TEMPLATE-ENGAGED AND FREE RNA POLYMERASE ACTIVITIES IN RAT LIVER NUCLEI AFTER CORTISONE INJECTION

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1. Introduction

Cortisone in vivo induces stimulation of the synthesis of ribosomal as well as heterogeneous nuclear RNA to about the same extent [1,2]. Three hours after cortisone injection the synthesis of these RNA is increased ~3-fold [1,2]. The observed increase in the rate of RNA synthesis may only partially be accounted for by the stimulation of the template activity of chromatin after cortisone injection, because the template capacity of chromatin is increased only to 20–25% [3,4]. Therefore, it may be suggested that cortisone stimulates the activity of both forms of the RNA polymerase (I and II).

After cortisone injection the activity of the RNA-polymerase I has been demonstrated to increase [5]. This increase is due to the modification of the enzyme to a more active species [6]. No changes in the level of RNA-polymerase II were found [5]. In these works the activity of only the engaged forms of the enzymes was studied. So it seemed of interest to investigate the possible changes in the activity of the free forms of the RNA-polymerases under cortisone injection. It may be mentioned that the changes in the ratio of free to bound forms of RNA-polymerase accompanying activation of transcription in the course of development have been reported [7,8].

Here, free and template-engaged RNA-polymerase activities were measured in nuclei from control and cortisone-stimulated rat livers. The both types of nuclei contain free and engaged α-amanitin-sensitive (RNA-polymerase II) and insensitive (RNA-polymerase I) activities. In nuclei isolated after cortisone injection (1.5 h and 3 h) the stimulation of RNA-polymerase I in engaged form is observed. Unlike it, the higher activity of RNA-polymerase II in the free form is observed after cortisone induction, the activity of bound form being unchanged.

2. Materials and methods

Male Wistar rats (100–120 g) were used. Hydrocortisone in 1 ml 0.14 M NaCl (5 mg/100 g body wt) was injected intraperitoneally 1.5 h or 3 h before the animals were sacrificed. Control rats received 1 ml 0.14 M NaCl. All animals had been fasted for 12 h. The injections of hydrocortisone were given always at the same time of the day (9:30 a.m.).

Rat liver nuclei were isolated by a modification of the Chauveau procedure [9]. Livers were homogenized in 4–5 vol. sucrose solution (SS: 2.2 M sucrose; 10 mM Tris; 2 mM $MgCl_2$; 25 mM KCl, pH 7.0). The homogenate was put on a 5 ml cushion of SS and centrifuged for 1 h at 135 000 × *g*. Highly hypertonic cold media used in this protocol prevents enzyme 'leakage' from the nuclei [10]. Integrity and purity of nuclei was checked by microscopic examination. The final pellet was resuspended to 3–4 mg DNA/ml in SS and was used immediately. We never used stored nuclei because after storage the nuclei exhibited variations in RNA-polymerase activity.

Template engaged nuclear RNA-polymerase activities were assayed in triplicate by the addition of 50 μl nuclear suspension (1 mg DNA/ml) to 50 μl reaction mixture containing 50 mM Tris–HCl (pH 7.9), 6 mM $MgCl_2$, 1 mM $MnCl_2$, 100 mM KCl, 2.5 mM dithiothreitol, 5 mM spermidine trichlorhydrate and 0.5 mM ATP, CTP and GTP. Usually the total concentration of UTP was 0.2 mM with 5 μCi [^{3}H]UTP.

α-Amanitin was used to distinguish between the multiple RNA-polymerases. Specifically RNA-poly-

merase II activity was equated with activity sensitive to low concentration (2.5 μg/ml) of α-amanitin [11]. The remaining activity is principally RNA-polymerase I but also contains a minor proportion of RNA-polymerase III activity. The RNA-polymerase III activity was estimated as resistant to high concentrations of α-amanitin (250 μg/ml). Free polymerase activities were assayed in the presence of actinomycin D (250 μg/ml) and poly[d(A–T)] (500 μg/ml) at low ionic strength [12]. Unless otherwise indicated, incubations were done for 30 min at 30°C, and the reaction was terminated by adding 50 μl 1 mg/ml RNA solution (as a carrier) and 0.5 ml 10% trichloroacetic acid containing 1% Na-pyrophosphate. The pellets were put onto Whatman GF/C glass microfibre filters and washed with 12 ml 10% trichloroacetic acid containing 1% Na-pyrophosphate. The radioactivity was measured in a Beckman LS-250 scintillation counter using KL-402 liquid scintillator (Koch-Light, England).

