DIURNAL RHYTHMICITY OF MAMMALIAN DNA-DEPENDENT RNA POLYMERASE ACTIVITIES I AND II: DEPENDENCE ON FOOD INTAKE

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SUMMARY

Diurnal variations of DNA-dependent RNA polymerase activities I and II have been found in rats maintained under controlled feeding schedules. RNA polymerase I has two peaks of activity in a 24-hours cycle: one 6 hours after the onset of dark period and a second one in the middle of the light period. Polymerase II shows only one peak coinciding with the first one of polymerase I. These diurnal fluctuations are not present in the liver of rats denied food on the day of the experiment. Both polymerases do not exibit different optima for divalent metal ions and ionic strength in the different feeding conditions studied.

INTRODUCTION

Considerable evidence has now been accumulated that many metabolic activities of rat liver are not constant during the 24-hours cycle, and vary in a predictable manner as a function of time of day (1-5).

A daily rhythm in hepatic tyrosine-amine-transferase was first observed by Potter et al. (6, 7) in rats whose access to food was restricted to 12 hours in each 24, and other enzyme activities have been shown since to fluctuate daily (8-11). The incorporation of labelled precursors into RNA (5), the endoplasmic rediculum (12) and DNA synthesis itself (13) have also shown diurnal variations.

More recent studies have demonstrated the association of these rhythms with cycles of light and darkness and food intake, indicat-

ing the need for controlled feeding and lighting schedules in experiments with the laboratory rat (14, 15).

Potter et al. (16) have reviewed the development of the controlled feeding schedules and the rational behind them.

We have studied the fluctuations of the two DNA-dependent RNA polymerase activities in the liver of rats maintained under controlled feeding schedules as the diurnal variations of some metabolic activities are mediated by RNA synthesis. Besides this, the potential dependence of RNA synthesis on food intake could prove itself to be a very useful experimental condition for further studies on the regulatory mechanisms of RNA synthesis in this mammalian tissue.

MATERIALS AND METHODS

Seven weeks old male albino rats of the Wistar strain obtained from the departmental animal house weighting 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 hours cycle. The food, a purine chow lab diet, was supplied just before the lights were switched off, and was removed 8 hours later according to the "8+16" feeding schedule developed by Potter et al. (16). Water was supplied ad libitum.

Rats were killed by decapitation at the time of day indicated in the single experiments.

Nuclei were prepared from the livers according to Widnell and Tata (17) and the two DNA-dependent RNA polymerase activities were assayed by the α-amanitine differential assay developed by Novello and Stirpe (18).

DNA was determined by the diphenylamine reaction of Burton (19).

RESULTS AND DISCUSSION

Figure 1 reports the patterns of DNA-dependent RNA polymerase I

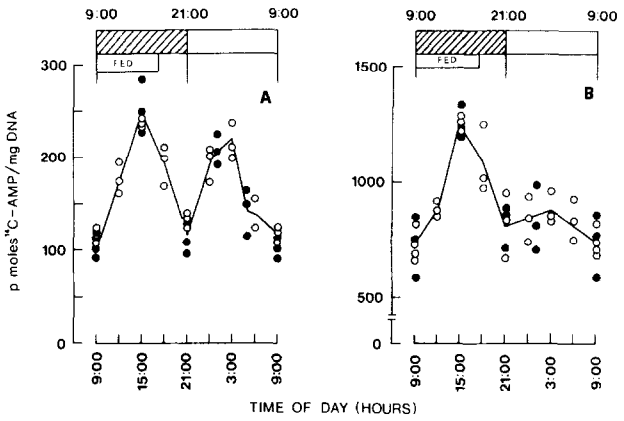

Fig. 1: Liver DNA-dependent RNA polymerase activities as a function of time of day. Purified nuclei (200-250 µg of DNA) were incubated for 15 min in the reaction mixture which contained in a final volume of 0.5 ml: for RNA polymerase I: 50 µmoles of Tris-HCl buffer, pH 8.0, 2 µmoles of $MgCl_2$, 7 µmoles mercaptoethanol, 3 µmoles of NaF, 0.3 µmole each of CTP, GTP and UTP, 0.015 µmole of non radioactive ATP, 0.005 µmole of 8-^{14}C-ATP, and 1 µg of α-amanitin; for RNA polymerase II: 50 µmoles of Tris-HCl buffer, pH 8.0, 2 µmoles of $MnCl_2$, 0.14 mmole of $(NH_4)_2SO_4$, 0.9 µmole each of CTP, GTP and UTP, 0.045 µmole of non radioactive ATP, and 0.015 µmole of 8-^{14}C-ATP, and 1 µg of α-amanitin (when present). The reaction was stopped by adding 1 mg of serum albumin and 5 ml of 0.5 M $HClO_4$. The precipitate was collected on Whatman GF/C glass fibre filters and the acid-insoluble radioactivity measured in a Nuclear-Chicago liquid scintillation spectrometer with a counting efficiency of 80%. Graph A: RNA polymerase I; Graph B: RNA polymerase II. Each point represents one rat (three determinations per animal), and the curves are drawn through the means. Open and close symbols represent separate experiments performed few months apart one from the other.

