Butyrate-Induced Changes in Nuclease Sensitivity of Chromatin Cannot Be Correlated with Transcriptional Activation

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We examined in the H4IIE rat hepatoma cell line the relationship between butyrate-induced changes in the nuclease sensitivity of chromatin and changes in transcriptional activity of specific genes. The butyrate-inducible metallothionein I (MT-I) gene underwent a dramatic increase in DNase I sensitivity after 3 h of butyrate treatment. However, genes not transcribed in H4IIE cells underwent the same changes in DNase I sensitivity. Thus, butyrate-induced increases in DNase I sensitivity are not sufficient for the transcriptional activation of a gene. Butyrate treatment has also been reported to alter the sensitivity of sequences to micrococcal nuclease (MNase) in a manner reflecting their tissue-specific expression. Butyrate exposure caused increased digestion of the MT-I gene by MNase. However, butyrate-induced MNase sensitivity also occurred for genes which are neither transcribed in untreated cells nor butyrate inducible. Moreover, cadmium, a potent transcriptional activator of the MT-I gene, does not alter the sensitivity of the MT-I gene to MNase. Thus, the butyrate-induced alterations in MNase sensitivity are neither sufficient for, necessary for, nor indicative of transcriptional activation.

Nuclease digestion of intact nuclei often reveals structural differences between transcriptionally active and inactive chromatin. Weintraub and Groudine (41) demonstrated that globin genes are preferentially digested by DNase I in chicken erythrocytes but not in cells which do not express these genes. Increased DNase I sensitivity of active genes has since been demonstrated in a variety of tissues (14-16). Micrococcal nuclease (MNase) also recognizes structural features associated with transcribed genes. MNase digestion of nuclei is reported to result in the preferential solubilization of chromatin containing actively transcribed sequences (3).

Sodium butyrate has been used to induce differentiated functions in a variety of cell culture systems (22, 28, 44). The basis of the action is not clear, since butyrate affects a multitude of nuclear processes (see reference 20 for a review). Included among the effects of sodium butyrate is an increase in the general sensitivity of chromatin to DNase I digestion (25, 33, 39). Thus, butyrate treatment leads to changes in chromatin which resemble alterations that accompany the packaging of chromatin for transcription. All the butyrate-induced changes in DNase I sensitivity reported to date consider the digestion of bulk chromatin after prolonged exposure to butyrate. However, in most cell culture systems, butyrate modulates the expression of relatively few genes (9, 23). The changes that occur throughout chromatin therefore may not be strictly coupled to changes in gene activity. Moreover, in some cases, butyrate can rapidly alter gene expression (11). To study those effects of butyrate which may be causal in modulating gene expression, it is important to identify butyrate-induced changes in defined regions of chromatin structure that precede or occur concomitantly with alterations in gene expression.

The metallothionein I (MT-I) gene offers an excellent opportunity to study the relationship between butyrate-induced nuclear changes and altered gene expression. We

have previously shown that butyrate induces an increase in the rate of synthesis and the accumulation of correctly initiated rat MT-I mRNA in the absence of concomitant protein synthesis (4). This response is rapid; MT-I transcriptional rates double within 3 h of butyrate addition, whereas the overall level of RNA synthesis is not altered. Our extensive characterization of the rat MT-I gene (2) now permits us to determine the relationship between changes in the sensitivity to nuclease digestion of a defined chromatin region and changes in gene activity.

MATERIALS AND METHODS

Cells and culture conditions. H4IIE rat hepatoma cells, clone Fao (12), were grown in alpha modified Eagle medium containing 10% fetal calf serum (Irvine Scientific). For butyrate induction, the medium was removed from the dishes and butyric acid (Sigma Chemical Co.) was added to a concentration of 5 mM. The medium was neutralized with NaOH and then returned to the dish.

