IN VITRO RNA SYNTHESIS ON THE CHROMATIN TEMPLATE FROM CORTISONE-TREATED RATS

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1. Introduction

It has been shown that following injection of cortisone qualitative changes in the population of nuclear RNA in liver cells occur [1–3]. The possible function of newly synthesized RNA as messengers for specific cortisone-induced enzymes was assumed [1–3].

To gain further insight into the changes of transcription pattern following cortisone administration we have studied *in vitro* transcription employing chromatin preparations from liver cells of cortisone-treated and control rats as templates. RNAs synthesized *in vitro* were isolated and their hybridization capacity compared.

Hybridization experiments revealed a higher relative content of RNA molecules synthesized on repetitive nucleotide sequences of DNA during *in vitro* transcription using chromatin templates isolated from cortisone-treated animals. The results obtained are interpreted to mean that cortisone induces specific activation of transcription of repetitive DNA sequences in liver cells.

2. Materials and methods

100–150 g rats were used in this work. Cortisone-acetate in 0.14 M NaCl (7 mg per 100 g of body weight) was injected intraperitoneally 4 hr before the animals were sacrificed.

Rat liver nuclei were isolated in 2.2 M sucrose with 0.5% sodium β-glycerophosphate and 0.003 M $CaCl_2$ [4]. Nuclei were washed 4–5 times with 0.14 M NaCl–0.001 M $MgCl_2$–0.01 M Tris, pH 8 [5], washed with 0.05 M Tris, pH 8 and finally suspended in 0.01 M Tris, pH 8. Chromatin thus obtained was used in preparative cell-free RNA synthesis. Deoxyribonucleoprotein (DNP) obtained by the method of Marushige and Bonner [6] was used for the determination of template activity.

DNA was isolated by the phenol–detergent method [3] and treated with RNase and pronase (Serva). RNA polymerase was isolated from *E. coli* according to Chamberlin and Berg [7].

The incubation mixture contained per 0.5 ml: 0.2 μmoles each of ATP, GTP, CTP and ^{14}C-UTP, 20 μmoles Tris, pH 8.0, 2 μmoles $MgCl_2$, 0.5 μmoles $MnCl_2$, 6 μmoles β-mercaptoethanol and 5–10 units of RNA polymerase. Incubation was at 37° for 10 min. In preparative RNA synthesis each sample (1–3 ml) contained 100 μg/ml of free DNA or DNA as chromatin. Incubation was at 30° for 40 min. The RNA synthesized was isolated by the phenol–detergent method and treated with DNase (Worthington) and pronase.

Hybridization was performed either on UV-gels of DNA [8] or on membrane filters HAWP (Millipore) or MF-50 (Sartorius) according to Gillespie and Spiegelman [9].

3. Results and discussion

Template activity of the chromatin preparations isolated from livers of normal (DNP_n) and cortisone-treated rats (DNP_c) was compared. As seen from fig. 1 the template capacity of DNP_c was 20–30% higher than that of DNP_n under identical assay conditions.

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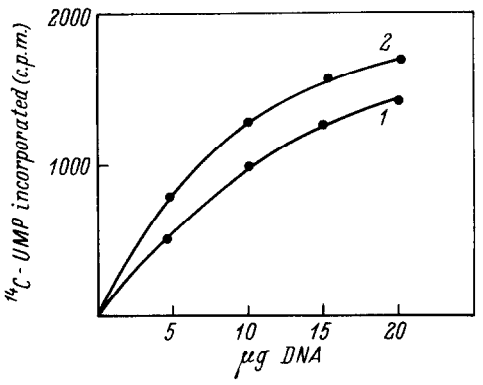

Fig. 1. Template activity of liver DNP, isolated from (1) control and (2) cortisone-treated rats. Abscissa: DNA as DNP per sample (μg). Ordinate: ^{14}C-UMP incorporated into acid-insoluble material (cpm per sample).

