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THE ROLE OF THE ANDROGEN-BINDING NONHISTONE PROTEINS IN THE TRANSCRIPTION OF PROSTATIC CHROMATIN

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SUMMARY

Non-histone proteins from rat prostate chromatin have been separated into three fractions: a salt-soluble fraction, a salt-insoluble fraction (the residual proteins), and a DNA–histone complex. A single injection of radioactive testosterone into the castrated rats resulted in an initially increased, and a subsequently reduced binding of labeled androgens to the salt-soluble nonhistone proteins. Concomittantly, the residual proteins showed an inverse pattern with androgen binding increasing late in the period. Analysis of the template activities of chromatins from castrated and normal rat prostates and comparison of the DNA-RNA hybridizations of RNAs transcribed from these chromatins indicate that the residual proteins are one of the determining factors in specifying the hormone responsive transcription of these chromatins.

INTRODUCTION

The action of androgens, and the other steroid hormones, is believed to be the activation of specific genes in their target organs [see Reviews 1,2]. Testosterone, in the prostate, is initially metabolized to its hormonally active form, 5α-dihydrotestosterone, which binds to a cytosol receptor [3–7]. This androgen–receptor complex is then transferred to the nucleus [3, 5, 8–10]. The translocated androgens bind to the acceptor(s) in the prostatic chromatin [3, 5, 11], leading to specific gene activation [12]. Extraction of either the androgen bound chromatin or nuclei with 0·3–0·4 M salt solutions releases an androgen-bound nonhistone protein fraction which has been considered to be the androgen acceptor fraction [3, 5, 13–19].

The nonhistone proteins of chromatin are complex and heterogeneous [2], and contain components that activate tissue-specific transcription of DNA [20–22] and chromatin [23–26]. The nonhistone protein binding of androgens is therefore consistent with the gene activation concept hypothesized for the action of the androgen-acceptors. The enhanced transcription *in vitro* of prostatic chromatin by the 0·4 M salt-soluble acceptor fraction [27] lends further support to this contention. However, a significant percent of the androgens also binds to the salt-insoluble nonhistone proteins associated with the DNA. In the work reported here, we show that the salt-insoluble nonhistone proteins (the residual proteins) of prostatic chromatin actively bind androgens following a single injection of labeled testosterone in the rat. The androgen-binding residual proteins are further shown to be indispensable for the androgen-specific transcription of prostatic chromatin.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats weighing 350–400 g were bought from Holtzman Co., Madison, Wis. and used in all experiments. Bilateral orchidectomy of rats was performed *via* the scrotal route under ether anesthesia. The animals were killed by decapitation 72 h after castration. Testosterone was administered to rats in one mg/0·1 ml peanut oil containing 10% ethanol. The time duration after a single intraperitoneal injection of testosterone is given in legends to the figures.

Materials. Testosterone, unlabeled ribonucleoside-5′-triphosphates and spermidine phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. Spray dried *Micrococcus lysodeikticus* cells, used for preparing RNA polymerase, were purchased from Miles Labs, Elkhart, Ind. Tritium labeled ribonucleoside-5′-triphosphates and [1,2-^{3}H]-testosterone (46 Ci/mmol) were procured from Schwarz–Mann, Orangeburg, N.Y. All other chemicals used were of reagent grade. Nitrocellulose membrane filters, type B-6, were purchased from Schleicher & Scheull, Keene, N. H.

Isolation of prostatic DNA and chromatin. DNA was isolated from prostates of normal rats by the method of Marmur [28] and purified by treatment with RNase and pronase [26]. Cell nuclei were isolated

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from prostates of either normal or castrated rats by the procedure of Blobel and Potter [29], from which the chromatin was isolated according to Seligy and Miyagi [30].

Fractionation and determination of prostatic chromatin proteins. The chromatin proteins were fractionated into three major fractions based on their extractability or solubility in salt solutions. The purified prostatic chromatin was initially extracted with 2 M NaCl containing 0·05 M Tris–HCl, pH 8·0, for 2 h. The 2 M NaCl extract was collected by centrifugation. This extraction was repeated twice and all three extracts were combined. The pooled 2 M NaCl extract was then diluted with 13 vol. of 0·02 M Tris–HCl, pH 8·0, to reduce the concentration of NaCl to 0·14 M. This step precipitated the DNA-histones [31] together with some nonhistone proteins [32], leaving most of the nonhistone proteins in solution which are referred to as the salt-soluble NHP. The chromatin residue from the 2 M NaCl extraction is referred to as the residual proteins [33].

