Studies on the Solubilized Ribonucleic Acid Polymerase from Rat Ventral Prostate Gland

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1. By using ultrasonic treatment in media of high ionic strength, the RNA polymerase activities associated with prostatic nuclei and nucleoli can be completely solubilized. Such enzyme preparations are entirely dependent on the provision of added DNA for full activity. 2. The solubilized enzymes from the nucleolar and extranucleolar regions can be separated by ion-exchange chromatography. 3. Based on differences in the optimum DNA templates, pH optima and the effects of ammonium sulphate on the activities *in vitro*, Mn^{2+} - and Mg^{2+} -specific enzymes are associated with both the nucleolar and extranucleolar regions of prostatic nuclei. 4. Androgenic hormones administered *in vivo* have a particularly pronounced effect on the activity of Mg^{2+} -dependent enzyme associated with the isolated prostatic nucleolus. 5. Time-course experiments *in vivo* show that androgens induce a rapid stimulation of the solubilized Mg^{2+} -dependent nucleolar enzyme before a pronounced activation of nucleolar chromatin can be measured. 6. The implications of these findings to the mechanism of action of androgenic steroids are discussed.

Many hormones are known to elicit a stimulation of nuclear RNA synthesis in hormone-responsive tissues (see review by Tata, 1967). In keeping with these general findings, the androgens are known to stimulate RNA synthesis directly in androgen-dependent tissues such as the seminal vesicle (Wicks & Kenney, 1964) and ventral prostate gland (Liao, Leininger, Sagher & Barton, 1965). Later work (Liao, Barton & Lin, 1966; Liao & Lin, 1967) showed that the RNA made after hormonal stimulation was predominantly ribosomal RNA. The activation of RNA synthesis could be effected by several processes: activation of the chromatin template, selective sequestering of essential bivalent cations, stimulation of the rate of synthesis of obligatory precursors such as the nucleoside 5'-triphosphates, or finally by direct activation of the enzyme RNA polymerase (EC 2.7.7.6). Activation of prostatic chromatin has been implicated by many authors (Liao & Lin, 1967; Mangan, Neal & Williams, 1968) but obtaining evidence for additional hormonal control at the level of the RNA polymerase enzyme has been hampered by the failure to solubilize the enzyme and thus free it from endogenous DNA template. RNA polymerase has been solubilized from a variety of highly differentiated cells (Furth & Loh, 1963; Ballard & Williams-Ashman, 1964) including the prostate (Doly, Ramuz, Mandel & Chambon, 1965) but such

preparations achieve only a partial solubilization of the total enzyme activity. Although it is true that a proportion of the RNA polymerase can be readily leached out from purified nuclei, the nucleolar-associated activity is so firmly bound that it has been suggested as a specific biochemical marker of nucleolar activity (Siebert *et al.* 1966). The complete and reproducible solubilization of all nuclear RNA polymerase has only recently been achieved (Roeder & Rutter, 1969, 1970*a,b*) and distinct enzymes have been identified in the nucleolar and extranucleolar fractions of nuclei from rat liver and sea-urchin oocytes. Our interest in accomplishing a similar solubilization of total RNA polymerase activities in the prostate was to assess whether the activation of the enzyme was an early event in the hormonal stimulation of the prostate, distinct from the activation of nuclear chromatin. In addition, the findings would provide a necessary foundation to future work in which the role of the specific androgen-binding proteins or 'receptors' in controlling genetic transcription could be investigated, perhaps in reconstituted cell-free systems. The androgen receptors are the centre of widespread interest at the present time, and although they have been partially characterized (Mainwaring, 1969*a,b*; Fang, Anderson & Liao, 1969; Baulieu & Jung, 1970) their biological function remains an open question.

EXPERIMENTAL

Animals. Adult male Sprague-Dawley rats, 8–10 weeks old, were used throughout this study. Bilateral orchidectomy was performed by the scrotal route under Fluothane anaesthesia. Groups of control animals were sham-operated without the final removal of the testes. When administered, testosterone phenylpropionate (2.5 mg) was given subcutaneously into the flank in oil (0.25 ml).