These data are in agreement with those previously reported by Dahmus and Bonner [10]. DNA was found to display 15–20 times higher template activity as compared to that of DNP.

The increase in template activity of DNP_c suggests the activation of the chromatin. To find out whether this activation involves qualitative changes of transcription we studied the nature of the RNA synthesized *in vitro* by the method of RNA–DNA hybridization.

It is known that the genome of eukaryotic organisms contains repetitive nucleotide sequences [11]. Hybridization conditions employed in this investigation provided evidence on the hybridization of RNA with repetitive sequences of DNA [12, 3]. The efficiency of binding of RNA molecules to DNA in excess DNA as well as hybridization kinetics can reflect the relative content of the RNA molecules synthesized on repetitive sequences of DNA [3, 12].

In the present work an attempt was made to study whether the RNA synthesized on chromatin from normal and cortisone-treated rats differs in the relative content of molecules synthesized on repetitive nucleotide sequences.

It was found that the hybridization capacity of RNA synthesized *in vitro* on DNP_c is considerably higher than that of RNA transcribed from DNP_n. As seen from fig. 2a about 20% of RNA synthesized on DNP_c is removed by DNA. However, with RNA synthesized on DNP_n the extent of hybridization is but some 5%.

The study of hybridization kinetics has shown that RNA synthesized on DNP_c hybridized at a higher rate than did RNA synthesized on DNP_n (fig. 2b).

Greater hybridizability and the higher rate of binding of RNA synthesized on DNP_c than on DNP_n indicates that RNA transcribed from DNP contains a

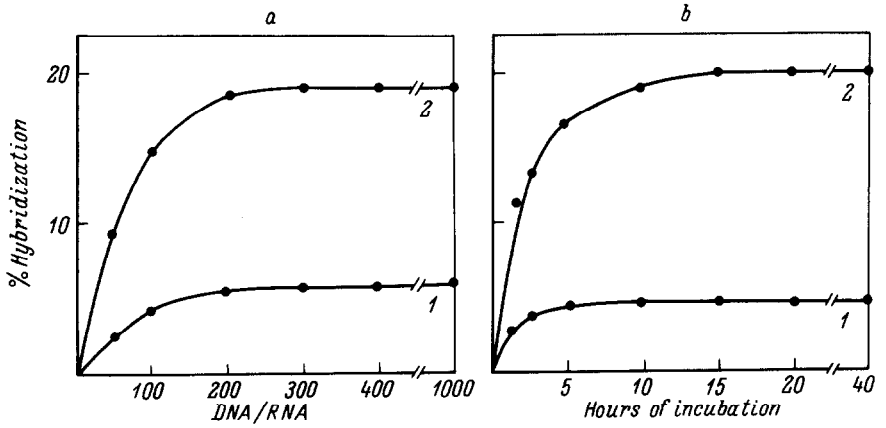

Fig. 2. Hybridization capacity of ^{14}C-RNA synthesized in cell-free system a) as a function of the DNA:RNA ratio in the sample and b) as a function of the incubation time. Chromatin isolated from livers of (1) control and (2) cortisone-treated rats was used as template. a) DNA per filter from 5–100 μg. ^{14}C-RNA per sample 0.1 μg (10,000 cpm). Incubation at 67° for 18 hr. b) DNA per gel, 300 μg. ^{14}C-RNA per sample, 0.1 μg (10,000 cpm). Incubation at 67°. Abscissa: a) DNA:RNA ratio, b) incubation time (hr). Ordinate: labelled RNA hybridized (%).

