SYNTHESIS OF HISTONE H1° IS NOT INHIBITED IN HYDROXYUREA-TREATED FRIEND CELLS

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1. Introduction

Until recently the notion of the stringent coupling of DNA and histone synthesis was accepted almost dogmatically [1]. It came mainly from studies on exponentially growing HeLa cells [2,3] or from studies where DNA-synthesis inhibition by hydroxyurea or cytosine arabinoside has been shown to be accompanied by histone synthesis inhibition [3–7]. A closer look at the literature data, however, shows that the coupling of DNA and histone synthesis is in many cases not absolute, some residual histone synthesis taking place even in the almost complete absence of DNA synthesis. A recent analysis of the interdependence of the two processes in HTC and HeLa cells under conditions of HU-inhibited DNA synthesis has led to the conclusion that the degree of coupling may be highly variable in different mammalian systems [8].

Here, HU-treated Friend cells are shown to exhibit also a significant degree of histone synthesis in the almost complete absence of DNA synthesis. In addition, the metabolic behaviour of histone H1° is shown to differ from that of the 5 common mammalian histones, its labelling remaining uninhibited by the treatment.

2. Materials and methods

Ostertag's erythroleukemic mouse spleen cells F4N were used [9]. The necessary final concentration of HU was achieved by adding calculated volumes of 10 mg/ml aqueous solution of HU to the culture medium. The amount of radioactivity incorporated into acid-insoluble material after labelling the cells with [^{3}H]thymidine or [^{3}H]lysine, [^{3}H]arginine, respectively was measured as in [10]. Nuclei were extracted with 0.25 N H_2SO_4, the histones fractionated by SDS–polyacrylamide gel electrophoresis [11] and the radioactivity in the separate fractions determined as in [10]. Protein was determined by the method in [12].

Abbreviations: HU, hydroxyurea; SDS, sodium dodecyl sulphate

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3. Results and discussion

3.1. *DNA synthesis in the presence of HU*

DNA synthesis in Friend cells is effectively inhibited by low concentrations of HU. Already 15 min after the addition of the drug (0.5 mM) the rate of [^{3}H]thymidine incorporation into acid-precipitable material drops to 5% of the control value and stays at this level for at least 3 h more. Lower concentrations of HU (0.2 mM) inhibit DNA synthesis to a lower degree (table 1). As HU is shown to produce only small changes in the TTP pool size [13], [^{3}H]-thymidine incorporation seems to be a valid measure for the rate of DNA synthesis.

3.2. *Protein synthesis in the presence of HU*

In order to study protein labelling under the conditions of inhibited DNA synthesis, cells were pretreated with HU for 15 min and then labelled with [^{3}H]lysine, [^{3}H]arginine for 2.5 h in the presence of the drug. During this period there was no measurable increase in the rate of DNA synthesis (see above). The data in table 1 show that the rate of radioactive pre-

Table 1
Effect of HU treatment on the labelling and the specific radioactivities of total nuclear protein and total histones

Treatment	DNA		Total nuclear protein				Total histones			
	Rate of labelling		Rate of labelling		Specific radio-activity		Rate of labelling		Specific radio-activity	
	cpm/10^4 cells	% inhib.	cpm/10^4 cells	% inhib.	cpm/μg	% decr.	cpm/10^4 cells	% inh.	cpm/μg	% decr.
None	262	0	732	0	1860	0	274	0	6600	0
HU 0.2 mM, 15 min	39	85	584	20	1450	22	97	65	2200	67
HU 0.5 mM, 15 min	14	95	571	22	1390	25	87	68	2110	68

cursor incorporation into total nuclear protein in the HU-treated cells was inhibited by ~20% in comparison with untreated controls. This inhibition led to a decrease in the specific activity of these proteins. At the same time there was a much more pronounced inhibition in the rate the labelled amino acids were incorporated into the histones (65–70%), reflected by a decrease in their specific radioactivity (table 1).

As we have made no measurements of the intracellular amino acid pools we cannot precisely evaluate how much the net protein synthesis was inhibited. The fact that the labelling of the total nuclear proteins was only slightly inhibited suggests that HU is not affecting the size of the amino acid pools significantly. In any case we can say for sure that the observed differences in the labelling of the two classes of proteins are real.

Thus, the almost complete inhibition of DNA synthesis is not accompanied by a complete shutting off of histone synthesis, similarly to the situation observed in experiments with other mammalian cells in culture [8,14–16].

