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Effects of glucocorticoid and cycloheximide on the activity and amount of RNA polymerase I in nuclei of rat liver

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The activity of the template-engaged form of RNA polymerase I from livers of adrenalectomized rats was about 50–60% of that of normal control rats, and increased about 2-fold at 6 h after the administration of dexamethasone. However, no change was found in the activity of the 'free' form of RNA polymerase I or the template-engaged form of RNA polymerase II. Immunochemical studies using guinea-pig anti-(RNA polymerase I) serum disclosed that the total number of RNA polymerase I molecules did not vary during the treatment with dexamethasone. Cycloheximide caused a rapid decrease in the template-engaged form of RNA polymerase I activity in normal rats and in dexamethasone-treated (6 h) adrenalectomized rats, to the value in adrenalectomized rats, but affected it only slightly in adrenalectomized rats. The elongation rate of rRNA-precursor synthesis in liver nuclei was not affected by a change in the concentration of circulating dexamethasone. From these results, it is concluded that about half the rRNA-precursor synthesis in rat liver is regulated by glucocorticoids, probably through the synthesis of short-lived protein(s) which may play a role in conversion of the 'dormant' form of RNA polymerase I into the 'engaged' form.

INTRODUCTION

It is well documented that the rate of synthesis of rRNA in mammalian tissues is subject to a wide variety of physiological and hormonal influences (for reviews see Chambon, 1975; Grummt, 1978; Miller & Sollner-Webb, 1982). The biochemical mechanism underlying this regulation, however, still remains unclear.

Administration of glucocorticoids caused a rapid increase in rRNA synthesis in liver (Greenman *et al.*, 1965; Dahmus & Bonner, 1965; Feigelson & Feigelson, 1965; Jacob *et al.*, 1969), whereas treatment of thymocytes, lymph cells and fibroblasts with glucocorticoids resulted in the inhibition of rRNA synthesis (Hallahan *et al.*, 1973; Borthwick & Bell, 1975, 1978; Cavanaugh & Thompson, 1983; Dembinski & Bell, 1984). Although the effect on pre-rRNA (rRNA precursor) synthesis appeared to be mediated by steroid-induced regulatory protein(s) (Sekeris & Schmidt, 1973; Bell & Borthwick, 1979), as with oestrogens (Nicolette & Babler, 1974) and androgens (Hosoya *et al.*, 1978), data were subsequently reported indicating that glucocorticoids might directly affect the transcription of the rRNA gene without obligatory participation of translational products which were sensitive to hormone (Frey & Seifart, 1982). In addition, other types of regulation were previously proposed, such as a modification (Sajdel & Jacob, 1971; Todhunter *et al.*, 1978) or a change in the activity (Yu & Feigelson, 1971, 1973) of RNA polymerase I (or A) (EC 2.7.7.6), or an activation of the processing of pre-rRNA by endonuclease (Dabeva & Ikonomova, 1982; Dabeva *et al.*, 1984).

Under these circumstances, we decided to examine whether or not template-engaged and 'free' form activities as well as the total amount of the enzyme in liver nuclei are affected by circulating glucocorticoid concentration and by cycloheximide administration, as studied in experiments on the effect of androgen on pre-rRNA synthesis in prostate (Suzuki *et al.*, 1984). The results indicate that the mode of glucocorticoid action on pre-rRNA synthesis in liver is very similar to that reported previously for androgens in prostate (Suzuki *et al.*, 1984, 1985).

MATERIALS AND METHODS

Chemicals

[5,6-^{3}H]UTP (sp. radioactivity 40 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. ATP, GTP, UTP and CTP were from Yamasa Shoyu Co., Choshi, Japan. Thioacetamide was from Merck, Darmstadt, Germany. Dexamethasone (9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione) and poly[d(A-T)] were from Sigma Chemical Co., St. Louis, MO, U.S.A. IgG Sorb (*Staphylococcus aureus*, Cowan I strain) was from the Enzyme Center Inc., Boston, U.S.A. All other chemicals were from Wako Chemical Industries Co., Osaka, Japan.

Experimental animals

Male Wistar albino rats (5–6 weeks old, 120–150 g), were adrenalectomized and maintained for 5–7 days on commercial food (Oriental MF, Osaka, Japan) and 0.15 M-NaCl *ad libitum*. Rats were housed under 12 h-light/12 h-dark lighting conditions, starved overnight and killed always at 10:00 h the next day. Cycloheximide was administered at a dose of 0.4 mg/100 g body wt. by intraperitoneal injection. Dexamethasone and other steroids were administered in saline (0.9% NaCl) containing 30% (v/v) ethanol by intraperitoneal injections.

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Isolation of nuclei

Rat liver nuclei were isolated by the hyperosmotic-sucrose method (Method II) as described previously (Hosoya *et al.*, 1978), except that the homogenizing medium was 2.2 M-sucrose containing 10 mM-$MgCl_2$ and 1 mM-phenylmethanesulphonyl fluoride and that the medium used to suspend the nuclear pellet was 0.34 M-sucrose containing 50 mM-Tris/HCl (pH 7.9), 0.5 mM-phenylmethanesulphonyl fluoride and 1 mM-dithiothreitol.

Assay for RNA polymerase I activities in isolated nuclei

Strictly speaking, the activity measured in this system contains the contribution from RNA polymerase III. However, the contribution was relatively small (usually < 5% of RNA polymerase I) and did not vary significantly under the conditions studied.