Chemicals. [5-³H]Uridine 5'-triphosphate (tetralithium salt; sp. radioactivity 11.1 Ci/mmol) was obtained from New England Nuclear Corp., Frankfurt, Germany. Dithiothreitol, ATP, CTP, GTP, UTP (sodium salts), calf thymus DNA (type IV), tris base (99.5% pure), crystalline bovine serum albumin and crystalline ox heart catalase were purchased from Sigma (London) Chemical Co., London S.W.6, U.K. Actinomycin D was a gift of Merck, Sharp and Dohme, Rahway, N.J., U.S.A. Amanitin (α -isomer) was obtained from C. F. Boehringer und Soehne, Ingelheim am Rhein, Germany. Microgranular DEAE-cellulose (type 52) was supplied by Whatman Biochemicals Ltd., Maidstone, Kent, U.K. Macherey-Nagel cellulose (type 2200 ff) was supplied by Camlab Ltd., Cambridge, U.K. Ammonium sulphate was 'Enzyme Grade', especially low in heavy metals. Remaining chemicals were AnalaR grade, and glass-distilled water was used in making up all solutions. *Escherichia coli* cells (strain M.R.E. 600) were generously provided by the Microbiological Research Establishment, Porton, Wilts., U.K. Bacterial RNA polymerase, containing sigma factor, was prepared by method A of Burgess (1969).

Isolation of subcellular fractions. All preparative procedures were conducted at temperatures as close to 0°C as possible.

(a) Nuclei. These were isolated by a previously described method (Mainwaring, 1969a) but with 1 mM-MgCl₂ replacing CaCl₂ in all preparative media. Based on DNA determinations, 60–70% of tissue nuclei were recovered in a purified form. Satisfactory purity was assessed by both microscopic and biochemical criteria (Widnell & Tata, 1964a). Pooled prostatic tissue from 12 animals provided 1.0–1.3 mg of DNA in the form of purified nuclei, with a nuclear protein/DNA ratio of 6–7:1.

(b) Nucleoli. Purified nuclei were washed once by centrifugation in 0.25 M-sucrose to remove most of the Mg²⁺ ions and evenly resuspended by gentle hand homogenization in 0.25 M-sucrose–5 mM-tris–HCl buffer, pH 7.4. The suspensions were sonicated for 25 s at 20 Hz in an MSE ultrasonic disintegrator and the nucleoli recovered by centrifugation in discontinuous sucrose density gradients (Muramatsu, Smetana & Busch, 1963). As judged by microscopic examination of specimens stained with Azure C (Ayres, 1948), 85–92% of the nuclei were disintegrated under these conditions. Of the initial nuclear DNA approx. 10–16% was recovered in the nucleoli, and the nucleolar protein/DNA ratio was 8.5–10:1. Purified nucleoli and nuclei were finally resuspended in 0.25 M-sucrose–1 mM-MgCl₂ except when used as for the preparation of soluble RNA polymerase, described below.

(c) Chromatin. This was prepared from purified nuclei (400–600 μ g of DNA) or nucleoli (40–80 μ g of DNA) by the procedure of T. Spelsberg (personal communication). At each successive extraction stage, the chromatin was

sedimented by centrifugation at 10000g for 10 min. The procedure consisted of two extractions with 80 mM-NaCl–20 mM-EDTA, titrated with citrate buffer to pH 6.3, two extractions with 0.35 M-NaCl and one extraction with 1.5 mM-NaCl in 0.15 M-sodium citrate buffer, pH 7.0. The final gel-like preparations were dissolved in water by gentle hand homogenization in all-glass TenBroeck homogenizers. Over 90% of the initial DNA was recovered in the final preparations. Prepared in this manner, prostatic chromatin contained 390 μ g of histone protein and 110 μ g of non-histone (acidic) protein/100 μ g of DNA. The latter determinations were done as described by Marushige & Bonner (1966).