3.3. *Effect of HU on the synthesis of individual histone fractions*

To compare the behaviour of the individual fractions, preparations of total labelled histones were fractionated by electrophoresis and the radioactivity of the individual stained protein bands determined. The densitogram of the stained gel is shown in fig.1. The identification of the fraction designated H1° is detailed in [17]. No other stained protein bands except the histones were seen. Radioactivity measurements of the sliced gels revealed no significant radio-

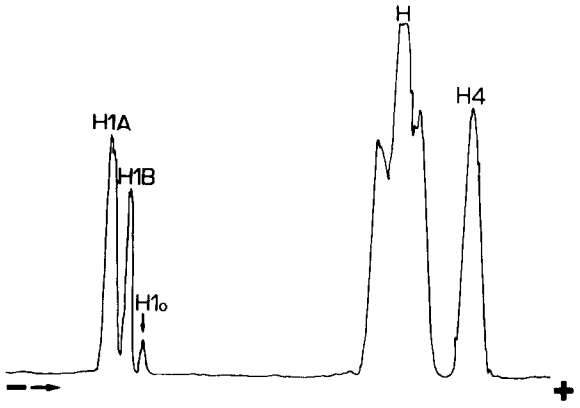

Fig.1. Gel electrophoretic analysis of the acid-extracted nuclear proteins from Friend cells: densitogram of the stained gel. Electrophoresis was performed in the presence of SDS; direction of migration was from left to right.

activity in the non-histone region of the gel. From these data it is evident that one cannot attribute the residual amino acid incorporation into the acid-extracted proteins observed in the presence of HU to incorporation in non-histone proteins of high specific activity as proposed in [18].

The results for the radioactive precursor incorporations into the individual histone fractions are presented in table 2. As seen, the overall reduction in the rate of total histone synthesis was the result of an unequal inhibition of the synthesis of the separate fractions. The strongest inhibition was observed for H4 and H (H2A+H2B+H3) followed by H1 (the two subfractions taken together). Strikingly, histone H1° showed a behaviour quite different from that of the other his-

Table 2
Radioactivity incorporated in the individual histone fractions in exponentially growing and HU-treated cells

Histone fraction	Exponentially growing cells		HU-treated cells (0.2 mM, 15 min)		HU-treated cells (0.5 mM, 15 min)	
	cpm/frac.	%	cpm/frac.	%	cpm/frac.	%
H1	13 670	100	5740	42	5130	38
$H1_o$	950	100	1280	135	1090	115
H	39 460	100	10 000	25	9620	24
H4	11 070	100	2080	19	1850	17
	65 150	100	19 100	29	17 690	27

The same amount of total histones was subjected to electrophoresis in all 3 cases. The yield of radioactivity in the gels was close to 100%. Histone fractions H2A, H2B and H3 were treated as 1 fraction as their separation on the gel was not satisfactory. The radioactivity in the 2 histone H1 subfractions (H1A, H1B) was also summed up. Qualitatively similar results were obtained in 2 other independent labelling experiments.

tones, its relative labelling being uninhibited. This situation is presented schematically in fig.2. Whether the high labelling of histone $H1^\circ$ is due to uninhibited synthesis or to a decreased rate of degradation remains to be established. As histone $H1^\circ$ accumulates during the induced erythroid differentiation of Friend cells [17] and since HU is also reported to be effective as an inducer [19], it would be interesting to see in further experiments whether $H1^\circ$ accumulates in the nucleus as a result of the HU-treatment.

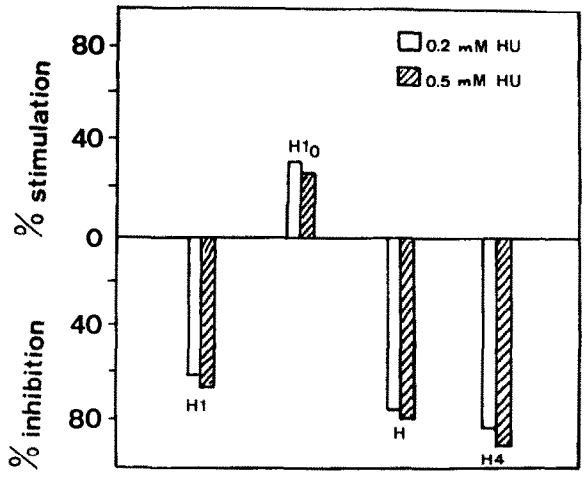

Fig.2. Schematic representation of the metabolic behaviour of the individual histone fractions in HU-treated Friend cells. The scheme is based on the data in table 2.

To our knowledge this is the first direct demonstration that histone $H1^\circ$ metabolism may be controlled independently of the metabolism of the other mammalian histones. The existence of such an independent control might have a bearing on the specific role of this histone in the terminal cellular differentiation.

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