Digestion of chromatin labeled in vivo. H4IIE cells were labeled for 20 h in medium containing 0.4 μ Ci of [³H]thymidine (Amersham Corp.; 54 Ci/mmol) per ml. Cells were washed with phosphate-buffered saline before fresh medium was added. Cells were harvested 24 h later, after either 0, 3, or 24 h of exposure to butyrate. Cells were washed twice with cold phosphate-buffered saline and suspended in cold phosphate-buffered saline containing 30% sucrose and 0.5% Nonidet P-40. After 5 min on ice, nuclei were collected by centrifugation, washed twice in DNase I digestion buffer (10 mM Tris, 10 mM MgCl₂, 250 mM sucrose [pH 8.0]), and suspended at a final chromatin concentration of 0.5 mg/ml (1 A₂₆₀ U = 50 μ g). Samples (approximately 400 μ l) were incubated for 10 min at 37°C after DNase I (Worthington Diagnostics) was added to the appropriate concentration. The reaction was terminated by the addition of 200 μ l of cold digestion buffer containing 60 μ g of carrier DNA. Three samples were withdrawn, precipitated with a 1/10 volume of 50% trichloroacetic acid, and incubated for 1 h on ice. Samples were centrifuged 20 min at 9,000 $\times$ g, the superna-

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FIG. 1. DNase I digestion of nuclei from butyrate-treated cells. H4IIE cells were labeled for 24 h with [3 H]thymidine and then exposed to 5 mM butyrate for 0, 3, or 24 h. Nuclei were prepared and digested with DNase I as described in Materials and Methods. Triplicate samples were removed for trichloroacetic acid (TCA) precipitation. The mean $\pm$ the standard error of the mean of the precipitable material is shown for control cells (●), cells treated for 3 h with butyrate (■), and cells treated for 24 h with butyrate (▲). Undigested DNA samples had greater than 300,000 cpm precipitated.

tant was withdrawn, and 200 μ l of DNase I digestion buffer was added to the pellets. All samples were digested at room temperature for 3 h with Protosol (New England Nuclear Corp.). Radioactivity was determined to a 2% error by liquid scintillation counting.

DNase I sensitivity of specific genes. The methods used in our laboratory to detect the DNase I sensitivity of specific genes have been described previously (36).

Chromatin fractionation with MNase. Nuclei were prepared as described above and washed twice in MNase digestion buffer (13). All subsequent procedures were done as described elsewhere (13). Digestions were done by using 20 or 50 U of MNase (Worthington) per mg of chromatin. Filters stripped for reprobing (37) were exposed to film for a length of time comparable to that used after probing, to ensure complete probe removal.

DNA probes. The rat MT-I cDNA (1) and the MT-I genomic intron probe (36) have been described elsewhere. To study the DNase I sensitivity of the MT-I gene, we used a 1,226-base-pair (bp) *EcoRI-BglII* fragment from the nontranscribed 5'-flanking region of the MT-I gene. Other probes and their sources were: pcTAT3, a rat tyrosine aminotransferase cDNA, obtained from Günther Schutz, Institute of Cell and Tumor Biology, Heidelberg, Federal

Republic of Germany; pGH1, a rat growth hormone (GH) cDNA, obtained from Ron Evans, Salk Institute for Biological Studies, San Diego, Calif.; mouse β -globin gene probe (30); and the human Alu probe (31). High-molecular-weight rat DNA was prepared from H4IIE cells as previously described (17).

RESULTS

DNase I sensitivity of chromatin after butyrate treatment.

Since in some instances butyrate can rapidly influence gene activity, we expected that the processes which are responsible for the modulation of such gene expression occur soon after the exposure of cells to butyrate. We have shown that butyrate doubles the rate of MT-I transcription in H4IIE cells after 3 h of treatment (4). We therefore investigated whether the change in DNase I sensitivity of chromatin that has been described after prolonged (24-h) exposure of cells to butyrate (25, 33, 39) is apparent at earlier times. As reported by others (25, 33, 39), a 24-h exposure to butyrate increased the sensitivity of chromatin to DNase I digestion (Fig. 1). At low concentrations of DNase I, nuclei from cells treated with butyrate for 24 h showed twice the rate of DNA digestion as nuclei from control cells did. Nuclei from cells treated for 3 h with butyrate also show enhanced rates of DNase I digestion. The difference in the sensitivity of DNase I digestion of nuclei from 3- and 24-h-treated cells, while statistically significant, was relatively minor. The fraction of DNA released from nuclei of 3-h-treated cells was, at almost all enzyme concentrations tested, more than 80% of that released from nuclei in 24-h-treated cells. Thus, structural changes that increase the general sensitivity of total chromatin to DNase I have largely taken place after just 3 h of exposure to butyrate.