Solubilization of RNA polymerase activities. This was accomplished by the procedure developed by Roeder & Rutter (1970a) for rat liver, with minor modifications to accommodate the smaller amount of starting material and available centrifuge rotors. In view of the relatively small amount of enzyme associated with prostatic nuclei, as a routine the purification of the enzyme was done as far as fraction 4 described by Roeder & Rutter (1970a). At this purity, however, the prostatic enzyme was entirely dependent on the provision of exogenous DNA template and was suitable for the main purpose of this investigation. Prostatic nuclei and nucleoli, derived from pooled tissue from 12 animals, were resuspended in 4.0 ml of medium A (10 mM-tris–HCl buffer, pH 7.9, containing 1.0 M-sucrose, 5 mM-MgCl₂ and 5 mM-dithiothreitol) and 0.4 ml of 3 M-(NH₄)₂SO₄ (adjusted to pH 7.9 with NH₃) and the viscous suspension was sonicated for four 10 s periods at 20 Hz, with 20 s intervals for cooling. After rapid dilution with 7.5 ml of medium B [50 mM-tris–HCl buffer, pH 7.9, containing 1 mM-MgCl₂, 0.1 mM-EDTA, 0.5 mM-dithiothreitol and 25% (v/v) glycerol] the samples were centrifuged at 100000g for 1 h. The clear supernatant was treated by the gradual addition of solid (NH₄)₂SO₄ to a total of 0.42 g/ml and after standing for 30 min, the precipitate was sedimented by centrifugation at 100000g for 30 min. The precipitate was taken up in 4 ml of medium B and dialysed for 2 h against this medium. Any precipitate formed during dialysis was removed by brief centrifugation at 100000g and the supernatant, containing the RNA polymerase activities, was either taken for further analysis or rapidly frozen in solid CO₂–acetone freezing mixture and stored at –20°C. The enzyme may be stored for 48 h without deleterious change. The final preparation derived from prostatic nuclei from 12 animals contained 4.0–4.4 mg of protein.

Assay of RNA synthesis. (a) Solubilized RNA polymerase. The standard assay system contained either 2.5 μ mol of MgCl₂ or 1.5 μ mol of MnCl₂, 3.5 μ mol of KCl, 0.2 μ mol of dithiothreitol, 0.1 μ mol each of ATP, CTP and GTP, 0.1 μ Ci of [³H]UTP and 2 nmol of UTP, 25 μ g of calf thymus DNA and 50 μ mol of tris–HCl buffer, pH 8.1, in a total volume of 0.5 ml. In certain experiments, thermally denatured DNA replaced native DNA as template. Denaturation was accomplished by heating at 95°C for 10 min, followed by rapid cooling in ice. Reactions were initiated by the addition of 0.2 ml of enzyme, followed by incubation for 15 min at 37°C. In assays containing MnCl₂, (NH₄)₂SO₄ (30 μ mol) was added 4 min after the beginning of the incubation (Widnell & Tata, 1966). The reactions were terminated by the addition of 2 ml of 5% (w/v) trichloroacetic acid containing 1 mM-Na₄P₂O₇, the

mixtures were cooled in an ice bath and carrier yeast RNA (250 μ g in 0.25 ml) was added. After 15 min at 0°C, the acid-insoluble material was collected on 25 mm Whatman GFA glass-fibre discs, washed twice with 5 ml of cold trichloroacetic acid- $\text{Na}_4\text{P}_2\text{O}_7$ and twice with cold ethanol and counted for radioactivity in 10 ml of phosphor (5 g of 2,5-diphenyloxazole/l of toluene) in a Nuclear-Chicago Mark II scintillation spectrometer. Quenching was determined by the channels-ratio procedure and the counting efficiency for ^3H was generally 45%. After correction for controls, in which DNA was omitted, the incorporation of UTP was calculated as d.p.m. of [^3H]-UTP incorporated/15 min per 100 μ g of protein. Accordingly, 120 d.p.m. was equivalent to the incorporation of 1 pmol of UTP into RNA.

(b) Nuclei and nucleoli. This was performed as described for the solubilized enzyme but the amount of carrier UTP per assay was raised to 20 nmol and the DNA and enzyme were replaced by 0.25 ml of nuclear or nucleolar suspension (20–80 μ g of DNA) in 0.25 M-sucrose–1 mM- MgCl_2 . The incorporation of [^3H]UTP into RNA was calculated in terms of d.p.m./15 min per 100 μ g of DNA or protein, after correction for zero-time controls.

