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INFLUENCE OF RECEPTOR COMPLEXES ON THE PROPERTIES OF PROSTATE CHROMATIN, INCLUDING ITS TRANSCRIPTION BY RNA POLYMERASE

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SUMMARY

Chromatin may be prepared in a manner that preserves the attachment of RNA polymerase. The administration of androgens to castrated animals, *in vivo*, specifically enhances the chromatin-associated RNA polymerase in the prostate gland. This activated chromatin also binds more actinomycin D than that from animals of lower androgenic status, suggesting that hormonally-modified chromatin contains more exposed regions of DNA.

The cytoplasmic 5α-dihydrotestosterone–receptor complex of the prostate gland changes its physicochemical properties on brief warming at 30°C in such a way that the "activated" complex associates more avidly with chromatin in reconstituted, cell free systems.

Androgen–receptor complexes, including the activated configuration, promote structural changes in chromatin *in vitro* such that transcription by prostate RNA polymerase is facilitated and the high affinity binding of actinomycin D is potentiated.

The elongation stage of the transcriptional process appears to be particularly enhanced following the incubation of chromatin with androgen receptor complexes in cell-free systems.

INTRODUCTION

As extensively reviewed by King and Mainwaring [1], the selective, high-affinity binding of biologically active steroids is a ubiquitous feature of steroid-sensitive cells. The proteins responsible for this binding phenomenon are generally termed steroid "receptors". A unique facet of the steroid-binding mechanism is the association of the steroid–receptor complex with chromatin and this may be simulated with considerable fidelity in reconstituted, cell-free systems [1]. Following a period of intensive investigation into the characteristic chemical and physical properties of steroid receptors, interest is now tending to focus on the means by which the steroid–receptor complexes control important metabolic processes, notably genetic transcription. Largely for reasons of practical facility and ease of experimental interpretation, such investigations are best conducted in cell-free systems, *in vitro*. Up to the present, however, there have been few reports of significant changes in the extent of transcription of DNA or chromatin promoted by steroid–receptor complexes under conditions, *in vitro*, but encouraging progress has been made in reconstituted systems derived from plants [2, 3] and, more recently, uterus [4] and prostate gland [5, 6]. The principal objective of the present study was to investigate further the structural changes in chromatin promoted by steroid–receptor complexes, particularly those relevant to an enhancement of the process of transcription. In common with receptor complexes derived from a diversity of sources [1], the cytoplasmic androgen receptor complex may be transformed or "activated" by an as yet ill-defined process such that the rate but not the extent of its association with chromatin, *in vitro*, is conspicuously enhanced [7]. Studies on the regulation of transcription by this activated receptor complex are also featured in this investigation.

METHODS AND MATERIALS

Animals

Male Sprague–Dawley rats (250–270 g body wt.) were castrated by the scrotal route under Fluothane anaesthesia. Unless specified to the contrary, animals killed by cervical dislocation 72 h after castration were used as a source of tissues for all preparations used in this study. Where indicated, testosterone phenylpropionate (2·5 mg in 0·25 ml arachis oil) was injected s.c. into the flank; control animals were injected with oil only.

Materials

[5-^{3}H]-Cytosine 5′-triphosphate (9·1 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, Bucks., U.K. [G-^{3}H] Actinomycin D (2·8 Ci/mmol) was supplied by Schwarz–Mann Co., Orangeburg, N.Y., U.S.A. The RNA polymerase inhibitor, AF/013, was generously donated by Gruppo Le Petit, Milan, Italy. Non-radioactive actinomycin D was supplied by Merck & Co., West Point, Penn., U.S.A. Tris base (99·5% pure), dithioerythreitol, 2-mercaptoethanol and nucleoside 5′-triphosphates

were supplied by Sigma (London) Chemical Co., London, S.W.6., U.K. $(NH_4)_2SO_4$ was enzyme grade, low in heavy metals (Fisons Chemical Co., Loughborough, Leics., U.K.); a saturated solution was adjusted to pH 7·4 with 1 N NH_4OH. Other chemicals were of Analar grade and glass distilled water was used in making up all solutions.