The activity of the template-engaged form of RNA polymerase I was assayed as described previously (Suzuki *et al.*, 1984) with the following modifications: (*a*) [^{3}H]UTP used was 1 μCi instead of 3 μCi; (*b*) the nuclear suspension was preincubated with α-amanitin (final concn. 1 μg/ml) at 25 °C for 5 min before other constituents were added and the mixture was incubated for 8 min instead of 10 min, in view of the time course shown in Fig. 6. The activity of the 'free' form of RNA polymerase I was also measured as described previously (Suzuki *et al.*, 1984), with the following modifications: (*a*) the total volume was decreased from 150 μl to 75 μl; (*b*) 3 μCi of [^{3}H]UTP, 30 μg of poly[d(A-T)] and 12 μg of actinomycin D were replaced with 1 μCi, 15 μg and 5 μg respectively; (*c*) 25 μl of the nuclear suspension was preincubated with α-amanitin (final conc. 1 μg/ml.) for 5 min at 25 °C and then mixed with 50 μl of other constituents, followed by incubation for 20 min. Subsequent procedures were the same as described by Suzuki *et al.* (1984). Enzyme activity was expressed as the amount of UMP incorporated into acid-insoluble materials per 100 μg of nuclear DNA in 8 min ('engaged' form) or per 25 μg of nuclear DNA in 20 min ('free' form).

Purification of RNA polymerase I and preparation of antiserum

Rat liver RNA polymerase I was purified by the method of Matsui *et al.* (1976), with modification at the following points. (*a*) As starting materials were used liver nucleoli of 40 rats which were previously injected intraperitoneally with thioacetamide (5 mg/100 g body wt.) 24 h before being killed (Anderson *et al.*, 1977; Leonard & Jacob, 1977). (*b*) The time of sonication was decreased from 60–80 s to 10 s by introducing a previous stirring for 60 min under high-salt conditions [0.3 M-$(NH_4)_2SO_4$]. (*c*) Removal of chromatin was performed by Sephadex G-50 gel filtration instead of centrifugation. (*d*) Poly(ethylene glycol) treatment was omitted and the step of dialysis was replaced by gel filtration on a PD-10 column (Pharmacia Fine Chemicals). (*e*) Glycerol-gradient centrifugation was replaced by the second phosphocellulose chromatography. The enzyme preparation obtained [sp. activity 650 units/mg of protein; 150–200 μg of protein in 0.5–1.0 ml of the buffer used for the second phosphocellulose column, i.e. 50 mM-Tris/HCl (pH 7.8)/0.5 mM-dithiothreitol/0.1 mM-EDTA/250 mM-$(NH_4)_2SO_4$/30% (v/v) glycerol] was emulsified with an equal volume of Freund's complete adjuvant and the mixture was injected to two guinea pigs biweekly. Antisera were obtained 12–16 weeks after the first injection. Non-immune IgG fractions which were used as control were prepared by $(NH_4)_2SO_4$ fractionation (0–40% saturation), followed by dialysis against 10 mM-Tris/HCl (pH 7.9) containing 0.9% NaCl. Double-diffusion analysis was performed as described by Ouchterlony (1949).

Immunochemical titration

RNA polymerase I employed for the immunochemical titration was prepared as follows. One group consisted of three rats. From pooled livers (20 g), nuclei were prepared as described above and then nucleoli were isolated by the method of Higashinakagawa *et al.* (1972). The crude nucleoli obtained were suspended in 0.5 vol. of 0.88 M-sucrose/0.05 mM-$MgCl_2$/0.2% Triton X-100, and the mixture was centrifuged at 2000 *g* for 30 min. The resulting pellets, which were almost devoid of chromatin, were suspended in 1.5 ml of 10 mM-Tris/HCl buffer (pH 7.9)/1 M-sucrose/5 mM-dithiothreitol and the mixture was gently stirred for 5 min. After addition of 1.5 M-$(NH_4)_2SO_4$ (pH 7.9) to give a final concentration of 0.3 M, the suspension was stirred for 30 min, followed by sonication (10 s at 200 W) with a Kubota Insonator 200 M with a micro-tip attachment.

The procedure for immunochemical titration was essentially the same as that described by Nakanishi & Numa (1970). The incubation mixture contained, in a total volume of 33 μl, guinea-pig anti-(RNA polymerase I) antiserum (10 μg of protein) and various amounts (30–300 munits) of sonicated nucleolar suspension mentioned above. Control tube contained non-immunized serum (10 μg of protein) of the animal instead of the antiserum. After incubation for 60 min at 0 °C, the solution was mixed with 33.3 μg of IgG Sorb dissolved in 1.33 μl of suspension buffer and further incubated for 20 min. The antigen–antibody precipitate was removed by centrifugation at 10000 *g* for 20 min and the supernatant was assayed for RNA polymerase I activity as described below. The reaction mixture, in a total volume of 60 μl, contained 2 μmol of Tris/HCl (pH 7.9), 0.6 μmol of $MgCl_2$, 19.8 nmol of dithiothreitol, 36 nmol of ATP, CTP and GTP, 1.8 nmol of UTP, 1 μCi of [^{3}H]UTP (40 Ci/mmol), 30 μg of bovine serum albumin, 10 μg of calf thymus DNA, 60 ng of α-amanitin and enzyme solution. $(NH_4)_2SO_4$ concentration in the assay medium was 150 mM. Incubation was carried out for 20 min at 30 °C and the incorporation of radioactive label into acid-insoluble materials was determined as described previously (Suzuki *et al.*, 1984). One unit of activity was defined as 1 pmol of UMP incorporated/min under the conditions specified above.