(c) Chromatin-primed synthesis. The priming ability of freshly prepared chromatin was determined in a manner based largely on the procedures of Marushige & Bonner (1966). The components of the assay medium (volume 0.2 ml) were as follows: 50 μ mol of tris-HCl buffer, pH 8.0, 2 μ mol of MgCl_2 , 0.25 μ mol of MnCl_2 , 0.1 μ mol each of ATP, CTP and UTP, 0.2 μ Ci of [^3H]GTP (sp. radioactivity 11.8 Ci/mmol) and 20 nmol of GTP, and 30 μ g of *E. coli* RNA polymerase. Reactions were started by the addition of 50 μ l of chromatin, containing 8–24 μ g of DNA, and after incubation at 37°C for 10 min the acid-insoluble radioactivity was measured as described above. The incorporation of [^3H]GTP was calculated as d.p.m./10 min per 50 μ g of DNA, after correction for zero-time controls and for the background incorporation in the total absence of DNA. The system can be saturated with chromatin in amounts in excess of 30 μ g of chromatin DNA per assay, but in the range suggested, incorporation was strictly proportional to the amount of chromatin DNA added. The percentage of the DNA transcribed with total prostatic chromatin was 10–11% of that achieved with an equivalent amount of purified thymus DNA as template. This value rose to 21–23% with nucleolar-associated chromatin. In the standard system, 6.10 nmol of GTP was incorporated per 50 μ g of thymus DNA. Chromatin itself was devoid of RNA polymerase activity.

Chromatographic separations. (a) DEAE-cellulose. Suspensions of Whatman DE52 DEAE-cellulose were stirred in medium B overnight, deaerated *in vacuo*, packed as 0.9 cm $\times$ 10 cm columns under gravity and equilibrated by washing with 250 ml of medium B. Solubilized RNA polymerase was applied to the adsorbent in a minimum volume of medium B and the column eluted with a linear gradient developed from 40 ml of medium B and 40 ml of medium B containing 0.8 M-KCl. Fractions (2 ml) were collected at a flow rate of 6 ml/h and assayed for RNA polymerase activity.

(b) DNA adsorbed on cellulose. DNA was purified from the prostate gland as described by Mainwaring (1968), including the treatment with deoxyribonuclease-free enzymes. DNA was then adsorbed on to Macherey-

Nagel cellulose as described by Alberts, Amodio, Jenkins, Gutmann & Ferris (1968). After freeze-drying overnight, the DNA-cellulose was washed twice in the cold with 10 mM-tris-HCl buffer (pH 7.4)–1 mM-EDTA and stored in small portions at –20°C. Small columns were packed with 250 mg of cellulose containing 250 μ g of adsorbed DNA and washed with 20 ml of medium C [20 mM-tris-HCl buffer, pH 8.1, containing 0.1 M-NaCl, 1 mM-EDTA, 1 mM-dithiothreitol, 250 μ g of bovine serum albumin/ml and 10% (v/v) glycerol]. Solubilized RNA polymerase was slowly run through the column and elution was carried out in a stepwise manner with medium C containing 0.3 M-, 0.5 M-, 0.6 M- and 0.8 M-KCl. Four fractions (2 ml each) were collected at each ionic strength at an overall flow rate of 8 ml/h.

Glycerol-density-gradient centrifugation. For these particular analyses, samples of solubilized RNA polymerase were dialysed against medium B containing only 5% (v/v) glycerol. Samples (0.5 ml) of dialysed enzyme were layered over 4.5 ml linear 15–30% (w/v) glycerol gradients made up in 20 mM-tris-HCl buffer, pH 8.0, containing 1 mM- MgCl_2 and 0.5 mM-dithiothreitol. One gradient out of each set of three was loaded with 0.2 mg of catalase ($s_{20,w}$ 11.4S) or 0.1 mg of *E. coli* RNA polymerase ($s_{20,w}$ 24S) as sedimentation markers. After centrifugation for 17 h in a swing-out 40 rotor of a Griffin-Christ ultracentrifuge at 33000 $g_{av.}$, rotor temperature 4°C, the gradients were mounted in a holder made to the design of McConkey (1967) and subdivided into 18 fractions. Samples (0.2 ml) were taken for RNA polymerase assay. The sedimentation markers were also located by measurement of enzymic activity.

Chemical analyses. DNA was determined by the procedure of Burton (1956) with calf thymus DNA as standard. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as reference.

RESULTS

Characterization of soluble prostatic RNA polymerase. Release of RNA polymerase from whole nuclei was dependent on the addition of ammonium sulphate to the isolation medium. If the activity released in the presence of ammonium sulphate at an overall concentration of 0.27 M is taken as 100%, only 24% of the activity was solubilized by sonication in the absence of added salt. The release of activity increased to 29%, 47%, 78% and 91% at final ammonium sulphate concentrations of 0.05 M, 0.10 M, 0.18 M and 0.22 M respectively.

