Sodium Butyrate Induces New Gene Expression in Friend Erythroleukemic Cells*

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Twenty-four hours after treatment of Friend erythroleukemic cells with millimolar concentrations of n-butyrate, the chromatin histones (particularly the nucleosome core histones H3 and H4) have become hyperacetylated. During this same time period, nucleic acid hybridization studies indicate that the butyrate-treated Friend cells accumulate a population of about 38% new RNA transcripts synthesized from unique sequences of mouse DNA. These butyrate-induced RNA transcripts are not detectable in control, non-fatty acid-treated cells. Furthermore, two-dimensional gel electrophoretic analysis of the butyrate-treated cells also indicates that the fatty acid-induced cells synthesize many new species of proteins that are not detectable in the control cells. These de novo changes in RNA and protein synthesis do not occur when other inducers of overt erythroid differentiation, such as dimethyl sulfoxide (which does not lead to histone hyperacetylation), are added to the Friend cells. Finally, all of these metabolic effects of short term treatment of cells with butyrate are readily reversible once the fatty acid is removed.

This remarkable coordinate effect of butyrate on the induction of histone hyperacetylation, the induction of new unique sequence gene RNA transcript accumulation, and the induction of new protein synthesis suggests that these three metabolic phenomena may be closely linked in Friend erythroleukemic cells. Furthermore, these findings are not inconsistent with the hypothesis that histone hyperacetylation (especially of H3 and H4 to the diacetylated and higher modified forms) may be a necessary, but obviously not sufficient, part of the biochemical mechanisms leading to new genomic expression in these cells.

Among the many documented effects of millimolar concentrations of sodium butyrate on mammalian cells growing in tissue culture is the induction of hyperacetylation of most of the histones found in the fundamental chromatin subunit, the nucleosome (1-3). For example, over 80% of histone H4 is converted to the mono-, di-, tri-, and tetraacetylated forms in a number of different types of cultured vertebrate cells after butyrate treatment (1-5). Furthermore, in all of these cell types, histone H3 also becomes hyperacetylated as do histones H2A and H2B in certain rat tissue culture cell lines (1). That these modified histones are actually present in chromatin nucleosomes has also been demonstrated (6). In addition, recent evidence clearly indicates that butyrate exerts its effect on histone hyperacetylation, both in vivo and in vitro, through the inhibition of the histone deacetylase enzymes rather than by a stimulation of the histone acetyltransferase enzymes (1, 4, 5, 7, 8). Perhaps of even more significance, it has been demonstrated that all of these effects of butyrate on histone acetylation are completely reversible once the short chain fatty acid has been removed from the cells after short term exposures (1, 2, 8).

Since a considerable body of indirect observational evidence seems to suggest that histone acetylation may play some important role in the control of chromatin transcriptional activity (for recent literature reviews, see Refs. 9 to 11) or chromatin assembly (12), or both, it was of some interest to investigate the nature of the genomic transcriptional responses of cells containing large quantities of hyperacetylated histones as a result of exposure to sodium butyrate.

In the present report, it is shown that in Friend murine erythroleukemic cells exposed for 24 h to n-butyrate, a complex but dramatic and characteristic series of changes occurs in both the types of de novo-synthesized proteins and in the types of unique sequence DNA transcripts found in the cells. Furthermore, it was found that other types of inducers of erythroid differentiation tested on these cells (for example, dimethyl sulfoxide which does not cause changes in normal histone acetylation patterns) do not result in the dramatic shifts in the types of newly synthesized molecules found in these cells after fatty acid treatment. Finally, it was found that all of these observed changes in synthesis patterns were readily reversible after removal of butyrate from the cells. Together, these results suggest that histone hyperacetylation may play an important necessary, but obviously not sufficient, role in the biochemical activation of new genomic expression in these mouse cells.