UMP/uridine analysis

Nuclei were incubated as described above, except that [^{3}H]UTP was present at 6.8 μCi per assay. After incubation, RNA was precipitated and hydrolysed, and the resulting nucleotides and nucleosides were absorbed on charcoal and re-extracted in pyridine/ethanol by the method of Barry & Gorski (1971), except that carrier uridine and UMP (10 μl of 10 mM solution) was added 30 min after the start of hydrolysis. In addition, radioactive uridine and UMP were omitted, since the present purpose was to estimate the elongation rate, and the separation of these materials was performed on

polyethyleneimine–cellulose thin-layer plates (Polygram Cel 300 PEI; Macherey–Nagel Co.) as described by Randerath & Randerath (1967), by using one-dimensional chromatography and 1.6 M-LiCl. Uridine and UMP spots were detected on the plates with an u.v. light, scraped off and counted for radioactivity after addition of 1 ml of 0.1 M-HCl followed by incubation at room temperature for 30–60 min and then mixing with 10 ml of scintillation fluid.

Determination of protein and DNA

DNA in the solubilized RNA polymerase I preparation was extracted with 5% (v/v) $HClO_4$ and determined by Burton's (1956) method, with calf thymus DNA as standard. Protein was determined by the Bio-Rad protein micro-assay method, based on the Coomassie Brilliant Blue method (Bradford. 1976), with bovine serum albumin as standard.

RESULTS

Effect of glucocorticoids *in vivo* on the activity of RNA polymerase I in isolated liver nuclei

Glucocorticoids were injected to adrenalectomized rats 6 h before death, and the activity of the template-engaged form of RNA polymerase I in isolated nuclei of liver was determined by the method described above. As shown in Table 1, all glucocorticoids used stimulated the enzyme activity to some extent, but the highest degree of enhancement was produced by administration of a synthetic glucocorticoid, dexamethasone (50 μg/100 g body wt.). The effect of various dexamethasone doses on liver nuclear RNA polymerase I activity was determined 6 h after the drug injection into adrenalectomized rats. It was found that even 5 μg of drug/100 g body wt. caused a marked increase in the activity, and to a plateau at 25 μg of drug/100 g body wt. Therefore 50 μg of the drug/100 g body wt. was used hereafter to examine the effect of glucocorticoids on the enzyme activity.

Table 1. Change in activity of template-engaged form of RNA polymerase I in isolated nuclei after administration of glucocorticoid hormones

Adrenalectomized rats were injected intraperitoneally with 50 μg of dexamethasone, 5 mg of cortisone acetate, 5 mg of cortisol or 1 mg of triamcinolone acetonide per 100 g body wt. 6 h before being killed. Adrenalectomized (control) rats were given the vehicle. RNA polymerase I activity in the isolated nuclei was assayed as described in the text. Experiments were done twice. The values are means of three determinations for each group. Values in parentheses indicate activity as a percentage of that of adrenalectomized (control) rats.

Treatment	RNA polymerase I activity (pmol of UMP incorporated) Expt. 1	Expt. 2
Adrenalectomized	26.2 (100)	23.9 (100)
+Dexamethasone	43.9 (168)	39.3 (164)
+Triamcinolone acetonide	53.0 (202)	42.2 (177)
+Cortisone acetate	43.0 (164)	47.1 (197)
+Cortisol	40.3 (153)	40.2 (168)

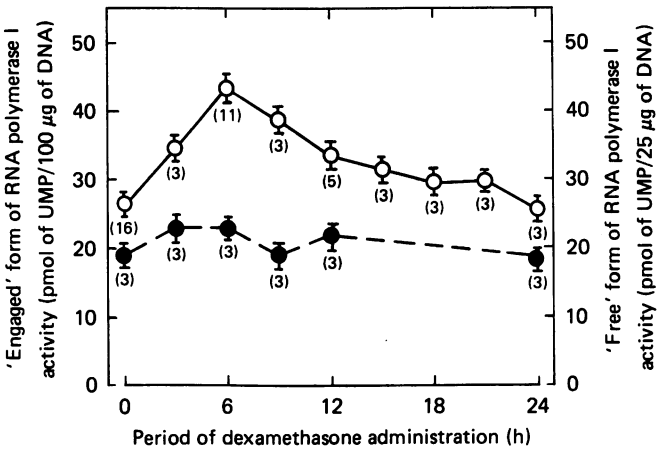

Fig. 1. Time course of hepatic RNA polymerase I activity after intraperitoneal administration of dexamethasone (50 μg/100 g body wt.) to adrenalectomized rats.

Nuclei were isolated and the activities of the 'engaged' (○) and the 'free' (●) form of RNA polymerase I were determined as described in the Materials and methods section. Each point represents the average of three to 16 independent experiments as indicated in parentheses, given with their S.E.M. (vertical bars).