A summary of the requirements for the incorporation of [^3H]UTP into an acid-precipitate form of polyribonucleotide is presented in Table 1. With Mg^{2+} as the activating cation, the incorporation of UTP was totally dependent on the presence of exogenous DNA template and all four ribonucleoside 5'-triphosphates. Suppression of incorporation by ribonuclease and deoxyribonuclease confirms that the reaction product, RNA, is synthesized on a DNA template. One outstanding anomaly observed

Table 1. *Properties of soluble prostatic RNA polymerase*

The soluble enzyme was assayed in the presence of 25 μ g of calf thymus DNA and either 5 mM-MgCl₂ or 3 mM-MnCl₂. Full details of the assay system are given in the Experimental section. Activity given is the mean of duplicate assays and calculated as d.p.m. of [³H]UTP incorporated/100 μ g of enzyme protein.

	RNA polymerase activity	
	Mg ²⁺ -activated	Mn ²⁺ -activated
Complete system	414	541
Minus DNA	105	137
Minus ATP, CTP, GTP	106	922
Minus activating cation	94	132
Plus 10 μ g of actinomycin D	118	139
Plus 10 μ g of ribonuclease	103	143
Plus 10 μ g of deoxyribonuclease	114	148

with the Mn²⁺-stimulated system, however, was the stimulation of the incorporation of [³H]UTP in the absence of the complementary nucleotides. This has also been found to a much lesser degree with [³H]GTP alone. Recently (F. R. Mangan & W. I. P. Mainwaring, unpublished work) we have established that homopolymer formation is totally suppressed by the presence of other nucleotides. Homopolymer formation in the presence of Mn²⁺ ions had been reported with certain other enzyme preparations from rat prostate (Hancock, Zelis, Shaw & Williams-Ashman, 1962) and with more purified enzyme preparations from rat liver (Roeder & Rutter, 1969) and, more notably, *E. coli* (Chamberlin & Berg, 1962). In the latter instances, however, the enzymes principally formed adenosine polymers. The optimum concentrations of activating cations (5 mM for magnesium chloride and 3 mM for manganese chloride) were determined in separate experiments, the optimum range for Mn²⁺ being particularly narrow.

When the effects of the mushroom toxin α -amanitin were investigated (Fig. 1), the Mn²⁺-stimulated activity was selectively inhibited. Even at maximum inhibition with 2 μ g of toxin, however, the Mn²⁺-stimulated activity from rat prostate was less inhibited than the enzyme prepared from rat liver. The reason for this difference remains unclear, but certainly the degree of inhibition found with the liver enzyme is in complete agreement with the findings of Jacob, Sajdel & Munro (1970). Under these experimental conditions, the RNA polymerase from *E. coli* was completely refractory to α -amanitin in the presence of Mn²⁺.

The solubilized RNA polymerase from whole

Fig. 1. Effect of α -amanitin on the soluble RNA polymerase enzymes from rat liver and rat prostate. ●, Liver; ○, prostate; ----, Mg²⁺-dependent activity; —, Mn²⁺-dependent activity. Activities (d.p.m./100 μ g of protein) in absence of toxin were as follows; prostate, 442 (Mg²⁺), 522 (Mn²⁺); liver, 561 (Mg²⁺), 649 (Mn²⁺).

prostatic nuclei was resolved into two discrete peaks when subjected to ion-exchange chromatography (Fig. 2). Similar separations were reported by Roeder & Rutter (1969) for the soluble liver enzyme with DEAE-Sephadex. In keeping with their results, peak I, the more rapidly eluted peak of enzymic activity, was derived from the nucleolus (lower curve, Fig. 2). Although peaks I and II were identified by assay in the presence of magnesium chloride, they also contained Mn²⁺-stimulated activity. The ratio of manganese chloride (plus ammonium sulphate)- to magnesium chloride-stimulated activities in peaks I and II was 2.4 and 3.3 respectively. These ratios are similar to those reported by Roeder & Rutter (1969). A similar separation of RNA polymerase into nucleolar and extranucleolar components was accomplished by the stepwise elution of enzyme from columns containing DNA adsorbed on cellulose. Two discrete peaks of activity were eluted with 0.3M- and 0.6M-potassium chloride. Of these, the more rapidly eluted peak largely contained enzyme derived from the nucleolus.