DNase I sensitivity of transcribed and silent genes after exposure to butyrate. The increased DNase I sensitivity of bulk chromatin that accompanies butyrate treatment may reflect either a minor increase in the DNase I sensitivity of the majority of cellular chromatin or larger changes in limited chromatin regions. We wished to characterize the changes in chromatin structure, revealed by DNase I digestion, that occur in a specific butyrate-induced gene after 3 h. Nuclei were treated with DNase I. The maximum amount of DNase I used (6.4 U/ml) released no more than 5% of the chromatin in nuclei from untreated cells. The DNA was then isolated and used for Southern analysis of the MT-I gene. The intensity of the MT-I gene band for DNA obtained from control cells was undiminished over the concentration range of DNase I used (Fig. 2A). In contrast, the MT-I gene became highly sensitive to DNase I after only 3 h of butyrate treatment. Digestion conditions which released less than 3% of the bulk chromatin from butyrate-treated cells almost eliminated the MT-I gene band (Fig. 2A).

To determine the extent to which butyrate changes the DNase I sensitivity of genes it does not transcriptionally activate, we stripped the filter shown in Fig. 2A and rehybridized it with additional probes. Figure 2B presents the superimposition of autoradiograms showing bands for the GH and β -globin genes, in addition to the band for the MT-I gene. No message for either the GH or β -globin gene was detectable in these cells either before or after butyrate treatment (data not shown). As with the MT-I gene, the intensity of the band containing the GH gene remained unchanged in DNA from control nuclei across the range of DNase I concentrations used. The same DNase I concentrations diminished the intensity of the GH gene band after

FIG. 2. DNase I sensitivity of genes in nuclei from butyrate-treated cells. (A) MT-I gene. Nuclei from control cells (Con) and cells treated for 3 h with 5 mM butyrate (But) were digested for 10 min at 37°C with (left to right) 0, 0.6, 1.2, 1.8, 2.4, or 3.0 μ g of DNase I per ml. DNA was prepared, digested with *Hind*III, and separated in a 0.7% agarose gel (10 μ g per lane). After being transferred to nitrocellulose, the filter was hybridized to a 32 P-labeled 1,200-base-pair *Eco*RI-*Bgl*II fragment which flanks the rat MT-I gene. Increasing concentrations of DNase I are indicated by the arrows. (B) Transcriptionally silent genes. The filter shown in panel A was reprobed with labeled cDNAs for GH and β -globin genes. The filter was stripped of all counts between hybridizations. The three autoradiograms have been superimposed, and the positions of the bands corresponding to the MT, GH, and β -globin (G) genes are indicated.

butyrate treatment. The β -globin gene did not show the same high degree of DNase I sensitivity in butyrate-treated cells that was observed with the MT-I or GH gene. However, an increase in sensitivity relative to that of the control was apparent. Analysis of DNA from nuclei isolated as described above but incubated without the addition of exogenous DNase I did not reveal any increased loss of specific fragments in DNA from butyrate-treated cells (data not shown); butyrate apparently does not stimulate endogenous nuclease activity. Regardless of the precise mechanism, we detected similar butyrate-induced increases in DNase I sensitivity not only in a region which is transcriptionally responsive to butyrate (the MT-I gene) but also for specific inactive regions (the GH gene and, to a lesser extent, the β -globin gene) of chromatin. The butyrate-induced structural changes associated with DNase I sensitivity therefore are not limited to the relatively few transcriptionally responsive regions of chromatin; they are more widespread and extend to transcriptionally inactive genes. We conclude that, while changes in DNase I sensitivity after butyrate treatment may precede transcriptional activation, they do not represent a causal step sufficient for butyrate stimulation of gene expression.