EXPERIMENTAL PROCEDURES

Cell Line and Culture Conditions—Friend erythroleukemic cells, line 745A (13), were a generous gift of Dr. David Kabat (University of Oregon Health Services Center, Portland, Oregon). The cells were maintained and passaged as previously described (1) in a medium containing 90% Dulbecco's Modified Eagle's Medium. 10% fetal calf serum, and 100 μg/ml each of sodium penicillin G and dihydrostreptomycin (DME/FCS medium). When required, butyric acid (neutralized with concentrated NaOH) was added to cultures to a final concentration of 5 mM. When cultures were labeled with L-[35S]-methionine (specific activity > 400 Ci/mM, New England Nuclear), cells in experimental cultures were harvested and transferred to "conditioned" DME/FCS medium containing the isotope at the desired concentrations. "Conditioned medium" is medium in which

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1 The abbreviations used are: DME/FCS medium, 90% Dulbecco's Modified Eagle's Medium, 10% fetal calf serum, and 100 μg/ml each of penicillin G and dihydrostreptomycin; SDS, sodium dodecyl sulfate.
logarithmic phase cells had been growing for 8 to 12 h before their subsequent removal (14). In all experiments, control and experimental (i.e. butyrate-treated) cell cultures were handled identically except for the presence of the short chain fatty acid in the medium of the latter.

As a standard experiment, exponentially growing cell cultures were divided into two lots, one sample acting as a control and the other being "induced" by addition of sodium butyrate. After 24 h of further growth, both the control and the induced cells were harvested and exposed to [35S]methionine-containing medium for various lengths of "pulse" time and with varying concentrations of isotope in the medium (depending on the experiment). For example, in dimensional experiments, the isotope concentration in the medium varied from 10 to 250 μCi/ml and the pulse times ranged from 30 min to 4 h. These various exposure conditions were performed in order to investigate possible variations in the rates of protein degradation and turnover in the cells under the experimental conditions employed (15–17), as explained under "Results". However, in each set of experiments described in the text, the control and butyrate-treated cells were labeled for the same lengths of time with the same concentrations of isotope, the electrophoretic gels in each case were loaded with equivalent amounts of radioactive proteins (with the same specific activities), and the autoradiographic exposures of the x-ray films applied to the film were identical (see below). The conclusions were the same in each case. These standardized procedures were employed so that direct comparisons could be made between the two-dimensional gels used for the analysis of the accumulation of newly synthesized proteins from the control and the butyrate-treated Friend cells (18, 19).

In butyrate-reversal experiments, cells that had been exposed to the fatty acid for 24 h were harvested and then transferred to fresh DME/FCS medium (without butyrate) and grown an additional 24 h before being used for further experimental analysis as described above.

Cell counts were made using a hemacytometer and cell viability was determined with trypan blue dye exclusion (14). Cells were periodically tested for possible mycoplasma contamination and consistently found to be negative.

Two-dimensional Gel Electrophoreses—Samples were prepared and electrophoresed as described by O'Farrell (18). The focusing time was 7500 V/h, and the second dimension's SDS gel contained 14.5% polyacrylamide at an acrylamide/bisacrylamide ratio of 100.1. Gels were fixed, stained, dried, and exposed to x-ray film (Kodak) as previously described (20). In most experiments, gels were exposed by the fluorographic method described by Laskey and Mills (21).

Comparisons between two-dimensional gel autoradiographs were always made at the same exposure settings of the x-ray films to ensure that both the major and minor protein spot patterns present in a given sample preparation were detected (22). Furthermore, gel protein spot pattern analyses on each autoradiograph were performed in three different ways: (1) often, gels were so topologically similar that the resulting autoradiographs could be directly superimposed on each other and the patterns between gels could be compared; (2) in other cases, slight topological variations between gels required the use of characteristic "reference constellations" of protein spots, along with various connecting coordinate lines, to normalize for the variations between gels (23); and (3) vinyl plastic transparents were marked with the appropriate autoradiographic spot patterns and then compared by visual inspection and by the use of polar coordinates around characteristic protein constellations. All three methods of analysis gave comparable results for both the control and the butyrate-induced patterns of proteins found in Friend cells.