Evidence for the existence of separate Mg²⁺- and Mn²⁺-dependent RNA polymerase enzymes in both solubilized nucleolar and whole nuclear enzyme was found in the differential effect of pH in the presence of either magnesium chloride or manganese chloride (Fig. 3). In both types of preparations, the optimum pH for the Mn²⁺-dependent enzyme was higher than that for the Mg²⁺-dependent enzyme. The presence of Mg²⁺- and Mn²⁺-specific enzymes in whole liver nuclei is well established (Widnell &

Fig. 2. Elution profile of soluble prostatic RNA polymerase from columns of DEAE-cellulose, with a linear gradient developed from 40 ml of medium A (for details see the Experimental section) and 40 ml of medium A containing 0.8M-KCl. Fractions were assayed for enzymic activity in the presence of $MgCl_2$. ●, Enzyme isolated from whole nuclei derived from 12 animals; ○, enzyme isolated from nucleoli derived from 12 animals; ----, concn. of KCl.

Tata, 1964b) and has been demonstrated in liver nucleoli (Jacob, Sajdel & Munro, 1969).

After brief dialysis to remove ammonium sulphate at the end of the enzyme preparation, the solubilized RNA polymerase had a sedimentation coefficient of approx. 20S (Fig. 4). Siefert & Sekeris (1969) have attributed a similar sedimentation coefficient to the enzyme prepared from rat liver by a different procedure. There was a marked decrease in the sedimentation coefficient of the prostatic enzyme after prolonged dialysis, to a final value of 13–14S. Purified nucleolar enzyme behaved similarly. We were unable to separate the solubilized enzyme into specific Mn^{2+} - and Mg^{2+} -dependent enzymes in density gradients, as reported by Liao, Sagher, Lin & Fang (1969). This failure is certainly attributable to our use of smaller (5ml) gradients, but despite this, there is excellent agreement on the final sedimentation value of the solubilized enzyme with their preparations from liver nuclei.

Effect of androgen treatment in vivo on the RNA polymerase activities in the prostate. The effects of

hormonal stimulation on the RNA polymerase activities in the prostate are of considerable importance to the further understanding of the mechanism of action of androgenic steroids. A more comprehensive study of the solubilized RNA polymerase activities is summarized in Table 2. The most striking outcome of castration is the pronounced loss of Mg^{2+} -dependent activity. This could be totally restored by the administration of testosterone *in vivo*. The Mg^{2+} -dependent activity showed a marginal preference for native DNA as template and was not stimulated by the further inclusion of ammonium sulphate in the assay medium. In contrast, the Mn^{2+} -dependent activity was not significantly altered by a decrease in the circulating concentrations of androgenic steroids, but showed an increase in activity with denatured DNA as template and was selectively stimulated by ammonium sulphate. This latter effect has been well documented in both whole liver nuclei (Widnell & Tata, 1964b) and solubilized liver enzyme (Stirpe & Novello, 1970). Replacement of native DNA by thermally denatured DNA as template had a less

Fig. 3. Effect of pH on the soluble Mg^{2+} - and Mn^{2+} -dependent prostatic RNA polymerase activities from (a) whole nuclei and (b) nucleoli. Each incubation mixture contained $185\mu g$ of protein (nuclei) or $35\mu g$ of protein (nucleoli). Buffers were of tris-HCl throughout. ●, Mg^{2+} -dependent activity; ○, Mn^{2+} -dependent activity.

significant effect on the Mg^{2+} - and Mn^{2+} -dependent activities than reported by other investigators (Liao *et al.* 1969).

From chromatographic studies on solubilized RNA polymerase from the prostate, a significant decrease in Mg^{2+} -dependent activity after castration is found in enzyme associated with both the nucleolus and the extranucleolar region (Fig. 5).