MNase digestion of a butyrate-responsive gene. Ferenz and Nelson (13) have demonstrated that the sequences for the active β -globin gene, but not for the untranscribed ovalbumin gene, are more readily solubilized by MNase soon after treatment of chick erythrocytes with butyrate. The effect of this treatment on the expression of the β -globin gene has not been reported. To determine whether such changes in MNase sensitivity accompany the butyrate induction of MT-I gene expression, we digested nuclei from control and 3-h-butyrate-treated H4IIE cells with MNase. The DNA was fractionated into solubilized and pelleted material as described elsewhere (13). Separation of these DNAs on agarose gels showed that the average size of the DNA was progressively reduced with increased times of digestion (Fig. 3A). To analyze the distribution of MT-I gene sequences in these preparations of chromatin, the DNA was transferred to nitrocellulose and hybridized to a rat MT-I intron fragment which hybridizes only to the MT-I gene (S. J. Taplitz and H. R. Herschman, unpublished observations). MT-I sequences remained primarily in pelleted material in the nuclei of control cells (Fig. 3B). Exposure of cells to butyrate for 3 h led to a shift of MT-I sequences into the

solubilized material (compare the amount of material solubilized at 3 and 5 min of digestion). Densitometry of these autoradiograms indicated that in nuclei of butyrate-treated cells, MT-I sequences were enriched more than twofold in the released chromatin fraction relative to that from untreated cells. Butyrate clearly alters the solubility or the susceptibility of the MT-I gene to digestion with MNase.

Release of MT sequences reflects increased nuclease digestion after butyrate treatment. Chromatin fragments containing highly acetylated histones show increased solubility compared to those of regions containing nonacetylated histones (27). The shift of MT-I gene-containing fragments from the pelleted material to the supernatant (Fig. 3) therefore could reflect a change in the solubility of the MT-I gene-containing region after butyrate treatment rather than any increased sensitivity to the nuclease. To distinguish between these alternatives, we analyzed the size of all MT-I gene-containing fragments produced by MNase digestion of nuclei from control and butyrate-treated cells without fractionating the digested chromatin on the basis of solubility. Figure 4A shows the size distribution of fragments containing the MT-I gene after digestion of nuclei from control or 3-h-butyrate-treated cells. As for Fig. 3, the hybridization probe contained sequences from the first intron of the MT-I gene, which detects only the unique MT-I gene. The MT-I gene-containing fragments generated by MNase digestion of nuclei from butyrate-treated cells were smaller than those from control nuclei. Scanning densitometry of a separate autoradiogram derived from these samples indicated that the midpoint of the distribution of MT-I gene-containing fragments was decreased by over 100 nucleotides after the butyrate treatment. In this experiment, we wanted to ensure that both sets of nuclei (control and butyrate treated) were subjected to identical digestion conditions. To this end, equivalent numbers of HeLa nuclei were added to nuclei from control and butyrate-treated H4IIE cells before digestion. After being probed for MT-I sequences, the filters represented in Fig. 4A were stripped and reprobed with the human Alu repeat sequence. Figure 4B demonstrates that the distribution of Alu sequences was identical for the two preparations, indicating that the exposures of control and butyrate-treated H4IIE nuclei to MNase were equivalent. The difference in sizes of MT-I gene-containing fragments in Fig. 4A therefore must represent an increased digestion of the MT-I gene after butyrate treatment. We conclude that

FIG. 3. MNase analysis of the MT-I gene. (A) Ethidium bromide-stained gels. Nuclei from control (Con) and 3-h-butyrate-treated (But) cells were suspended at a chromatin concentration of 3.5 mg/ml and digested at 37°C with MNase (1 U/20 µg of chromatin). Samples were removed at (left to right) 1, 3, 5, 10, 20, and 40 min and separated into solubilized (S) and pelleted (P) material. DNA was prepared, separated in a 1% agarose gel, and stained with ethidium bromide. DNA (3.5 µg) was loaded in the lanes containing the supernatant of 1-min digestions (only limited amounts of solubilized DNA were available after the early time points). All other lanes contain 15 µg of DNA. The positions of lambda *Hind*III fragments are indicated at the far right (top to bottom, 23.1, 9.4, 6.5, 4.3, 2.3, 2.0, and 0.5 kilobases). (B) MT-I gene sequences in MNase-fractionated chromatin. The stained DNA was transferred to nitrocellulose and probed with a fragment from the first intron of the rat MT-1 gene (see Materials and Methods). This fragment hybridizes only to the rat MT-I gene.

the shift of MT-I gene-containing fragments from the pellet to the supernatant (Fig. 3 and subsequent figures) represents the increased susceptibility of the MT-I gene to MNase digestion after exposure of cells to butyrate.

