Biochem. J. (1983) **210**, 837–844
Printed in Great Britain

Control of activation of liver RNA polymerase I occurring after re-feeding of protein-depleted mice

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(*Received 8 September 1982/Accepted 9 November 1982*)

Shortly after feeding protein-depleted mice with a meal containing protein, the RNA polymerase I activity in isolated liver nuclei shows a 2-fold increase over the values in the nuclei of either normal or protein-depleted mice. The activity of the RNA polymerase I solubilized from nuclei of re-fed mice was slightly enhanced, probably reflecting an increase in enzyme amount. However, this increase only accounts for about 30% of the stimulation of transcription in the intact nuclei. Administration of pactamycin, an inhibitor of protein synthesis, to normal or protein-depleted mice has almost no inhibitory effect on the RNA polymerase I activity in the isolated nuclei. On the contrary, within 15 min after treatment with the drug, the stimulated activity in nuclei from re-fed mice declines towards the values in normal or protein-depleted mice and then remains constant. The activity of the solubilized enzyme remains slightly elevated for at least 2½h after re-fed mice are treated with pactamycin. These observations indicate that the stimulation of the RNA polymerase I activity in the intact nuclei after re-feeding is controlled by mechanisms other than an increase in the enzyme amount and suggest the presence of short-lived proteins required for inducing an activated state of transcription.

We have previously shown that in the livers of protein-depleted animals there is a decrease of about 50% of the ribosome mass. When these animals are fed with an adequate diet, the ribosome content is restored after 1 day of re-feeding. This dramatic increase can be quantitatively explained by the enhancement of rRNA transcription and the suppression of ribosome degradation (Conde & Franze-Fernández, 1980). In the present study we analyse the mechanism underlying the increase in rRNA transcription after re-feeding. This is a suitable system for studying the control of transcription in normal cells, under conditions in which cell division is not pronounced (Conde & Franze-Fernández, 1980).

Regulation of the cellular rates of rRNA transcription may be accomplished via alteration of cellular RNA polymerase I (RNA nucleotidyltransferase, EC 2.7.7.6) concentration. In addition, regulatory factors may modulate the activity of the RNA polymerase I by modification of the enzyme itself or the chromatin template. Alterations in enzyme amounts may reflect long-term adaptive changes. This control, for instance, appears to be responsible for the stimulation of rRNA synthesis in the liver of rats treated with chemical carcinogens (Leonard & Jacob, 1977) and for the elevated degree of transcription in various hepatomas as compared with normal liver (Duceman & Jacob, 1980). On the other hand, the rapid increase in activity of RNA polymerase I in isolated liver nuclei after corticosteroid administration appears to be mediated by a modification of the enzyme (Sajdel & Jacob, 1971; Todhunter *et al.*, 1978). The present studies show that the stimulation of the RNA polymerase I activity in the intact nuclei after re-feeding is not due to an increase in the enzyme amount and suggest the presence of short-lived proteins required for maintaining the activated state of transcription.

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0306-3283/83/030837-08$2.00

Experimental

Diets

Purina Rat Chow (pellets) containing 23% (w/w) protein was from Purina de Argentina S.A., Buenos Aires, Argentina. The Protein Depletion Diet (powder) was prepared as indicated in U.S.P. XV, except that dextrin was replaced by the same amount of sucrose. This is a protein-free diet.

Chemicals

[5,6-^{3}H]UTP (52 Ci/mmol) and L-[1-^{14}C]leucine (50 Ci/mol) were from The Radiochemical Centre, Amersham, Bucks., U.K. Calf thymus DNA (type I) was from Sigma Chemical Co. The α-amanitin was kindly provided by Professor Th. Wieland of the Max Planck Institute, Heidelberg, W. Germany, and pactamycin was a gift of Dr. John Douros of the National Institutes of Health, Bethesda, MD, U.S.A.

