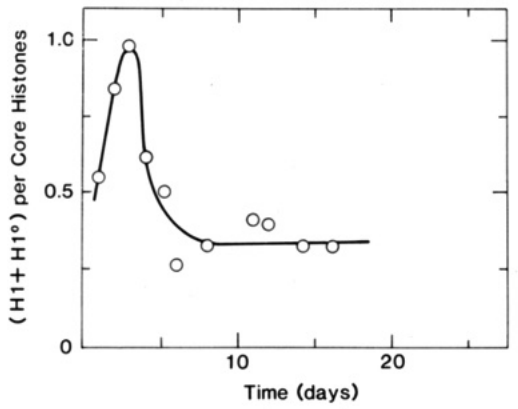

FIGURE 2: Changes in the ratio of H1abc^0 to core histones during a 16-day butyrate treatment. The amounts of the four H1 subfractions and the four core histones were measured by scanning densitometry of Coomassie Blue stained gel lanes containing 0.4 N H_2SO_4 extracts of proteins from cells sampled during treatment with 5 mM butyrate. The H1abc^0/core histone ratio is based on stain and does not represent the molar amounts of those proteins present.

that was maintained through the remaining 8 days of the experiment. Such a change in the H1^0/H1abc ratio could have been the result of either an increase in H1^0 or a decrease in the other H1's. Analysis of the various H1's relative to the core histones at the end of a 7-day butyrate treatment showed that H1^0 had increased relative to the core histones, while the ratios for the other H1's were the same as they were at the start of the butyrate treatment (Table I). The increase in the H1^0/H1abc ratio is therefore an increase in the amount of H1^0, not a decrease in the other H1 subfractions. A surprising observation was made when the H1/core histone ratio was measured at 1-day intervals during 16 days of butyrate treatment. The H1/core histone ratio almost doubled in the first 2 days of treatment and then decreased to a level slightly higher than that observed before treatment (Figure 2). As shown below, the transient peak in the accumulation of H1 histone was the result of an inhibition of core histone synthesis that was more abrupt than the inhibition of H1 synthesis.

Synthesis of H1 Variants Is Differentially Regulated To Increase the Level of H1^0. To determine how cells brought about an increase in H1^0 on their chromatin in response to butyrate treatment, we studied rates of synthesis of the individual H1 histones. Cells were pulse-labeled for 3 h with [^{14}C]lysine (3.3 μCi/mL) during the first 24 h of butyrate treatment. The histones were isolated from the cells after the pulse and analyzed by SDS–polyacrylamide gel electrophoresis followed by autoradiography. The four components recognized by two-dimensional electrophoresis (Pehrson & Cole, 1982) were analyzed by a one-dimensional system as H1ab, H1c, and H1^0, as was done previously (Pehrson & Cole, 1982). An autoradiogram (Figure 3A) shows that the synthesis pattern of the H1 histones during this period shifted to favor the synthesis of H1^0. As shown in Figure 4, the most dramatic shift in relative synthesis among the H1 subfractions occurred during the first 24 h of butyrate treatment. An increasing proportion of the H1 synthesis was due to H1^0 and H1c, and consequently a corresponding decrease on the part of H1 synthesis was due to H1ab. During the remainder of the 7-day time course the percent incorporation into H1c remained elevated while those of H1ab and H1^0 tended toward their original levels.

Table I: Changes in the H1/Core Ratio for the H1 Subfractions in Neuroblastoma Cells after a 1-Week Treatment with Butyrate[a]

treatment	H1ab/core	H1c/core	H1^0/core	H1abc^0/core
rapidly growing cells (untreated)	0.13 ± 0.06	0.06 ± 0.02	0.02 ± 0.01	0.21 ± 0.09
7-day butyrate cells	0.12 ± 0.03	0.04 ± 0.01	0.08 ± 0.02	0.24 ± 0.06

[a] Values in this table are expressed as ratios of the areas of stained gel bands as measured by densitometry ± average deviations.