Butyrate alteration of MNase sensitivity of genes not induced by butyrate. Butyrate acts additively with dexamethasone (an inducer of the MT-I gene) in stimulating MT-I message levels in H4IIE cells (4). Like the MT-I gene, the tyrosine aminotransferase (TAT) gene is expressed in H4IIE cells and is induced by glucocorticoid hormones. In contrast to the MT-I gene, however, the amount of cytoplasmic message for the TAT gene in H4IIE cells is not increased by butyrate; indeed, butyrate blocks the glucocorticoid-induced increase in TAT message levels (4). We determined the effect of butyrate on the MNase sensitivity of a gene (TAT) for which steroid induction, though not basal expression, is blocked. The TAT gene showed increased MNase sensitivity after butyrate treatment (Fig. 5A). The enrichment of TAT sequences in the released chromatin fraction after butyrate treatment, relative to that in the pelleted material, was at least as great as that observed for the MT-I gene (compare Fig. 5A with Fig. 3B). Thus, while butyrate modulates the expression of these two genes in strikingly different manners, the alterations in structure in response to butyrate, as detected by MNase, are similar.

We examined next the MNase sensitivity of a gene which is neither expressed in control H4IIE cells nor induced by butyrate. After butyrate treatment, GH sequences displayed the same enrichment in the released material as observed with MT-I and TAT genes (Fig. 5B). Thus, in H4IIE cells the structural changes assayed by MNase digestion are not limited either to genes expressed in the control cells or to genes that are butyrate responsive.

Filters from this experiment were subsequently probed with ³²P-labeled high-molecular-weight DNA to examine the distribution of repeated sequences. These sequences were evenly distributed between the solubilized and pelleted material regardless of butyrate treatment (Fig. 5C). Butyrate did not alter the distribution of repeat sequences in DNA between the two chromatin fractions. Differences observed with gene-specific probes therefore reflect butyrate-induced changes in MNase sensitivity of specific chromatin regions.

We also analyzed the effect of butyrate on the β-globin gene in MNase-digested H4IIE chromatin (Fig. 6). MT-I sequences were, once again, shifted to the released material after butyrate treatment. In contrast, β-globin sequences did not undergo a change in sensitivity to MNase after exposure of these cells to butyrate. Rather, β-globin sequences remained in the pelleted material. Thus, although neither the GH nor the β-globin gene is expressed in H4IIE cells,

FIG. 4. Size analysis of total chromatin from MNase-digested nuclei. Nuclei were prepared from control (Con) or 3-h-butyrate-treated (But) H4IIE cells as described in the text. Nuclei from untreated HeLa cells were added to represent 10% of the total amount of chromatin in the digestions. Each reaction contained 2 mg of chromatin at 3.5 mg/ml and was digested with 80 U of MNase. After a 4-min digestion, total DNA was prepared (i.e., without fractionation based on solubility) and then size fractionated and blotted as described in the text. (A) MT-I sequences were identified by hybridization to a rat MT-I intron probe. (B) After being strip washed, the filter shown in panel A was hybridized to a human Alu gene probe. The positions of DNA size markers are indicated in kilobases.

butyrate causes a shift of GH gene sensitivity to MNase but has no effect on the sensitivity of the β -globin gene. A summary of the expression of these genes and their MNase sensitivity is presented in Table 1.