[5,6-^{3}H]UTP (spec. act. 46 Ci/mmol) was purchased from Radiochemical Centre, Amersham. Unlabeled nucleoside triphosphates, α-amanitin, actinomycin D and poly[d(A–T)] were obtained from Boehringer Mannheim. All other chemicals were of analytical grade and were obtained from Sigma (St Louis MO).

3. Results and discussion

Fig.1 shows the kinetics of the total and α-amanitin-insensitive bound forms of activities of RNA-polymerases in nuclei from control and cortisone-stimulated rat livers. The activity of the engaged enzymes in normal nuclei and in nuclei isolated 1.5 h or 3 h after cortisone injection is depicted in fig.2. One can see, that the cortisone treatment leads to an increase of the activity of the engaged RNA-polymerase I both at 1.5 h and 3 h after cortisone injection, while the activity of the bound RNA-polymerase II is not stimulated.

Not all the RNA-polymerases in nuclei are, however, in the form of engaged transcription complexes [12,13]. Addition of exogeneous DNA to avian erythrocytes stimulated their capacity to synthesize RNA in vitro [14], suggesting that a part of the population of RNA-polymerase molecules is either free or released from the chromatin during incubation. It has also been observed that in rat liver nuclei a part of the RNA-polymerases I and II population is also free [15].

After cortisone injection, the activity of RNA-polymerase I in the free form is increased only slightly (table 1, fig.2), while the activity of the free RNA-polymerase II (α-amanitin-sensitive) is significantly stimulated at both studied intervals after cortisone injection (fig.2).

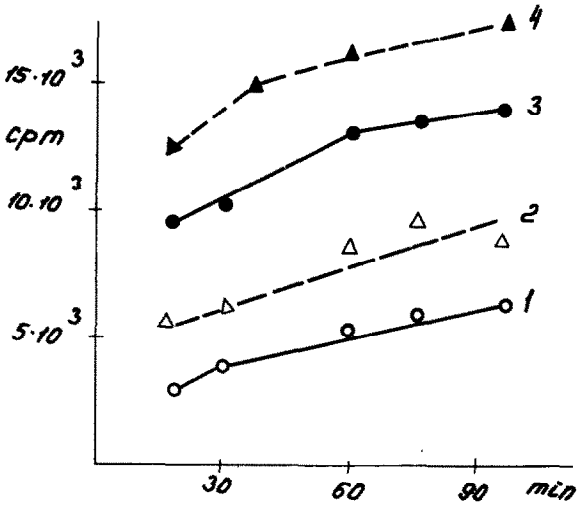

Fig.1. The kinetics of the total (3,4) and α-amanitin (2.5 μg/ml)-resistant (1,2) RNA-polymerase activity in rat liver nuclei: (1,3) normal rats; (2,4) cortisone-treated rats (1.5 h).

It is also seen from fig.2, that the total RNA-poly-

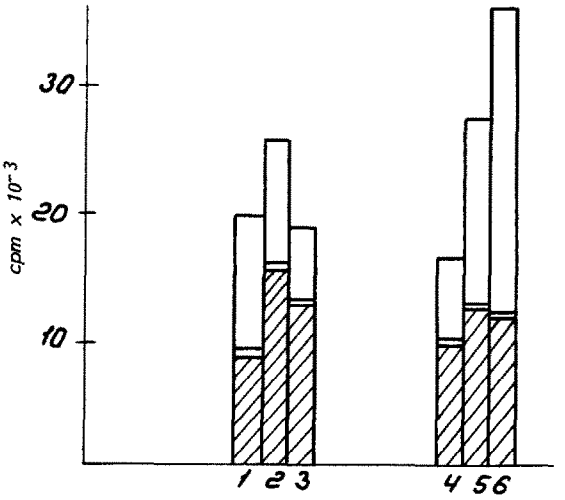

Fig.2. The activity of the free and bound forms of RNA-polymerase I and II in the nuclei of normal and cortisonestimulated rat livers: (1–3) bound forms of activity; (4–6) free forms of activity; (1,4) control rats; (2,5) 1.5 h after cortisone injection; (3.6) 3 h after cortisone injection; (▨) RNA-polymerase I; (▭) RNA-polymerase II.