Nucleic Acid Hybridizations—High specific activity, 3H-labeled, unique sequence gene DNA from exponentially growing Friend cells was isolated by the following procedures: actively growing cells were harvested and exposed to [3H]thymidine (specific activity, 80 to 100 Ci/mmol, New England Nuclear), the nuclei were isolated, and the [3H]DNA was extracted by described methods (24). The [3H]DNA was then treated with RNase A (Worthington, DNase-free) at 50 μg/ml (in 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl2) for 45 min at 30°C. The DNA solution was then precipitated with ethanol (100 μl/ml) and resuspended in a solution containing 1 mg/ml BSA, 100 μg/ml of proteinase K (Calbiochem), and 1 mM EDTA. The sample was digested for 1 h at 37°C, and the resulting mixture incubated at 37°C for 1 h. The solution was then deproteinized by repeated phenol/chloroform/isoamyl alcohol (25:25:1) extractions. The RNAse treatment was repeated to ensure complete removal of contaminating RNA and the final deproteinized [3H]DNA was precipitated by ethanol addition and collected by centrifugation (24).

Unique sequence [3H]DNA was isolated from the total purified DNA preparation by described hydroxyapatite chromatographic procedures (24, 25). Briefly, the [3H]DNA was dissolved in 0.12 M phosphate buffer, pH 7.0, and sheared by sonication to fragment lengths about 250 to 700 bases (24). The single-stranded [3H]DNA was recycled through this procedure a second time, chromatographed on a column of Sephadex G-25 with an underlying bed of Chelex-100 mixed with Dowex-50 (24) and finally collected by ethanol precipitation. This [3H]DNA single-stranded, Friend cell DNA corresponds to the in vivo, unique sequence gene DNA of mouse cells (26–29). The unique sequence DNA used in these studies had a specific activity of 2.3 x 106 cpm/μg.

Total cellular (nuclear ativ) RNA was isolated from either control or 24-h butyrate-treated cells by described procedures (30). The purified RNAs were chromatographed on a step column of Sephadex G-25/Chelex-100/Dowex-50 before being used in nucleic acid hybridization reactions as described above.

In hybridization reactions where total Friend cell RNA (from either control or butyrate-induced cells) was annealed to unique sequence [3H]DNA, the unlabeled RNA was normally at 20 μg/ml and the labeled mouse DNA at 1.5 μg/ml, giving an RNA/DNA ratio of 13,333:1. In additivity experiments, on the other hand, 10 μg/ml of total RNA from control cells was mixed with 10 μg/ml of total RNA from butyrate-induced cells and this combined RNA population annealed to 1.5 μg/ml of unique sequence [3H]DNA. For all reactions, the hybridization solution was 0.2 M phosphate buffer (pH 6.8), 1 mM EDTA, and 0.1% SDS.

In general, 50-μl aliquots of reaction mixtures were sealed in siliconized glass capillary tubes and heated to 100°C for 10 min prior to incubation at 65°C until desired Rd values for annealing were reached. Rd is the product of RNA concentration (in moles per liter of nucleotides) and time (in seconds). The hybridization reactions were terminated by diluting the contents of a capillary into 2 ml of 30 mM phosphate buffer (pH 6.8) at 4°C. Duplex RNA/[3H]DNA formation was monitored by standard hydroxyapatite chromatography procedures (24) followed by the digestion of the hydroxyapatite-retained hybrids with S1 nuclease (31) to remove any single-stranded regions of nucleic acids. The final nuclease-resistant duplex molecules were then precipitated with cold 10% trichloroacetic acid and collected on Whatman GF/C filters and the resulting acid-insoluble radioactivity counted in an Isocap liquid scintillation counter (Nuclear Chicago).

