The Effects of Oestradiol-17 β on the Ribonucleic Acid Polymerases of Immature Rabbit Uterus

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Measurements of the endogenous RNA polymerase activities of nuclei isolated from immature rabbit uteri have shown that prior treatment of the animals with oestradiol-17 β has a profound effect on the apparent activities of both RNA polymerases A and B. Within 1 h of hormone treatment, the activity of RNA polymerase A is increased and continues to rise until about 4 h when it reaches a plateau and remains steady until at least 8 h. The activity of RNA polymerase B increases sharply after oestradiol treatment reaching an early maximum at 30–45 min. Thereafter this activity declines until by 1–2 h it approaches control values but a second increase in activity then occurs with a maximum at 3–4 h. Treatment of the rabbits with α -amanitin before the administration of oestradiol inhibits the hormone-induced stimulation of RNA polymerase A activity in isolated nuclei but when the administration of α -amanitin is delayed until after the early rise of RNA polymerase B activity, the oestradiol-induced stimulation of RNA polymerase A is retained. Similar results have been obtained in experiments with cycloheximide suggesting that the stimulation of RNA polymerase A activity by oestradiol is dependent on the hormone-induced stimulation of RNA polymerase B and the subsequent synthesis of protein using the RNA product of the early increase in RNA polymerase B activity. Measurement of the activities of RNA polymerases A and B after isolation of the enzymes from immature rabbit uterine nuclei before and after oestradiol treatment failed to show any differences. Therefore it would appear that the changes in the observed activities of RNA polymerases A and B in isolated nuclei are consequences of changes in the structure and function of chromatin rather than the results of modifications in the RNA polymerases themselves.

Within a few hours of treatment with oestradiol, there is a considerable increase in the synthesis of RNA in the uteri of immature animals (Mueller *et al.*, 1958; Gorski *et al.*, 1968; Hamilton, 1968; Billing *et al.*, 1969; Jensen & DeSombre, 1973). Although quantitatively the changes are most marked in rRNA, and commence 4–6 h after administration of the hormone (Billing *et al.*, 1969; Knowler & Smellie, 1971), evidence is accumulating that this may be preceded by, and possibly dependent on, the synthesis of mRNA. Earlier studies on the base composition of rapidly labelled nRNA formed in response to oestradiol suggested that it was ribosomal in nature (Hamilton *et al.*, 1968) but more recent experiments have demonstrated the appearance of very-high-molecular-weight RNA in the uteri of immature rats 30–45 min after oestradiol treatment (Knowler & Smellie, 1971; Luck & Hamilton, 1972). This high-molecular-weight RNA shows all the characteristics of nRNA and

oestrogen-stimulated increases in incorporation of labelled precursor are found in RNA species with a wide range of molecular weights (Knowler & Smellie, 1973). These observations are therefore consistent with the suggestion that an early stimulation of the synthesis of some species of mRNA may be necessary for the synthesis of a small number of uterine proteins which in turn initiate the increased production of ribosomes and other proliferative changes.

It has been known for some years that administration of oestradiol to immature or ovariectomized animals causes increases in the activities of DNA-dependent RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase: EC 2.7.7.6) (Gorski, 1964; Hamilton *et al.*, 1968) and of the apparent template activity of uterine chromatin (Barker & Warren, 1966; Hamilton, 1968; Church & McCarthy, 1970; Glasser *et al.*, 1972).

More recent studies on the RNA polymerases of eukaryotic cells have revealed the presence of at least two classes of enzyme, RNA polymerase A and RNA polymerase B (Chambon *et al.*, 1970;

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Roeder & Rutter, 1970; Chesterton & Butterworth, 1971; Chesterton *et al.*, 1972). Previous measurements of the RNA polymerase activities of nuclei from the uteri of immature or ovariectomized rats have shown that oestradiol causes an increase in the endogenous RNA polymerase A activity 1–2 h after hormone treatment although no changes were detected in the activity of RNA polymerase B during the first 12 h (Barry & Gorski, 1971; Raynaud-Jammet *et al.*, 1972).

The present paper reports the results of measurements of the RNA polymerase activities in nuclei isolated from the uteri of immature rabbits before and after treatment with oestradiol-17 β and measurements of the activities of isolated RNA polymerases A and B from control and responding uteri.

