MAMMALIAN RNA POLYMERASES I AND II: INDEPENDENT DIURNAL VARIATIONS IN ACTIVITY

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SUMMARY

Diurnal variations in the activities of nucleolar (I) and non-nucleolar (II) DNA-dependent RNA polymerases of rat liver were observed. Both polymerase I and II displayed diphasic shifts in activity levels. However, the patterns of the individual shifts were not synchronous. Polymerase I activity was highest between 0100-1100 hours and lowest between 1700-2000 hours while polymerase II peaks between 2400 and 0500 hours with a low point at 1000 hours. The daily fluctuations and the dissimilar patterns of polymerase I and II activity may be of such significance as to require reevaluation and redesign of studies dealing with regulation of enzyme activity.

INTRODUCTION

The description of significant circadian changes in the activity levels of several hepatic enzymes that metabolize amino acids has demonstrated the necessity for controlling the time factor in studies of enzyme regulation (1). It would follow that if the product of specifically directed protein synthesis show cyclicity then the biochemical steps prefatory to enzyme activity, i.e., enzyme synthesis, synthesis of specific species of RNA, might also exhibit this endogenous rhythmicity.

Circadian rhythms have, in fact, been described for DNA synthesis (2), RNA synthesis (3), mitotic index (4), alterations in endoplasmic reticulum (5), as well as for a number of liver enzymes (1, 6-9). These internal variations of biologic processes are prone to direction and synchronization by external correlates. In these cases the external synchronizer or "Zeitgeber" (11) modulating the induction of these processes has been shown to be light.

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Additional refinements of these variations are due to patterns of food intake and glucocorticoid synthesis and release (1).

When the rate of RNA synthesis and subsequent protein synthesis is altered either nutritionally or hormonally, the change should be reflected in the level of DNA-dependent RNA polymerase activity. There are, however, no reports of which the authors are aware concerning the periodocity of the activity of the endogenous DNA-dependent RNA polymerase enzyme. Knowledge of such potential fluctuation of this activity may prove vital to the proper evaluation of the effects of various endogenous and exogenous factors on RNA synthesis.

This paper reports the presence of separate diurnal variations which exist in the activity patterns of two mammalian DNA-dependent RNA polymerases, i.e., polymerase I (nucleolar, α-amanitin insensitive, producing r-RNA) and polymerase II (extranucleolar, α-amanitin sensitive, producing DNA-like RNA). (12).

MATERIALS AND METHODS

Male rats (Charles River CD strain) were purchased at 150-170 grams body weight. They were housed 6 to a colony cage, under standard conditions of temperature and humidity. The light cycle was programmed to provide 12 hours of light and 12 hours of darkness; lights automatically went on at 0700 hours and off at 1900 hours. Food (Purina Laboratory Chow) and water were available at all times but the feeding schedule was such that the animals were observed to consume 65-75% of their daily intake during the period of 2000 to 0100 hours. The rats were adapted to these standardized conditions for 3 weeks.

Randomly selected rats were killed by cervical dislocation, every hour on the hour throughout a 24 hour period. From 1900 through 0600 hours rats were killed in total darkness. A minimum of 4 rats were killed at each time period Livers were removed immediately and a 5 gm section of the lower quadrants of each was used for isolation of nuclei.

