Induction of histone H1° differs with different treatments among different cell lines

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Received 29 May 1985; revised version received 16 July 1985

Histone H1° is an H1 subfraction whose level on the chromatin is inversely correlated with the mitotic index of the cell. We investigated the increase in H1° in 6 cell lines when cell division was blocked by 4 methods: 5 mM butyrate, 2% DMSO, serum withdrawal or density inhibition. The 6 cell lines (HeLa S1 and S3, CHO, 3T6, NIE-115 and Vero) responded differently to the treatments as regards the amount of increase in H1° and comparisons among the lines reveal no obvious similarities between the lines in the differential effects of the various treatments on H1° levels.

Histone H1° *Cell culture* *Butyrate* *DMSO* *Serum withdrawal* *Density inhibition*

1. INTRODUCTION

H1° is an H1 histone subfraction that was first characterized by its presence in tissues with low mitotic indices [1]. Measurements of H1° levels during tissue regeneration support the idea that H1°, while present in limited amounts in dividing cells, is greatly increased in tissues where there is little or no cell division [2–4]. Substantial H1° levels seem to require both the terminal state of differentiation, and the concomitant non-dividing state [5]. In some cases [5], loss of differentiation of hormone-dependent tissues by hormone deprivation was correlated with a decrease in H1° levels. The following question then arises. If different cell types had the non-dividing state in common, and were treated with a common set of H1° inducers, would the cells show a common pattern of response? If so, a relatively simple and direct connection might be implied between H1° levels and the termination of differentiation in the non-dividing state.

In addition to the tissue studies mentioned above, H1° has been studied in cultured cells. H1° has been observed to increase in Friend [6], CHO [7], neuroblastoma and HeLa [8] cells in response to different treatments that blocked cell division; in 2 cell lines blocking division was accompanied by recognizable differentiation. Such cell culture systems provide the opportunity to test for a common pattern of response among different cell types exposed to the same set of H1° inducing treatments, and this report describes such experiments.

2. MATERIALS AND METHODS

The following cell lines and cell culture conditions were used in these studies: (A) mouse neuroblastoma NIE-115 cells and (B) African Green Monkey kidney (Vero) cells were grown in Dulbecco's Modified Eagle's Media supplemented with 10% fetal calf serum and 2 mM glutamine; (C) HeLa S3 cells were grown in Dulbecco's Modified Eagle's Media supplemented with 5% calf serum; (D) HeLa S1 cells were grown in Dulbecco's Modified Eagle's Media supplemented with 10% calf serum; (E) Chinese hamster ovary (CHO) cells were grown in F-12 supplemented with 10% fetal calf serum and 2 mM glutamine; (F)

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Published by Elsevier Science Publishers B.V. (Biomedical Division)

Swiss albino mouse embryo fibroblast (3T6) cells were grown in Dulbecco's Modified Eagle's Media supplemented with 10% calf serum, and 5 mM glutamine. For the inhibitor studies this medium was supplemented with 500 μg/ml sodium butyrate (5 mM) or 1 ml spectrograde DMSO per 50 ml culture medium (250 mM). All cells were grown in 100 × 20 mm tissue culture dishes at 37°C in 10% CO_2. The culture media were changed every day for the first 4 days of each treatment, and every other day thereafter. To measure DNA synthesis, the medium was removed from the 100 × 20 mm tissue culture dish and the cells were washed once with phosphate buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4). The cells were then incubated at 37°C for 3 h in media supplemented with [^{3}H]thymidine (NEN) at a concentration of 50 μCi/ml. This medium was also supplemented with 10 μM deoxycytidine, to insure that dCTP was not limiting.

2.1. *Histone extraction*

Outer cell membranes were lysed by incubating the cells for 3 min at room temperature in 10 ml buffer (0.25 M sucrose, 50 mM Tris base, 25 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.5) containing 0.1% Triton X-100. The cells were washed off the plate in this buffer and the nuclei were pelleted by a 5 min centrifugation at 1000 × g and then resuspended in 1 ml of 0.4 N H_2SO_4. Following an overnight incubation at 4°C, cell debris was pelleted by a 10 min centrifugation at 12000 × g and the histones in the supernatant were precipitated by addition of trichloroacetic acid to 20%. The precipitate was washed once with ethanol and air dried.