Alteration in MNase sensitivity is not required for induction of gene expression. Butyrate induces both transcription of the MT-I gene (4) and a chromatin alteration detectable by MNase (Fig. 3). Cd produces a more rapid and a greater overall MT-I mRNA induction than does butyrate (4). Hypersensitivity studies show that Cd^{2+} induces substantial alterations in chromatin containing the MT-I gene (36). We next asked whether changes in chromatin structure similar to those induced by butyrate accompany the transcriptional activation of the MT-I gene by a different mechanism. Nuclei were prepared from control and 3-h-Cd-induced cells. At this time there is a nearly sixfold increase in MT-I gene transcription (4). The chromatin was digested with MNase, and the fraction of material released by nuclease digestion was determined by measuring A_{260} . Such measurements indicated that Cd treatment, like butyrate treatment, does not increase the overall sensitivity of chromatin to MNase (data not shown). DNA from control and Cd-induced cells was

FIG. 5. Distribution of additional sequences in MNase-fractionated chromatin. The filters shown in Fig. 3B were stripped and reprobed sequentially with TAT cDNA (A), GH cDNA (B), and high-molecular-weight DNA prepared from H4IIE cells (C). The filters were exposed to film after each round of stripping to confirm the complete removal of the probes. The abbreviations are described in the legend to Fig. 3.

subjected to agarose electrophoresis and transferred to nitrocellulose. Hybridization with an MT-I gene-specific probe demonstrated that there was no shift to the soluble fraction after Cd induction of the MT-I gene (Fig. 7). Butyrate-treated cells prepared simultaneously did demonstrate the increase in MNase sensitivity of the MT-I gene (data not shown). Thus, Cd treatment, which increases MT-I gene activity and DNase I hypersensitivity, does not detectably alter the sensitivity of the MT-I gene to MNase.

DISCUSSION

Butyrate treatment leads to multiple changes in chromatin, some of which are likely to be responsible for the diverse effects of butyrate on gene expression. These changes include a variety of chemical modifications of existing histone (6, 42, 43) and nonhistone chromosomal proteins (24) as well

TABLE 1. Expression and MNase sensitivity of selected genes in H4IIE cells

Gene	Basal expression	Modulation of transcription by butyrate	MNase sensitivity
MT-I	Yes	Induced	Yes
TAT	Yes	Hormone induction blocked	Yes
GH	No	None	Yes
β -Globin	No	None	No

FIG. 6. Distribution of β -globin sequences in MNase-digested chromatin after butyrate treatment. Chromatin from control (con) and 3-h-butyrated-treated (but) cells was digested with MNase, fractionated as described above, and blotted to nitrocellulose. The filters were probed with a rat MT-I genomic fragment (A) or a mouse β -globin cDNA (B). DNA (15 μ g) from the supernatant (S) and pellet (P) of the 5-min MNase digestion is shown.

as changes in the amounts or specific activity of chromatin-associated proteins (10, 18). We have measured rapidly occurring butyrate-induced changes in chromatin to minimize the influence of the changes that occur after those that directly alter gene expression.

Transcriptionally active genes generally exist in a conformation that exhibits enhanced sensitivity to digestion with DNase I (14–16, 41). Butyrate treatment increases the sensitivity of bulk chromatin to DNase I. It therefore has been suggested that butyrate modulates gene expression by making the DNA more accessible for transcription (32). Changes in DNase sensitivity occur rapidly after exposure of cells to butyrate; increased sensitivity is apparent within 3 h (Fig. 1). Thus, the butyrate-sensitive events responsible both for the alterations in bulk chromatin structure and for increased expression of the MT-I gene take place rapidly after exposure to butyrate. The effect of butyrate on levels of histone acetylation also occur rapidly. The fraction of histones

showing the most rapid modification appear hyperacetylated after 1 h of butyrate treatment (7, 8). Interestingly, the chromatin released by DNase I digestion (which preferentially attacks active genes) is enriched in hyperacetylated histones (26, 32).