Table 1
Free and bound RNA-polymerase activity in rat liver nuclei

	Control rats		Cortisone treatment				Experiment to control ratio	
	cpm[a]	% total act.	cpm[a]		% total act.		1.5 h	3 h
			1.5 h	3 h	1.5 h	3 h		
Total bound activity	20 400	100	26 200	19 720	100	100	1.28	0.97
Bound form I + III	8200	40.2	15 100	11 760	57.5	59.5	1.84	1.43
Bound form II	12 200	59.8	11 100	7960	42.5	40.5	0.91	0.65
Bound form III		5			5	5		
Total free activity	16 600	100	28 700	35 050	100	100	1.73	2.12
Free form I + III	10 000	60.2	12 100	12 300	42	35	1.21	1.23
Free form II	6600	39.8	16 600	22 750	58	65	2.51	3.44
Free form III		4.5			5	5		
Total free/total bound	0.82		1.08	1.78				
Free I/bound I	1.22		0.8	1.05				
Free II/bound II	0.54		1.50	2.86				

[a] Each value is the mean of 3 parallel measurements

merase activity in the free form is stimulated to a higher extent than the total activity of the engaged enzyme. The main part of the stimulation of the activity of the total free enzyme is due to the increased activity of the free form of the RNA-polymerase II (fig.2). The activity of RNA-polymerase III is negligible in comparison with that of RNA-polymerase I and II (table 1), and the activity of RNA-polymerase III does not change after cortisone injection.

Different tendencies in the changes of the activities of free and bound forms of RNA-polymerases I and II at various intervals after cortisone injection are observed (table 1): 1.5 h after hormone injection, the total activities of the bound (128%), as well as the free form (173%) of the enzyme are stimulated; 3 h after cortisone injection an increase in the total activity of only the free form of the enzyme is found (212%) whereas total bound activity is practically unchanged in comparison with the control. The activity of the bound form I is increased to a higher extent 1.5 h after cortisone induction (184%), than 3 h after hormone treatment (143%). Unlike it, the activity of the free form II is stimulated to higher extent after 3 h (344%), than after 1.5 h from the cortisone injection. Thus, the changes in the activity of form I are more pronounced at shorter times of cortisone action (1.5 h), than the changes of the activity of form II, which are more significant 3 h after the treatment.

Our results support the notion that the changes in gene expression which occur after cortisone injection result from a combination of changes both in functional characteristics of the chromatin template [3,4] and in the levels of RNA-polymerase activities. The rat liver nuclei isolated by this method contain a normal complement of these enzymes. After cortisone injection, an increased activity predominantly of RNA-polymerase I in the bound form and of RNA-polymerase II in the free form occurs.

The former observation is in good agreement with what could be expected from cortisone stimulation of ribosomal RNA synthesis in vivo. This result is also consistent with earlier data: that the activity of the engaged RNA-polymerase I is stimulated after cortisone injection [5,6]. Unlike the type I enzyme, nuclear RNA-polymerase II activity, not template-engaged, but free, increases principally after cortisone injection at the time when the synthesis of the RNA is stimulated to a large extent in vivo. The in vivo increase of the synthesis of heterogeneous nuclear RNA occurs to about the same extent (2–3-times) as ribosomal RNA at the intervals studied and could not be explained by the much lower stimulation of the template capacity of chromatin (20–25%) isolated from the livers of cortisone-treated rats. Thus, in rat liver nuclei the activity of the free RNA-polymerase II may reflect the activity of the enzyme that is actually active in vivo. It may be assumed that the elongation rate is increased after the cortisone injection and, as the result of this increase, a higher proportion of the enzyme molecules is released per time unit from the enzyme–template

complex and can be detected in the cell-free system as the free enzyme. A similar suggestion was put forward earlier for free RNA-polymerase activity in the loach embryonic nuclei [16].

Other assumptions may be made; i.e., that cortisone induces certain changes in the molecular structure of RNA-polymerase II. This question is under investigation now.

The observed differences in the character of the changes of the two types of RNA-polymerase after cortisone stimulation may be due to different mechanisms underlying increases of their activities or the intrinsic properties of different types of RNA-polymerase connected with peculiarities of the chromatin structure of nucleolar and other parts of chromatin.

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