Isolation of subcellular fractions

These were prepared at temperatures as close to 0°C as possible. (a) *Chromatin.* Two isolation procedures were adopted, the first of which has been described in detail formerly [8]. This means of isolation was generally used, providing chromatin preparations of low endogenous RNA polymerase activity. The second procedure [9] was employed whenever it was essential to retain significant chromatin-associated RNA polymerase activity. Irrespective of the means of preparation, chromatin could be satisfactorily stored for up to 2 weeks at −20°C without deleterious change. (b) *Cytoplasmic androgen receptor.* Tissue homogenates were prepared in the medium described by Mainwaring and Peterken [8], additionally containing 10% (v/v) glycerol, and with the mandatory maintenance of thorough cooling [10]. After centrifugation at 10,000 *g* for 10 min, 5α-dihydrotestosterone was added to the supernatant fraction in ethanol: 1,2-propanediol, 1:4 (v/v) to an overall concentration of 1×10^{-8} M. Following centrifugation at 150,000 *g* for 30 min, additional 5α-dihydrotestosterone was added to provide a calculated concentration of 5×10^{-8} M in the final supernatant preparation. After 1 h, the preparation was gently stirred magnetically in an ice-bath and one half volume of saturated $(NH_4)_2SO_4$ was added dropwise. Exactly 10 min later, the fine precipitate was collected by centrifugation at 8000 *g* for 5 min and dissolved in a volume of glycerol-containing medium [8] equal to that of the 150,000 *g* supernatant fraction from which it was derived. It is imperative that the preparation be conducted at 0°C throughout. Due to the instability of the 8S androgen–receptor complex in extracts derived from long-term castrated animals [11], tissues were taken 24 h after castration. Control preparations were supplemented with vehicle only (ethanol–propanediol mixture). Spleen extracts were similarly prepared but female human serum, a convenient source of the sex steroid-binding β-globulin (SBG), was used directly; all preparations were diluted to a protein concentration equal to that of the prostate androgen receptor complex (approx. 2 mg/ml).

Prostate form B RNA polymerase

This was isolated by a modification of the procedure devised by Kedinger *et al.*[12]. In our hands, however, satisfactory preparations of prostate enzyme could only be achieved using fresh tissue and provided that the preparative medium [12] contained a minimum of 30% (v/v) glycerol. The enzyme was completely stable at −70°C for up to 1 month.

Assay of RNA polymerase

(a) *Endogenous chromatin-associated enzyme.* This was conducted in an assay volume of 0·25 ml [9], but with ^{3}H-CTP as tracer. Each assay contained 75–100 μg of chromatin DNA and 0·2 M $(NH_4)_2SO_4$.

(b) *Solubilized RNA polymerase.* Each assay, total volume 0·1 ml, contained 4 μmol of Tris–HCl buffer, pH 7·9; 15 μmol of KCl; 2 μmol of $MgCl_2$; 0·5 μmol of $MnCl_2$; 0·25 μmol of dithioerythreitol; 40 n mol each of ATP, GTP and UTP; 0·4 μCi of ^{3}H-CTP plus 2 nmol of unlabelled CTP; chromatin (equiv. to 30–50 μg of DNA) and partially purified prostate form B RNA polymerase. Incubation was for 40 min at 37°C. In this assay and (a) above, sodium dodecyl sulphate 10% (w/v) was added to an overall concentration of 0·5% and incubation continued for a further 5 min. Aliquots (100 μl) of the assay mixture were then pipetted directly onto DEAE cellulose discs (Whatman DE81; 2·5 cm dia.) and dried under an I.R. lamp. Up to 50 discs were placed directly in 250 ml of 5% (w/v) Na_2HPO_4 and gently stirred for 5 min. After decantation, this washing process was repeated twice more with Na_2HPO_4 and finally once with 50% (v/v) ethanol. The discs were dried and counted for radioactivity.

(c) *Stages of transcription.* An attempt was made to delineate the stages of the transcriptional process by judicious use of the inhibitor, AF/013. This was dissolved in dimethylformamide and diluted nine-fold to a final concentration of 1 mg/ml. Components necessary for each postulated stage of transcription were preincubated at 37°C for 5 min when AF/013 (10 μg) was added, followed by the remaining constituents in (b) above necessary for the completion of RNA synthesis. Incubation was then continued at 37°C for 30 min. Preincubation was conducted as follows: enzyme binding, in the absence of nucleoside 5′-triphosphates; initiation; in the presence of only ATP and GTP; elongation; presence of all nucleoside 5′-triphosphates except ^{3}H-CTP. Comparison of the incorporation of radioactivity after these different conditions of preincubation gave some indication of the relative extent of each stage of the transcriptional process.