Experimental

Reagents

Oestradiol-17 β , cycloheximide and calf thymus DNA were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Rabbit liver DNA was prepared by the method of Kay *et al.* (1952). Actinomycin D and α -amanitin were purchased from Calbiochem Ltd., London W1H 1AS, U.K. and rifampicin and poly(dA-dT) from the Boehringer Corp. (London) Ltd., Ealing, London W5, U.K. DE-52 and CM-52 substituted celluloses were the products of Whatman Biochemicals Ltd., Maidstone, Kent, U.K. [5-³H]UTP (1 Ci/mmol), [5-³H]uridine (28 Ci/mmol), [8-³H]guanosine (16 Ci/mmol), [4,5-³H]lysine (5.5 Ci/mmol) and L-[4,5-³H]leucine (1 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Toluene scintillator was 0.5% (w/v) 2,5-diphenyloxazole in toluene.

Experimental animals

Female Dutch rabbits were purchased from Hyline Rabbit Farms, Northwich, Cheshire, U.K. and used when 7–8 weeks old within a weight range of 1.2–1.4 kg.

Oestradiol-17 β (10 μ g/kg body weight) was administered as a single intravenous injection by the ear vein in 0.9% NaCl containing 2.5% (v/v) ethanol. This stereoisomer was used throughout. Control animals received saline-ethanol alone. Inhibitors and radioactive isotopes were all injected intraperitoneally in saline.

Isolation of nuclei

All procedures were carried out at 0–4°C unless otherwise specified. The nuclei were isolated and purified by the following modification of the method of Blobel & Potter (1966). Excised

uteri were finely chopped and homogenized in 5–10 vol. of 0.25 M-sucrose in TKM buffer [50 mM-Tris-HCl (pH 7.4)–25 mM-KCl–5 mM-MgCl₂]. Homogenization was carried out in an Ultra-Turrax type TP18/2 homogenizer (Janke and Kunkel, Staufen, W. Germany) run at 60 V for 40 s. The homogenate was filtered through four layers of muslin cloth and the filtrate was mixed with 2 vol. of 2.3 M-sucrose in TKM buffer. This solution was underlaid with 10 ml of 2.4 M-sucrose in TKM buffer and centrifuged for 90 min at 105 000 g. The pellet which contained purified nuclei was resuspended in a small volume (1–2 ml) of TGED buffer [50 mM-Tris-HCl (pH 7.9)–0.1 mM-EDTA–0.5 mM-dithiothreitol–25% (v/v) glycerol].

RNA polymerase assay

RNA polymerase activity was assayed in isolated nuclei in either high salt to measure RNA polymerase B activity or in low salt plus α -amanitin to measure RNA polymerase A activity. The reaction mixture contained in a volume of 0.05 ml, 0.075 μ mol each of ATP, CTP and GTP, 0.005 μ mol of [³H]UTP (0.5 μ Ci), 1.25 μ mol of Tris-HCl, pH 7.9, 0.5 μ mol of 2-mercaptoethanol and 5% (v/v) glycerol. Under low-salt conditions, the same volume of reaction mixture also contained 0.2 μ mol of MgCl₂, 2.5 μ mol of KCl and 1 μ g of α -amanitin. Assays carried out in high-salt conditions contained 0.2 μ mol of MnCl₂ and 20 μ mol of (NH₄)₂SO₄ in the same volume of reaction mixture.

The reaction mixture for the soluble RNA polymerase preparations contained the same final concentrations of reactants as shown above in a total volume of 0.025 ml, but contained in addition 10 μ g of rabbit liver or calf thymus DNA as template.

A unit of enzyme activity is defined as the amount of enzyme which catalyses the incorporation of 1 pmol of UMP into acid-insoluble material per mg of protein in 30 min. Assays were started by adding 0.05 ml of either a suspension of nuclei or soluble RNA polymerase preparation to the reaction mixture at 0°C and this mixture was incubated at 37°C for 30 min.

The reaction was stopped by immersing the incubated samples in crushed ice. Samples (0.05 ml) of the incubation mixtures were spotted on 2.5 cm discs of Whatman no. 1 filter paper and washed for 30 min with occasional stirring in 10% (w/v) trichloroacetic acid containing 1% (w/v) tetrasodium pyrophosphate. The filters were washed twice more with 5% (w/v) trichloroacetic acid containing 1% (w/v) pyrophosphate, twice with alcohol, once with alcohol-ether (1:1) and once with ether. After being dried in a stream of air each disc was incubated for 20 min at 60°C in a counting vial

with 0.5 ml of 1 M-Hyaminate 10-X, 10 ml of toluene scintillator was added and radioactivity was measured by scintillation counting in a Philips model PW 4510 liquid-scintillation spectrometer with a tritium counting efficiency of 20–24%. Quench correction was performed by using an external standard.