(nucleolar, α-amanitin insensitive) and II (nucleoplasmic, α-amanitin sensitive)(20) activities in a 24-hours cycle, as assayed in purified whole rat liver nuclei. In our experimental conditions polymerase I (Fig. 1A) shows diurnal changes characterized by two separate peaks of activity: one at 3:00 p.m. (15:00), and a second one around 2:00 a.m. (2:00). Both peaks of activity are twofold the background level of 115 pmoles of ^{14}C-AMP incorporated per mg DNA. A similar diurnal variation has also been found for polymerase II (Fig. 1B). The daily pattern is characterized by only one peak of activity at 3:00 p.m. (15:00) again reaching the range

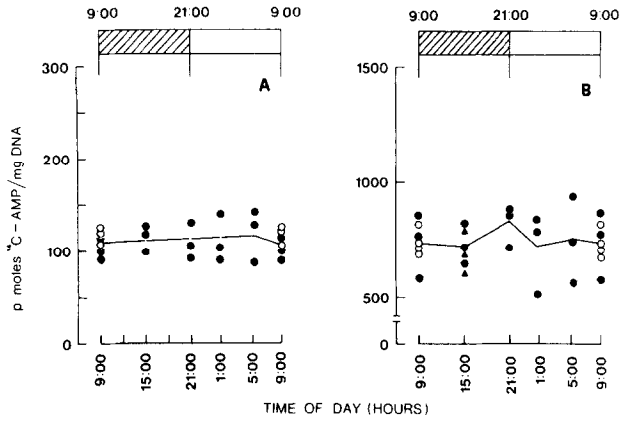

Fig. 2: Liver DNA-dependent RNA polymerase activities as a function of time of day in rats denied food on the day of the experiment. Graph A: Polymerase I; Graph B: Polymerase II. Experimental conditions were as described in the legend to Fig. 1 and in the text.

of twofold its background level of 700 pmoles of ^{14}C-AMP incorporated per mg DNA. We were unable to find a second peak of activity during the light period like for polymerase I.

It is worth noticing that both polymerase I and II show a fluctuation of their activity peaking 6 hours after the onset the dark period coinciding with the beginning of food availability. The remarkable possibility of a dependence relationship between food intake and the induction of either polymerase activity has been investigated by measuring the two reactions in the liver of rats denied food on the day of the experiment. The results are reported in Fig. 2. Both polymerase I (Fig. 2A) and II (Fig. 2B) do not show any more daily fluctuations in their activity, and the background level is maintained throughout the day. Therefore, the food intake is responsible for the rising phase of the diurnal variations of both RNA polymerase activities although not necessarily it can be ascribed to the absorption of a nutrient from the diet.

We have also studied the metal ion and ionic strength requirements for the optima of the reactions at 9:00 a.m. and at 15:00 (3:00 p.m.) in both rats fed since 9:00 and denied food on the day

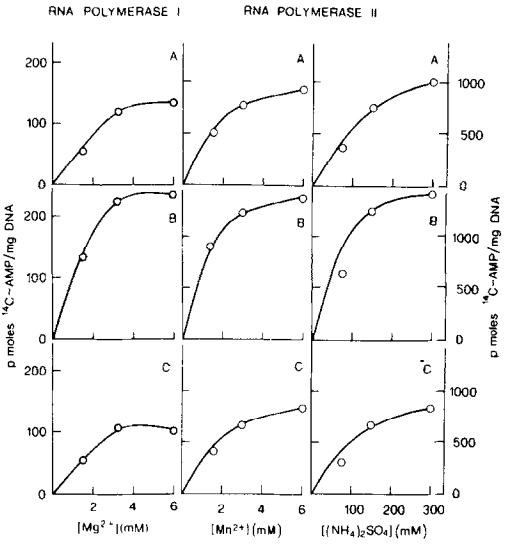

Fig. 3: Influence of divalent cations and ionic strength on liver DNA-dependent RNA polymerase activities I and II as a function of time of day and of feeding. Graph A: rats at 9:00 a.m.; Graph B: rats at 3:00 p.m. (15:00) fed since 9:00 a.m.; Graph C: rats at 3:00 p.m. (15:00) denied food on the day of the experiment. Experimental conditions were as described in the legend to Fig. 1 and in the text.

of the experiment. The results are reported in Fig. 3. In these experimental conditions no difference has been found in either polymerase activity in respect to their sensitivity to the *in vitro* concentration of Mg^{2+} ions (polymerase I), Mn^{2+} ions and ionic strength (polymerase II) showing that the food intake is not able to affect it.

Our findings, by presenting clear evidence for constant diurnal rhythms of both nucleolar and nucleoplasmic RNA synthesis and for their dependence on food intake, emphasize the need for a precise knowledge and control of the feeding schedules on which the animals are maintained in order to obtain more useful informations from the experiments. While these experiments were in progress, other authors (11) reported a rhythmic periodicity of the two RNA polymerase activities. Nevertheless it must be pointed out that their feeding schedule was practically unknown as it was a "natural one", being the food always available. Le Magnen and Tallon (21) have

in fact published a detailed study that demonstrated the irregular pattern of eating by rats in both light and dark periods with about 60% of the food eaten nocturnally.

Beyond this, it would be premature speculating whether the food-dependent diurnal fluctuations of both nucleolar and nucleoplasmic RNA polymerase activities are due to an increased activity and/or synthesis of the enzymes, and/or to a larger availability of DNA template for the polymerases, although there are indications (22) supporting this last possibility.

Nevertheless the present data indicate that the feeding status can be utilized as a useful experimental condition for studying the mechanisms of regulation of nuclear RNA synthesis in the mammals.

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