To analyze the change in synthesis of the H1 subfractions in more detail, we measured specific activities in the incorporation of [^{3}H]lysine into each H1 subfraction during the first 24 h of butyrate treatment. Cells were pulsed with [^{3}H]lysine (30 μCi/mL) for 3 h at various times during butyrate treatment; after the pulse the histones were isolated and analyzed on a 12.5% SDS gel by staining and scanning to measure protein levels. The bands were excised from the gel, and radioactivities were measured. The radioactivity per unit of stain was used as a measure of the specific activity. As seen in Figure 5, the specific activity of all three H1 subfractions

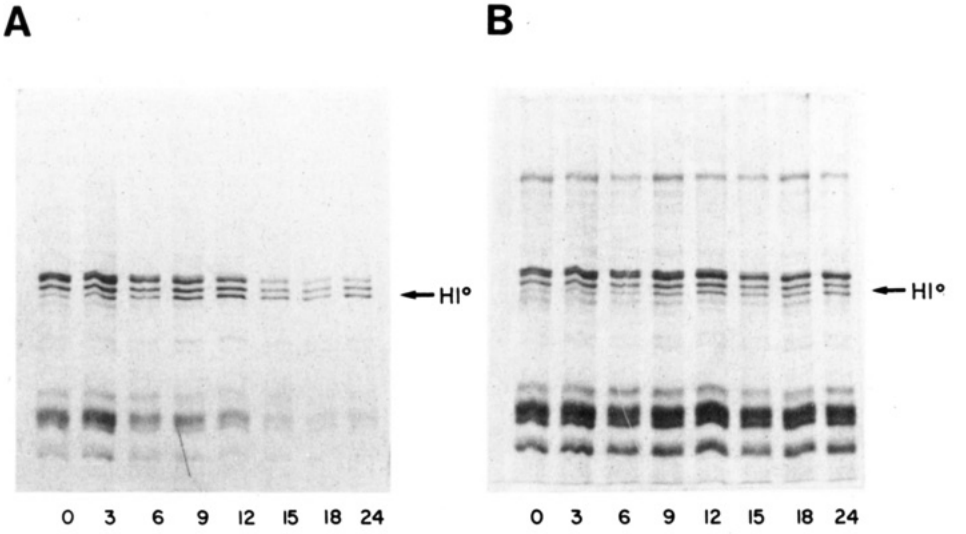

FIGURE 3: 12.5% SDS gel and autoradiogram of [^{14}C]lysine-labeled histones from cells pulsed at various times during the first 24 h of butyrate treatment. Cells were labeled with [^{14}C]lysine (3.3 μCi/mL) at various times during the first 24 h of butyrate treatment. The period of labeling started at the time indicated and lasted for 3 h. At the end of the 3-h pulse the histones were isolated and electrophoresed on a 12.5% polyacrylamide–SDS gel, which was stained with Coomassie Blue and autoradiographed. (A) Autoradiogram of the polyacrylamide gel in panel B. (B) 12.5% Coomassie Blue stained 12.5% polyacrylamide–SDS gel.

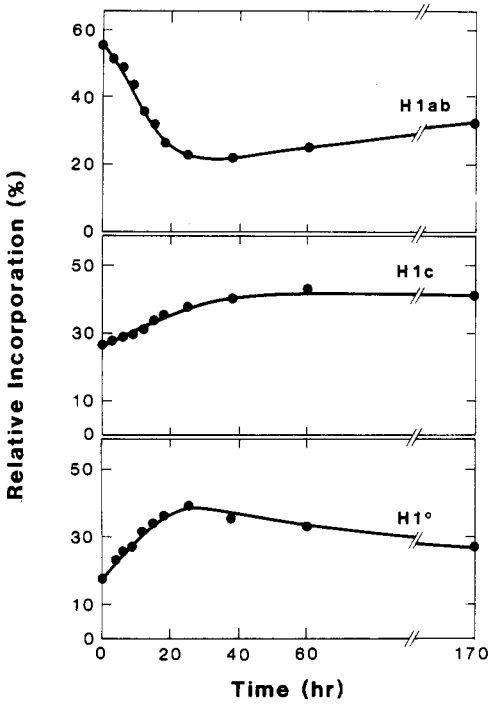

FIGURE 4: Relative incorporation of [^{14}C]lysine into the H1 subfractions at various times during butyrate treatment. Cells were labeled with [^{14}C]lysine at various points during butyrate treatment, and the histones were extracted and run on a 12.5% polyacrylamide–SDS gel. The gel was autoradiographed, and the H1 region of the autoradiogram was scanned. The percent of the total cpm in the H1 region represented by each of the H1 subfractions was plotted as a function of time in butyrate. Top, H1ab; middle, H1c; bottom, H1^0.