Fig. 1 shows the changes in the activities of 'engaged' and 'free' forms of RNA polymerase I after a single injection of dexamethasone (50 μg/100 g body wt.). The 'engaged'-form activity reached a peak (about 100% increase over control) 6 h after administration of dexamethasone, whereas no significant change in the 'free'-form activity was observed during the time studied.

Determination of amounts of rat liver RNA polymerase I molecules in normal, adrenalectomized and dexamethasone-primed rats

Attempts have been made to examine whether or not the change in activity of RNA polymerase mentioned above is related to the amount of enzyme molecules in liver nucleoli. To accomplish this, the antibody against the enzyme was raised in the sera of guinea pigs by using the enzyme preparation obtained by the method described in the Materials and methods section. The purification method is based on the procedures previously reported (Matsui *et al.*, 1976), but is more simple and efficient: it took only 40 h after the extraction of nucleoli by sonication to obtain the final preparation, which contained 150–200 μg of protein, starting from usually 40 rats.

Antibodies raised in guinea-pig sera showed a single precipitation line against liver RNA polymerase I (Fig. 2). The antisera were able to inhibit the enzyme activity, and immunochemical titrations as depicted in Fig. 3 showed that a stoichiometric relationship existed between the enzyme and antibody, since the breaking points (57.4, 94.4 and 162.2 munits) showing the amount of enzyme removed in the enzyme–antibody precipitate were almost proportional to the amounts of antisera added (5, 10 and 20 μg of protein respectively).

When RNA polymerase I from normal, adrenalectomized, adrenalectomized plus 6 h-dexamethasone-treated or adrenalectomized plus 24 h-dexamethasone-treated rats was tested by immunochemical titration, it was found

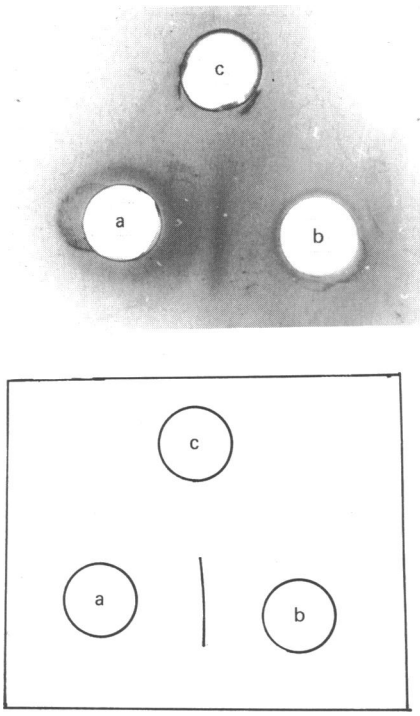

Fig. 2. Ouchterlony double-diffusion pattern of rat liver RNA polymerase I

Well a, 30 μg of partially purified RNA polymerase I (DEAE-Sephadex step); well b, 300 μg of protein of anti-(RNA polymerase I) serum; well c, 300 μg of protein of non-immunized serum.

that they had almost the same breaking points (Fig. 4, Table 2). This suggests that the amount of immunoreactive enzyme protein did not vary under these conditions. Table 2 also reveals that the specific activities in the extracts from the nucleoli were almost constant under the various conditions, except that DNA-based specific activity from 24 h-dexamethasone-treated rats was slightly higher than the control value. These data suggest that, except in 24 h-dexamethasone-treated rats, the amount of enzyme was not altered under the conditions used.

Effect of cycloheximide *in vivo* on the activity of template-engaged form of RNA polymerase I in liver nuclei

Fig. 5 shows the effect of cycloheximide administration on the template-engaged form of RNA polymerase I in isolated nuclei from liver. In these experiments, a low dose of cycloheximide (0.4 mg/100 g body wt.) (Suzuki *et al.*, 1984) was given, at the time indicated before death, to normal (○), adrenalectomized (△), or adrenalectomized plus 6 h-dexamethasone-primed (●) rats; the activity of the template-engaged of RNA polymerase I of normal and of adrenalectomized plus 6 h-dexamethasone-treated rats decrease rapidly after the injection of cycloheximide, to the value in adrenalectomized rats within 120 min. Thus the situation seems to be quite similar to that in prostate, reported previously (Suzuki *et al.*, 1984), although the half-life of the enzyme activity (about 15 min) was shorter than that in prostate (60 min).

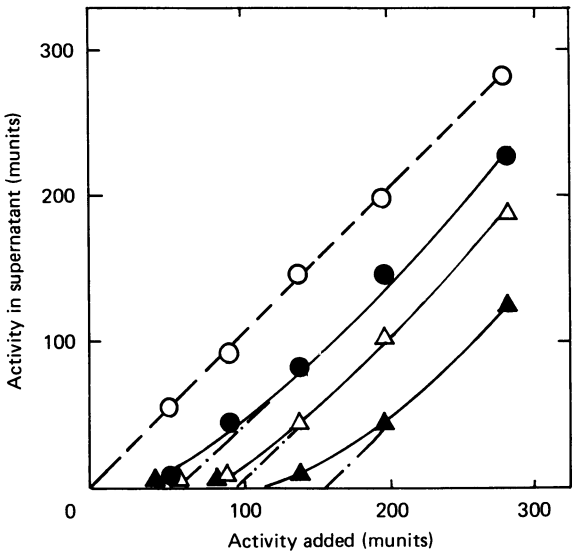

Fig. 3. Relationship between amount of antiserum added and amount of RNA polymerase I activity removed

Increasing amounts of solubilized RNA polymerase I from normal liver nucleoli were added to antiserum, (●, 5 μg; △, 10 μg; ▲, 20 μg of protein) or to non-immune serum (○; 20 μg of protein) as described in the Materials and methods section.