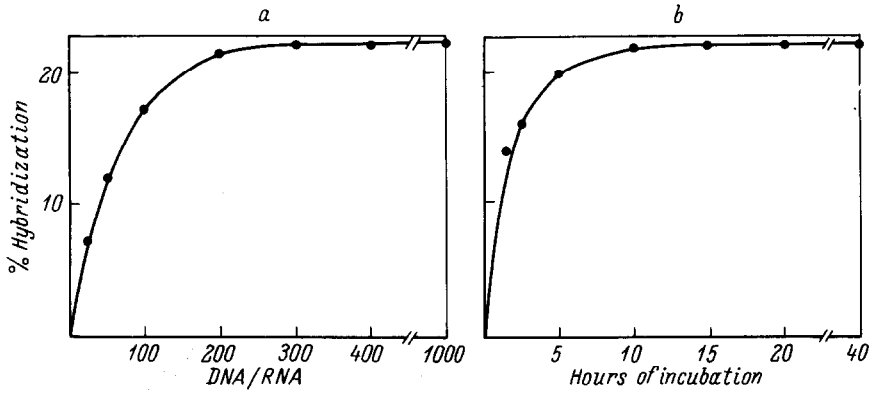

Fig. 3. Hybridization capacity of RNA synthesized on free rat liver DNA a) as a function of the DNA:RNA ratio and b) as a function of the incubation time. All experimental conditions as in fig. 2. Abscissa: a) DNA:RNA ratio, b) incubation time(hr). Ordinate: labelled RNA hybridized (%).

higher relative amount of RNA classes transcribed from repetitive DNA nucleotide sequences.

It has been shown also [13] that nuclear RNA isolated from livers of cortisone-treated rats displays a higher hybridization capacity with the DNA fraction renaturing at moderate rates (C_0t 0.4–5.4 × 10^2) than does control RNA.

The observed differences in the hybridization capacity can hardly be accounted for by the preferential synthesis of DNA-like RNA on the activated chromatin template because it has been shown [3] that, 4 hr after administration, cortisone stimulates the DNA-like RNA synthesis in liver cells to approximately the same extent as that of ribosomal RNA.

It is interesting that the hybridization capacity and the rate of binding of the RNA synthesized on purified DNA (fig. 3) are very close to those observed with RNA transcribed from DNP_c. The similar hybridization behaviour of RNA synthesized on free DNA and DNP_c does not imply that the same repetitive nucleotide sequences of the genome are transcribed. It only means that the relative content of RNA transcribed from repetitive DNA sequences is about equal.

Thus, hybridization experiments indicate that the cortisone-induced increase in template activity of chromatin involves qualitative changes in transcription. It can be assumed that cortisone preferentially activates transcription of repetitive DNA sequences in liver cells compared to less repetitive and unique sequences. Whether this activation is due to the specific increase in the rate of transcription of already active repetitive sequences or to the greater amount of repetitive sequences involved in transcription is now being studied.

We have already shown that the increase of chromatin template activity during early embryogenesis is also connected with qualitative changes in transcription [14]. Here, however, a different situation is observed; in embryo chromatin less repetitive and unique sequences of DNA are preferentially activated.

It might be assumed that the observed changes in transcription are accompanied by the changes of chromatin composition. But it appeared that the protein content as well as the ratio of histone to non-histone proteins within the chromatin remains unchanged during hormonal activation (table 1).

However, more delicate changes of chromatin components seem to be involved. Thus, we observed changes in the electrophoretic pattern of histone distribution [15]. According to Allfrey [16], cortisone induces selective changes in the synthesis of some acidic nuclear proteins in liver cells. Experiments are under way to further investigate the nature of cortisone-induced changes in the chromatin structure.

The function of the RNA's newly synthesized on repetitive DNA sequences under the action of cortisone remains to be investigated. These RNA's may serve as regulator RNA or messengers for regulator proteins. The possibility of the functioning of these RNA's as messengers for hormone-inducible enzymes

Table 1
Chemical composition of rat liver DNP.

	DNP_n	DNP_c
DNA	1.0	1.0
RNA	0.033	0.035
Histones	1.08	1.04
Non-histone proteins	0.73	0.70

Values represent the average of 3 experiments.

seems an equally attractive explanation. In which case the inducible enzymes might be coded for by repetitive nucleotide sequences and the enzyme induction by the hormone would involve activation of additional repetitive sequences.

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