Methods for the isolation and purification of nuclei, as well as for the

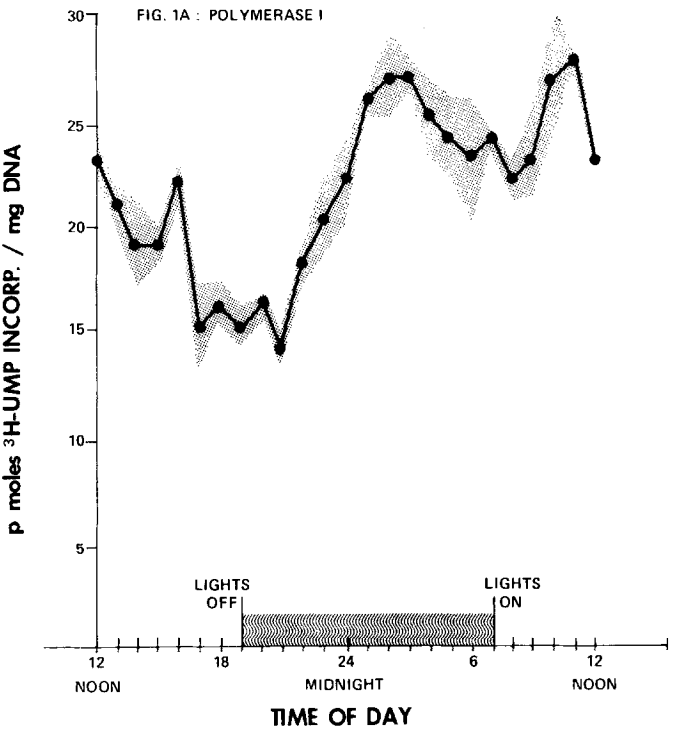

Fig. 1 (A): Fluctuations in the activity of the nucleolar DNA-dependent RNA polymerase (I) of rat liver during a 24 hour period. Rat liver nuclei were prepared as follows: Unless otherwise specified, all procedures were carried out at 4° C. Fresh liver tissue was homogenized in a Brinkman Pt-10 Polytron at 1/3 full speed in 25 vol. of 2.2 M sucrose in TKM buffer (0.01M Tris-HCl + 2 mM $MgCl_2$ + 25 mM KCl, pH 7.5). Nuclei were isolated and purified by modifications of the method of Blobel and Potter (15, 16). The assay for the endogenous RNA polymerase activity in isolated nuclei is a modification of the method of Roeder and Rutter (12) and is described elsewhere (26). Briefly, 100λ of nuclei (≈100 μg DNA) are resuspended in 25% glycerol in 0.01 M Tris HCl + 1 mM $MgCl_2$ (pH 7.9) and incubated at 15° C in the reaction mixture which contained: 10 μmoles Tris-HCl (pH 7.9), 0.4 μmole of mercaptoethanol, 0.16 μmole each of GTP, CTP and ATP, 0.05 μmoles ^{3}H-UTP (49 μCi/mole), 0.1 μmoles KH_2PO_4-NaOH buffer, pH 7.5, 2 μmoles KCl, 0.4 μmole $MnCl_2$, 0.5 μmole $MgCl_2$ in a total volume of 250λ. The reaction was incubated for 10 min. at 15° C. Under these conditions the rate of incorporation of ^{3}H-UTP into an acid-insoluble product was linear. Incorporation was dependent on all 4 nucleotides, divalent cations, DNA template and temperature.

Reactions containing low salt (0.01 M KCl) with 0.2 μg α-amanitin (Fig. 1 A) synthesized RNA with an AU/GC value of 0.75 while reactions containing high salt (0.25 M $[NH_4]_2 SO_4$) (Fig. 1 B) synthesized RNA with an AU/GC value of 1.10. RNase activity has been shown to be absent with incubation at 15° C (12, 17). The product (acid-insoluble radioactivity) was shown to be labile to RNase treatment and KOH hydrolyses.

The sensitivity of the reactions was optimized by terminating the reaction with 1 ml of cold 10% TCA, centrifuging, washing the pellet with 2 ml cold 5% TCA containing 1% $Na_2P_2O_7$ and recentrifuging. The pellet resuspended in the TCA-$Na_2P_2O_7$ solution was placed on filters (Millipore, 0.45μ pore size) The filters were washed with the TCA-$Na_2P_2O_7$ solution, dried, washed again with toluene to remove TCA, and counted in 5 ml of toluene based PPO-POPOP fluor in a scintillation spectrometer. The filters were removed from vials,

dried, hydrolyzed in 0.5 ml of 0.3 N $HClO_4$ at 90° C for 30 min. to remove DNA (18, 19). The DNA was quantitated by the diphenylamine reaction (20) and CPM/mg DNA calculated. This eliminates variations due to losses of precipitated nuclei (containing some labeled RNA) on walls of tubes and pipettes during the TCA washes. See section on Methods for details of animal handling and excision of liver. Each point represents triplicate analyses of at least 4 animals ± the standard error of the mean. The S.E.M. is represented by the shaded area about each point.

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assay of the DNA-dependent RNA polymerases are described in the legend of Fig. 1.

RESULTS AND DISCUSSION

A diurnal variation in the activity of both DNA-dependent RNA polymerases, I and II, is readily apparent in the isolated nuclei of rat liver (Fig. 1A and 1B). Under the specific standardized conditions of our animal quarters the activity of polymerase I in isolated rat liver nuclei peaks at approximately 0100-0300 hours at a mean level of 27 pmoles ^{3}H-UMP incorporated per mg DNA (Fig. 1A). Polymerase I activity then appears to fall gradually until it is apparently restimulated by the beginning of the light cycle (0700 hours). The variability in the activity of polymerase I (0100-0700 hours) is not statistically significant, therefore we assume that polymerase I remains within peak activity levels for approximately 10 hours. It then falls to a low activity plateau between 1700 and 2100 hours. The onset of the dark phase (1900 hours) initiates the feeding period and increased physical activity. Polymerase I then rises rapidly from 15 pmoles ^{3}H-UMP incorporated/mg DNA to the activity peak of 27 at 0100 hours.