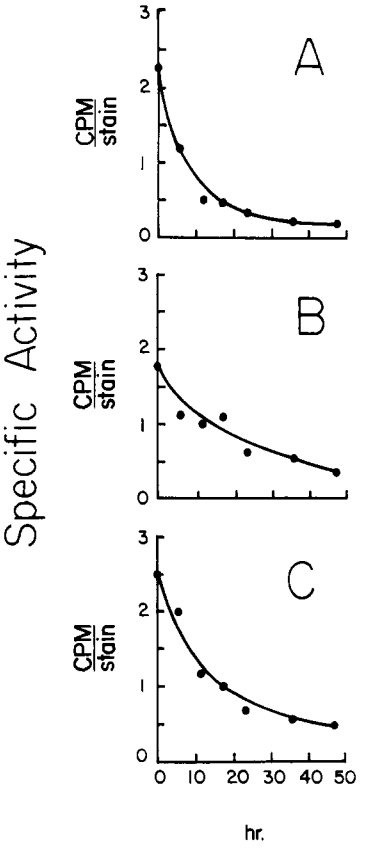

FIGURE 5: Specific activity of the H1 subfractions during the first 50 h of butyrate treatment. Cells were given a 3-h pulse of [^{3}H]lysine (30 μCi/mL) at various times during butyrate treatment. Histones were isolated and analyzed as described under Experimental Procedures. (A) H1ab; (B) H1c; (C) H1^0.

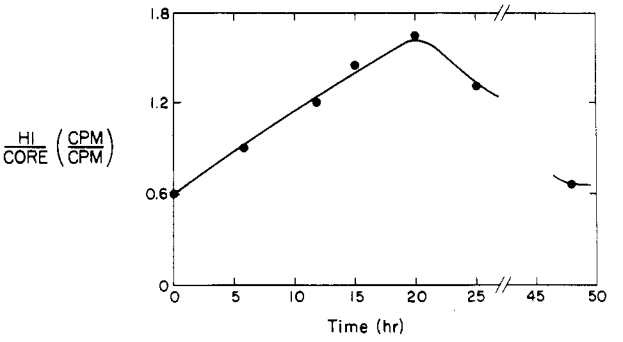

FIGURE 6: Ratio of counts incorporated into the H1's and the core histones during butyrate treatment. Cells were given a 3-h pulse of [^{3}H]lysine (30 μCi/mL) at various times during butyrate treatment. Histones were isolated and analyzed.

declined during the first 24 h of butyrate treatment. This was expected, since butyrate is known to inhibit DNA synthesis and the synthesis of H1 histones has been shown to be coupled to DNA synthesis to a large extent (Robbins & Borun, 1967; Plumb et al., 1984). However, the H1 histone subfractions decreased their synthesis to different extents with butyrate treatment. The synthesis of the H1ab subfraction decreased the most, with a 90% decrease, while H1c and H1^0 synthesis decreased only about 75%.

The incorporation of label into the core histones was monitored during an extended period of butyrate treatment. Figure 6 shows that the incorporation of [^{14}C]lysine into the H1 histones relative to that into the core histones increased almost 3-fold during the first 20 h of butyrate treatment and then declined to the levels found in rapidly growing cells by 48 h of treatment. This indicates that the decline in synthesis was greater for the core histones than for the H1's during the first 20 h of butyrate treatment. Evidently the synthesis of the core histones is more tightly coupled to DNA synthesis than that of the H1's. It may be that during the change in the recipe of H1 subtypes in a nondividing cell, H1 synthesis initially declines less than that of the core histones in order to provide a pool of excess H1 that can be manipulated to reapportion the H1 subtypes.

Preferential Stability of H1^0 toward Turnover Is a Major Factor in H1 Accumulation. That selective turnover played a role in the increase of H1^0 deposited on the chromatin was demonstrated in pulse–chase experiments. Cells were pulsed for 3 h with [^{14}C]lysine on the first day of butyrate treatment and then chased for 3 days in media containing nonradioactive lysine. The fraction of the total H1 radioactivity accounted for by H1^0 increased from 40% in the day 1 pulse to 70% after a 3-day chase (Figure 7, right panel). To further demonstrate the preferential stability of H1^0 relative to that of the other H1's, cells were grown in the presence of [^{3}H]lysine (30 μCi/mL) for 24 h prior to beginning butyrate treatment and then kept in labeled media for the first 24 h of butyrate treatment. After this long pulse in which a majority of the histones should have been labeled, the cells were chased for 3 days in unlabeled media. Cells were harvested and analyzed on each successive day of the chase. As seen in Figure 7, left panel, H1^0 had a much longer half-life (360 h) than did H1ab (90 h) or H1c (42 h). Previously we reported (Pehrson & Cole, 1982) that in rapidly dividing neuroblastoma H1c had a half-life of about 2 days, but H1ab as well as H1^0 showed negligible turnover in 2 days.