For the determination of chromatin proteins, histones were extracted by 0·4 N H_2SO_4 in the cold for 20 min. A total of three extractions were made, and the pooled acid-extract is assumed to represent the total histone. The acid-insoluble residue was dissolved in 0·1 N NaOH and was determined as the total nonhistone protein. Protein content was determined by the procedure of Lowry *et al.* [34], using bovine serum albumin and calf thymus histone as standards, respectively, for nonhistone proteins and histones. DNA and RNA were determined on separate chromatin samples by the methods of Burton [35] and Lusena [36], respectively.

Reconstitution of chromatin. Chromatin was reconstituted from the DNA-histones, the salt-soluble NHP and the residual proteins following the procedure of Bekhor *et al.* [37]. The three chromatin fractions were dialyzed overnight against 2 M NaCl-5 M urea containing 0·01 M Tris–HCl, pH 7·5, and combined. This mixture was then sequentially dialyzed against the following NaCl solutions containing 5 M urea and 0·01 M Tris–HCl, pH 7·5: 2 M NaCl for 1 h; 1 M NaCl for 2 h; 0·8 M NaCl for 2 h; 0·6 M NaCl for 2 h and 0·4 M NaCl overnight. The mixture was finally dialyzed against 0·01 M NaCl in 0·01 M Tris–HCl, pH 7·5. The reconstituted chromatin was pelleted at 78,000 *g* for 30 min and washed three times with 0·01 M Tris–HCl, pH 7·5, before use.

Assay for template activity of chromatin. Previous studies of the transcription of rat prostatic chromatin using rat liver polymerase B has shown that the template activity of chromatin isolated from prostates of castrated rats is greater than that of normal rats [12]. When the experiment was subsequently repeated in *M. lysodeikticus* RNA polymerase reaction, similar result showing a higher template activity of chromatin from castrated than that from normal rat prostate was also observed. In the present study, therefore, *M. lysodeikticus* RNA polymerase was employed for determining the template activity of prostatic chromatin and of reconstituted chromatin.

The standard assay system of Nakamoto *et al.* [38] was used to assay the capacity of chromatin in RNA synthesis *in vitro*. The reaction mixture, in 0·5 ml, contained the following: 50 μmol of Tris–HCl, pH 7·5, 0·80 μmol of spermidine phosphate, 12·5 μmol of $MnCl_2$, 0·40 μmol each of ATP, CTP, GTP and [^{3}H]-UTP, 2 units of *M. lysodeikticus* RNA polymerase, and varying amounts of chromatin as indicated in the figures. The reaction mixture was incubated at 30° for 10 min. At the end of incubation, the reaction was terminated by chilling in ice-water, followed in succession by 0·1 ml of 50% and 2 ml of 5% trichloroacetic acid with mixing. The acid-insoluble precipitate was collected on Millipore filter (HA 0·45 μm) and washed six times with 5 ml of cold 5% trichloroacetic acid. Ten ml of scintillation fluid (333 ml of Triton X-100, 667 ml of toluene, 5·5 g of 2,5-diphenyloxazole and 0·1 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene) were added to the sample in a vial and the radioactivity was counted in a Packard liquid scintillation spectrometer.

Hybridization of RNA transcribed from chromatin in vitro. To synthesize RNA *in vitro* for hybridization, the standard RNA polymerase reaction mixture as described above was increased 40-fold. The reaction mixture, in a final volume of 10 ml, contained 1–1·5 mg DNA-equivalent chromatin, all four tritium-labeled ribonucleoside triphosphates, 1000 units of RNA polymerase and other constituents as indicated in the standard assay. The reaction mixture was incubated at 30° for 30 min. An additional 500 units of RNA polymerase were then added to the reaction mixture, and the reaction was continued for 30 min. At the end of the incubation, sodium dodecylsulfate and NaCl were added to final concentrations of 0·5% and 0·14 M, respectively. The *in vitro* synthesized [^{3}H]-RNA was extracted with redistilled phenol and precipitated by 2 vol. of ethanol. Purification of the [^{3}H]-RNA by treatments with DNase and pronase has been described elsewhere [26].