Animals and treatments

Mice (male, 6 weeks old) were from a white strain bred by the Instituto Nacional de Farmacologia, Buenos Aires, Argentina. On arrival, the animals were housed at 25°C in a room illuminated from 07:00 to 19:00 h, and fed with Purina Rat Chow and water *ad libitum*. They were kept for at least 1 week under these conditions. They represent the normal control animals. For protein depletion, animals were fed *ad libitum* with the Protein-Depletion Diet for $3\frac{1}{2}$ days. At this time some of the mice were again re-fed with Purina Rat Chow for the indicated time. A similar decrease in liver protein and ribosome content was obtained when mice were protein-depleted with our powdered diet or with a commercial pelleted diet obtained from ICN Pharmaceuticals, Cleveland, OH, U.S.A. Pactamycin was dissolved as described by Scornik (1974*a*) and administered by intraperitoneal injection at the doses indicated. Control animals received injections of the solvent alone.

Solutions

Buffer A: 0.34 M-sucrose, 0.02 M-Tris/HCl (pH 7.9), 0.025 M-KCl, 0.01 M-$MgCl_2$. Buffer B: 1 M-sucrose, 0.02 M-Tris/HCl (pH 7.9), 0.01 M-$MgCl_2$. Buffer C: 0.05 M-Tris/HCl (pH 7.9), 0.01 M-$MgCl_2$, 0.1 mM-EDTA, Dextrans T70, T150 and T250 (0.3 mg/ml each), 30% (v/v) glycerol.

All buffers were prepared in double-distilled water and were made 1 mM in dithiothreitol and 1 mM in phenylmethanesulphonyl fluoride immediately before use.

Preparation of purified nuclei

Mice were killed by cervical dislocation. The livers from four or five mice for each experimental condition were quickly removed, thoroughly washed with cold iso-osmotic saline (0.9% NaCl; pH 7.3), immersed in 1.5 vol. of buffer A per g of liver and the pooled livers were homogenized. The homogenate was filtered through three layers of cheesecloth and quickly diluted with 2 vol. of 2.4 M-sucrose in buffer A. Nuclei were sedimented as described by Weaver *et al.* (1971). The nuclear pellet was resuspended in buffer B (1 ml/g of original liver). The DNA recovery was 55.6 ± 1.2% (S.E.M.) of the total tissue DNA in all the experimental conditions used.

Preparation of crude nuclear and cytoplasmic fractions

Livers were perfused with 5 ml of cold iso-osmotic saline (pH 7.3) before removal. Homogenization was performed in 1.8 vol. of buffer A containing 20% glycerol. After filtration through cheesecloth, nuclei were sedimented for 2 h at 100000 ***g*** in a MSE Prespin 50 centrifuge, with an 8 × 14 titanium angle rotor. The pellet, resuspended in buffer B (1 ml/g of original liver), constitutes the crude nuclear fraction; the supernatant is the cytoplasmic fraction.

Solubilization and chromatography of the RNA polymerases

The purified nuclear suspension was adjusted to 0.1 M-$(NH_4)_2SO_4$ by the addition of the appropriate amount of a 3.5 M stock solution (pH 7.9) and sonicated in 4 ml batches for about 14 s in 7 s bursts (Heat Systems–Ultrasonic Inc. sonicator, model W 225R, large probe, setting 5–6). This suspension was then adjusted to 0.32 M-$(NH_4)_2SO_4$, diluted with 2 vol. of buffer C, stirred for 15 min and centrifuged for 1 h at 100000 ***g*** in an 8 × 14 titanium angle rotor of a MSE Prespin 50 centrifuge. The supernatant was termed fraction F_1. The pellet showed no detectable enzymic activity in any of the different experimental situations. Similar yields of RNA polymerase I activity in the F_1 fraction were obtained when the nuclear suspension was disrupted as indicated or sonicated for another 15 s after adjustment to 0.32 M-$(NH_4)_2SO_4$.

Fraction F_1 was diluted with buffer C to an $(NH_4)_2SO_4$ concentration of 0.05 M, and a portion corresponding to 2–4 mg of DNA of the original nuclei was applied to a DEAE/Sephadex A-25 column (0.9 cm × 10 cm). The enzymes were eluted with a linear $(NH_4)_2SO_4$ gradient as previously indicated (Cereghini & Franze-Fernández, 1977). The peak of RNA polymerase activity eluted between 0.090 M- and 0.15 M-$(NH_4)_2SO_4$ was insensitive to 200 μg of α-amanitin/ml, and therefore corresponds to the RNA polymerase I (Kedinger *et al.*, 1970; Lindell *et al.*, 1970). The fractions containing RNA polymerase I activity were pooled, constituting fraction F_2. All the activity in the F_1 fraction was recovered after DEAE-Sephadex chromatography in the different experimental con-

ditions. Fraction F_2 can be stored at −70°C for at least 7 days with no loss of activity.