Protein determinations were carried out by the method of Bramhall *et al.* (1969) and DNA determinations by the method of Burton (1956).

Extraction of RNA polymerases from rabbit uteri

All procedures were carried out at 0–4°C unless otherwise specified. The method was based on that described by Sugden & Keller (1973). Rabbit uteri were homogenized in 5–10 vol. of 10 mM-Tris-HCl (pH 7.9)–1 mM-EDTA–5 mM-dithiothreitol in an Ultra-Turrax homogenizer run at 60 V for 45 s. The homogenate was filtered through four layers of muslin cloth and the filtrate adjusted to a final concentration of 25 mM-Tris-HCl, pH 7.9, 0.5 mM-EDTA, 3.5 mM-dithiothreitol, 5 mM-MgCl₂, 10% (w/v) sucrose, 20% (v/v) glycerol and 0.4 M-(NH₄)₂SO₄ in twice the volume of the filtrate. The viscous suspension was cooled to 0°C, left for 8 h and centrifuged for 10 h at 105 000g. The supernatant from this high-speed centrifugation was saturated with (NH₄)₂SO₄ by the slow addition of 0.38 g of solid (NH₄)₂SO₄ per ml of solution. The mixture was stirred mechanically for 6 h at 0°C and the precipitate was collected by centrifugation at 40 000g for 30 min. After the supernatant had been decanted, the pellet was resuspended in a small volume of TGMED buffer [50 mM-Tris-HCl (pH 7.9)–5 mM-MgCl₂–0.1 mM-EDTA–0.5 mM-dithiothreitol–25% (v/v) glycerol] and dialysed overnight against 100 vol. of TGMED buffer containing 0.08 M-(NH₄)₂SO₄. After dialysis, the solution was clarified by centrifugation for 1 h at 120 000g. The supernatant was decanted and applied to a column (12 cm × 0.9 cm) of DEAE-cellulose which had been equilibrated with TGMED buffer containing 0.08 M-(NH₄)₂SO₄. After sample application, the column was washed with 5 column volumes of 0.08 M-(NH₄)₂SO₄, 5 column volumes of 0.14 M-(NH₄)₂SO₄ and finally eluted by using a linear gradient of 10 column volumes of 0.14–0.4 M-(NH₄)₂SO₄ all in TGMED buffer. Fractions (2 ml) were collected and assayed for RNA polymerase activity.

CM-cellulose chromatography

The protein which did not bind to DEAE-cellulose was dialysed overnight at 4°C against PGED buffer [10 mM-potassium phosphate (pH 7.5)–0.1 mM-EDTA–0.5 mM-dithiothreitol–15% (v/v) glycerol] and passed on to a column (10 cm × 0.9 cm) of CM-cellulose which was equilibrated with PGED buffer.

The column was washed with 5 column volumes of PGED buffer followed by a linear gradient of 10 column volumes of PGED buffer containing 0–0.4 M-KCl.

Experiments in vivo with inhibitors of RNA and protein synthesis

In some experiments, animals were treated with actinomycin D (0.5 mg/kg body weight), α -amanitin (0.05 mg/kg body weight) or cycloheximide (0.4 mg/kg body weight). These concentrations were sufficient to inhibit RNA synthesis by 95% in the case of actinomycin D and α -amanitin and protein synthesis by 98% in the case of cycloheximide as measured by the incorporation of [³H]uridine and [³H]guanosine or [³H]leucine and [³H]lysine into acid-insoluble material.

Results

Effects of oestradiol on the RNA polymerase activities in isolated uterine nuclei

Measurements of RNA polymerase activity have been made in nuclei from the uteri of immature rabbits which had been treated with oestradiol-17 β for various periods of time including intervals of less than 1 h which have not usually been included in other investigations. RNA polymerase activities were determined in either low-salt or high-salt conditions and correspond to RNA polymerase A and B respectively.