2.2. *Gel electrophoresis and analysis*

SDS-polyacrylamide gels were run according to the method of Laemmli [9], using a 12.5% acrylamide resolving gel. Gels were stained for 1 h in stain no.1 (0.03% Coomassie blue, 25% isopropanol, 10% acetic acid) followed by 12 h in stain no.2 (0.0015% Coomassie blue, 5% isopropanol, 5% acetic acid). Gels were then destained by incubation for 24 h in 10% acetic acid. Gels were scanned on a Kratos SD-3000 Xenon lamp spectrodensitometer set at 520 nm. The output was quantified by a Hewlett-Packard 3380A integrator.

3. RESULTS

This line of research was initiated by the observation that in the NIE-115 cell line, different

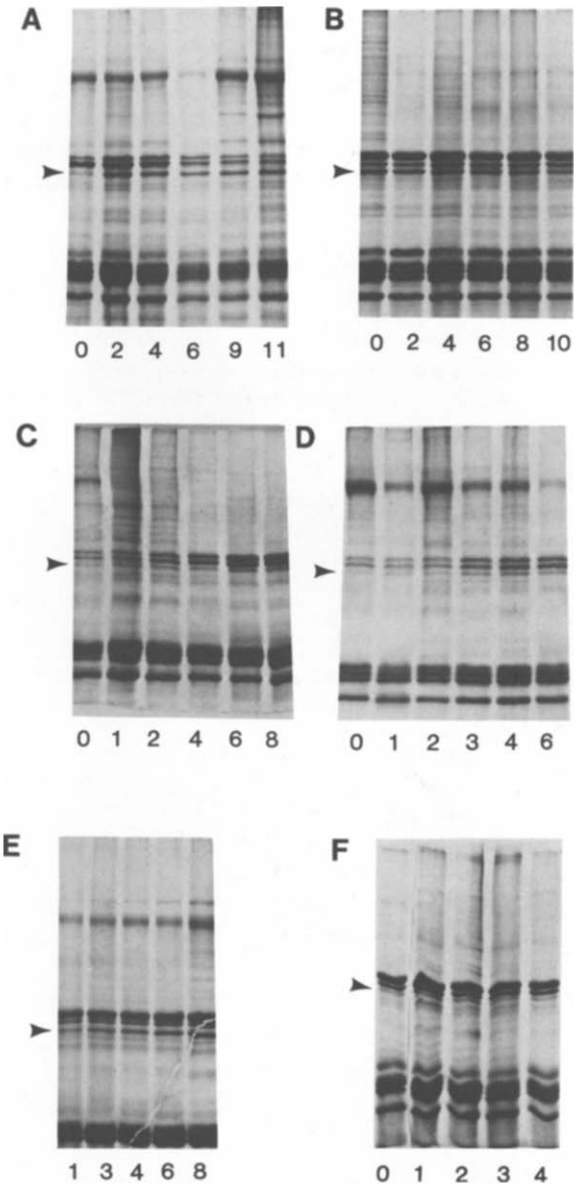

Fig.1. SDS-polyacrylamide gels showing the histone proteins during the time course of one treatment for each cell line. (A) CHO cells, butyrate treatment; (B) Vero cells, no serum; (C) HeLa S1, density inhibition; (D) HeLa S3, no serum; (E) NIE-115 cells, butyrate treatment; (F) 3T6 cells, DMSO. The numbers under each lane represent the days of each treatment. The position of histone H1° is marked by an arrowhead on each gel.