The RNA polymerases in the crude nuclear fraction were extracted and chromatographed by the procedure described above for the purified nuclei. The cytoplasmic fraction was adjusted to 0.05 M-$(NH_4)_2SO_4$, directly applied to the DEAE-Sephadex A-25 column and chromatographed by the procedure described by Cereghini & Franze-Fernández (1977). The poly(U) polymerase activity (Hayashi & MacFarlane, 1979) present in the crude nuclear and cytoplasmic fractions was not retained in the DEAE-Sephadex column.

To ascertain the absence of leakage of RNA polymerase I during nuclear purification, the enzymic activity in fraction F_2 obtained from purified nuclei was compared with those in the crude nuclear fraction and in the cytoplasmic fraction (Table 1). The results show that, for a given experimental situation, the activity extracted from purified nuclei was almost identical with that solubilized from the crude nuclear fraction. No preferential leakage to the cytoplasmic fraction was observed with livers from either protein-depleted or re-fed mice. No difference in the yield of RNA polymerase I was obtained irrespective of whether the nuclei were purified as described above or by the hyperosmotic-sucrose method described by Yu (1975).

RNA polymerase I assay

DNA-dependent RNA polymerase I in isolated nuclei was assayed by measuring the incorporation of [^{3}H]UTP (200–400 Ci/mol) into RNA as described by Iapalucci-Espinoza *et al.* (1977) in the presence of 15 μg of α-amanitin/ml. No further inhibition of the activity was found when 200 μg of α-amanitin/ml was added, indicating that in our nuclei preparation the α-amanitin-insensitive activity corresponds to RNA polymerase I (Weil & Blatti, 1976). Unless otherwise indicated, assay mixtures were incubated for 5 min at 30°C. All assays were performed in triplicate.

The assay of isolated RNA polymerase I was performed as described previously (Cereghini & Franze-Fernández, 1974) at an $(NH_4)_2SO_4$ concentration of 40 mM and in the presence of 15 μg of α-amanitin/ml. Unless otherwise indicated, assays were performed at two concentrations of the enzyme with saturating amounts of calf thymus DNA (100 μg/ml). Samples were incubated for 5 min at 30°C; reactions were linear for at least 10 min. Fraction F_1 and F_2 prepared from purified nuclei and fraction F_2 from both crude nuclear and cytoplasmic fractions were completely dependent on the addition of DNA for activity.

Transcription by isolated RNA polymerase I without reinitiation

The procedure described by Leonard & Jacob (1977) was applied with the following modifications: reaction mixtures, containing 50 mM-Tris/HCl (pH 7.9), 2 mM-$MnCl_2$, 6 mM-NaF, 0.7 mM-dithiothreitol, 0.6 mM-ATP and -GTP, different concentrations of calf thymus DNA and 15 μl of RNA polymerase I (corresponding to 17 μg of DNA of the original nuclei), were preincubated at 30°C for 5 min in a total volume of 60 μl. After the initiation complex was formed, $(NH_4)_2SO_4$, CTP and [^{3}H]UTP (400 Ci/mol) were added to final concentrations of 160 mM, 0.6 mM and 0.01 mM respectively. The reaction mixture (70 μl total volume) was further incubated for 5 min at 30°C; the reaction was then terminated by chilling and by adding 10 μl of a solution containing 3% sodium dodecyl sulphate and 0.15 M-sodium pyrophosphate; UMP incorporation was determined as described previously (Cereghini & Franze-Fernández, 1974).

Incorporation of radioactive amino acids into proteins

For the estimation of protein synthesis, mice were injected intraperitoneally with [^{14}C]leucine (10 μCi/100 g body wt.) and killed 15 min later by cervical dislocation. The livers were homogenized as indicated above and 0.1 ml samples of the liver homogenate were processed as indicated by Scornik (1974*b*) for radioactivity counting.