Hyperacetylation Induced by Butyrate Is Unnecessary for Maintenance of H1^0. One of the best characterized effects of millimolar concentrations of butyrate is the acetylation of

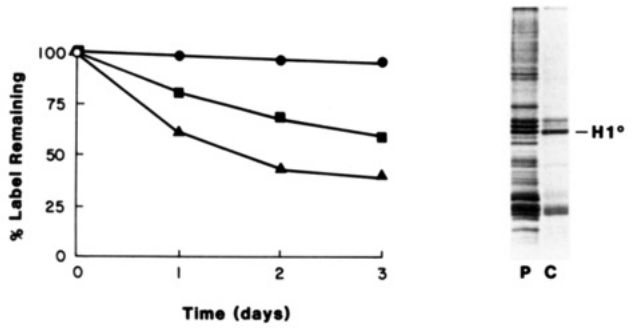

FIGURE 7: Pulse labeling of the H1 histones followed by a 3-day chase during butyrate treatment. Left: Cells were pulsed with [^{3}H]lysine (30 μCi/mL) for 48 h (24 h in normal media and 24 h in butyrate-containing media) and then chased for 3 days in media containing unlabeled lysine and butyrate. Samples were taken at the end of the pulse and at 1-day intervals during the chase. The total cpm in the H1's was broken down as the percent cpm in each subfraction and plotted as a function of the chase interval. ▲, H1ab; ■, H1c, ●, H1^0. Right: Cells were pulsed for 3 h with [^{14}C]lysine (3.3 μCi/mL) after 24 h in butyrate and then chased for 3 more days in media containing unlabeled lysine. Histones were isolated and run on a 12.5% polyacrylamide–SDS gel, and the gel was autoradiographed. P, autoradiogram of gel from cells pulsed for 3 h after 24 h in butyrate. C, autoradiogram of gel from cells pulsed as in P and then chased for 3 days in media containing unlabeled lysine.

the core histones (Sealy & Chalkley, 1978; Candido et al., 1978). A connection between H1^0 accumulation and core histone acetylation seemed possible, and two experimental approaches were taken to test this possibility. In both approaches, acetylation was analyzed by electrophoresis of histones in gels that resolve H4 into the unacetylated and mono-, di-, tri-, and tetraacetylated forms of H4, referred to as H4^0, H4^1, H4^2, H4^3, and H4^4, respectively. It was shown by Covault et al. (1982) that acetylation of all of the core histones proceeds in parallel and that H4 acetylation alone is a convenient marker with which to monitor overall core histone acetylation. Rapidly growing neuroblastoma cells had only H4^0 and H4^1. Increases in the concentration of butyrate in 4-day treatments produced increases in the more highly acetylated forms of H4, and those increases were correlated with increases in H1^0 levels (Figure 8). This correlation would seem to support the possibility that increases in acetylation of core histones and H1^0 accumulation may be triggered by the same concentration-dependent mechanism. A second experimental approach, however, demonstrated that acetylation was not required for maintenance of high H1^0 levels on the chromatin. Cells were grown in butyrate for 2 days to achieve elevated levels of H1^0 and highly acetylated core histones. Cells were then switched to media containing 2% Me_2SO as the blocking agent for another 6 days. Me_2SO does not cause acetylation, but it does block the cells from dividing and it does maintain elevated H1^0 levels (data not shown). Although H1^0 levels remained elevated in the cells that were switched to Me_2SO, the acetylation levels dropped to those of rapidly growing cells within 2 days of the switch (Figure 9). This demonstrated that acetylation was not required for maintenance of elevated levels of H1^0 on the chromatin; although it seems unlikely, it remains possible that the initial induction of H1^0 accumulation was directly related to the acetylation of the core histones, even though maintenance of H1^0 levels was not.