Polymerase II activity (Fig. 1B) is notable for its sharp fall from a plateau of 400 pmoles ^{3}H-UMP incorporated/mg DNA (2400-0500 hours) to a low of 240 at 1000 hours. Between 1000 and 1100 hours the activity of polymerase II rises 125% (540 pmoles ^{3}H-UMP incorporated/mg DNA). This peak is sustained through 1300 hours, falling to 335 before rising steadily again to a broad activity peak (500) between 1900 and 2200 hours. It is of interest that a similar pattern of diurnal variation has been observed for template capacity

of mouse liver chromatin (11). Whether fluctuations in the activity of polymerase II are related to such variations remains to be determined.

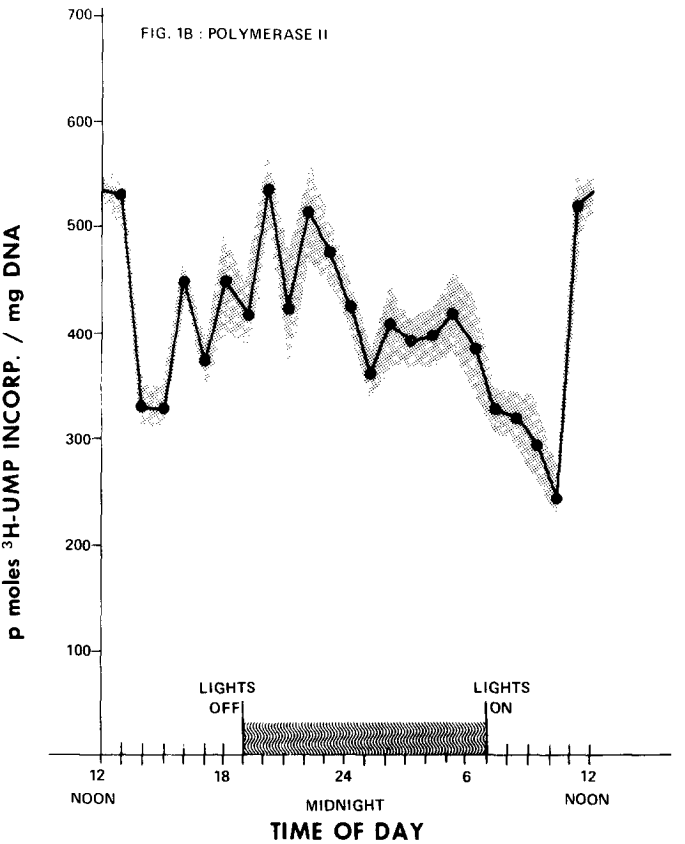

Fig. 1 (B): Fluctuations in the activity of the non-nucleolar DNA-dependent RNA polymerase (II) of rat liver during a 24 hour period. The conditions set down for Fig. 1 A also apply to this figure.

Comparison of Figs. 1A and 1B discloses a unique feature in the diurnal patterns of RNA polymerase activity in isolated liver nuclei. Polymerase I, which has been assigned the production of r-RNA (12) is most active in the early morning hours and least active at the end of the light phase. Polymerase II, which directs the synthesis of DNA-like RNA (12), shows its peak activity during the latter half of the light phase and falls at the time that polymerase I activity is at its peak. These data pose a series of relevant questions. What is the advantage to the animal of these patterns of RNA

polymerase activity? Are the peak activity periods independent of each other (polymerase I vs polymerase II) or are they integrated in some way? Is the activity of each polymerase further modulated by different series of exogenous synchronizers and would the modulation of a particular synchronizer or external factor be the same at different times? For instance, does the regime of food intake influence activity or is it the onset of the dark period which causes these variations?

Figure 2 attempts to correlate our polymerase findings with other biochemical processes involved in the regulation of protein synthesis. Our data and protocol, as well as all other data used in this figure, have been integrated with the schedules reported by Potter et al. (13) and Wurtman et al. (14). They have described the dependency of DNA synthesis and the activity of certain hepatic enzymes on light-dark directed feeding patterns and substrate availability.