For all hybridization experiments, controls were performed to determine: 1) the stability of the RNA during, and at the end of, the annealing reactions; 2) the amount of self-reassociated [3H]DNA present in each reaction mixture at the end of an experiment—this amount of radioactivity (usually only 1.5 to 2% of the input amount) was always subtracted as a background from the experimental hybridization points, 3) the sensitivity of the annealing reactions to hydrolysis of the input RNA (by treatment with either NaOH or RNase) before the hybridization reactions were initiated; and 4) the melting temperature profile of the resulting RNA/[3H]DNA hybrids formed during the reactions as determined by standard procedures (32). Cot values for both [3H]DNA released from the reassociated duplex molecules (in 0.12 phosphate buffer) at 5°C-increments from 65° to 100°C. The percentage of single-stranded and double-stranded molecules present in experimental aliquots at each temperature point during the melt was determined by digestion of the mixture with S1 nuclease (as described above) followed by precipitation of the acid-insoluble radioactivity with cold 10% trichloroacetic acid and counting in a liquid scintillation counter.

RESULTS

n-Butyrate Induction of Unique Sequence Gene Transcripts—If the accumulated reports that indirectly relate an increase in histone acetylation to new gene expression (9–11) have any validity in terms of reflecting a possible causal relationship between the two phenomena, it might be anticipated that an artificial increase in the amount of histone
hyperacetylation induced by butyrate treatment of mammalian cells would noticeably alter their normal mode of gene expression. To investigate this possibility, Friend erythroleukemic cells were grown for 24 h in 5 mM sodium butyrate (by which time the nucleosomal histones H3 and H4 are very highly acetylated (1)) and total cellular RNA was extracted and purified as described under “Experimental Procedures.” As a control, total RNA from untreated, logarithmically growing Friend cells was extracted and purified as well. These two RNA populations were then separately annealed, under conditions of vast excess, to trace quantities of high specific activity, unique sequence \(^{3}H\)DNA isolated from uninduced Friend cells. This type of hybridization protocol is required to test for the possible presence of new unique sequence gene expression at the level of transcription since we must be concerned with both the assay of new nuclear precursor RNA transcripts (i.e. primary gene products) as well as those transcripts contained in functionally active cytoplasmic polysomes (33-35).

The saturation curves shown in Fig. 1 illustrate the results of one such hybridization experiment. Double reciprocal plot analysis of this data (36) indicates that both hybridization curves follow first order reaction kinetics. Extrapolation of the curves to infinite \(R_{o}\) values gives a saturation plateau value of about 2% of the unique sequence DNA being hybridized by total RNA from untreated, control Friend cells. On the other hand, similar analysis for the hybridization of total RNA extracted from butyrate-treated cells gives a saturation plateau value of about 2.8% annealing for the nonrepetitive DNA sequences. This large difference in the RNA saturation levels indicates that an enormous number of new RNA transcripts are present in the fatty acid-treated cells that are not detectable in the control Friend cells (see “Appendix” for approximate calculations of this number). In fact, the amount of difference between the types of transcripts found in the control cells and the butyrate-treated Friend cells is of the same order of magnitude as the differences found in the unique gene RNA transcripts occurring in several different tissues of fetal and adult mice in vivo (26-29).

Additivity RNA Saturation Hybridizations—Experiments in which equal amounts of total cellular RNAs from control and butyrate-treated Friend cells were mixed together and then annealed to saturation to trace quantities of nonrepetitive \(^{3}H\)DNA also indicate that the fatty acid-treated mouse cells contain a considerable number of new RNA transcripts not found in control cells. Fig. 2 shows the results of such an experiment. In this type of RNA-driven reaction, if the RNA sequence population present in the control Friend cells is completely different from the population of RNA sequences present in the butyrate-treated cells, one would expect that the final saturation plateau of annealing of the mixed RNA populations would be a simple summation of the two independent plateaux shown in Fig. 1. On the other hand, if the two separate RNA populations shared some sequences in common but each still contained sequences not found in the other, the final saturation level of the mixture would be expected to be above that of the butyrate-treated cell RNA plateau shown in Fig. 1 by an amount that is proportional to the number of different sequences in the two independent RNA populations (33). However, as is evident from Fig. 2, neither of these two possibilities applies in this experiment. Rather, it is seen that in this additive experiment (in which control and butyrate-derived RNAs were mixed), the final saturation plateau for annealing (as determined by a double reciprocal plot of the data) is about 2.9% of the single copy DNA hybridized. This level, within error, is the same as that obtained with butyrate-treated cell RNA alone (Fig. 1), although the kinetics of annealing in this mixed RNA experiment are slower than with the fatty acid-derived RNA alone.