Binding of ^{3}H-actinomycin D

Chromatin (30–50 μg of DNA) was diluted to a total volume of 0·25 ml in medium A (50 mM Tris–HCl buffer, pH 7·6, containing 0·1 mM EDTA). ^{3}H-Actinomycin D was added in medium A up to a total of 60 μl/assay, and the samples allowed to stand at ambient temperature for 30 min. After the addition of 0·75 ml of medium B (medium A plus 0·15 M KCl), samples of chromatin were collected on glass fibre discs (Whatman GF/A; 2·5 cm. dia.), washed three times with 3 ml of medium B and counted for radioactivity.

Reconstituted systems

Partially purified receptor preparations in the form of protein precipitated at 33% saturation with respect

to $(NH_4)_2SO_4$ were resuspended in medium C (50 mM Tris–HCl buffer, pH 7·4, containing 0·1 mM EDTA, 0·5 mM dithioerythreitol and 10% (v/v) glycerol) to a final protein concentration of 2 mg/ml. Chromatin (30–50 μg of DNA) was diluted to a total volume of 0·25 ml with medium C in small glass tubes, capacity 1 ml, and 0·25 ml of receptor preparation (or other proteins in medium C) was added. After standing for 30 min in an ice-bath, the tubes were centrifuged at 10,000 *g* in a microcentrifuge (Micro-chemical Specialities, Inc., Berkeley, Cal., U.S.A.). After decantation of the supernatant, the sedimented chromatin was resuspended in a medium appropriate for subsequent studies. There were no losses of chromatin during incubation at 0°C and the procedure has the advantage that only proteins firmly associated with the chromatin during incubation are present in ensuing analyses. This is particularly important when transcription of chromatin is to be conducted.

Analytical procedures

DNA was determined by the diphenylamine procedure of Burton[13], with calf thymus DNA serving as the standard. Radioactivity was counted in a Nuclear Chicago Mark II scintillation spectrometer in an aqueous phosphor [8] with a counting efficiency of 40% for ^3H retained on discs, either glass fibre or DEAE cellulose.

RESULTS

The activation of prostate cytoplasmic–receptor complex

In an earlier publication from this laboratory [8], it was suggested that the 5α-dihydrotestosterone formed by the metabolism of testosterone within the prostate gland was first bound to a cytoplasmic–receptor protein of sedimentation coefficient approx. 8S. Somewhat later, this complex was converted to a form of sedimentation coefficient 4·5S which was recoverable by extraction of nuclei in media of high ionic strength [8, 14]. Further studies [7] established that this change in the physicochemical properties of the 8S cytoplasmic–receptor complex could be mimicked simply by warming at 30°C for 10 min, *in vitro*. Since it has been widely established that the nuclear binding of 5α-dihydrotestosterone in whole cells is a temperature-dependent process [15, 16], the thermally-induced change in the 8S cytoplasmic–receptor complex becomes of some importance in our understanding of the overall binding mechanism for androgenic steroids, notably of 5α-dihydrotestosterone. This transformation process may be summarized briefly; (a) the 8S receptor complex may be converted at 30°C to a sedimentation coefficient of 4·5S and isoelectric point at pH 6·5 such as to be indistinguishable from the nuclear receptor complex [7, 8, 14]; (b) the activated complex is able to bind to chromatin in reconstituted systems at a greater rate but not overall extent than the initial 8S cytoplasmic–receptor complex [7, 8]. Until such times as the 8S receptor complex is available in a purified form approaching homogeneity, it remains to be established whether these thermally-promoted changes are an intrinsic property of the receptor protein or simply a reflection of the presence of proteolytic or other enzymic contaminants in crude receptor preparations. Overall, the current but admittedly indirect evidence suggests that this temperature-dependent change in the physicochemical properties of the 8S receptor complex plays an integral and necessary part in the high affinity binding process for androgens in the prostate gland.

Changes in the properties of chromatin promoted by androgenic stimulation in vivo

Before embarking on any investigation of the changes evoked by androgen receptor complexes, *in vitro*, it seemed advisable to assess the extent of changes in the properties of prostate chromatin promoted by androgenic stimulation, *in vivo*. Such studies, particularly during the early phase of androgenic stimulation, when the receptor complex is accumulating in chromatin [8, 14], would provide an indication of the extent of the changes to be expected in reconstituted, cell-free systems.