Fig. 1(a) shows that after treatment of the animals with oestradiol the activity of RNA polymerase A remains at control values for the first 30 min and then begins to increase, reaching a maximum about five times the control value at 2 h. Thereafter the activity remains constant until at least 3 h. RNA polymerase B activity, however, increases rapidly to a peak of about three times the control value within 30–45 min of hormone treatment and then declines to about control value by 2 h, before beginning to rise for a second time at 3–4 h.

Figs. 2(a) and 2(b) show the results of experiments carried out over a period of 12 h after oestradiol treatment. It is evident that after reaching a maximum at 1–2 h the activity of RNA polymerase A remains elevated until at least 12 h. The early rise in RNA polymerase B activity is again apparent but it can be seen that there is a biphasic response in that the activity diminishes between 30 min and 2 h and then undergoes a second increase over the period 2–5 h to a maximum of about six times the control which declines slowly thereafter.

The early stimulation of RNA polymerase B in response to oestrogen has also been observed in rat uteri (Glasser *et al.*, 1972) and correlates

Fig. 1. Effect of duration of treatment with oestradiol on the RNA polymerase activities in uterine nuclei

Rabbits were treated with hormone for periods up to 3 h, and the nuclei were prepared from uteri and assayed for RNA polymerase activity as described in the Experimental section. Each point shows the range of values obtained from at least four determinations. (a) RNA polymerase A activity in oestrogen-treated (●) and control (■) uteri. (b) RNA polymerase B activity in oestrogen-treated (●) and control (■) uteri.

Fig. 2. Effect of extended time of treatment with oestradiol on the RNA polymerase activities in uterine nuclei

Rabbits were treated with hormone for periods up to 12 h, and the nuclei were prepared from uteri and assayed for RNA polymerase activity as described in the Experimental section. Each point shows the range of values obtained from at least four determinations. (a) RNA polymerase A activity in oestrogen-treated (●) and control (■) uterine nuclei. (b) RNA polymerase B activity in oestrogen-treated (●) and control (■) uterine nuclei.

exactly with the observation of stimulation of the synthesis of hnRNA in rat uterus in response to oestradiol (Knowler & Smellie, 1973). The

change in RNA polymerase B activity precedes the increase in RNA polymerase A activity after hormone treatment and confirms the findings of

DeAngelo & Gorski (1970), using inhibitors, that an early increase in RNA polymerase B activity is essential for subsequent biochemical changes in responding uteri.

There have been indications that the stimulation of uterine rRNA synthesis in response to oestradiol is dependent on protein synthesis (DeAngelo & Gorski, 1970), and to determine whether the stimulation of RNA polymerase A activity is dependent on the synthesis of some product of the early increase in RNA polymerase B activity, experiments were performed in which animals were treated *in vivo* with inhibitors of RNA and protein synthesis before or after the administration of oestradiol.

Figs 3(a) and 3(b) show the effect of administering actinomycin D to the rabbits 30min before the injection of oestradiol. This treatment abolishes not only the oestrogen-induced increase in RNA polymerases A and B but also the control polymerase activity, and the results are compatible with the mechanism of action of actinomycin D in binding to the DNA template and preventing further transcription (Jain & Sobell, 1972).

Treatment of the animals *in vivo* with α -amanitin 30min before the administration of oestradiol (Fig. 4a) prevents the oestrogen-induced rise in the activity of RNA polymerase A but does not inhibit the control activity. By contrast, the toxin not only abolishes the oestrogen-induced increase in RNA polymerase B activity but it totally inhibits this activity in both control and treated animals (Fig. 4b).

These findings are consistent with the action of α -amanitin in binding to one subunit of the RNA polymerase B molecule (Cochet-Meilhac & Chambon, 1974), but it is noteworthy that the oestrogen-induced increase in RNA polymerase A is also abolished. This observation is compatible with those of Tata *et al.* (1972) who found that the synthesis of all species of rRNA is inhibited in rat liver after treatment with α -amanitin.

Figs. 5(a) and 5(b) show the results of experiments in which the rabbits were treated with α -amanitin 30min after the administration of oestradiol, at a time when the early increase in RNA polymerase B activity had already occurred. This treatment with α -amanitin did not prevent the oestrogen-induced rise in RNA polymerase A activity (Fig. 5a) nor did it affect the activity of this enzyme in nuclei from control animals. However, although the early rise in RNA polymerase B activity was observed as expected, the administration of α -amanitin after 30min rapidly and totally abolished the RNA polymerase B activity in both control and test animals.