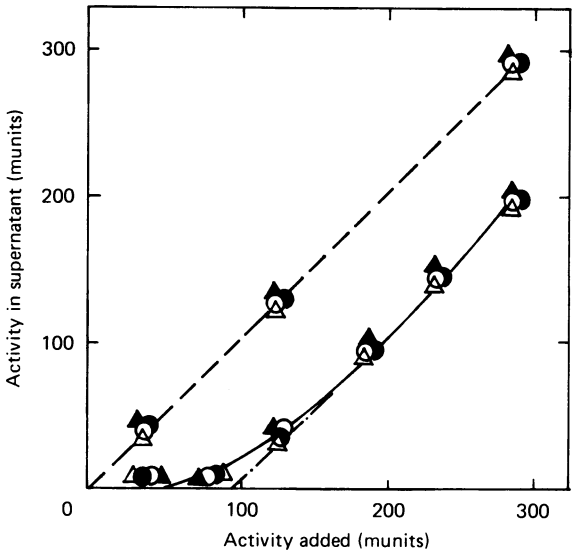

Fig. 4. Immunochemical analysis of RNA polymerase I in liver nucleoli from normal, adrenalectomized and adrenalectomized plus 6 h- or 24 h-dexamethasone-primed rats

Increasing amounts of RNA polymerase I solution containing the activities indicated were added to antiserum (continuous lines) or non-immune serum (broken lines) (each 10 μg of protein). Other details were the same as in Fig. 3. ○, Normal (untreated) rats; ●, adrenalectomized rats; △, adrenalectomized plus 6 h-dexamethasone-primed rats; ▲, adrenalectomized plus 24 h-dexamethasone rats.

Administration of cycloheximide to adrenalectomized rats appeared to cause a small but distinct decrease in RNA polymerase I activity, which is discussed below.

Table 2. Immunochemical titration and activity of RNA polymerase I in liver nucleoli from normal (not treated), adrenalectomized and dexamethasone-primed rats

Each group consisted of three rats. Equivalence points were obtained from the experiments shown in Fig. 3. Total activity of RNA polymerase I was obtained by measuring the activity, DNA and protein for the solubilized enzyme fraction from nucleoli as described in the text. The values are means ± S.E.M. of three experiments, with three determinations for each preparation. Statistical significance of differences between experimental groups and controls: *not significant; † $P < 0.05$ by Student's t test.

Treatment	Equivalence point (munits/10 μg of antiserum)	RNA polymerase I activity (munits/μg of DNA)	RNA polymerase I activity (munits/μg of protein)
Normal (not treated)	92.5±3.2	240.5±22.5	34.2±3.0
Adrenalectomized	95.9±0.6*	197.0±12.6*	31.9±2.5*
+Dexamethasone (6 h)	93.4±2.7*	222.8±20.6*	32.1±2.5*
+Dexamethasone (24 h)	95.9±1.6*	358.5±40.9†	36.6±6.2*

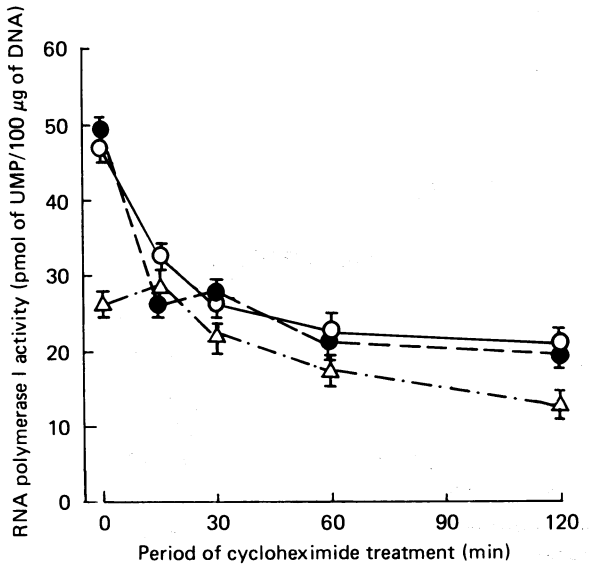

Fig. 5. Effect of cycloheximide *in vivo* on RNA polymerase I activity of rat liver *in vitro*

Animals were killed at 0, 15, 30, 60 or 120 min after cycloheximide administration. Nuclei were isolated and RNA polymerase I activity was determined as described in the Materials and methods section. For each time point, the mean ± S.E.M. (vertical bars) for three experiments with three determinations is shown. ○, Normal rats; △, adrenalectomized rats; ●, adrenalectomized plus 6h-dexamethasone-primed rats.