Discussion

In these studies on the alteration of H1 subfraction recipes during the differentiation of neuroblastoma cells, all subfractions changed their proportions, but the most dramatic

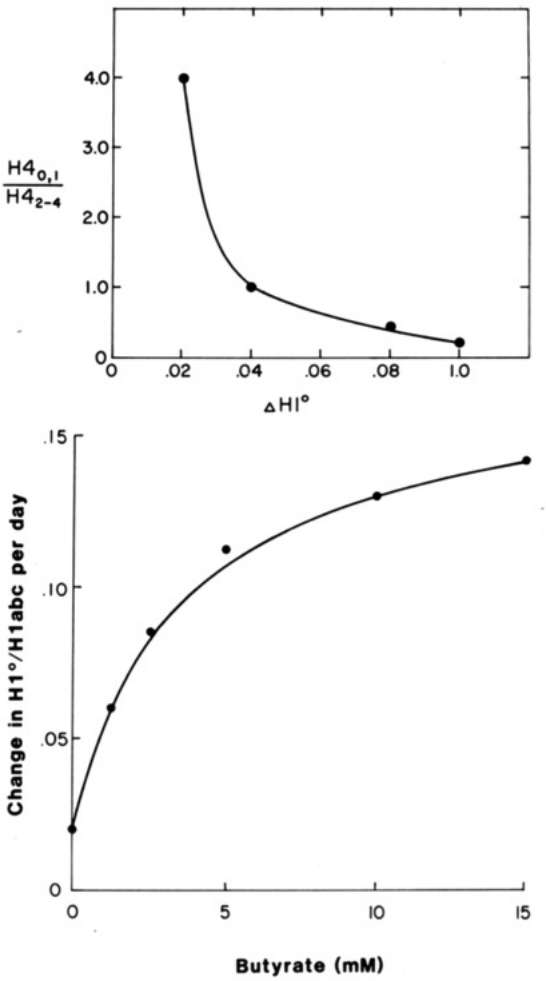

FIGURE 8: Decrease in unacetylated and monoacetylated forms of H4 as a function of the increase of H1^0 at different butyrate concentrations. Top: The amounts of unacetylated (H4$_0$), monoacetylated (H4$_1$), diacetylated (H4$_2$), triacetylated (H4$_3$), and tetracetylated (H4$_4$) histone H4 were measured from cells treated for 24 h with various butyrate concentrations and plotted as a function of the increase in H1^0 relative to H1abc (bottom panel) for the same butyrate concentrations. The lowest point for ΔH1^0 represents cells blocked from dividing by serum deprivation, which results in some increase in H1^0 but no increase in acetylation over that found in rapidly growing cells. Bottom: The increase in H1^0 relative to H1abc during the first 4 days of treatment was measured and plotted as a function of butyrate concentration.

change was in H1^0. The H1 histone subfraction H1^0 first attracted attention due to its prominence in nondividing tissues (Panyim & Chalkley, 1969). Studies on the levels of H1^0 during tissue regeneration confirmed an inverse relationship between H1^0 levels and mitotic index (Varricchio et al., 1977; Benjamin, 1971). Tissue-culture cell systems allowed more sensitive analysis of changes in H1^0 levels in cell lines such as Friend (Keppel et al., 1977), CHO (D'Anna et al., 1982), and HeLa (Pehrson & Cole, 1980) and in all cases demonstrated that H1^0 levels can be increased by blocking cell division. Butyrate was shown in several of the cell-culture systems to increase H1^0 levels, and in fact in the CHO line H1^0 was first identified as BEP (butyrate-enhanced protein) until identification as H1^0 was made (D'Anna et al., 1980b). Knowing that H1^0 would show a substantial response, we chose to use butyrate to induce differentiation in neuroblastoma cells.

The present study showed that the adjustment in H1^0 accumulation to higher levels was complete in about 4 days at 5 mM butyrate and was sustained until at least the 12th day. This was a considerably longer period than that studied by