(a) *RNA polymerase activity*

The procedure devised by Butterworth *et al.*[9] was suitable for preparing prostate chromatin containing associated RNA polymerase. Further, as shown from the results presented in Table 1, prostate

Table 1. The selective enhancement of chromatin-associated RNA polymerase in the prostate gland by androgenic stimulation, *in vivo*

Androgenic stimulation (h)	Chromatin-associated RNA polymerase (c.p.m. ^3H-CTP incorporated/50 μg of DNA) (a) Prostate gland	(b) Liver	(c) Spleen
None (controls)	540 ± 20	2760 ± 70	200 ± 10
0·5	590 ± 40	2840 ± 120	170 ± 20
1	720 ± 40	2910 ± 30	210 ± 20
12	1630 ± 100	2710 ± 30	180 ± 10
24	1210 ± 90	2430 ± 70	200 ± 30

At 72 h after castration, a large group of rats was given 2·5 mg of testosterone/animal s.c.; 8 animals received an injection of oil only and served as controls. At timed intervals, tissues were pooled from 8 animals and chromatin prepared in a manner preserving endogenous RNA polymerase activity (see Methods section for details). Enzyme activity was determined in triplicate and the results given as mean ± S.D.

Table 2. The properties of chromatin-associated RNA polymerase

Conditions of assay	Chromatin-associated RNA polymerase (c.p.m. ^{3}H-CTP incorporated/50 μg of DNA)
Standard conditions	1230
Plus 0·1 M $(NH_4)_2SO_4$	470
Plus 0·3 M $(NH_4)_2SO_4$	1100
Minus $(NH_4)_2SO_4$	170
Minus $MnCl_2$	110
Minus ATP, GTP and UTP	240
Plus α-aminitin (10 μg)	140
Plus AF/013 (20 μg)	1170

The chromatin used in these experiments was prepared from the prostate gland of normal (non-castrated) animals. The standard assay system contained 0·2 M $(NH_4)_2SO_4$ (see Methods section for details).

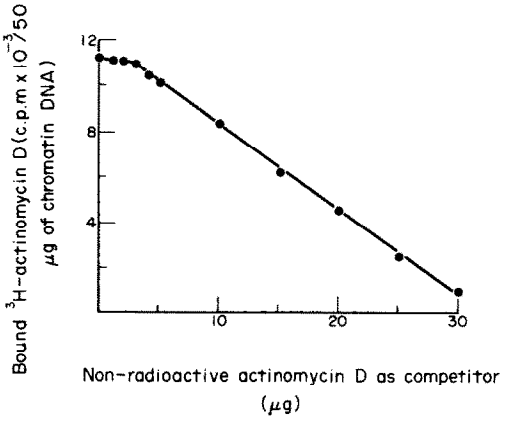

Fig. 1. The presence of two sets of binding sites for actinomycin D in chromatin.

Liver chromatin was either incubated with ^{3}H-actinomycin D alone (50,000 c.p.m., 50 ng) or additionally with increasing amounts of non-radioactive actinomycin D. The chromatin was collected on glass fibre discs, washed and counted for radioactivity.

chromatin-associated RNA polymerase is markedly enhanced by androgenic stimulation, *in vivo*. This stimulation of RNA polymerase activity was stringently tissue-specific and was not evident in chromatin derived from spleen or liver of hormonally-stimulated animals. The androgen-mediated increase in the transcription of prostate chromatin was detectable within 1 h of hormonal stimulation when the level of 5α-dihydrotestosterone–receptor complex in prostate chromatin is approaching a maximum [8, 14].

The properties of the chromatin-associated RNA polymerase activity are summarized in Table 2 and are strictly harmonious with previously published data on preparations derived from liver [9]. In particular, the activity essentially requires assay conditions of high ionic strength and the acute sensitivity of the enzyme to α-aminitin suggests that it is of the form B type [17]. As reported by others [9], the chromatin-associated enzyme is essentially refractory to the rifamycin derivative, AF/013, suggesting that the enzyme is recovered at sites representing RNA chain elongation rather than RNA chain initiation [18].