treatments used to block cell division resulted in different increases in the level of H1° [10]. We set out to determine whether this pattern was general by examining several other cell lines. Four treatments were used to block cell division. These were: 5 mM butyrate; 2% DMSO; withdrawal of serum and density inhibition. Six cell lines were tested, including 2 isolates of the human HeLa cell line, one of which, S3, was selected on the basis of its ability to grow in suspension, while the S1 line, like the other cell lines in this study, normally adheres to a plastic surface during growth. Two mouse lines were used, one of embryonic fibroblast origin (3T6), the other of neural tumor origin (NIE-115). Finally, a monkey kidney line (Vero) and a hamster ovary line (CHO) were used. As examples, fig.1 shows electrophoretic patterns of the histones sampled during the course of one treatment for each of the 6 cell lines. As expected [8], the H1 histone subfractions show electrophoretic heterogeneity among the different lines, however, in all 6 lines the levels of H1°, the fastest migrating H1 species, can be observed to increase during the treatments. H1° was identified

Table 1

Changes in the H1°/H1ab ratio among 6 cell lines blocked from dividing with 4 different treatments

Cell line	Treatment	Day 0 (H1°/H1ab)	*n*	Day 4 (H1°/H1ab)	*n*	Day 8 (H1°/H1ab)	*n*	Fold increase
S1	NaBU	0.05 ± 0.01	5	0.13 ± 0.00	3	0.12 ± 0.01	4	2.4
	DMSO			0.17 ± 0.02	3	0.20 ± 0.01	5	4.0
	NS			0.08 ± 0.00	5	0.10 ± 0.00	5	2.0
	DI			0.16 ± 0.02	5	0.18 ± 0.02	5	3.6
S3	NaBu	0.13 ± 0.02	5	0.33 ± 0.03	5	0.30 ± 0.07	5	2.3
	DMSO			0.33 ± 0.00	5	0.33 ± 0.06	5	2.5
	NS			0.25 ± 0.02	5	0.23 ± 0.01	5	1.8
	DI			0.37 ± 0.13	3	0.28 ± 0.01	4	2.2
CHO	NaBu	0.08 ± 0.00	5	0.86 ± 0.09	5	0.83 ± 0.12	5	10
	DMSO			0.32 ± 0.02	3	0.42 ± 0.01	4	5.3
	NS			0.85 ± 0.20	5	0.56 ± 0.16	5	7.0
	DI			0.40 ± 0.03	5	0.41 ± 0.06	5	5.1
3T6	NaBu	0.06 ± 0.01	5	0.21 ± 0.03	5	0.23 ± 0.02	5	3.8
	DMSO			0.21 ± 0.03	5	0.31 ± 0.03	5	5.2
	NS			0.21 ± 0.01	5	0.20 ± 0.02	5	3.3
	DI			0.17 ± 0.01	5	0.14 ± 0.01	3	2.3
Vero	NaBu	0.32 ± 0.03	5	0.97 ± 0.04	4	0.89 ± 0.03	5	2.8
	DMSO			0.58 ± 0.03	5	0.78 ± 0.07	5	2.4
	NS			0.42 ± 0.03	5	0.62 ± 0.02	4	1.9
	DI			0.49 ± 0.05	5	0.55 ± 0.02	5	1.7
NIE-115	NaBu	0.08 ± 0.01	5	0.42 ± 0.04	5	0.63 ± 0.06	5	7.9
	DMSO			0.20 ± 0.02	5	0.30 ± 0.03	5	3.7
	NS			0.19 ± 0.02	5	0.29 ± 0.03	5	3.0
	DI			0.19 ± 0.02	5	0.27 ± 0.03	5	3.4

Treatments NaBu, 5 mM butyrate; DMSO, 2% DMSO; NS, serum withdrawal; DI, density inhibition. *n* is the number of independent plates analyzed for each value obtained. The error in measurement reported is the SD for each value. Fold increase refers to the factor of increase in the Day 8 value compared with the Day 0 value for each treatment

with authentic bovine H1° as the fastest moving H1 in acetic acid/urea gels as well as on SDS-containing gels. In the acetic acid/urea system, more extended electrophoresis showed that H1° could be resolved as a doublet in all lines (not shown), as has also been observed in the case of calf kidney H1° [11].