For hybridization experiments, one μg of prostatic DNA was denatured in alkali and immobilized on 25 mm nitrocellulose membrane filters according to Gillespie and Spiegelman [39]. Annealing of [^{3}H]-RNA transcribed *in vitro* on DNA was performed essentially by the procedure of Tan and Miyagi [40]. Varying amounts of the *in vitro* synthesized [^{3}H]-RNA in 1·0 ml of 30% formamide, 0·30 M NaCl and 0·030 M sodium citrate were incubated at 37° for 24 h in screw cap vials. Each vial contained two DNA filters and two blank filters. At the end of the incubation period, the filters were washed with 50 ml of 0·30 M NaCl–0·030 M sodium citrate, and incubated with pancreatic RNase (100 μg/5 ml saline–citrate) for 1 h at room temperature. The incubated filters were washed with 75 ml of the same saline citrate per each side, dried, and counted in a liquid scintillation counter.

For double-saturation hybridization of the *in vitro* synthesized [^{3}H]-RNA, two blank filters and the DNA filters annealed with saturating amount of [^{3}H]-RNA synthesized from one chromatin template processed as above were washed with saline–citrate, blotted on a filter paper, and placed in a vial containing [^{3}H]-RNA transcribed from a different chromatin, 30% formamide, 0·30 M NaCl and 0·030 M sodium citrate. The filters were further incubated at 37° for another 24 h. At the end of this incubation, the filters were washed, treated with RNase and counted as described above.

Binding of [^{3}H]-androgen to chromatin proteins in vitro. The *in vitro* binding of [^{3}H]-androgen to chromatin proteins was carried out by incubation of prostate nuclei with [^{3}H]-androgen-cytosol. To prepare the prostate cytosol, prostates from 10 castrated rats were minced and homogenized in 0·25 M sucrose containing 0·05 M Tris–HCl, pH 7·4, 0·025 M KCl and 0·005 M $MgCl_2$. The homogenate, after filtering through glass wool, was centrifuged at 105,000 ***g*** for 2 h to yield the cytosol. The cytosol, in 10 ml of the sucrose-buffer medium, was incubated with [^{3}H]-testosterone (0·5 mCi) at 37° for 20 min. After the incubation, the cytosol was chilled in ice, and dialyzed against 0·02 M Tris–HCl, pH 7·5, with three changes of the buffer. The dialyzed [^{3}H]-androgen-cytosol was concentrated by lyophilization and dialyzed against 0·32 M sucrose in 0·02 M Tris–HCl, pH 7·5, with two changes of the sucrose-Tris buffer. The dialyzed solution was centrifuged at 10,000 ***g*** for 10 min to remove some insoluble material and the clear supernatant was used as the [^{3}H]-androgen-cytosol receptor source.

Nuclei were freshly prepared from rat prostates as described previously and incubated with the [^{3}H]-androgen-cytosol in the sucrose–Tris buffer at 37° for 20 min. The mixture was chilled in ice and the nuclei were pelleted at 1000 ***g***. The nuclei were washed with 0·5% Triton X-100 in the sucrose–Tris, followed by two washings with the sucrose–Tris buffer. The [^{3}H]-androgen-chromatin was isolated from the washed nuclei as described previously.

RESULTS

Binding of [^{3}H]-androgen in vivo *to chromatin proteins following injection of [^{3}H]-testosterone.* The relative compositions of prostatic chromatins isolated from castrated and normal rats are shown in Table 1. The histone content of chromatin from the castrated rats was reduced by approximately 18% as compared to that of the normal prostate; while the acid-insoluble nonhistone proteins did not show any apparent difference between normal and castrated animals. The residual proteins contained significant amount of acid-soluble proteins, assumed to be histones (Table 1b). The nonhistone protein content of the residual proteins from normal rat prostatic chromatin appears to be higher than that from castrated chromatin; whereas the nonhistone protein content in the salt-soluble chromosomal proteins shows the reverse pattern. These results may explain the apparent similarity between non-histone protein content in the chromatins of normal and castrated rats.