The time-course of the restoration of the biochemical activities in prostatic nuclei after the administration of testosterone *in vivo* to castrated animals is given in Fig. 6. At 2 h after the administration of hormone, there was a very pronounced rise in the Mg^{2+} -dependent RNA polymerase activity in the nucleolar fraction that was far higher than in whole nuclei (Fig. 6a). This rise was equally significant when the enzyme activity was calculated with reference to either DNA or protein. In both nucleoli and whole nuclei, the increase in the Mn^{2+} -dependent activity was both slower and less marked. The speed and selective nature of the response in whole nuclei are in keeping with the results of Liao *et al.* (1965). When the template activities of nucleolar and total nuclear chromatin were examined (Fig. 6b), the increase in transcribable sites after hormonal stimulation was much slower than the increase in overall RNA synthesis. Nevertheless, the overall rise in template activity

is in agreement with the longer-term response of prostatic chromatin reported by Mangan *et al.* (1968). In contrast, the stimulation of solubilized RNA polymerase, measured in the presence of magnesium chloride, was at a rate similar to that reported for whole nuclei and nucleoli. Despite the rapidity of this response, the actual rise in activity was not as high as expected from the earlier experiments reported in Table 2. In this experiment, there was a 45% stimulation of the Mg^{2+} -dependent activity derived from whole nuclei, compared with a 120% increase shown in Table 3. It should be emphasized, however, that this experiment was performed on nuclear fractions derived from only three animals, as against 12 in Table 3. The lower degree of stimulation may well reflect a higher rate of inactivation of the labile RNA polymerase enzyme at lower concentrations of protein. Notwithstanding this, the response of solubilized RNA polymerase is more in keeping with the rate of steroid-induced stimulation of RNA synthesis than the activation of nuclear chromatin.

Although a decrease in the activity of the solubilized Mg^{2+} -dependent enzyme occurs after castration (see Fig. 5) a final series of experiments suggests that androgenic steroids not only control the activity of available RNA polymerase but also its efficient utilization (Table 3). When completely

Fig. 4. Sedimentation profile of solubilized RNA polymerase from rat prostate in linear 4.0ml 15-30% (w/v) glycerol gradients. Gradients were centrifuged for 17h, 4°C, at 33000g_{av}. and individual fractions were assayed for RNA polymerase in the presence of MgCl₂. Direction of centrifugation was from right to left. C, sedimentation marker, catalase (11.4S); ●, enzyme immediately after preparation; ○, enzyme after overnight dialysis.

Table 2. Activity of soluble RNA polymerase from prostatic nuclei in the presence of either 5mM-magnesium chloride or 3mM-manganese chloride

Either native or thermally denatured calf thymus DNA (25µg) was used as template. Full details of the assay system are given in the Experimental section. The animals were either sham-operated or used 24h after bilateral orchidectomy. Where indicated, testosterone (2.5mg) was given subcutaneously at the time of castration. The results are the means of triplicate determinations. Each experimental group contained 12 animals.

Bivalent cation ...	RNA polymerase activity (d.p.m. of [³ H]-GTP incorporated/100µg of enzyme protein)		
	MgCl ₂	MnCl ₂	MnCl ₂ + (NH ₄) ₂ SO ₄
Native DNA as template			
Sham-operated animals	790 ± 37	960 ± 31	1769 ± 191
Castrated animals	302 ± 11	990 ± 52	1996 ± 104
Castrated, androgen-treated animals	910 ± 30	898 ± 20	1870 ± 100
Denatured DNA as template			
Sham-operated animals	630 ± 27	1070 ± 100	2074 ± 176
Castrated animals	150 ± 13	1000 ± 87	2420 ± 271
Castrated, androgen-treated animals	792 ± 31	1210 ± 43	2241 ± 132

non-repressed template is added to purified nuclei or nucleoli *in vitro*, in the form of thymus DNA, there is a small stimulation of RNA polymerase activity. This is increased by several orders of magnitude, however, when the experiments are repeated with nuclear fractions isolated from

castrated animals. The stimulation by thymus DNA *in vitro* is decreased by the administration of testosterone *in vivo* to castrated animals. Similar results were reported by Liao & Fang (1969) in prostatic nuclei, but the effects on nucleoli are far more striking.

Fig. 5. Elution profile of soluble prostatic RNA polymerase from columns of DEAE-cellulose (see Fig. 2 for complete details). ●, Enzyme isolated from nuclei derived from 12 sham-operated animals; ○, enzyme from nuclei derived from 12 animals 24 h after bilateral orchidectomy.