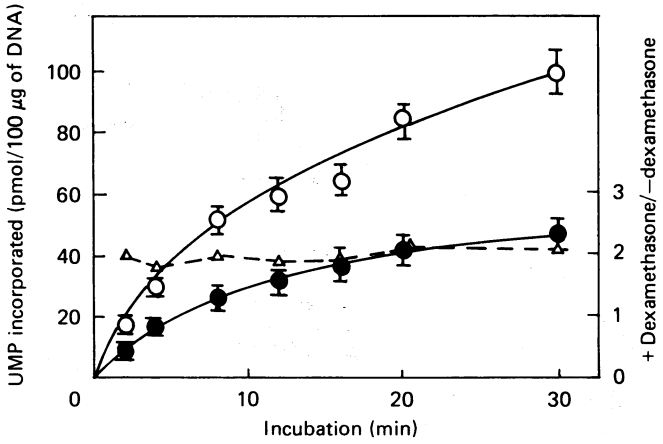

Fig. 6. Comparison of kinetics of UMP incorporation in isolated nuclei from rat liver

The time course of RNA polymerase I activity in isolated nuclei from adrenalectomized rats (●) and from 6 h-dexamethasone-primed adrenalectomized rats (○) were measured for up to 30 min incubation: △, ratio dexamethasone-treated/adrenalectomized. For each time point, the mean ± S.E.M. (vertical bars) for three experiments with three determinations is shown.

Effect of dexamethasone on the elongation rate of pre-rRNA chains

Whether dexamethasone affects the elongation rates of pre-rRNA chains in isolated nuclei or not was examined by using two methods. One takes advantage of the time courses of incorporation of labelled nucleotides into acid-insoluble materials in isolated nuclei from adrenalectomized rats and from adrenalectomized plus 6 h-dexamethasone-primed rats, taking ratios of labelled radioactivities at each point. As shown in Fig. 6, the ratios were almost constant until 30 min after the beginning of the reaction.

Another method is based on chromatography of hydrolysis products of labelled RNA (Barry & Gorski, 1971; Cox, 1976), the ratio of ribonucleoside monophosphate to ribonucleoside found by this method being proportional to the rate of elongation. As shown in Table 3, the value for adrenalectomized plus 6 h-dexamethasone-primed rats was almost the same as that for adrenalectomized rats. From these results, the elongation rate of the rRNA chain in isolated nuclei is considered to remain constant under the conditions used.

DISCUSSION

Although the effect of glucocorticoids *in vivo* on the 'engaged' form of RNA polymerase I activity in isolated liver nuclei has been studied in several laboratories, the time course was reported only by Todhunter *et al.* (1978). According to them, a single injection of cortisol (3 mg/100 g body wt.) to normal rats caused a rapid increase in the activity, the maximum (about 2-fold over control) being attained at 4 h, followed by a decrease to the control value at 6 h. Using normal and dexamethasone-treated (50 μg/100 g body wt.) rats, we were unable to reproduce such a marked elevation in the activity.

Table 3. Effect of dexamethasone on the chain length of RNA synthesized by the template-engaged form of RNA polymerase I

Portions of liver nuclei from adrenalectomized and adrenalectomized plus dexamethasone-treated (6 h) rats, containing 100 μg of DNA, were incubated, and hydrolysis products of RNA were measured as described in the text. Values are means ± S.E.M. for three experiments, with three determinations for each preparation.

Treatment	^{3}H (d.p.m./μg of DNA) recovered in		
	Uridine	UMP	[^{3}H]UMP/[^{3}H]uridine
Adrenalectomized	13.92 ± 0.90	805.0 ± 0.90	60.5 ± 3.7
+ Dexamethasone (6 h)	18.32 ± 1.83	1203.9 ± 3.42	61.8 ± 9.5

Moreover, the changes in activity tended to vary from experiment to experiment, probably owing to individual differences in stress. Therefore we decided to use adrenalectomized rats instead of normal rats in the present experiments. The results thus obtained (Fig. 1) show clearly that the maximum activity was attained at 6 h after administration of dexamethasone. This was about twice the original value in adrenalectomized rats which was in turn about 50% of that in normal rats.

The dexamethasone-induced increase in activity of the 'engaged' form of RNA polymerase I was promptly abolished by the administration of cycloheximide (Fig. 5). Administration of cycloheximide to normal rats also decreased the enzyme activity, to that of adrenalectomized rats. These results imply that normal rat liver has two types of pre-rRNA synthesis catalysed by the enzyme, approximately half being insensitive to cycloheximide and the other half being dependent on glucocorticoids and sensitive to cycloheximide. Thus the situation is essentially similar to the regulation by androgen and oestrogen of transcription of rRNA genes in prostate (Hosoya *et al.*, 1978; Suzuki *et al.*, 1984) and uterus (Nicolette & Babler, 1974) respectively. A slight difference between glucocorticoids and androgen effects appears in that the administration of cycloheximide to adrenalectomized rats caused a small but significant decrease in the 'engaged' form of enzyme activity (Fig. 5), in contrast with practically no inhibition found in castrated rats. This may be ascribed to the fact that pre-rRNA synthesis in liver is subject to regulation by other hormones, such as androgen, thyroid hormone and growth hormone (Tata & Widnell, 1966).