(b) *Binding of actinomycin D*

As elegantly demonstrated by Kleiman and Huang[19] using spectrophotometric procedures, chromatin contains two sets of binding sites for actinomycin D. This was confirmed in the present study (Fig. 1), using ^{3}H-actinomycin D. There appear to be a small set of high affinity binding sites of limited capacity and a large number of low affinity sites of high capacity. In experiments conducted later in this study where ^{3}H-actinomycin D was employed as a convenient means of studying hormonally-induced changes in chromatin structure, it is most likely that the occupation of the high affinity binding sites was measured.

Androgenic stimulation, *in vivo*, provoked tissue-specific changes in prostate chromatin such that more actinomycin D binding sites were exposed (Fig. 2). From inspection of the measurements of RNA polymerase activity (see Table 1) it may be seen that the enhanced extent of transcription of prostate chromatin was concomitant with a large increase in the binding of actinomycin D. Furthermore, liver and spleen chromatin was not activated by androgenic stimulation either in the sense of RNA polymerase activity or the binding of the antibiotic.

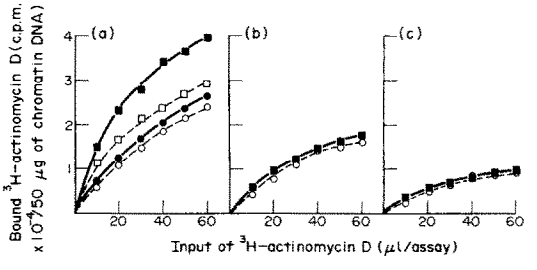

Fig. 2. Enhanced binding of ^{3}H-actinomycin D to prostate chromatin after androgenic stimulation, *in vivo*.

Chromatin was prepared from animals after varying periods of androgenic stimulation, *in vivo*, as described in the legend to Table 1. Binding of ^{3}H-actinomycin was conducted at increasing levels of the labelled compound (5×10^4–3×10^5 c.p.m.). Chromatin from prostate gland, a; liver, b and spleen, c. In all Figs., chromatin from castrate controls, ○; and after 0·5, ●; 1; □ and 12 h, ■, of androgenic stimulation. The data in Figs. b and c were essentially identical and for clarity, only two sets of symbols are presented. Each 10 μl of ^{3}H-actinomycin D solution contained 50,000 c.p.m. of radioactivity.

Table 3. The stimulation of the transcription of chromatin by 5α-dihydrotestosterone–receptor complexes *in vitro*

Chromatin	Cytoplasmic preparation	5α-Dihydrotestosterone	RNA synthesis (c.p.m. ^{3}H-CTP incorporated/50 μg of chromatin DNA)
Prostate (−enzyme)	None (controls)		80 ± 10
(+enzyme)			330 ± 20
Spleen (−enzyme)			60 ± 10
(+enzyme)			130 ± 30
Prostate	Prostate	−	320 ± 10
		+	530 ± 20
	Prostate (activated)	−	290 ± 20
		+	510 ± 10
Prostate	Spleen	−	140 ± 10
		+	130 ± 20
Prostate	Human serum	−	400 ± 10
		+	380 ± 10
Spleen	Prostate	−	140 ± 10
		+	150 ± 20
Spleen	Spleen	−	110 ± 20
		+	100 ± 40

Chromatin was prepared with low endogenous RNA polymerase activity. Cytoplasmic extracts were prepared in the presence (+) and absence (−) of 5×10^{-8} M 5α-dihydrotestosterone. Activation of receptor complex was accomplished by warming at 30°C for 10 min. Chromatin was incubated with the cytoplasmic preparations, collected by sedimentation at 10,000 *g* and resuspended in a medium suitable for the assay of RNA synthesis in the presence of prostate form B RNA polymerase. Full details of these procedures may be found in the Methods section. Two types of controls were run; (a) without added RNA polymerase or cytoplasmic preparations to assess residual chromatin-associated enzyme or (b) without cytoplasmic preparations. Determinations were performed in triplicate; results are given as mean ± S.D.