This provides support for the suggestion that a product of the early RNA polymerase B reaction may be required for the stimulation of RNA polymerase

Fig. 3. Effect of treatment with actinomycin D *in vivo* on the RNA polymerase activities in uterine nuclei

Animals were treated with 500µg of actinomycin D/kg body wt. 30min before the administration of 10µg of oestradiol or saline. Nuclei were isolated and assayed for RNA polymerase activity as described in the Experimental section. (a) RNA polymerase A activity in oestradiol-treated animals (●), oestradiol+actinomycin D (○), control animals (■) and control+actinomycin D (□). (b) RNA polymerase B activity in oestradiol-treated animals (●), oestradiol+actinomycin D (○), control animals (■) and control+actinomycin D (□).

Fig. 4. Effect of treatment with α -amanitin *in vivo* on the RNA polymerase activities in uterine nuclei

Animals were treated with 100 μg of α -amanitin/kg body wt. 30 min before the administration of 10 μg of oestradiol or saline. Nuclei were isolated and assayed for RNA polymerase activities as described in the Experimental section. (a) RNA polymerase A activity in oestradiol-treated animals (●), oestradiol + α -amanitin (○), control animals (■) and control + α -amanitin (□). (b) RNA polymerase B activity in oestradiol-treated animals (●), oestradiol + α -amanitin (○), control animals (■) and control + α -amanitin (□).

Fig. 5. Effect of treatment with α -amanitin *in vivo* on the RNA polymerase activities in uterine nuclei

Animals were treated with 100 μg of α -amanitin/kg body wt. 30 min after the administration of 10 μg of oestradiol or saline. Nuclei were isolated and assayed for RNA polymerase activities as described in the Experimental section. (a) RNA polymerase A activity in oestradiol-treated animals (●), oestradiol + α -amanitin (○), control animals (■) and control + α -amanitin (□). (b) RNA polymerase B activity in oestradiol-treated animals (●), oestradiol + α -amanitin (○), control animals (■) and control + α -amanitin (□).

A activity and hence for the increased synthesis of rRNA. Such a product could be a species of RNA or a protein derived from it and attempts were made to distinguish these possibilities by carrying out experiments in which the animals were treated with cycloheximide to inhibit the synthesis of protein.

When cycloheximide was administered 30 min before oestradiol (Fig. 6a), the oestrogen-induced stimulation of RNA polymerase A activity was almost completely abolished. Cycloheximide, however, did not affect the control activity of RNA polymerase A or the early stimulation of RNA polymerase B by oestradiol and indeed the activity of this enzyme seemed to decline slightly less rapidly in animals treated with this compound (Fig. 6b).

Figs. 7(a) and 7(b) show the results obtained when cycloheximide was administered to the rabbits 30 min after oestradiol treatment. This no longer prevents the oestradiol-induced increase in RNA polymerase A activity and again seems to delay the decline from the early peak of activity of RNA polymerase B.

The fact that cycloheximide, administered before oestradiol, abolishes the oestrogen-induced stimulation of RNA polymerase A but not that of RNA polymerase B suggests that protein synthesis is required before the activity of RNA polymerase A can respond to oestrogen treatment, whereas the increased activity of RNA polymerase B is independent of protein synthesis. The findings in Figs. 7(a) and 7(b) are consistent with this conclusion and suggest that the increased activity of RNA polymerase A is not a consequence of new synthesis of the enzyme itself. Thus a protein factor derived from an RNA product of RNA polymerase B might be responsible for activating existing RNA polymerase A molecules. To test this idea, cytoplasm prepared from the uteri of animals treated for various periods with oestradiol-17 β , was fractionated on columns of CM-cellulose and the individual fractions were added to assays of the RNA polymerase A and B activities in nuclei isolated from untreated rabbits. There is a fraction, present only in the cytoplasm of uteri from animals treated with oestradiol 30–45 min previously, which stimulates the RNA polymerase A but not the RNA polymerase B activity of the isolated nuclei (Fig. 8). This observation provides further support for the hypothesis that an RNA product of RNA polymerase B activity serves as a messenger for the synthesis of a protein factor which in turn progressively activates RNA polymerase A.