This result indicates that the control (untreated) Friend cell total RNA is a subpopulation of sequences contained within the total RNA sequences derived from butyrate-treated cells. However, the results also indicate that the fatty acid-treated cells contain, in addition, a large set of new transcripts derived from single copy DNA that is not present in the control cells.

Reversal of the Butyrate Effect—If Friend cells that have been exposed to millimolar concentrations of butyrate for 24 h are subsequently transferred to fresh culture medium without this fatty acid, after a short lag period (8), the cells resume their normal growth and cell divisions and the hyperacetylation of the nucleosomal histones is reversed (1-3,5,8). A similar reversal is seen in the types of unique sequence gene RNA

![Fig. 1. Saturation hybridization curves from experiments in which total cellular RNA from either control (●) or butyrate-induced (○) Friend erythroleukemic cells was annealed, under conditions of vast excess, to trace quantities of \(^{3}H\)-labeled unique sequence DNA from control Friend cells.](http://www.jbc.org/)

![Fig. 2. Additivity experiment in which equal quantities of total cellular RNAs from both control and butyrate-induced Friend cells were mixed together and then annealed, under conditions of vast excess, to trace quantities of unique sequence \(^{3}H\)DNA from Friend cells until saturation of the DNA was approached.](http://www.jbc.org/)
transcripts found in Friend cells allowed to recover from butyrate treatment by growth for 24 h in normal medium. Fig. 3 shows the saturation hybridization curve obtained when total RNA from such butyrate-reversed cells is hybridized to unique sequence DNA. In this case, extrapolation to infinite Rot values gives a plateau of saturation of the nonrepetitive DNA of about 2%, the same as for RNA from control cells that have never been exposed to butyrate. Thus, the effects of butyrate on RNA transcription are labile and reversible.

As described under “Experimental Procedures,” a number of control experiments were performed to verify the specificity, fidelity, and reproducibility of the hybridization procedures used in this study. One of the most stringent of these controls is shown in Fig. 4. This figure shows the thermal melt profile of a butyrate-treated Friend cell RNA/[3H]DNA hybrid molecule population. The single transition melt curve with a temperature of 81°C for dissociation of half of the hybrid molecules (T_{m,1/2}) indicates that the fidelity of base-pairing in the RNA/DNA duplex molecules is very high with little mismatching of sequences. Thus, the results of Figs. 1 to 3 cannot be the result of nonstringent hybridization conditions (37).

Two-dimensional Gel Electrophoresis of Proteins—Since the saturation hybridization experiments discussed above for control and butyrate-treated cells involved the annealing of total (nuclear and cytoplasmic) cellular RNAs to isolated unique DNA sequences, the results do not, a priori, necessarily indicate a difference in the types of functional messenger RNA molecules present in the two cell populations. Rather, they clearly indicate that a fair percentage of the primary unique gene transcripts differ in the two populations, but this information, without further evidence, might simply reflect a
difference in the various nuclear RNA species (such as heterogeneous nuclear RNAs) found in the two cell populations which, due to their rapid rates of degradation, might never leave the nucleus as functional messenger molecules (33-35). For Friend erythroleukemic cells in particular, this alternative is a distinct possibility since it has recently been demonstrated that the nucleus of these cells contains at least 5 times as many different kinds of unique sequence gene transcripts as are present on the active cytoplasmic polysomes (38-40).