We examined changes in DNase I sensitivity of specific sequences by probing blots of DNA prepared from control and butyrate-treated cells. The technique used here differs from the measurements of the digestion kinetics for bulk chromatin presented by us (Fig. 1) and others (25, 33, 39) in that the specific fragment being analyzed will disappear with the first cut by DNase I that occurs internal to the restriction sites defining the fragment. This technique, then, measures the relative speed with which the first DNase I cut occurs in a particular genomic fragment, and this speed is assumed to reflect the overall accessibility of that particular chromatin region to the nuclease. Each of the specific fragments we examined demonstrated increased sensitivity to DNase I after butyrate treatment. However, butyrate does not cause uniform structural changes throughout chromatin, as demonstrated by the fact that the fragments identified with the β -globin probe are less sensitive to DNase I after butyrate treatment than are those from the MT-I or GH gene. This is not surprising, since the repression of chromatin domains may involve stable conformations which limit the accessibility of the DNA (reviewed in reference 40). Clearly, however, increased sensitivity to DNase I after butyrate treatment does not indicate either that a gene is transcriptionally active or that it is responsive to butyrate. The inactive GH gene shows the same change in DNase I sensitivity in response to butyrate as does the MT-I gene, which is butyrate inducible. Thus, the butyrate-induced alteration in structure responsible for increased DNase I sensitivity is not sufficient to lead to increased gene expression.

A direct relationship between the sensitivity to MNase of a particular gene and its rate of transcription has been described for several systems (19, 29). For example, the rate of chick oviduct ovalbumin transcription decreases upon estrogen withdrawal (35). This phenomenon is accompanied by a decrease in the extent to which MNase releases ovalbumin-containing sequences (5). The increased solubility of MT-I sequences we detected after butyrate treatment is consistent with these observations, since butyrate is able to stimulate MT-I gene transcription. However, Cd, an even more effective inducer of MT-I gene transcription, does not alter the sensitivity of the MT-I gene to MNase. These data indicate that the structural changes detected by the nuclease

FIG. 7. MNase sensitivity of the MT-I gene after Cd exposure. Chromatin from control cells (Con) and cells treated for 3 h with 3 μ M CdCl₂ (Cd) was prepared, isolated, exposed to MNase, and fractionated as described in the text. DNA (15 μ g per lane) was loaded except for the 1-min (1- μ g) and 3-min (11.7- μ g) supernatant samples of control cells and the 1-min (1.3- μ g) and 3-min (2.5- μ g) supernatant samples of the Cd-induced cells. S, Supernatant; P, pellet. The numbers across the top indicate exposure times.

in this procedure do not reflect differences associated with different rates of transcription. In addition, these data lead us to conclude that increased MNase sensitivity is not a necessary aspect of increased transcriptional activity. MNase sensitivity is neither a sufficient nor even a necessary correlate of induction of MT-I gene expression.

Ferenz and Nelson reported that the nonexpressed ovalbumin gene from butyrate-treated erythrocytes did not demonstrate enhanced digestion with MNase, which suggests some specificity to this response (13). Similarly, we found no change in MNase digestion of β -globin sequences with butyrate treatment of H4IIE cells. However, we demonstrated that the same butyrate-induced increase in nuclease sensitivity that occurs for an active, butyrate-induced gene (MT-I) occurs in an active gene not induced by butyrate (TAT) and an inactive gene not inducible by butyrate (GH). Several possible explanations exist for the apparent discrepancy in these observations.

The increased sensitivity of the GH gene after butyrate treatment could reflect the influence of neighboring sequences. The nuclease sensitivity of chromatin extends well beyond the boundaries of the particular regulated gene (34); the sensitivity of the GH gene may reflect the activity of a neighboring gene. The β -globin gene, located in a cluster of untranscribed genes, is in effect buffered from transcriptionally active regions of the genome and lacks sensitivity to MNase both before and after butyrate treatment. Further, the erythrocytes examined by Ferenz and Nelson were severely limited in transcriptional activity (38) and contained nuclear RNA which represented only 1.5% of the single-copy component of the genome (21). The differences we observed could indicate that such measurements in erythrocyte chromatin are not representative of processes in other tissues.

Neither the mechanism by which butyrate alters chromatin structure nor the nature of the structures recognized by the nucleases is understood. We are unable to relate either of these processes to changes in transcription in a causal fashion. Cd induces MT-I gene expression without altering the generalized sensitivity of the gene to DNase I (data not shown) or MNase (Fig. 6). In the same cells, butyrate leads to altered sensitivity to both nucleases of the GH gene, which is not expressed.

All these data suggest that although distinct regions of chromatin display differential nuclease sensitivity after exposure to butyrate, no butyrate-induced alterations in nuclease sensitivity can be identified that are sufficient for, necessary for, or even indicative of transcriptional activation in H4IIE cells.

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