DNA measurement

DNA content was determined as described by Fleck & Munro (1962).

Table 1. *RNA polymerase I activity solubilized from isolated nuclei and liver homogenates after nutritional changes*

The RNA polymerases present in the cytoplasmic fraction and those solubilized from the purified nuclei and the crude nuclear fraction were chromatographed in DEAE-Sephadex columns as described in the Experimental section. The fractions containing RNA polymerase I activity were combined and assayed as indicated. The enzymes were prepared from the livers of five animals for each experimental condition.

Source of RNA polymerase I	Activity (pmol of UMP/mg of DNA) Protein-depleted mice	Re-fed mice, 5 h
Purified nuclei	119	147
Crude nuclear fraction	122	145
Cytoplasmic fraction*	25	23

* Estimated for an amount of cytoplasmic fraction corresponding to 1 mg of DNA in the crude nuclear fraction.

Results

RNA polymerase I activity in isolated nuclei and amounts of extractable enzyme after nutritional changes

We have previously demonstrated that after protein-depleted mice are fed with a meal containing protein the liver responds by increasing rRNA transcription (Conde & Franze-Fernández, 1980). The results in Fig. 1(*a*) show that 5 h after re-feeding, the RNA polymerase I activity in isolated liver nuclei increases about 2-fold over that in nuclei from normal or protein-depleted livers. No increase in activity was found at 3 h after the shift in diet (result not shown). As shown in Fig. 1(*b*), the enhanced activity in nuclei from re-fed mice is also observed when the assay is performed in the presence of aurintricarboxylic acid to prevent initiation of new RNA chains (Samal *et al.*, 1980). On the other hand, the results of an 8 min chase with the unlabelled precursor indicate that the RNA synthesized by liver nuclei from animals fed with the different diets has the same stability. Therefore the enhanced UMP incorporation in liver nuclei after re-feeding reflects an increase in either the number of growing RNA chains or the rate of RNA-chain elongation by RNA polymerase I molecules that were initiated *in vivo* before isolation of nuclei. It is noteworthy that, when liver nuclei of re-fed and protein-depleted mice were mixed and then incubated in the RNA-synthesizing system, the incorporation of UMP was equal to the theoretical value obtained by adding the activities of both nuclei when determined separately (results not shown). This observation rules out the presence of stimulatory or inhibitory factors in the nuclei preparation.

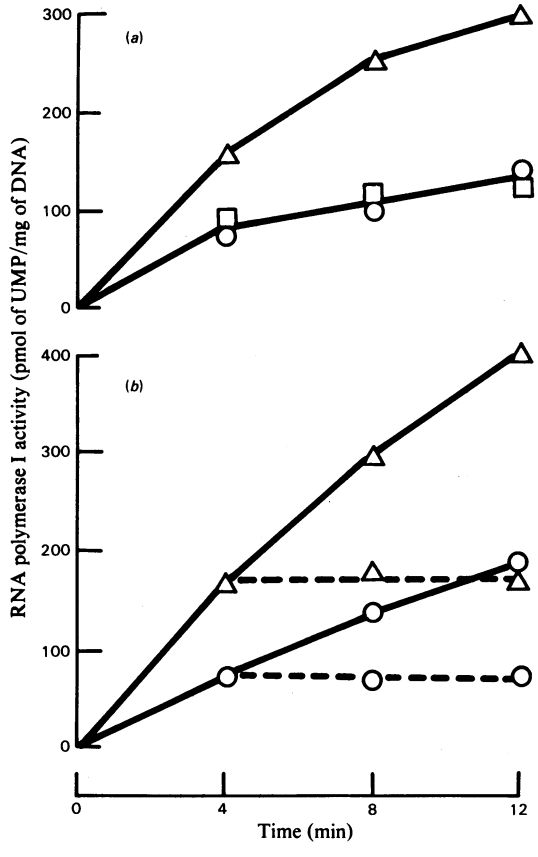

Fig. 1. *Kinetics of RNA polymerase I activity in liver nuclei after nutritional changes*

Nuclei were isolated from normal mice (□), mice fed with Protein Depletion Diet for 3½ days (○), or protein-depleted animals re-fed with the normal diet for 5 h (△). (*a*) The RNA polymerase I activity was assayed as indicated in the Experimental section or (*b*) nuclei were preincubated with 180 μg of aurintricarboxylic acid/ml for 4 min at 0–4 °C and the reactions were then initiated by the addition of the ribonucleoside triphosphates. Reaction mixtures were incubated for the times indicated (——). After 4 min, a 50-fold excess of unlabelled UTP was added to one part of the assay and the incubation was continued for the indicated times (– – – –).