However, since the degree of difference in the RNA populations between control and butyrate-treated cells is so dramatic as detected by nucleic acid hybridizations, if only a small fraction (i.e. one-sixth or less) of the total newly accumulated, butyrate-induced, RNA transcripts emerged from the nucleus as functional messenger RNAs, a noticeable change in protein synthesis patterns might be expected in the treated cells compared to the controls. That such a marked change in the constellation of the major de novo-synthesized proteins does indeed occur in Friend cells exposed to butyrate for 24 h is shown in Figs. 5 to 8.

Fig. 5 shows the two-dimensional electrophoretic separation on O'Farrell gels (18) of the [35S]methionine-labeled total cellular proteins from control and butyrate-treated Friend cells. A selected region of the two-dimensional gels from matched sets of control and butyrate-treated cells are shown in the panels, with reference to the protein spots marked with an “X” being the same on all of the gels. A, total cell proteins from control cells; B, total cell proteins from cells treated for 24 h with 5 mM butyrate. In A and B, 4 X 10⁶ cpm of cellular proteins were applied to the first dimension of each of the gels and the autoradiographic exposures were for 4 weeks, C and D, protein patterns obtained from control (C) and butyrate-treated (D) cells in an entirely different experiment from those shown in Panels A and B. In each of these gels, 3.5 X 10⁶ cpm of total cellular proteins were applied to the first dimension of the gels and autoradiographic exposures were for 6 weeks. IEF, isoelectric focusing.

The apparent decrease in the rate of synthesis or accumulation, or both, of certain cellular proteins, relative to others, in response to the treatment of cells with butyrate may well result from the fact that this fatty acid inhibits DNA synthesis and cell division in these cells (2,8), which very likely leads to some differential degradation and altered rates of turnover of some cellular proteins (15-17). This type of altered rates of accumulation of some normally occurring logarithmic phase cell proteins also occurs in Friend cells which have been induced to terminally differentiate into nondividing hemoglobin synthesizing cells by treatment with dimethyl sulfoxide. In this instance too, where cells stop active proliferation, certain proteins decrease markedly in their relative concentration within the cells; however, when analyzed carefully, it can be demonstrated that the control and the dimethyl sulfoxide-induced Friend cells actually contain similar constellations of synthesized proteins. In this instance, no proteins completely disappear from the dimethyl sulfoxide-treated cells relative to the controls and neither do any new types of proteins appear in the treated cells relative to the controls (20, 42).

The most important observations to be made from Fig. 5, while over 50% of the major protein spots on these underexposed gel autoradiographs are the same for both the control and butyrate-treated cells, an appreciable number (approximately 20%) of the spots present on the control gels (Fig. 5A) have apparently “disappeared” below the level of detectability on the gels of butyrate-treated cell proteins (Fig. 5B). However, such an apparent absence of these control cell proteins from the butyrate-treated cell protein profiles is really only illusory since by a careful analysis of many such gels, having varying autoradiographic exposure times and containing proteins with various different specific activities (20, 22), most of the “absent control cell proteins” can generally be detected as minor spots on the butyrate gels (Fig. 6).²

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IEF, isoelectric focusing.

Fig. 7. Diagrammatic representation of the major de novo synthesized total cellular proteins from control Friend erythroleukemic cells as seen on O'Farrell gels (18) (solid spots). Superimposed on this standard control Friend cell protein pattern are the de novo-synthesized protein spots found on O'Farrell gels of butyrate treated-Friend cell total proteins (open spots) but not detectable in the control cells. IEF, isoelectric focusing.

However, concern the large number of new, de novo-synthesized, and accumulated proteins that appear on the gels from butyrate-treated cells (Fig. 5B) but which are not detected on nontreated, control cells (Fig. 5A) regardless of the techniques used for the analysis of these gels (see “Experimental Procedures” for the various methods of analysis). The reproducibility and authenticity of these new “butyrate-induced” proteins is attested to by the further results shown in Fig. 6 which shows a restricted region of two-dimensional gels for total cellular proteins from control and butyrate-treated cells taken from entirely independent experiments. The autoradiographs in Fig. 6 depict the results obtained with increasing lengths of exposure (relative to Fig. 5) of the control and butyrate-derived protein gels to fluorography (21).