To determine the extent of androgens binding to chromatin fractions, the chromatin proteins were fractionated into the salt-soluble NHP, DNA-histones, and the residual proteins as described in Methods. Table 2 shows a time course of the relative distribution of [^{3}H]-androgens bound *in vivo* to prostate chromatin proteins after a single injection of [^{3}H]-testosterone into rat. It can be seen that the binding of [^{3}H]-androgens to the salt-soluble NHP was immediate after the hormone administration and was also the highest among the three chromatin fractions. The salt-soluble NHP, prepared by extraction of the prostate chromatin with 2·0 M NaCl, would include the 0·4 M KCl-soluble chromatin proteins, and hence, contains the androgen acceptor fraction which has been described by other investigators [17–19]. The initial active binding of [^{3}H]-androgens by the salt-soluble NHP *in vivo* is therefore consistent with the evidence of nuclear retention of [^{3}H]-androgens through binding to the chromatin acceptors [8–13, 17–19]. The binding of radioactive androgens to the salt-soluble NHP decreased steadily with time.

Table 1. Compositions of chromatins isolated from normal and castrated rat prostates

(a)

	DNA	Protein: Histones	Protein: Non-histone proteins	RNA
Normal [9]	1·00	1·37 ± 0·06	0·75 ± 0·04	0·081
Castrate [3]	1·00	1·13 ± 0·05	0·78 ± 0·01	0·069

(b)

	DNA	Chromosomal proteins: Acid-soluble	Chromosomal proteins: Acid-insoluble
Salt-soluble proteins			
Normal [4]	1·00	1·10 ± 0·03	0·32 ± 0·01
Castrate [3]	1·00	0·83 ± 0·04	0·44 ± 0·02
Residual proteins			
Normal [4]	1·00	0·26 ± 0·02	0·43 ± 0·01
Castrate [3]	1·00	0·30 ± 0·06	0·32 ± 0·02

Values in parenthesis represent number of determinations. The deviations represent: Mean ± S.D.

Table 2. Distribution of tritium among chromatin proteins after a single injection of [^{3}H]-testosterone into rats

	Salt-soluble NHP		Residual proteins		"DNA-histone"	
	d.p.m./mg	%	d.p.m./mg	%	d.p.m./mg	%
20 min	93·6 ± 1·2*	40·0	70·1 ± 4·3	30·0	70·3 ± 3·6	30·0
1 h	68·0 ± 1·4	34·0	66·8 ± 1·2	33·4	65·2 ± 2·4	32·6
2 h	53·7 ± 2·3	30·0	60·1 ± 1·3	33·6	65·2 ± 2·2	36·4
8 h	52·4 ± 1·3	28·5	63·4 ± 3·5	34·4	68·3 ± 1·1	37·1
12 h	43·4 ± 0·8	22·2	86·8 ± 4·8	44·3	65·6 ± 1·5	33·5

* The values are averages of three experiments, mean ± S.D.

On the other hand, the binding of androgens to the residual proteins, after decreasing during the first 2 h and maintained at a steady level until 8 h after injection of the hormone, increased 22% above its initial value. The relationship of androgen-binding by these two chromatin protein fractions is better illustrated by plotting their percentage distribution of bound androgens vs time, as shown in Fig. 1. Since the binding of androgens to the DNA-histones remained relatively constant throughout the time period, the results suggest that after translocation of the androgen–receptor complex to the nucleus, there is a sequential transfer of the bound [^{3}H]-androgens from the salt-soluble NHP to the residual proteins in the chromatin.