DISCUSSION

The results on the solubilized RNA polymerase from prostate are in general agreement with the results obtained with rat liver enzyme (Roeder & Rutter, 1969, 1970*a,b*). Solubilization of all the available enzyme represents a significant advance on previous work with the prostate (Doly *et al.* 1965) since it enables the early response of the tissue to hormonal stimulation to be identified more precisely. In addition, it enables the effect of androgens on the RNA polymerases and chromatin fractions to be studied separately. In keeping with the work of Jacob *et al.* (1969) on the cortisol-induced changes in RNA synthesis in liver, the earliest response in the prostatic nucleus to androgenic stimulation is found in the nucleolus, at the level of the Mg^{2+} -dependent RNA polymerase itself. This stimulation occurs before the activation of nucleolar-associated chromatin, yet after the binding of hormone at specific nuclear sites is at a maximum (Mainwaring, 1969*a*). The androgens appear to control the efficient utilization of RNA polymerase and also its activity. It is not yet clear whether the hormones

control the activity of the enzyme directly or by means of control of the rate of enzyme synthesis. We hope to clarify this point in the near future by studying the effects of inhibitors of protein synthesis, such as cycloheximide, on the hormonal stimulation of solubilized nucleolar RNA polymerase. It is tempting to implicate the specific androgen 'receptors' for 5α -dihydrotestosterone in this selective activation process. Certainly none of the stimulations described can be simulated by adding 5α -dihydrotestosterone or other androgenic steroids directly to the assay systems *in vitro*. This suggests that factors other than the steroids themselves are involved. In this regard, it should be added that we have been unable to repeat the direct stimulation of the incorporation of $[^3H]$ cytidine into RNA in prostatic nuclei by dihydrotestosterone *in vitro* as claimed by Bashirelahi, Chader & Villee (1969). Many proteins have been isolated from bacteria that have the specific function of controlling genetic transcription (Burgess, Travers, Dunn & Bautz, 1969; Roberts, 1969). One of these factors specifically controls the synthesis of ribosomal RNA (Travers, 1970), and the receptor-steroid complexes

Fig. 6. Stimulation of activities in prostatic nuclei after the administration of testosterone *in vivo*. Beginning 36 h after castration, groups of six animals were given 2.5 mg of testosterone subcutaneously at timed intervals before all were killed a total of 48 h after orchidectomy. Three animals from each group were untreated and served as controls. A total of 36 animals was used in each of the following three experiments. Details of the assay procedures and the calculation of activities are given in the Experimental section. In each experiment, measurements were made on preparations from nuclei or nucleoli derived from tissue from three animals. (a) Stimulation of RNA polymerase activities: ●, nucleoli; ○, whole nuclei; —, Mg²⁺-stimulated activity; ----, Mn²⁺-stimulated activity. (b) Stimulation of DNA template activity for *E. coli* RNA polymerase: ●, nucleolar chromatin; ○, total nuclear chromatin. (c) Stimulation of solubilized Mg²⁺-stimulated activity: ●, nucleoli; ○, whole nuclei.

Table 3. Stimulation of RNA synthesis in prostatic nuclei and nucleoli by the addition of calf thymus DNA

Details of the assay system are given in the Experimental section. Where indicated, 50 μg of thymus DNA was added to duplicate assay systems.

	RNA polymerase activity (pmol of UTP/15 min per 100 μg of particulate DNA)	
	Nuclei	Nucleoli
Sham-operated animals		
Minus DNA	28.7	240.2
Plus DNA	40.2	316.5
Castrated animals		
Minus DNA	20.1	80.2
Plus DNA	35.3	218.0
Testosterone-treated castrated animals		
Minus DNA	29.4	200.3
Plus DNA	41.3	277.7

may play such a role. Certainly the nucleolus is the principal site of ribosomal RNA synthesis in differentiated cells (Perry, 1967) and the RNA formed in response to hormonal stimulation is largely of this type (Liao & Lin, 1967; Jacob *et al.* 1969). Recently (F. R. Mangan & W. I. P. Main-

waring, unpublished work) the formation of mainly ribosomal RNA by the prostatic nucleolus has been confirmed by using nearest-neighbour-frequency analysis. Further, steroid-receptor complexes can react with the template for RNA synthesis directly (Mainwaring & Mangan, 1971) and Hough, Arnaud & Mousseron-Canet (1970) have shown that the activity of RNA polymerase in uterine nuclei may be modified *in vitro* by a soluble receptor-oestradiol-17β complex.

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