The inhibition of pre-rRNA synthesis in mammalian cells by cycloheximide or other antibiotics was previously reported by several investigators, leading to the hypothesis that the normal rate of transcription of the rRNA genes is supported by continuous synthesis of some protein(s) with a rapid turnover (Tsukada & Lieberman, 1965; Muramatsu *et al.*, 1970; Gross & Pogo, 1974; Franze-Fernández & Fontanive-Sengüesa, 1973; Lampert & Feigelson, 1974; Gross & Pogo, 1974; Lindell *et al.*, 1978). Although some workers questioned the idea (Farber & Farmer, 1973; Grummt & Grummt, 1976; Stoyanova & Dabeva, 1980), Mishima *et al.* (1979) and Haim *et al.* (1983), using cycloheximide and pactamycin respectively, presented data supporting the participation of short-lived protein(s) in pre-rRNA synthesis. On the basis of the results reported here (Fig. 5) as well as those obtained by using puromycin (results not shown), we hold the same view concerning the drugs, but we differ from the previous workers who studied pre-rRNA synthesis in liver (Muramatsu *et al.*, 1970; Yu & Feigelson, 1972; Benecke *et al.*, 1973; Sekeris & Schmidt, 1973; Onishi *et al.*, 1977) in that we find that the drugs do not affect the total pre-rRNA synthesis, but only the hormone-induced stimulation of pre-rRNA synthesis (Fig. 5). In this respect, the results agree with our previous results concerning androgen and the prostate (Hosoya *et al.*, 1978; Suzuki *et al.*, 1984) and resemble those obtained by Perrone-Bizzozero *et al.* (1985), using cultured cells (BALB/c 3T3), that cycloheximide decreased the enhanced RNA polymerase I activity in serum-stimulated cells without affecting the activity in resting cells.

No change in the total activity of RNA polymerase I in liver nuclei after administration of cycloheximide to normal rats was previously reported (Benecke *et al.*, 1973; Sekeris & Schmidt, 1973; Onishi *et al.*, 1977). The present study also demonstrated that the total activity of the enzyme in liver remained unchanged by adrenalectomy and for 6 h after a single injection of glucocorticoids to adrenalectomized rats (Fig. 4 and Table 2). The invariability in the total amount of the enzyme protein was further confirmed for the first time in liver by immunochemical studies (Table 2). In addition, the activity of the 'free' form (Fig. 1) as well as the elongation rate of pre-rRNA chains (Table 3 and Fig. 6) were found to be scarcely changed, but only the activity of the 'engaged' form was increased during the time studied after the hormone injection (Fig. 1). These findings force us, as in the case of the regulation by androgen of rRNA synthesis in prostate (Suzuki *et al.*, 1984), to assume the presence of the third type of RNA polymerase I, bound to rRNA genes so that it can transcribe neither endogenous nor exogenous template in the absence of glucocorticoid but can transform to the 'engaged' form under the influence of the hormone.

By administering cortisone to normal rats, Yu & Feigelson (1971) found that the activity of the 'free' form of RNA polymerase I in liver nuclei increased by 38–316% over controls. The reason of the discrepancy between their results and ours is not clear, but it may be partly due to the difference in methods used: the values of the 'free'-form activity tend to fluctuate from experiment to experiment if great care is not taken with the assay conditions, as described previously (Suzuki *et al.*, 1984) and in the present paper. Moreover, if the 'free'-form activity of the enzyme is really increased by glucocorticoid, the total activity and amount of the enzyme after solubilization from nucleoli would be augmented, but this was not the case in the present experiments.

In studying the effects of extracts from lymphosarcoma

cells and from cells treated with dexamethasone on the transcription of cloned mouse rRNA, Cavanaugh & Thompson (1985) found that extracts from the latter cells were capable of forming stable preinitiation complexes, but were unable to initiate transcription, and that initiation was restored by the addition of initiation factor (TFIC) prepared from control cells. This suggests that glucocorticoids regulate the transcription of rDNA in the cells by decreasing the amount of TFIC. Although the direction of the glucocorticoid effect is reversed in liver cells, the situation seems to be very similar if we suppose that preinitiation complexes in liver cells are converted into true initiation complexes by combination of the 'dormant' RNA polymerase I with protein factors which are dependent on glucocorticoids, as discussed above, although much remains to be done to verify this hypothesis.

In conclusion, there are two modes of regulation in the transcription of ribosomal genes in liver nuclei. About half of the rRNA synthesis, which represents the basal amount, i.e. that in adrenalectomized rats, is not affected by the administration of cycloheximide, whereas the other half, which is dependent on glucocorticoids, is inhibited by injection of the drug, probably by cessation of synthesis of short-lived protein(s). The role of the protein(s) is probably to convert the 'dormant' type of the enzyme into an active form.