Changes in the properties of prostate chromatin promoted by 5α-dihydrotestosterone receptor complexes in vitro

(a) *Transcription of chromatin by exogenous prostate RNA polymerase.* From the results presented in Table 3 it may be seen that incubation of prostate chromatin with partially purified androgen–receptor complexes promoted an increase in transcription by exogenous prostate form B RNA polymerase. This effect was achieved with a concentration of 5α-dihydrotestosterone compatible with the physiological levels of androgens. This response was highly specific. First, it could not be simulated in spleen chromatin. Second, the stimulation of the template activity of prostate chromatin could not be evoked by human serum or cytoplasmic preparations from spleen. Serum contained some unknown factor that marginally stimulated transcription but this was not potentiated by the presence of 5α-dihydrotestosterone. In every respect, the stimulation of transcription followed precisely the extent and specificity of the transfer of 5α-dihydrotestosterone into chromatin, as described in our original development of this reconstituted, cell-free system [8].

Certain aspects of the enhancement of the template activity of prostate chromatin warrant further comment. It was imperative that the chromatin was recovered by sedimentation after incubation with the androgen receptor preparation and prior to an assessment of the transcriptional process. Contrary to the findings of other investigators [5, 6] we were unable to enhance the transcription of prostate chromatin in the presence of the partially purified receptor complex when these two prostate subcellular fractions were mixed directly in the assay medium for RNA synthesis. Under these experimental conditions, transcription was severely impaired and the potentiating effect of 5α-dihydrotestosterone was not observed. It is probable that the crude receptor preparations contained some type of nuclease activity. Specificity was also evident in the source of the exogenous RNA polymerase and despite a very high degree of enzyme parity, the enhancement of transcription could not be achieved with RNA polymerase from *Escherichia coli*. This finding is again at variance with other published reports [6].

(b) *Binding of actinomycin D.* The use of this antibiotic as a probe for subtle changes in the structure of chromatin was particularly evident from the data presented in Fig. 3. After incubation of prostate chromatin with the androgen–receptor complex, *in vitro*, it was found that there was an increase in the number of binding sites for actinomycin D. These studies were an invaluable counterpart to the studies conducted on transcription by exogenous RNA polymerase (see Table 3) and again reflected the extreme specificity in the potentiation of the activity of prostate chromatin by the 5α-dihydrotestosterone–receptor complex. In particular, the increase in the binding of actinomycin D in spleen chromatin was minimal by comparison and no change in binding properties could be detected after the incubation of chromatin with cytoplasmic preparations derived from spleen.

(c) *The possible stage of transcription enhanced by androgen–receptor complexes.* The precise nature of the various steps of the transcriptional process in eukaryotic organisms has not been unequivocally eluci-

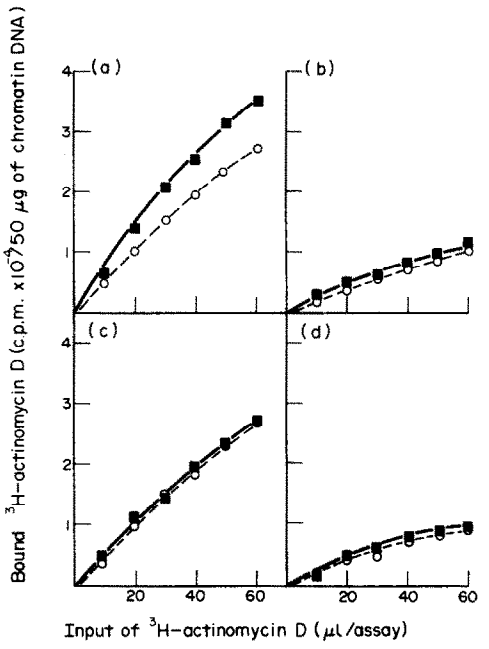

Fig. 3. A stimulation in the binding of ^{3}H-actinomycin D to chromatin by 5α-dihydrotestosterone–receptor complexes, *in vitro*.

Prostate chromatin and cytoplasmic extracts were prepared as described in the legend to Table 3. After incubation with cytoplasmic extracts, samples of chromatin were incubated with increasing amounts of ^{3}H-actinomycin D. The chromatin was collected on glass fibre discs, washed and counted for radioactivity. Fig. a, prostate chromatin and prostate cytoplasm; b, spleen chromatin and prostate cytoplasm; c, prostate chromatin and spleen cytoplasm, and d, spleen chromatin and spleen cytoplasm. In all Figs. cytoplasmic extracts were either prepared in the presence, ■, or absence, ○, of 5×10^{-8} M 5α-dihydrotestosterone. Each 10 μl of added ^{3}H-actinomycin D solution contained 50,000 c.p.m. of radioactivity.