The activity of the RNA polymerase I solubilized from liver nuclei of normal, protein-depleted and re-fed mice was then examined (Table 2). The RNA polymerases were extracted from purified nuclei and, after chromatography in DEAE-Sephadex columns, the fractions containing RNA polymerase I activity were pooled and assayed with saturating amounts of intact calf thymus DNA as template. The results showed a small and significant ($P < 0.05$) enhancement in the activity of the enzyme extracted from re-fed mice as compared with that from either

Table 2. *RNA polymerase I activity in the isolated nuclei and in the solubilized enzymes after nutritional changes*

Nuclei were isolated from four or five pooled livers of normal mice, mice fed for 3½ days with the Protein Depletion Diet and protein-depleted animals re-fed for 5 h with the normal diet. The RNA polymerases were solubilized and purified by DEAE-Sephadex chromatography as described in the Experimental section. The RNA polymerase I activity in the intact nuclei and in the solubilized enzymes were determined as indicated in the Experimental section. The results are given as means ± S.E.M. for the numbers of independent experiments indicated in parentheses.

	RNA polymerase I activity (pmol of UMP/mg of DNA)	
Condition	Isolated nuclei	Solubilized enzyme
Normal	107 ± 11 (5)	110 ± 14 (5)
Protein-depleted	104 ± 9 (12)	116 ± 14 (7)
Re-fed, 5 h	242 ± 20 (8)	149 ± 15 (6)

protein-depleted or normal animals. The increase in the activity of the liver enzyme from re-fed mice was independent of the incubation time (5 to 10 min), the enzyme concentration and the replacement of DNA by poly(dA-dT) as template. Furthermore, when the assay mixture contained saturating amounts of UTP (0.1 mM) instead of the usual limiting amounts (0.01 mM) (Cereghini & Franze-Fernández, 1977), the UMP incorporation increased by about 3-fold, but the ratio of RNA polymerase I activity in re-fed mice to that in either protein-depleted or normal mice remained unaltered (results not shown). In none of these cases did the increase observed in the solubilized enzyme activity explain the 2-fold ($P < 0.05$) activation in the intact nuclei after re-feeding. On the other hand, assays performed in a mixture of the solubilized enzymes similar to those described above for intact nuclei resulted in an UMP incorporation very similar to the predicted values, thus indicating the absence of stimulatory or inhibitory factors from the enzyme preparations. The enhanced activity of the solubilized enzyme after re-feeding reflects either an increase of RNA polymerase I concentration or a higher activity of the enzyme.

The activity of RNA polymerase I in both the intact nuclei and the solubilized enzyme were then measured at different times after protein-depleted mice were transferred to the normal diet. The results in Table 3 show that the activity of the solubilized enzyme remains slightly elevated for at least 36 h after re-feeding, whereas the stimulated activity in the intact nuclei begins to decline at about 12 h after the shift in diet.

Table 3. *RNA polymerase I activity in the isolated nuclei and in the solubilized enzyme at different times after protein-depleted mice are transferred to the normal diet*

Protein-depleted mice were either re-fed with normal diet before the experiment or maintained on the Protein Depletion Diet. Nuclei were isolated from the pooled livers of five animals for each experimental condition and the RNA polymerases were extracted and purified in DEAE-Sephadex columns as indicated in the Experimental section. Values in parentheses are percentages of those for protein-depleted mice.