Binding of [^{3}H]-androgens to chromatin proteins in vitro. To determine whether the binding *in vivo* of [^{3}H]-androgens by the residual proteins can be demonstrated *in vitro*, the [^{3}H]-androgen–cytosol receptor complex was incubated with the prostate nuclei of castrated rats and the chromatin was isolated from the incubated nuclei as described in Methods. The chromatin, with its bound [^{3}H]-androgens, was extracted with 0·4 M NaCl and then with 2·0 M NaCl. The 2·0 M NaCl extract was diluted with 13 vol. of 0·02 M Tris–HCl, pH 7·5, to precipitate the DNA-histones, similar to the fractionation procedure described above. Altogether, four chromatin protein fractions were obtained. The radioactivity of each fraction was determined. As shown in Table 3, the 0·4 M NaCl- and 2·0 M NaCl-soluble NHP, which together make up the salt-soluble NHP in Table 1 and Fig. 1, account for 22·4% of the total chromatin-bound [^{3}H]-androgens. Most of these bound [^{3}H]-androgens were in the 0·4 M NaCl-soluble NHP, the acceptor fraction. The largest amount of the chromatin bound [^{3}H]-androgens (45·8%) was in the residual protein fraction. These values of the distribution of the binding of [^{3}H]-androgen–cytosol receptor complex to chromatin *in vitro* are comparable to those obtained in the *in vivo* experiment. The data support the results from the previous *in vivo* experiments which suggest that the interaction of [^{3}H]-androgen–cytosol receptor complex with chromatin may involve two hormonally responsive acceptor fractions, the 0·4 M salt-soluble NHP and the residual proteins.

The template activity of prostatic chromatin lacking the residual proteins. The active binding of androgens by the residual proteins, and the parallel temporal increase in both the residual protein-bound androgens and the synthesis of uracil-rich nuclear RNA [12] suggest that the residual proteins may play

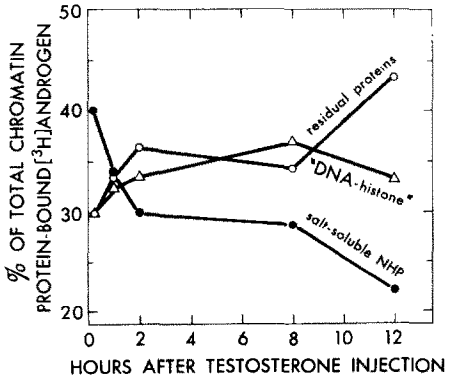

Fig. 1. Time course of the binding of tritium-labeled androgen to prostate chromatin protein fractions after injection of [^{3}H]-1,2-testosterone to castrated rats. One mg of the labeled testosterone was injected intraperitoneally into each rat at the indicated time intervals before sacrifice. Chromatin was prepared from the nuclei of the excised prostates and fractionated into salt-soluble NHP, DNA-histones and the residual proteins as described under Methods.

Table 3. Binding of [^{3}H]-androgen *in vitro* by chromosomal proteins isolated from rat prostate chromatin

Chromatin protein fraction	[^{3}H]-androgen bound (d.p.m.)	% bound androgen
0·4 M NaCl NHP	12,192	14·4
2·0 M NaCl NHP	6775	8·0
DNA-histones	26,925	31·8
Residual proteins	38,778	45·8

Nuclei containing 1·8 mg DNA isolated from castrated rat prostates were incubated with [^{3}H]-androgen-cytosol (12 mg protein) in 0·32 M sucrose and 0·02 M Tris–HCl, pH 7·5, at 37° for 20 min. After incubation, the nuclei were re-isolated and washed with the sucrose–Tris three times, from which the chromatin was prepared. The chromatin proteins were fractionated and the radioactivities of the bound androgens in each fraction determined. Experimental conditions were as described under Methods. Total radioactivity input of [^{3}H]-androgen–cytosol was 246,930 c.p.m. and the radioactivity recovered in the chromatin was 84,670 c.p.m., representing 34·3% of the input androgens bounds to chromatin.

a role in the androgen responsive gene activity in the prostatic chromatin. To ascertain this role of the residual proteins, chromatin was reconstituted from the salt-soluble NHP and DNA-histones, with or without the residual proteins, of the prostates of normal and castrated rats. The reconstituted chromatins were then examined for their template activities in RNA synthesis *in vitro*. As shown in Fig. 2a, the template activity of chromatin reconstituted from the chromatin constituents of castrated rat prostates was greater than that reconstituted from chromatin fractions of normal rat prostates. Both template activities were similar to those observed with their corresponding native chromatins [12]. However, when chromatins were reconstituted in the absence of the residual proteins (i.e., chromatin reconstituted with only the salt-soluble NHP and DNA-histones) the template activity of the reconstituted chromatin from castrate fractions became less than that reconstituted from normal prostate fractions (Fig. 2b). This result indicates that the characteristic higher template activity of castrated rat chromatin as compared with chromatin of normal rat is dependent upon the presence of the residual proteins. The data suggest that the residual proteins are a determining factor in the correct transcriptional expression of prostatic chromatin.