The panels in Fig. 6 represent matched sets of control and butyrate-treated cell protein gels exposed to autoradiography for either 4 (Fig. 6, A and B) or 6 weeks (Fig. 6, C and D). The spots marked with an “x” in these panels are reference proteins that are the same on all of the gels and therefore can be used for orientation. As can be clearly seen on these autoradiographs, regardless of the length of exposures, the butyrate-treated cells (Panels B and D) always contain numerous new protein spots not found on the gels from control cells (Panels A and C). Furthermore, the patterns or constel-
lutions of protein spots found on both the control and the butyrate-treated cell gels are remarkably constant from gel to gel although with increasing lengths of autoradiographic exposures, more spots become visible on all of the gels.

Another important observation that can be made from the gels shown in Figs. 5 and 6 is that the newly expressed butyrate-induced proteins are seen to be distributed throughout the two-dimensional gels (cf. the open spots in Fig. 7) and thus occur in all molecular weight size classes and are present in all areas of the pH range of the first dimension isoelectric focusing gels. Therefore, the newly appearing proteins cannot be the result of artificial protein charge heterogeneity induced by butyrate treatment, the result of premature polypeptide chain terminations or protein degradations, or the result of protein clumpings or pH gradient breakdown in the gels (cf. Refs. 18 and 19). Furthermore, as mentioned above, it is probably quite significant, from the point of view for possible molecular mechanisms that might control gene expression in mammalian cells, that the constellation pattern of butyrate-induced protein spots is practically identical each time this fatty acid is added to the cells. This point will be discussed elsewhere.3

Fig. 7 illustrates several of the above points well for it diagrammatically depicts a composite representation of the major de novo-synthesized proteins found on gels from control Friend cells (solid black spots) and, in addition, shows the locations of the apparently new proteins resulting from a 24-h treatment of the cells with butyrate (open spots) From this diagram, and from the gels shown in Figs. 5 and 6, it is evident that in butyrate-treated cells many of the proteins detected by two-dimensional gel electrophoretic analysis are not readily detected on the nontreated control Friend cell gels. These results for total cellular proteins are in good agreement with the findings from the nucleic acid hybridization experiments reported above.

The dramatic changes seen in the types of proteins synthesized after butyrate treatment are not confined to changes in total cellular proteins, for, as seen in Fig. 8, two-dimensional gel analysis of isolated cytoplasmic proteins from control (Fig. 8A) and from butyrate-treated (Fig. 8B) Friend cells show similar degrees of difference in patterns, as do the total cellular proteins from the same cells shown in Figs. 5 and 6. It is evident that in butyrate-treated cells many of the proteins detected by two-dimensional gel electrophoretic analysis are not readily detected on the nontreated control Friend cell gels. These results for total cellular proteins are in good agreement with the findings from the nucleic acid hybridization experiments reported above.

The present report documents a number of interesting and highly suggestive findings concerning the coordinate effect of butyrate treatment of Friend erythroleukemic cells on the hyperacetylation of nucleosomal histones and the concomitant induction of new gene expression in these cells. Thus, after 24 h of fatty acid treatment, when both histones H3 and H4 are in very highly acetylated forms, it is seen that the induced cells contain about 38% new species of RNA transcripts derived from unique sequence DNA and, at the same time, are synthesizing many new proteins not found in the uninduced control cells. Furthermore, these newly induced metabolic changes are not merely a result of inhibition of cell growth and division by the butyrate, and all of these changes are readily reversible once the fatty acid has been removed from the cells. In addition, similar changes in the types of synthesized RNA and proteins are not observed in these cells when other inducers of differentiation, such as dimethyl sulfoxide, are added to the Friend cells. Together, these findings suggest that all of the observed metabolic changes induced by butyrate are closely linked in the Friend cells and may be causally related to each other in some way. For example, the results are not inconsistent with the hypothesis that hyperacetylation of the nucleosome core histones (particularly of H3 and H4 to the diacetylated and higher forms) may play an important necessary, but not sufficient, role in the biochemical activation of new gene expression in these murine cells in culture. Such a situation would be consonant with the recent findings of others (5, 43) that the DNA sequences in chromatin that have artificially increased levels of hyperacetylated histones as a result of butyrate treatment also show increased sensitivity to digestion by pancreatic DNase I which preferentially hydrolyzes the DNA of transcriptionally active, or potentially active, genes (44).