Studies on the isolated RNA polymerases of immature rabbit uterus

Analysis of the soluble RNA polymerases from immature rabbit uteri on columns of DEAE-cellulose

Fig. 6. *Effect of treatment with cycloheximide in vivo on the RNA polymerase activities in uterine nuclei*

Animals were treated with 400µg of cycloheximide/kg body wt. 30 min before the administration of 10µg of oestradiol or saline. Nuclei were isolated and assayed for RNA polymerase activities as described in the Experimental section. (a) RNA polymerase A activity in oestradiol-treated animals (●), oestradiol+cycloheximide (○), control animals (■) and control+cycloheximide (□). (b) RNA polymerase B activity in oestradiol-treated animals (●), oestradiol+cycloheximide (○), control animals (■) and control+cycloheximide (□).

Fig. 7. Effect of treatment with cycloheximide *in vivo* on the RNA polymerase activities in uterine nuclei

Animals were treated with 400µg of cycloheximide/kg body wt. 30min after the administration of 10µg of oestradiol or saline. Nuclei were isolated and assayed for RNA polymerase activities as described in the Experimental section. (a) RNA polymerase A activity in oestradiol-treated animals (●), oestradiol+cycloheximide (○), control animals (■) and control+cycloheximide (□). (b) RNA polymerase B activity in oestradiol-treated animals (●), oestradiol+cycloheximide (○), control animals (■) and control+cycloheximide (□).

Fig. 8. Fractionation of cytoplasm from uteri of animals treated with oestradiol

Cytoplasm prepared from immature rabbit uteri at various times after treatment with oestradiol as described in the Experimental section was fractionated on columns of CM-cellulose. Nuclei prepared from uteri of untreated animals were assayed for RNA polymerase activities in a total volume of 0.1 ml in the presence of 0.02 ml samples of each fraction obtained by CM-cellulose chromatography of the cytoplasm. (a) RNA polymerase A activity in nuclei assayed with cytoplasm from uteri treated with oestradiol for 30min (●) and 60min (○). (b) RNA polymerase B activity in nuclei assayed with cytoplasm from uteri treated with oestradiol for 30min (■) and 60min (□).

(Fig. 9) gives rise to three peaks of activity. The first, which is eluted with the bulk of the cellular protein in the column wash, has been designated RNA polymerase C. The second peak, eluted with 0.14M-(NH₄)₂SO₄, is insensitive to the action of α -amanitin and is designated RNA polymerase A and the third component, eluted by 0.22–0.25M-(NH₄)₂SO₄, is sensitive to α -amanitin and is designated RNA polymerase B.

If the original tissue homogenate is centrifuged for 15min at 800g immediately after filtration, it is possible to obtain a crude preparation of uterine nuclei but this fraction contains only two peaks of RNA

Fig. 9. Multiple forms of DNA-dependent RNA polymerase from rabbit uterus

Immature uteri were homogenized and the total protein was extracted by salt as described in the Experimental section; 80–100 mg of uterine protein was passed on to a DEAE-cellulose column which was developed with an $(\text{NH}_4)_2\text{SO}_4$ gradient. Each fraction was assayed in duplicate for RNA polymerase activity and protein content in the column buffer at the salt concentration at which it was eluted. ●, RNA polymerase activity; ·····, E_{610} ; △, salt concentration.

Fig. 10. Multiple forms of RNA polymerase in uteri from animals treated with oestradiol

RNA polymerases were isolated from immature rabbit uteri as described in the Experimental section; 40 mg samples of uterine protein prepared at each time-interval after oestradiol treatment were applied to identical columns ($10\text{cm} \times 0.9\text{cm}$) of DEAE-cellulose, and column chromatography was carried out as described in the Experimental section. Each fraction was assayed in duplicate in column buffer for RNA polymerase activity. RNA polymerases from uteri were treated with oestradiol for (a) 30 min; (b) 1 h; (c) 2 h; (d) 4 h.

Table 1. *Characteristics of uterine RNA polymerases A, B and C*

(a) RNA polymerase A and B (10units) and RNA polymerase C (5units) were assayed in various ionic and salt conditions. The results show the concentrations which gave maximal enzymic activity. The optimum concentrations of $(\text{NH}_4)_2\text{SO}_4$ were 20 and 60mM when the assays were performed in the presence of Mg^{2+} and Mn^{2+} ions respectively. (b) RNA polymerase A and B (10units) and RNA polymerase C (5units) were assayed in the standard RNA polymerase assay mixture in the presence of α -amanitin (1 μg). The results are expressed as a percentage of activity in controls. (c) RNA polymerase A and B (10units) and RNA polymerase C (5units) were assayed in the standard RNA polymerase assay mixture in the presence of either 10 μg of DNA or poly(dA-dT). Results are expressed as percentage of the activity, with double-stranded rabbit liver DNA as the template.