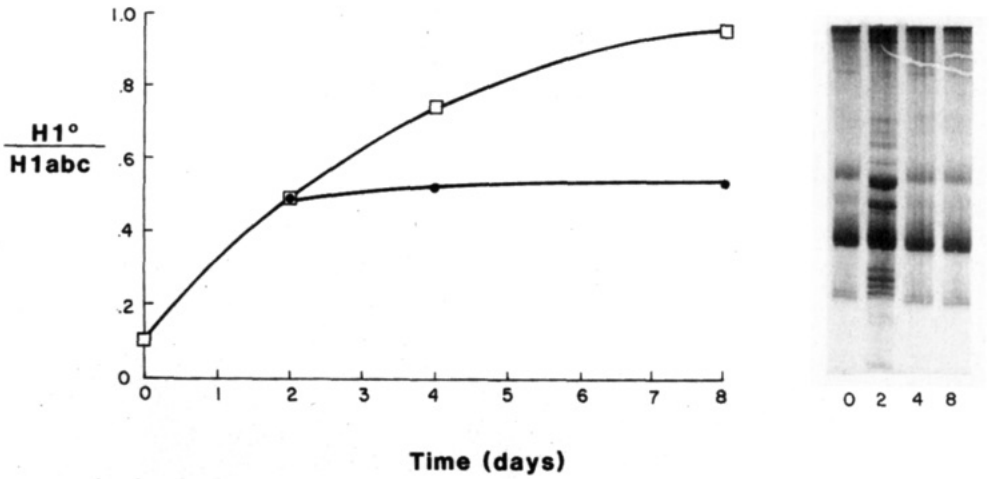

FIGURE 9: Cells switched from butyrate-containing medium to Me_2SO-containing medium lose their hyperacetylation but retain elevated $H1^0$ levels. Cells were grown in 5 mM butyrate for 2 days and then either maintained in this medium for another 6 days or switched to media containing 2% Me_2SO as the blocking agent for the remaining 6 days. Cells were harvested at 0, 2, 4, and 8 days in each case, and the histones were isolated and analyzed for their $H1^0$/H1abc ratio and for hyperacetylation of histone H4. Left: graph of $H1^0$/H1abc ratio during these treatments. □, butyrate treatment; ●, Me_2SO treatment. Right: acetic acid/urea gel of histones from the switch treatment. 0, untreated cells; 2, 2 days of butyrate treatment; 4, 2 days of butyrate treatment followed by 2 days of Me_2SO treatment; 8, 2 days of butyrate treatment followed by 6 days of Me_2SO treatment.

other workers (Keppel et al., 1977; D'Anna et al., 1980a), and the $H1^0$ reached a higher level than that in previous work. The difference in $H1^0$ levels between this and earlier works is probably due to differences in cell types (Hall et al., 1985).

One possible way in which butyrate might have mediated $H1^0$ accumulation was the hyperacetylation of the core histones, which appears to be a universal effect of millimolar concentrations of butyrate (Sealy & Chalkley, 1978; Candido et al., 1978). The initial rate of accumulation of acetyl groups on the core histones in the neuroblastoma cells was dependent on butyrate concentration; thus the two parameters of $H1^0$ accumulation and core histone acetylation might have been interrelated, as cause and effect, or their changes might be the effects of a common cause. It was tempting to imagine that increased levels of $H1^0$ might represent a compensation for acetylation of the core histones, since acetylation would otherwise presumably loosen the general chromatin structure. However, since our experiments on switching cells from butyrate-containing media to media containing Me_2SO to maintain the block in the cell cycle showed that high $H1^0$ levels were maintained even in the absence of core histone hyperacetylation, if a relationship exists between hyperacetylation and $H1^0$ it must be manifested only in the initial accumulation and not in the maintenance of $H1^0$ on the chromatin.

Whatever does initiate the process, the mechanism for $H1^0$ accumulation includes changes in both synthesis and turnover of the H1 subfractions that favor the accumulation of $H1^0$ on the chromatin. Synthesis of all the H1 subfractions declined in the first 24 h of butyrate treatment, with a more drastic inhibition of H1ab synthesis than of either $H1^0$ or H1c synthesis. Turnover and changes in turnover rate during the first 4 days of butyrate treatment favored degradation of H1ab and H1c, with $H1^0$ being much less sensitive to turnover. $H1^0$ turns over very little, and H1c turns over rapidly ($t_{1/2} \sim 42$ h) whether cells are butyrate treated or not, but H1ab turnover is changed from a negligible rate in rapidly growing cells to a 90-h half-life by butyrate treatment. Thus the change in the recipe of H1 subfractions on the chromatin brought about by butyrate treatment involved selective changes in both synthesis and turnover that result in a large (ca. 5-fold) increase in $H1^0$ accumulation and a modest (30%) increase in H1c.