To quantify the apparent increase in H1° during the treatments seen in fig.1, the histones were extracted and analyzed from multiple plates of each line grown for 4 or 8 days in each treatment. Using scanning densitometry of the H1 histones resolved by SDS-polyacrylamide gel electrophoresis the ratio of H1° to the other H1 histones was calculated and compared with the ratio in untreated cells. The analysis is shown in table 1. The H1°/H1ab ratio increased with all 4 treatments in all 6 cell lines. However, further examination reveals that the amount of the increase in the H1°/H1ab ratio differed among the different cell lines and that the relative effectiveness of the specific treatments on the H1°/H1ab ratio differed from one cell line to another.

A comparison of the H1°/H1ab ratios before any treatment was begun reveals that while 4 of the lines (CHO, NIE-115, 3T6 and S1) had about the same H1°/H1ab ratio (0.5–0.8), the plated HeLa cells (S3) had twice the initial H1° (0.13) of the spinner HeLa cells (S1), and the Vero cells had 4-times the starting H1°/H1ab ratio of the first 4 cell lines (0.32). Once treatment began, the overall increase in the H1°/H1ab ratio differed substantially among the different lines with differing treatments. Butyrate treatment of CHO cells resulted in the most dramatic increase in the H1°/H1ab ratio, a 10-fold increase over rapidly growing cells, while butyrate treatment of NIE-115 cells showed the second largest increase (7.9-fold). However, attempts to discern general trends in these data have proven futile. The relative increases in H1°/H1ab ratios were not simply a reflection of different extents of inhibition of DNA synthesis. For NIE-115 cells, after 4 days, butyrate, DMSO and no serum treatments showed identical 93% inhibitions of DNA synthesis rates and yet the relative effectiveness of the 3 treatments in H1° induction was 2.6:1.2:1, respectively. As shown in table 2, in which the treatments are ranked for each cell line in terms of their effect on the H1°/H1ab ratio based on a statistical analysis of the data from table 1, there are no generalities which can be drawn about specific treatments and the increase in the H1°/H1ab ratio. All 6 lines have different patterns of response to the treatments, including the 2 HeLa lines, which might have been expected to be very similar in most respects.

Table 2

Ranking of Various Treatments for Induction of H1°

Cell line	Effectiveness
S1	DMSO, DI > NaBu > NS
S3	NaBu, DMSO, DI > NS
3T6	NaBu, DMSO, NS > DI
Vero	NaBu > DMSO > NS, DI
NIE	NaBu > NS, DMSO, DI
CHO	NaBu, NS > DMSO, DI

The rankings in this table were established by pooling the data of day 4 and 8 for each treatment listed in table 1. The data were then analyzed at the 99% confidence level by using Student's *t*-test

4. DISCUSSION

The levels of H1° were measured in 6 cell lines when cell division was blocked by 4 kinds of treatment. No matter what the cell type there was an increase in the H1° ratio when cells were blocked from dividing. Thus, qualitatively, the correlation between H1° levels and mitotic quiescence held true among mouse, human, monkey and hamster cells of a variety of original tissue origins. However, quantitatively, the increase differed among the different lines from 2- to 10-fold, with final values of H1°/H1ab ratios ranging between 0.10 and 0.89. Moreover, it is striking that for a given cell line the amount of increase in the H1°/H1ab ratio was dependent on the method used to block the cells from dividing. Not only did the 4 treatments differ from each other in the extent to which they induced H1° in any cell lines, but the cell lines differed in their patterns of response to the various treatments. For example, for S1 cells density inhibition was in the most effective category and serum withdrawal in the least effective, while the opposite was true for CHO cells. The quantitative aspects of control of H1°

levels, therefore, are dependent upon the nature of the inducer but they are also dependent on the differentiated nature of the cell. H1° accumulation is not just a matter of mitotic quiescence [1] and of the termination of differentiation [5] but it is also affected by the particular pattern of differentiation in any cells. Clearly, then, the regulation of H1° is complex and the connection between an inducer and H1° induction is probably not direct.

ACKNOWLEDGEMENTS

This work was supported by NIH grants GMS20338, NIEHS ITES07075, and ES01896 and by the Agricultural Research Station.

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