REGULATION OF RNA SYNTHESIS IN THE LIVER OF RATS MAINTAINED UNDER CONTROLLED FEEDING SCHEDULES

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1. Introduction

It has been recently found [1] that both nucleolar and nucleoplasmic RNA synthesis measured in whole purified nuclei show daily systematic variations in the liver of rats maintained under the controlled feeding schedules developed by Potter et al. [2].

The food intake is responsible for the modulation of RNA synthesis in these experimental conditions [1]. However it is not known whether this regulation is achieved by a larger template availability for RNA polymerase, and/or by an increased activity and/or availability of the enzyme itself.

In the present paper we have evaluated the template availability of chromatin and the total amount of DNA-dependent RNA polymerase II after extraction from the liver nuclei at the times of the day in which RNA synthesis is maximal or minimal, in rats maintained under controlled feeding schedules.

2. Materials and methods

Seven-week-old male albino rats of the Wistar strain obtained from the departmental animal house weighing 200–220 g have been used in these experiments. The rats were housed, since weaning, in an air-conditioned windowless room with an inverted and displaced lighting schedule in which lights were on from 9.00 p.m. (21.00) to 9.00 a.m. (9.00) in a 24 hr cycle. The food, a purina chow lab diet, was supplied just before the lights were switched off, and was removed 8 hr later according to the '8 + 16' feeding schedule developed by Potter et al. [2]. Water was supplied ad libitum.

DNA dependent RNA polymerase II was solubilized according to the Jacob et al. [3] method from the liver nuclei purified according to Widnell and Tata [4]. Rat liver chromatin was extracted and purified from the purified nuclei as indicated by Butterworth et al. [5]. Deproteinized DNA was obtained from the purified chromatin [5]. Spectroscopic titration of the DNA, whole nuclei or chromatin with actinomycin D, was performed by the method described by Ringertz and Bolund [6]. DNA has been determined by the diphenylamine reaction of Burton [7] and proteins by the method of Lowry et al. [8].

3. Results and discussion

Fig. 1 reports some characteristics of the DNA-dependent RNA polymerase II solubilized at different hours of the day and in different feeding conditions.

We extracted the enzyme at 9.00 a.m. because in our experimental conditions, at this time of the day, RNA synthesis measured in whole purified nuclei is at its background level. The enzyme was solubilized also at 15.00 (3.00 p.m.). At this time of the day in fact RNA synthesis reaches its maximum level, after the rats have been allowed to feed for 6 hr (since 9.00 a.m.).

Addition of DNA is an absolute requirement for activity of the soluble enzyme in both the experimental conditions studied (fig. 1, graph B). In all preparations, the enzyme activity of 0.6 mg enzyme protein