However, to unambiguously demonstrate a causal relationship between histone hyperacetylation and the activation of new gene activity in eukaryotic cells, it will probably be necessary to find genetic mutations involving either the histone genes themselves or genes controlling the levels of acetylated histones in particular cells for detailed biochemical analysis. The search for such mutants is in progress.

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APPENDIX

The calculation of the number of unique sequence genes expressed in butyrate-induced Friend cells is as follows. Assuming that the haploid mouse genome contains 1.8 \( \times 10^{12} \) daltons of DNA (40) and that about 70% of this is unique sequence DNA (25), then the approximate haploid complement of unique sequence DNA per cell is about 1.26 \( \times 10^{12} \) daltons. In control Friend cells, about 1.9% of this DNA is expressed as RNA transcripts (Fig. 1) which means that about 2.5 \( \times 10^{10} \) daltons of DNA are expressed in these cells. However, since, in mouse cells, there are at least 5 times more different kinds of unique sequence RNA transcripts found in the nucleus than in the cytoplasm (38, 39), it is not unreasonable to assume that only about one-sixth of the total expressed sequences, or about 4.2 \( \times 10^{9} \) daltons of DNA, might function as templates for protein synthesis in the control cells. Furthermore, if it is assumed that the median size for a cytoplasmic mRNA molecule is about 1200 nucleotides (or about 4.2 \( \times 10^{9} \) daltons (33)), then the uninduced Friend cells might be expressing about 10,000 different genes in protein synthesis. This value for the number of different cytoplasmic mRNA products is in reasonable agreement with the findings of others for Friend erythroleukemic cells (33, 45) and also for the number of different proteins synthesized in certain mouse tissues (40). If similar calculations are made for the 2.8% of
the unique sequence DNA transcripts found in the butyrate-induced cells (Fig. 1), it is found that these cells may be expressing about 13,800 unique gene mRNAs in their cytoplasmic polysomes. Since the butyrate-treated cells also probably contain all of the gene transcripts found in the control cells (Fig. 2), this would suggest that the butyrate-treated cells are expressing about 38% (3,800/10,000) new unique gene products not found in the control cells. A similar degree of difference between the control and induced cells is seen if the calculations are made solely on the basis of the total RNA transcripts found in the two cell types (regardless of their locations within the cells) or if the average molecular size of a cytoplasmic mRNA is taken to be 2000 nucleotides (46) rather than 2000.

Nevertheless, such simplistic calculations must be viewed with a great deal of reservation since so many of the values used are only gross approximations and since other variables not considered here (such as the fact that the Friend cells are not true diploids and the recent findings that some mouse cell unique sequence genes contain DNA inserts in the coding regions (47) that are transcribed into RNA but which are not found in the polysomal messenger fractions) would undoubtedly affect the numbers determined. However, the degree of difference calculated here between the control and the butyrate-treated cells remains the same regardless of the numerical machinations and is within reasonable agreement with the degree of difference between the two cell types detected by two-dimensional gel electrophoresis of the newly synthesized proteins.

REFERENCES
34. Lewin, B. (1975) Cell 4, 11-20
35. Lewin, B. (1975) Cell 4, 77-93
Sodium butyrate induces new gene expression in Friend erythroleukemic cells.
R Reeves and P Cserjesi


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