dated, but as an acceptable working hypothesis it may be schematically represented as follows [18]

Initial stage: Enzyme + template DNA → primary complex

Enzyme binding: Primary complex $\xrightarrow{37°C}$ stable complex

Initiation: stable complex + ATP or GTP → initiation complex

Elongation: initiation complex + complementary 5′-triphosphates → RNA synthesis

Since it has been established that RNA synthesis with chromatin as template becomes increasingly less sensitive to AF/013 when the rifamycin derivative is added at increasing longer intervals of time after the beginning of the incubation [9], we pursued the possibility that this would provide a possible means for delineating the stage of transcription most influenced by the presence of the androgen–receptor complex. The series of preincubation media described in the Methods section was developed to investigate this premise. As indicated in Table 4, it seems possible that the elongation phase of transcription is particularly enhanced by the presence of the androgen receptor complex. This conclusion must be accepted tentatively until such time as the stages of transcription in eukaryotic cells have been unequivocally established.

DISCUSSION

The experimental findings are consistent with the view that the androgen receptor complexes modify the structure of chromatin in a subtle manner such transcription may proceed more extensively. This conclusion is upheld by three independent lines of evidence. After incubation of the receptor complex with chromatin: (1) more sites on the DNA template are available for exogenous RNA polymerase, (2) more of the high affinity binding sites for actinomycin D are exposed and (3) the elongation phase of transcription is particularly enhanced. It could be argued that the receptor complex either displaces chromatin-associated proteins in such a manner that more template sites on DNA are made more available or alternatively that the DNA is partially unwound and thus converted to a more active template. A further possibility is that the receptor complex in some way enables the RNA polymerase to traverse freely across

Table 4. An investigation into the stage of transcription particularly enhanced by 5α-dihydrotestosterone receptor complexes, *in vitro*

Stage of transcription	5α-Dihydrotestosterone	RNA synthesis (c.p.m. ^{3}H-CTP incorporated/50 μg of chromatin DNA)
None (controls)		80 ± 10
Enzyme binding	−	140 ± 10
	+	130 ± 20
Chain initiation	−	290 ± 10
	+	340 ± 20
Chain elongation	−	570 ± 20
	+	840 ± 10

Prostate chromatin and cytoplasmic extracts, with (+) or without (−) 5×10^{-8} M 5α-dihydrotestosterone, were prepared as described in the legend to Table III. After incubation with cytoplasmic extracts, chromatin was sedimented by centrifugation at 10,000 *g* and resuspended in media capable of supporting only restricted stages of the transcriptional process (see text for details). The inhibitor AF/013 was added, followed by prostate form B RNA polymerase and other components necessary for permitting RNA synthesis to proceed to completion. In the experimental controls, AF/013 was added from the beginning of the incubation of chromatin and the components necessary for RNA synthesis in order to assess the background incorporation of ^{3}H-CTP.

certain nucleotide sequences which in its absence act as termination regions for the growing polyribonucleotide chain. These possibilities remain in conjecture until the chromatin site occupied by the receptor complex can be identified with more precision. There is certain evidence that the receptor complex preferentially occupies regions of prostate chromatin depleted in histones [20] but with the advent of labelled 5α-dihydrotestosterone from the Radiochemical Centre, Amersham, U.K. at a remarkably high specific radioactivity it may be possible to confirm this conclusion by means of autoradiography at the level of the electron microscope. Notwithstanding this possibility, further insight into the location of the receptor complex is of outstanding importance to future developments in this area of contemporary endocrinology conducted at the molecular level.

The present data on transcriptional and structural changes in prostate chromatin are in strict accord with our earlier investigations [8] on the specific transfer of 5α-dihydrotestosterone into chromatin in cell-free systems. The acute specificity of the transfer process is reflected in the specific nature of the changes reported here. First, only prostate chromatin is activated, *in vitro*, as is consistent with the hypothesis that prostate chromatin contains unique acceptor sites for the receptor complex [8]. Second, the activation of chromatin, *in vitro*, essentially requires the presence of the androgen receptor protein and cannot be effected by receptor-free preparations of spleen or human serum. The latter is an important control (see reference 1), because SBG binds 5α-dihydrotestosterone with high affinity but is unable to fulfil other characteristic properties of receptors, notably the transfer of 5α-dihydrotestosterone into chromatin [7].

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