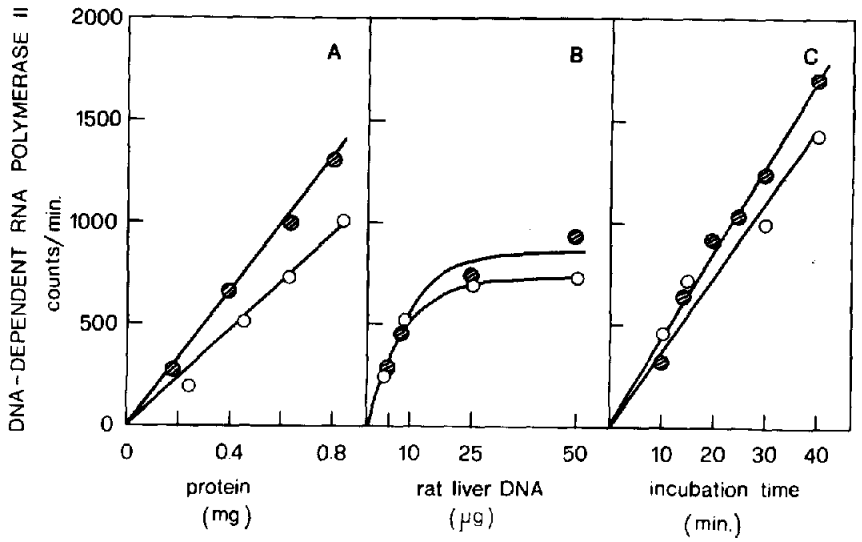

Fig. 1. Characteristics of DNA-dependent RNA polymerase II solubilized from rat liver nuclei at two different times of day. A – Relationship between enzyme concentration and activity. B – Relationship between template concentration and polymerase activity. C – Time–course of the reaction. (●-●-●,) enzyme at 9.00 a.m.; (o-o-o,) enzyme at 15.00 hr.. 0.75 ml of the reaction mixture contained: Tris–HCl, (pH 8.0) 40 μmoles; $MnCl_2$, 1.5 μmoles; NaF, 2.0 μmoles; GTP, UTP and CTP at 0.9 μmoles; ATP at 0.045 μmoles and [^{14}C] ATP at 0.015 μmoles. $(NH_4)_2SO_4$ was included at a final concentration of 0.06 M; the salt was added after 5' of preincubation of the reaction mixture. 0.6 mg of enzyme protein was incubated except for experiments in graph A in which several concentrations were tested. 50 μg of purified rat liver DNA was used except for experiments in graph B. The soluble enzyme was incubated for 15 min at 37°C except for experiments in graph C in which several incubation times were tried. The reaction was stopped by addition of 1 mg of albumin and 5 ml of 0.5 M $HClO_4$. The precipitate was collected on Whatman GF/C filters and the radioactivity was measured in a Nuclear-Chicago liquid scintillation spectrometer with a counting efficiency of 80%.

is also proportional to the amount of DNA and is almost saturated at about 25 μg of DNA.

At a fixed DNA concentration (50 μg), the soluble polymerase activity is proportional to the enzyme concentration (fig. 1, graph A). It appears also a different slope of the two curves. This indicates a lower specific activity of the enzyme solubilized from the liver of fed rats, although this could be ascribed to an increase of other proteins in the crude preparation.

The time–course experiments have shown the reaction rate to be constant for about 40 min (fig. 1, graph C) in all enzyme preparations studied. Again the different slope of the two curves indicate a lower activity of the enzyme solubilized from 6 hr-fed rats.

In order to have an estimate of the amount of DNA-dependent RNA polymerase II solubilized from the rat liver nuclei at different time of day, we have measured the amount of enzyme activity extracted per mg DNA. The results are reported in table 1. At a time of day, when a low rate of RNA synthesis is found (i.e. 9.00 a.m.), 170 units of enzyme activity are solubilized per mg DNA, and 148 are extracted at 15.00 hr, a time of day when maximum values are reached by the liver RNA synthesis. This result shows that the enzyme availability cannot account for the daily variations of RNA synthesis found in the rat liver [1].

On the other hand, an estimate of the degree of chromosomal repression and DNA template availability can be obtained by measuring the amount of actinomycin D that binds to chromatin [6, 9, 10]. This measurement cannot give absolute results because of the different nature of the binding (i.e. antibiotic and enzyme). Nevertheless the relative differences found in different experimental conditions have been shown to be a valid estimate of template availability [11, 12].

The data relative to the spectroscopic titration of the actinomycin D binding to the DNA, whole nuclei, and chromatin are reported in table 2. The results show a larger template availability either in whole nuclei or in the chromatin obtained at 15.00 hr. A similar nocturnal rise in the liver chromatin template

Table 1
DNA-dependent RNA polymerase II solubilized from rat liver nuclei at two different times of day.

Time of day	RNA synthesis in whole purified nuclei (see ref. 1)	Units solubilized/mg liver DNA
9.00 hr	Low	170 ± 12 [7]
15.00 hr	High	148 ± 10 [6]

Data are expressed as units solubilized/mg of liver DNA ± S.E.M. In parentheses are the number of experiments performed. One unit of enzyme activity is defined as the amount of enzyme able to catalyze the incorporation of one picomole of [^{14}C] AMP into the acid-insoluble product in 15 min using rat liver DNA as template.

activity has also been found in mice, by measuring the capacity to be transcribed by a bacterial enzyme [13].

The present results show that the systematic daily fluctuations of liver RNA synthesis can be ascribed to a regulatory system involving the DNA template rather than the enzyme availability. This point of view is also supported by recent results [14] showing a very low turnover rate of the mammalian liver enzyme, and hence the impossibility of regulating the short-term variations of liver RNA synthesis through the RNA polymerase availability.

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Table 2
Optical titration of actinomycin D binding to DNA, whole nuclei, and chromatin of rat liver at two different times of day.

Time of day	RNA synthesis in whole purified nuclei (see [1])	DNA	Whole nuclei (μg/mg DNA)	Chromatin
9.00 hr	low	302 ± 24 [4]	47.3 ± 3.1 [7]	30.6 ± 2.3 [6]
15.00 hr	high	–	70.4 ± 5.2 [6]	56.9 ± 2.9 [6]

Solutions of rat liver DNA (50 μg), whole nuclei (250–300 μg of DNA), or chromatin (250–300 μg of DNA) in 3 ml of 0.7 mM sodium phosphate buffer, pH 6.8, were titrated by successive additions of 2 μg quantities of actinomycin D. Data are expressed as μg of the antibiotic bound/mg DNA ± S.E.M.; in parentheses are the number of experiments performed on different occasions.

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