The residual proteins and specific transcription of prostatic chromatin. To further ascertain that the residual proteins are indispensable constituents for the accurate transcription of chromatin, the prostate chromatin proteins of castrated and normal rats were reconstituted with and without the residual proteins,

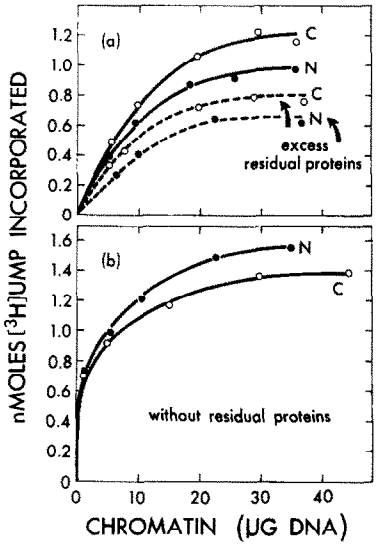

Fig. 2. Comparison of the template activities of castrated and normal chromatins reconstituted from chromatin proteins of prostates (a) with and (b) without the residual proteins. In the case where excess residual proteins were used for chromatin reconstitution, the residual proteins isolated from two extra rats were added to the normal complement of the residual proteins before the gradient dialysis. Isolation of chromatin proteins, reconstitution of chromatin, and assay for the template activity of chromatin are described in Methods.

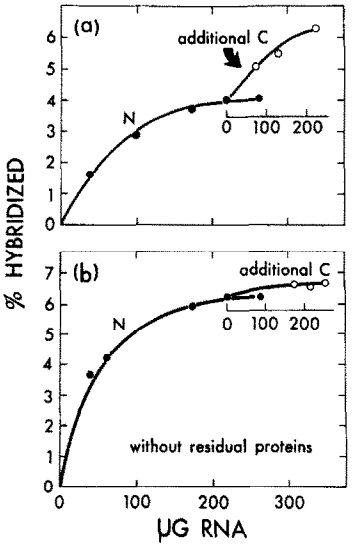

Fig. 3. Double-saturation hybridization of DNA with [^{3}H]-RNAs synthesized from chromatins reconstituted from castrate and normal prostate chromatin proteins (a) with and (b) without the residual proteins. In the upper figure (a), chromatin was reconstituted from the salt-soluble NHP, DNA-histones and the residual proteins isolated from normal rat prostate. RNA transcribed from this reconstituted normal prostate chromatin (N) was hybridized to prostate DNA to saturation. The saturated DNA was further hybridized with [^{3}H]-RNA transcribed from chromatin reconstituted from castrate chromatin proteins (C). In the bottom figure (b), similar double-saturation hybridization experiments were carried out using [^{3}H]-RNA transcribed from normal chromatin which was reconstituted in the absence of the residual proteins (N), and [^{3}H]-RNA transcribed from chromatin reconstituted from castrated chromatin without residual proteins (C). Reconstitution of chromatin, synthesis of RNA using the reconstituted chromatin as template, and DNA-RNA hybridization are described in Methods.

and their transcripts were studied by double-saturation DNA-RNA hybridization. The results are shown in Fig. 3. As indicated in Fig. 3a, when the [^{3}H]-RNA transcribed from the chromatin which was reconstituted from a complete supplement of chromatin proteins isolated from normal rat prostate was hybridized to prostatic DNA, there was 4% DNA-RNA saturation hybridization. Further annealing of this RNA-saturated DNA with [^{3}H]-RNA synthesized from chromatin reconstituted from chromosomal proteins of castrated rat prostate yielded an additional 2·3% DNA-RNA hybrid, resulting in a total of 6·3% DNA-RNA hybrid formation. This value is the same as that obtained using the transcripts of native chromatins from the prostates of castrated and normal rats (data not shown). It is also consistent with a value of 6·0% DNA-RNA hybrid formation obtained by annealing normal chromatin transcripts with DNA saturated with castrated chromatin transcripts [12].