	A	B	C
(a) Salt and ionic requirements			
Optimal Mg^{2+} concn. (mM)	8	6	5
Optimal Mn^{2+} concn. (mM)	2-3	3	3
Optimal $(\text{NH}_4)_2\text{SO}_4$ concn. (mM)			
In the presence of Mg^{2+}	20	110	80
In the presence of Mn^{2+}	60		
(b) Sensitivity to α -amanitin (% of control activity)	100	0	80
(c) Template requirements			
Native rabbit liver DNA	100	100	100
Denatured rabbit liver DNA	68	212	118
Native calf thymus DNA	94	109	93
Denatured calf thymus DNA	64	286	127
Poly(dA-dT)	223	180	138
DNA omitted	0	0	0

polymerase activity corresponding to RNA polymerases A and B. Since polymerase C is absent from nuclei it seems likely that it is either soluble and leached out of the nuclei during isolation or that it is cytoplasmic in origin or that it is inactivated during the isolation of the nuclei. The possible presence of a cytoplasmic RNA polymerase has already been described (Seifart *et al.*, 1972).

All three RNA polymerases required the four ribonucleoside 5'-triphosphates and their other properties are summarized in Table 1. The ionic requirements, sensitivities to α -amanitin and template requirements of the uterine RNA polymerases A and B are similar to those of RNA polymerases A and B from other sources (Chambon *et al.*, 1970; Roeder & Rutter, 1970). RNA polymerase C, however, differs significantly from both RNA polymerase A or B.

Effect of oestradiol, administered in vivo, on the activities of isolated RNA polymerases

RNA polymerases were isolated from the uteri of immature rabbits that had been treated with

oestradiol for various periods of time. A series of columns of DEAE-cellulose of identical size were prepared and identical amounts of soluble protein prepared from uteri at different times after oestradiol treatment were applied to the columns. After elution the fractions were assayed for RNA polymerase activity by using double-stranded rabbit liver DNA as the template. The results are summarized in Figs. 10(a)–10(d). No new species of RNA polymerase was detected and there was no significant change in the activities of any of the RNA polymerases isolated from the uteri of rabbits which had been treated with oestradiol for periods of 30min to 4h.

Since these results were in sharp contrast with those with isolated nuclei (Figs. 1 and 2) it seemed possible that in the course of isolating the RNA polymerases some factor, which is required to activate one or other of them in response to hormone treatment, is lost or inactivated. To examine this possibility the protein eluted from the DEAE-cellulose columns was fractionated on columns of CM-cellulose as had been done in the experiments on isolated nuclei (Figs. 8a and 8b). Each fraction was assayed for its effect on the soluble RNA polymerases A and B by using double-stranded rabbit liver DNA as a template. None of these fractions exerted any stimulatory effect on the isolated RNA polymerases.

In other experiments cytoplasm from the uteri of animals responding to oestradiol treatment were fractionated on CM-cellulose as described in the experiments in Figs. 8(a) and 8(b) and the fractions were tested for their effect on the isolated RNA polymerases A and B. None of these fractions stimulated either of the RNA polymerases.

There have been reports (Seifart *et al.*, 1973) of specific mammalian protein factors which are capable of enhancing the activity of RNA polymerase B *in vitro* but as yet there have been no reports of the occurrence of similar factors for RNA polymerase A. Our failure to detect stimulatory protein factors for soluble preparations of RNA polymerases A or B from rabbit uteri does not rule out the possible existence of such factors but does make their existence seem less likely and this is especially so in view of our finding (Figs. 8a and 8b) that the RNA polymerase A activity of isolated nuclei can be enhanced by a protein fraction from the cytoplasm of uteri responding to oestradiol.

On the basis of our present results it seems unlikely that oestradiol exerts a direct effect on the RNA polymerases A and B. The increased activities of these enzymes observed in isolated nuclei responding to the hormone seem more likely to be consequences of changes in the uterine chromatin. In the case of RNA polymerase B the activation might arise directly from the interaction of oestradiol and its receptor protein with chromatin whereas

for RNA polymerase A a protein factor or factors synthesized on a mRNA derived from RNA polymerase B might be the effector substance.

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