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REFERENCES

Anderson, M. W., Ballal, N. R. & Busch, H. (1977) Biochem. Biophys. Res. Commun. **78**, 129–135
Barry, J. & Gorski, J. (1971) Biochemistry **10**, 2384–2390
Bell, P. A. & Borthwick, N. M. (1979) J. Steroid Biochem. **11**, 381–387
Benecke, K. J., Ferencz, A. & Seifart, K. H. (1973) FEBS Lett. **31**, 53–58.
Borthwick, N. M. & Bell, P. A. (1975) FEBS Lett. **60**, 396–399
Borthwick, N. M. & Bell, P. A. (1978) Mol. Cell. Endocrinol. **9**, 269–278
Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
Burton, K. (1956) Biochem. J. **62**, 315–323
Cavanaugh, A. H. & Thompson, E. A. (1983) J. Biol. Chem. **258**, 9768–9773
Cavanaugh, A. H. & Thompson, E. A. (1985) Nucleic Acids Res. **13**, 3357–3369
Chambon, P. (1975) Annu. Rev. Biochem. **44**, 613–538
Cox, R. F. (1976) Cell **7**, 455–465
Dabeva, M. D. & Ikonomova, R. N. (1982) Mol. Cell. Endocrinol. **28**, 263–273
Dabeva, M. D., Dineva, B. B. & Ikonomova, R. N. (1984) Cell Biol. Int. Rep. **8**, 231–237
Dahmus, M. E. & Bonner, J. B. (1965) Proc. Natl. Acad. Sci. U.S.A. **54**, 1370–1375
Dembinski, T. C. & Bell, P. A. (1984) J. Steriod Biochem. **21**, 497–504
Farber, J. L. & Farmer, R. (1973) Biochem. Biophys. Res. Commun. **51**, 626–630
Feigelson, M. & Feigelson, P. (1965) Adv. Enzyme Regul. **3**, 11–27
Franze-Fernández, M. T. & Fontanive-Sengüesa, A. V. (1973) Biochem. Biophys. Acta **331**, 71–80
Frey, A. & Seifart, K. H. (1982) Mol. Cell. Endocrinol. **28**, 161–172
Greenman, D. L., Wicks, W. D. & Kenny, F. T. (1965) J. Biol. Chem. **240**, 4420–4426
Gross, K. J. & Pogo, A. D. (1974) J. Biol. Chem. **249**, 568–576
Grummt, I. (1978) in The Cell Nucleus (Busch, H., ed.), vol. 5, pp. 373–414, Academic Press, New York
Grummt, I. & Grummt, F. (1976) Cell **7**, 447–453
Haim, L., Iapalucci-Espinoza, S., Conde, R. & Franze-Fernández, M. T. (1983) Biochem. J. **210**, 837–844
Hallahan, C., Young, D. A. & Munck, A. (1973) J. Biol. Chem. **248**, 2922–2927
Higashinakagawa, T., Muramatsu, M. & Sugano, J. (1972) Exp. Cell Res. **71**, 65–74
Hosoya, T., Nagai, Y., Inagaki, T. & Hayashi, M. (1978) J. Biochem. (Tokyo) **84**, 1519–1528
Jacob, S. T., Sajdel, E. M. & Munro, H. N. (1969) Eur. J. Biochem. **7**, 449–453
Lampert, A. & Feigelson, P. (1974) Biochem. Biophys. Res. Commun. **58**, 1030–1038
Leonard, T. B. & Jacob, S. T. (1977) Biochemistry **16**, 4538–4544
Lindell, T., O'Malley, A. F. & Puglishi, B. (1978) Biochemistry **17**, 1154–1160
Matsui, T., Onishi, T. Muramatsu, M. (1976) Eur. J. Biochem. **71**, 351–360
Miller, K. G. & Sollner-Webb, B. (1982) in The Cell Nucleus (Bush, H., ed.), vol. 7, pp. 69–100, Academic Press, New York
Mishima, Y., Matsui, T. Muramatsu, M. (1979) J. Biochem. (Tokyo) **85**, 807–818
Muramatsu, M., Shimada, N. & Higashinakagawa, T. (1970) J. Mol. Biol. **53**, 91–106
Nakanishi, S. & Numa, S. (1970) Eur. J. Biochem. **16**, 161–173
Nicolette, J. A. & Babler, M. (1974) Arch. Biochem. Biophys. **163**, 263–270
Onishi, T., Matsui, T. & Muramatsu, M. (1977) J. Biochem. (Tokyo) **82**, 1109–1119
Ouchterlony, Ö. (1949) Acta Pathol. Microbiol. **26**, 507–515
Perrone-Bizzozero, N., Iapalucci-Espinoza, S., Medrano, E. E. & Franze-Fernández, M. T. (1985) J. Cell. Physiol. **124**, 160–164
Randerath, K. & Randerath, E. (1967) Methods Enzymol. **12**, 323–347
Sajdel, E. M. & Jacob, S. T. (1971) Biochem. Biophys. Res. Commun. **45**, 707–715
Sekeris, C. E. & Schmidt, W. (1973) in Endocrinology (Scow, R. O., ed.), pp. 408–414, American Elsevier Publishing Co., New York
Stoyanova, B. B. & Dabeva, M. D. (1980) Biochim. Biophys. Acta **608**, 358–367
Suzuki, N., Hosoya, T., Igarashi, T. & Muramatsu, M. (1984) J. Biochem. (Tokyo) **95**, 1389–1398
Suzuki, N., Matsui, H. & Hosoya, T. (1985) J. Biol. Chem. **260**, 8050–8055
Tata, J. R. & Widnell, C. C. (1966) Biochem. J. **98**, 604–620
Todhunter, J. A., Weissbach, H. & Brot, N. (1978) J. Biol. Chem. **253**, 4514–4516
Tsukada, K. & Lieberman, I. (1965) J. Biol. Chem. **240**, 1731–1736
Yu, F.-L. & Feigelson, P. (1971) Proc. Natl. Acad. Sci. U.S.A. **68**, 2177–2180
Yu, F.-L. & Feigelson, P. (1972) Proc. Natl. Acad. Sci. U.S.A. **69**, 2833–2837
Yu, F.-L. & Feigelson, P. (1973) Biochem. Biophys. Res. Commun. **53**, 754–760

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