If, as suggested by the previous data, the residual proteins play a specific role in the correct transcription of prostatic chromatin, then reconstituted

chromatin without residual proteins should transcribe unrestrictedly, deviating from the DNA-RNA hybridization pattern as illustrated in Fig. 3a. As shown in Fig. 3b, when [^{3}H]-RNA transcribed from chromatin reconstituted from normal rat chromosomal proteins but without the residual proteins was hybridized to prostatic DNA, there was a 6·2% DNA-RNA hybrid formation. This is equivalent to the combined hybrids formed between prostatic DNA and the RNAs synthesized from normal and castrated rat chromatins. Further annealing of this RNA-saturated DNA with [^{3}H]-RNA transcribed from chromatin reconstituted from complete supplements of castrated chromosomal proteins yielded insignificant additional DNA-RNA hybrid (0·4%). The results thus support the interpretation that prostatic chromatin does not transcribe correctly without the residual proteins as part of the chromatin constituents. Furthermore, since reconstituted chromatin without the residual proteins transcribes more RNA species, the residual proteins appear to restrict transcription of prostatic chromatin. Indeed, if chromatins were reconstituted in the presence of excess residual proteins, the template activities of these reconstituted chromatins showed a decreased capacity for RNA synthesis *in vitro* as compared to chromatins reconstituted from normal complements of chromosomal proteins (Fig. 2a). These data, taken together, show that characteristic transcription of prostatic chromatin from normal rat and castrate are contingent upon the presence of the residual proteins.

DISCUSSION

In the present study, the residual proteins of prostatic chromatin are shown to actively bind [^{3}H]-androgens in a temporal sequence after a single injection of labeled testosterone into the castrated rats. Since the residual proteins are not released by 2 M NaCl, they do not contain the nuclear or chromatin acceptor molecules obtainable with 0·3–0·4 KCl or NaCl. The seemingly quantitative precursor-product relationship of androgen binding between the salt-soluble NHP and the residual proteins after testosterone administration suggests a possible multiple-step mechanism of chromatin acceptor reaction in androgenic action.

The residual proteins also contain some histones. Since the binding of androgens by the DNA-histone complex, which perhaps was partly due to the presence of nonhistone proteins, remained practically constant throughout the experimental period, it is unlikely that the histones in the residual proteins contributed to the elevated androgen-binding activity of the residual proteins. The histones in the residual proteins, however, were probably one of the causes for the lowered template activity of the chromatins reconstituted with excess residual proteins. As histones do not determine the specific transcription of chromatin, the characteristic transcription of castrated and normal prostatic chromatins, as shown by the hybridization results here, is specified, at least in part, by the residual proteins.

The above interpretation does not, however, imply that the residual proteins are the only nonhistone proteins involved in specific transcription of chromatin. In a previous study of regenerating rat liver, we have shown that the salt-soluble NHP are also indispensable chromosomal proteins for the selective transcription of chromatin [41]. Thus, it can be stated that both the salt-soluble NHP and the residual proteins contain specific nonhistone proteins that determine characteristic transcription of chromatin, and both may act as androgen acceptors in the rat prostatic chromatin.

Castration evidently causes a profound change in the state of prostatic chromatin. Chung and Coffey [42] have reported a reduction in histone F1 in the ventral prostate of castrated rats as compared with the normal rats. This reduction in histone F1 in castrated rat chromatin could result in the unmasking of DNA sequences, making them available for transcription and perhaps accounting, in part, for the higher template activity of castrated rat chromatin as compared to that of the chromatin from normal rat prostates.

As shown by the present data, the RNA synthesized from castrated rat chromatin contains RNA species transcribed from DNA sequences that are different from that transcribed from the normal prostatic chromatin. While DNA-RNA hybridization technique detects only highly repetitive DNA sequences, and also, bacterial RNA polymerase, which was used for synthesizing sufficient amount of *in vitro* RNA, does not transcribe specific DNA regions [42, 43], the technique does provide a basis for comparing transcription of two chromatins. In this respect, the additional transcription indicates gene activation. On the other hand, normal prostatic chromatin also contains transcribable DNA sequences that are not available in castrated chromatin. Hence, the effect of castration is both gene activation and gene repression in the prostatic chromatin. This and the correlation of the temporal binding of androgens to the salt-soluble NHP and then later to the residual proteins with the activation of different nuclear RNAs [12] and the early increase and later decrease in template activity of testosterone-treated prostatic chromatin, indicate differential gene regulation in the rat prostate induced by the androgens.

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