Condition	RNA polymerase I activity (pmol of UMP/mg of DNA)	
	Isolated nuclei	Solubilized enzyme
Protein-depleted	108 (100)	126 (100)
Re-fed, 5 h	237 (219)	149 (118)
Re-fed, 7½ h	233 (216)	158 (125)
Re-fed, 12 h	193 (179)	161 (128)
Re-fed, 24 h	170 (157)	161 (128)
Re-fed, 36 h	159 (147)	150 (119)

Effect of protein-synthesis inhibition on the RNA polymerase I activity after nutritional changes

Reports from different laboratories have shown that rat liver rRNA transcription *in vivo* and the activity of the RNA polymerase I in the isolated nuclei are rapidly inhibited after cycloheximide administration (Muramatsu *et al.*, 1970; Yu & Feigelson, 1972; Coupar *et al.*, 1978). This has led to the postulate that rRNA synthesis in rat liver is stringently coupled to protein synthesis (Muramatsu *et al.*, 1970). More recent results, however, suggested that the observed inhibition of rRNA transcription may result not from the suppression of protein synthesis by cycloheximide but from deleterious effects of the drug (Stoyanova & Dabeva, 1980). These observations prompted us to use pactamycin as an alternative drug for protein-synthesis inhibition.

We first investigated the amount of pactamycin that inhibits protein synthesis in mouse liver, and found that, within 15 min after administration of 0.3 mg of pactamycin/100 g body wt., protein synthesis is inhibited by 90% or more and the effect lasts for at least 5 h (results not shown). The effect of pactamycin administration on the normal extent of transcription was then studied. As shown in Fig. 2(*a*), treatment of normal mice with 0.5 mg of pactamycin/100 g body wt. has only a slight inhibitory effect on the RNA polymerase I activity in the intact nuclei. No further inhibition of transcription was observed after longer periods of exposure to pactamycin (up to 5 h) or after administration of higher doses of the drug (up to 2 ml/100 g body wt.) (results not shown).

The results in Fig. 2(*b*) indicate that in the protein-depleted mice, as in the normal animals, the activity of the RNA polymerase I in the intact nuclei is hardly affected after pactamycin administration. On the contrary, the stimulated RNA polymerase I activity in liver nuclei from re-fed mice declines, after inhibition of protein synthesis, towards that observed in the protein-depleted animals. Since the RNA synthesized by liver nuclei from pactamycin-treated animals is stable (Fig. 2*b*), and under the conditions of the assay there is no initiation of new RNA chains, the decreased UMP incorporation by liver nuclei after pactamycin administration to re-fed mice should reflect a decline in either the number or the activity of RNA polymerase I molecules that were initiated *in vivo*.

The time course of the effect of pactamycin on the RNA polymerase I activity after re-feeding was also examined (Fig. 3). The results showed that, within 15 min after pactamycin administration to re-fed mice, the enzymic activity in the intact nuclei decreases towards the values in nuclei of protein-depleted mice and then remains constant. Confirming the above findings, almost no effect of the