Finally, during the initial period of treatment with butyrate the synthesis of the core histones was shut off more abruptly than the synthesis of the H1 histones. This resulted in a net overproduction of H1 histone and a transient increase in H1 subfractions on the chromatin. The original ratio of H1/core histone synthesis was eventually reestablished approximately, but the period of transition demonstrated that the chromatin of a cell can tolerate an excess of H1, at least over a short period. The H1/core ratio can vary among different cells, and in the case of the chicken red blood cell, the overall levels of H1 + H5 (H5 is a variant of H1) may reach 1.3 molecules per nucleosome (Bates & Thomas, 1981). Initially, butyrate seems to uncouple H1 and core histone synthesis, and the possibility remains that this may bring about the adjustment in H1 subfraction levels more rapidly.

In conclusion, the accumulation of $H1^0$ on the chromatin of butyrate-treated cells and the change in proportions of H1ab and H1c were brought about by selective changes in both synthesis and turnover of all the H1 subfractions. The cellular determination of H1 histone subfraction levels was thus a complex phenomenon in which each of the three H1 fractions analyzed displayed a different behavior. Enrichment of chromatin with $H1^0$ and H1c was first brought about by the inhibition of synthesis of core histones and H1ab more abruptly than that of $H1^0$ and H1c. The accumulation of $H1^0$ was further favored by its slow turnover and the rapid turnover of H1c, while the latter was favored over H1ab by a change in the turnover of H1ab from slow to fast when the cells stopped dividing. Zweidler (1980) has proposed an attractive classification of H1 variants based on biological functions related to chromatin dynamics. In addition to H1 subtypes linked to DNA replication, Zweidler proposed that there are three sets of histone variants independent of replication: a set for H1 replacement, a set of meiosis (particularly during spermatogenesis), and a set used in oogenesis to accumulate a reserve. Lennox (1984) has classified H1 subtypes further according to evolutionary stability. It becomes increasingly clear that the differential regulation of these classes of H1 variants is very complicated. Not only do the H1 subtypes respond differentially to hormones (Hohmann & Cole, 1971; Gjerset et al., 1982) and the kinds of treatments described in this report, but the mechanisms of response involve different degrees of coupling to DNA replication and selective changes in turnover as well as synthesis.

Registry No. Butyrate, 107-92-6.

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A Nonuniform Distribution of Excision Repair Synthesis in Nucleosome Core DNA†

Suey Y. Lan and Michael J. Smerdon*

Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4660

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ABSTRACT: We have investigated the distribution in nucleosome core DNA of nucleotides incorporated by excision repair synthesis occurring immediately after UV irradiation in human cells. We show that the differences previously observed for whole nuclei between the DNase I digestion profiles of repaired DNA (following its refolding into a nucleosome structure) and bulk DNA are obtained for isolated nucleosome core particles. Analysis of the differences obtained indicates that they could reflect a significant difference in the level of repair-incorporated nucleotides at different sites within the core DNA region. To test this possibility directly, we have used exonuclease III digestion of very homogeneous sized core particle DNA to "map" the distribution of repair synthesis in these regions. Our results indicate that in a significant fraction of the nucleosomes the 5′ and 3′ ends of the core DNA are markedly enhanced in repair-incorporated nucleotides relative to the central region of the core particle. A best fit analysis indicates that a good approximation of the data is obtained for a distribution where the core DNA is uniformly labeled from the 5′ end to position 62 and from position 114 to the 3′ end, with the 52-base central region being devoid of repair-incorporated nucleotides. This distribution accounts for all of the quantitative differences observed previously between repaired DNA and bulk DNA following the rapid phase of nucleosome rearrangement when it is assumed that linker DNA and the core DNA ends are repaired with equal efficiency and the nucleosome structure of newly repaired DNA is identical with that of bulk chromatin. Furthermore, the 52-base central region that is devoid of repair synthesis contains the lowest frequency cutting sites for DNase I in vitro, as well as the only "internal" locations where two (rather than one) histones interact with a 10-base segment of each DNA strand.

The process of nucleotide excision repair of DNA involves a "cut-and-patch" mechanism where a small region (containing the lesion) is excised and the resulting gap is filled by repair synthesis [see reviews by Lieberman (1976), Hanawalt (1977), Hanawalt et al. (1979), and Setlow (1980)]. Since the DNA in eukaryotes is organized into chromatin [for recent reviews on chromatin structure, see Cartwright et al. (1982), Igo-Kemenes et al. (1982), and Reeves (1984)], an integral feature of the repair process must be the interaction of repair enzymes with this tightly folded structure. Indeed, several different laboratories have now shown that, following damage of

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