The pattern of polymerase II (producing DNA-like RNA) activity levels

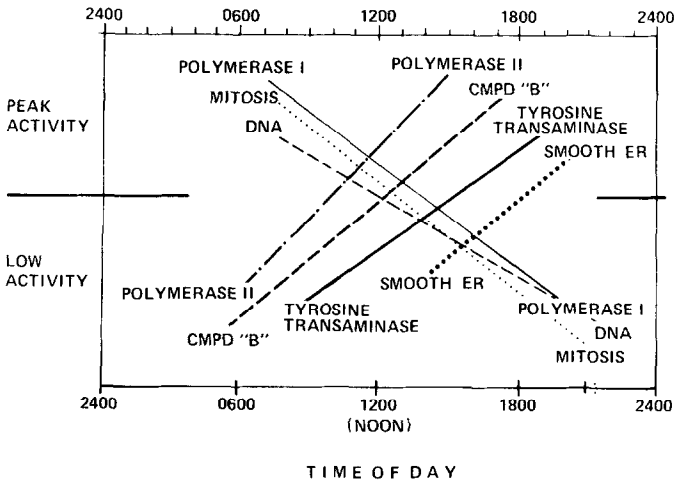

Fig. 2: Diurnal variation in a number of biochemical processes, occurring in rat liver, related to and dependent on protein synthesis. All results, including the polymerase I and II data cited in this paper, were normalized, for comparative purposes, to the light-dark and feeding cycles cited for DNA synthesis (2). Other data includes: mototic index (4), plasma levels of cortico sterone or compound "B" (7), activity of tyrosine transaminase (14) and appearance of smooth endoplasmic reticulum (5).

correlates with that of hepatic enzymes and smooth endoplasmic reticulum. This is of interest since the polymerase II enzyme is believed to synthesize RNA which translates, at least in part, for the enzymes and other "non-ribosomal" genes. It is apparent that a correlation exists between the diurnal variation in each of the endogenous RNA polymerases and certain specific biochemical processes (Fig. 2).

Finally one must inquire if these daily variations are of academic interest only or must they be considered in protocol design? When the hormonal induction of polymerase activity is reported, do the data actually reflect an action of the hormone on the enzyme or is the rise which is assigned to the hormone fortuitous but actually due to the daily rhythmicity in the enzyme's synthesis and activity? Thus, even when proper controls are included, animals assayed during periods of peak polymerase activity (either I or II) may not be as responsive to various stimuli as are animals used during the trough periods of enzyme activity.

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REFERENCES

1. Black, I.B. and Axelrod, J. in Biochemical Actions of Hormones, G. Litwack, Ed., Academic Press, N.Y. (1970), p. 135.
2. Barbiroli, B. and Potter, V.R. Science 172, 738, 1971.
3. Barnum, C.P., Jardetzky, C.D. and Halberg, F. Am. J. Physiol. 195, 301, 1958.
4. Jaffee, J.J. Anat. Rec. 120, 935, 1954.
5. Chedid, A. and Nair, V. Science 175, 176, 1972.
6. Rapport, M.I., Feigin, R.D., Burton, J. and Beisel, W.R. Science 153, 1642, 1966.
7. Civen, M., Ulrich, R., Trimmer, B.M. and Brown, C.B. Science 157, 1563, 1967.
8. Radzealowski, F.M. and Bousquet, J. Pharmacol. Exp. Therap. 163, 229, 1968.
9. Colas, A., Gregonis, D. and Moir, N. Endocrinology 84, 165, 1969.
10. Aschoff, J. Naturwissenschaften, 38, 506, 1951.

11. Steinhart, W.L. Biochim. Biophys. Acta 228, 301, 1971.
12. Roeder, R.G. and Rutter, W.J. Biochemistry 9, 2543, 1970.
13. Potter, V.R., Gebert, R.A., Pitot, H.C., Peraino, C., Lamar, Jr., C., Lesher, S. and Morris, H.P. Cancer Res. 26, 1547, 1966.
14. Wurtman, R.J., Shoemaker, W.J. and Larin, F. Proc. Natl. Acad. Sci. U.S.A. 59, 800, 1968.
15. Blobel, G. and Potter, V.R. Science 154, 1662, 1966.
16. Glasser, S.R., Chytil, F.C. and Spelsberg, T.C. Submitted, Biochem. J.
17. Chambon, P., Ramuz, M., Mandel, P. and Doly, J. Biochim. Biophys. Acta 157, 504, 1968.
18. Spelsberg, T.C. and Hnilica, L.S. Experentia 26, 136, 1970.
19. Spelsberg, T.C. and Hnilica, L.S. Biochim. Biophys. Acta 228, 217, 1971
20. Burton, K. Biochem. J. 62, 315, 1956.