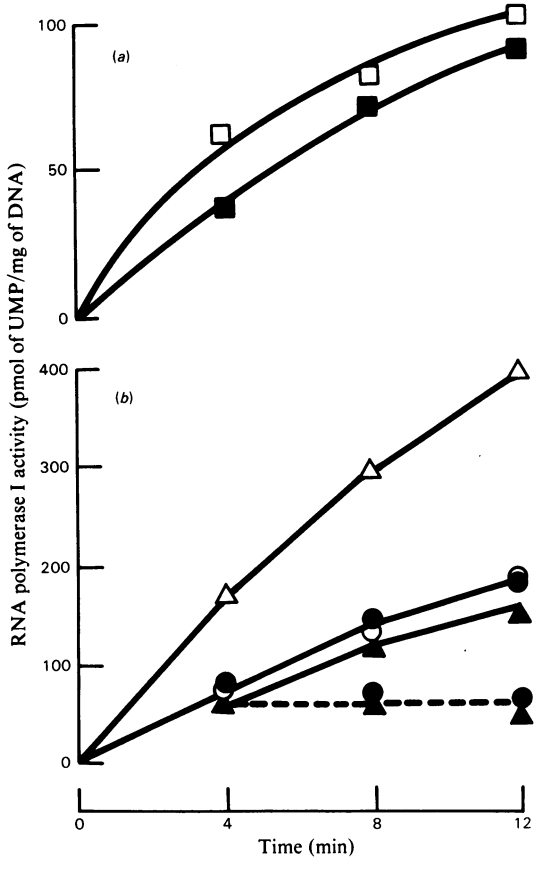

Fig. 2. *Effect of pactamycin administration on the RNA polymerase I activity in the intact nuclei*
(*a*) Nuclei were isolated from normal mice (□) or from normal mice injected with 0.5 mg of pactamycin/100 g body wt. 30 min before killing (■), and the RNA polymerase I activity was assayed as indicated in the Experimental section. (*b*) Mice were fed with the Protein Depletion Diet for 3½ days (○, ●). Then some of the animals were again fed with the normal diet for 5 h (△, ▲). Pactamycin (0.5 mg/100 g body wt.) was injected, 30 min before killing, to some of the animals in each condition (■, ●, ▲). Nuclei were isolated and the RNA polymerase I activity was assayed in the presence of 180 μg of aurintricarboxylic acid/ml as indicated in the legend to Fig. 1. Reactions were incubated for the times indicated (——). After 4 min, a 50-fold excess of unlabelled UTP was added to one part of the assay, and the incubation was continued for the indicated times (– – – –).

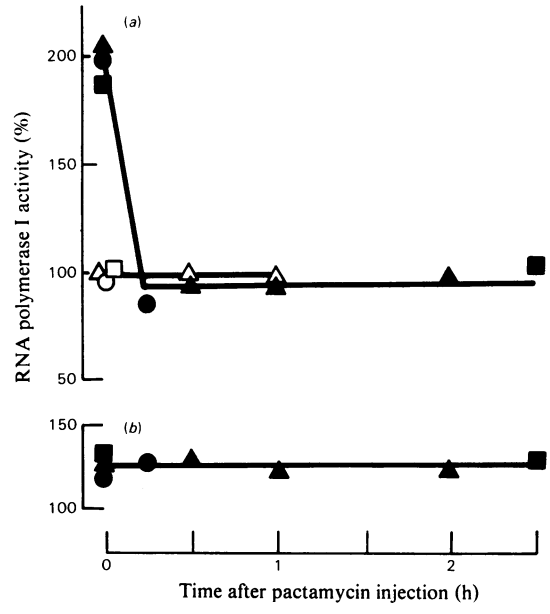

Fig. 3. *Time course of the effect of pactamycin on the RNA polymerase I activity after nutritional changes*
Animals were fed with the Protein Depletion Diet for 3½ days (○, △, □). Then some of the animals were returned to the normal diet for 5 h (●, ▲, ■). At zero time, pactamycin (0.5 mg/100 g body wt.) was injected into some of the protein-depleted and re-fed animals and, at the indicated times, mice were killed, and liver nuclei were isolated. (*a*) The RNA polymerase I activity was assayed in a fraction of the nuclear preparation. (*b*) The RNA polymerases were solubilized from the remaining nuclei and purified by DEAE-Sephadex chromatography. The fractions containing RNA polymerase I activity were pooled and assayed as indicated in the Experimental section. The different symbols correspond to independent experiments. The values are expressed as percentages of those in protein-depleted mice injected with 0.9% NaCl.

drug on the transcriptional capacity in starved nuclei was observed. With regard to these results it should be mentioned that pactamycin administration to protein-depleted animals at the time of re-feeding prevents the RNA polymerase I activation in the intact nuclei that takes place otherwise (results not shown).

The data in Fig. 3 also showed that the activity of the solubilized RNA polymerase I remains slightly elevated after re-fed mice are treated with pactamycin. Considering that RNA polymerase I has a long half-live (Benecke *et al.*, 1973; Chesterton *et al.*, 1975; L. Haim, unpublished work), this result is to be expected if the slight enhancement of the solubilized enzyme activity after re-feeding reflects an increase in the amount of enzyme. To test this point, the experiment depicted in Table 4 was performed. Samples of RNA polymerase I purified from re-fed or protein-depleted animals, corresponding to the same amount of DNA in the nuclei, were pre-

Table 4. *Assay of RNA polymerase I activity with different amounts of DNA*

Enzyme assay was performed under conditions inhibitory to reinitiation, as described in the Experimental section. Samples of RNA polymerase I (F_2 fraction) from liver nuclei from protein-depleted and re-fed mice corresponding to 17 μg of DNA of the original nuclei were used.

DNA concn. (μg/ml)	RNA polymerase I activity (pmol of UMP incorporated)	
	Protein depleted	Re-fed, 5 h
3	0.12	0.13
6	0.36	0.32
10	0.86	0.80
20	1.08	1.34
50	1.00	1.32
100	0.99	1.32

incubated with ATP and GTP and increasing concentrations of DNA in order to form the initiation complexes. This was followed by the addition of CTP and labelled UTP to allow RNA-chain elongation, together with an amount of $(NH_4)_2SO_4$ that prevents initiation of new RNA chains (Leonard & Jacob, 1977). The results show that at limiting DNA concentration the activity was identical in both enzyme preparations. However, at saturating concentrations of DNA, the difference in activity between the enzymes became evident. This result lends support to the idea of an increase in the enzyme amount after re-feeding. At 3–4h after the shift to the normal diet, liver protein synthesis increases by about 20–25% over the values in protein-depleted livers (results not shown). Thus the enhanced RNA polymerase I amount might simply reflect an increase in general protein synthesis.

Discussion

The results in this paper show that, shortly after protein-depleted mice are fed with a meal containing protein, there is a 2-fold stimulation of the RNA polymerase I activity in the isolated liver nuclei. The activity or, most probably, the amount of the enzyme extracted from nuclei of re-fed mice is also enhanced. The increase of the solubilized enzyme, however, is much lower than could be expected from the degree of stimulation of transcription in the intact nuclei and does not appear to be related to the mechanism controlling this stimulation. This last notion is supported by the observation that pactamycin administration to re-fed mice has a different effect on the activity of RNA polymerase I as measured in the intact nuclei or in the solubilized enzyme (Fig. 3) and by the finding that there is no parallelism between both activities at different times after re-feeding (Table 3). On the other hand, the rapid decline of the transcriptive capacity in nuclei of re-fed mice after inhibition of protein synthesis is not compatible with the half-life of the RNA polymerase I (Benecke *et al.*, 1973; Chesterton *et al.*, 1975) and suggests that short-lived proteins may be implicated in the maintenance of the high degree of transcription after re-feeding. It must be pointed out that the variations of the RNA polymerase I activity are only detected in the intact nuclei, in which RNA is synthesized by enzyme molecules that were initiated *in vivo*, but not under the non-specific assay used for the solubilized enzyme. Thus it is not possible to assert whether the enzyme itself or the chromatin template, or both, are involved in the control.

It was also shown that pactamycin administration to normal and protein-depleted mice does not measurably decrease the nuclear RNA polymerase I activity, suggesting that short-lived proteins are not required for the normal extent of transcription by the liver enzyme. This is in contrast with previous proposals based on results obtained in rats treated with high doses of cycloheximide (Muramatsu *et al.*, 1970; Benecke *et al.*, 1973), but is in agreement with a subsequent report which showed that the rate of rRNA synthesis in rat liver *in vivo* is not affected after treatment with low amounts of cycloheximide that inhibited protein biosynthesis (Stoyanova & Dabeva, 1980). The stimulated activity of the RNA polymerase I after re-feeding is, however, stringently coupled to protein synthesis.

It has been reported that the increase of the RNA polymerase I activity in rat liver nuclei after corticosteroid administration may be mediated by modification of the RNA polymerase I (Sajdel & Jacob, 1971; Todhunter *et al.*, 1978). In this case, however, the stimulation of the enzyme takes place even in the presence of protein synthesis inhibitors (Jacob *et al.*, 1975). Thus it appears that the early changes in RNA polymerase I activity in response to steroid hormones are different from those observed in the experimental situation reported here.

This work was supported in part by grants from Consejo Nacional de Investigaciones Cientificas y Tècnicas (CONICET), Secretaria de Ciencia y Técnica and Fundación Alberto Roemmers. We thank Professor Th. Wieland for giving us the α-amanitin and Dr. A. C. Paladini for the critical revision of the manuscript. M. T. F.-F. and R. C. are established investigators of CONICET. L. H. is recipient of a Fellowship from the same institution.

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