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Rat Ventral Prostate Chromatin. Effect of Androgens on Its Chemical Composition, Physical Properties, and Template Activity†

R. M. Couch‡ and K. M. Anderson*

ABSTRACT: The effect of castration and hormone replacement on the chemical composition, physical properties, and template activity for *Escherichia coli* RNA polymerase of rat ventral prostate chromatin was examined. Chromatin isolated by two methods, one with and the other without intentional shearing, from normal rats (N), 3-day castrates (C), and castrates treated with testosterone propionate for either 4 (CT_4) or 72 (CT_{72}) hr, was compared. Chromatin solubilized by mechanical shearing contained four major fractions, but when intentional shearing was avoided, it included two major components, as judged by analytical ultracentrifugation and sucrose gradient centrifugation. Hormone-dependent increases in protein and RNA content occurred within the first 4 hr of androgen administration. Paradoxically the template activity of prostate chromatin from normal rats or castrated rats treated for 3 days with testosterone propionate was less than that from castrates. However, chromatin from 3-day castrates treated for 4 hr with androgen was 80% more active than C chromatin. These results did not depend upon demonstrable differences in the activities of nucleases, histone protease, or ribonucleoside triphosphatase. The marked increase in nonhistone protein content and stimulation of template activity following brief exposure to androgens may reflect hormone-induced changes in the structure of prostate chromatin.

Many cellular constituents and enzymatic activities of male accessory reproductive glands are altered by changes in the level of circulating androgens. In the prostate, castration leads to decreased protein and RNA synthesis, and subsequent involution of cytoplasmic structures (Price and Williams-Ashman, 1961). Hormone-dependent changes in the macromolecular composition of prostate cytoplasm and nuclei (Liao, 1965; Anderson *et al.*, 1970; Chung and Coffey, 1971) and the effect of androgens on prostatic RNA synthesis, both *in vivo* (Fujii and Vilee, 1968, 1969) and *in vitro* (Liao *et al.*, 1965; Liao and Fang, 1969), have been described.

Two hours after a subcutaneous injection of testosterone propionate to rats castrated for 70 hr, RNA synthesis in isolated prostate nuclei, incubated under conditions of low ionic strength, increased 40%. The formation of ribosomal RNA or its precursors was particularly stimulated (Liao *et al.*, 1966).

When the template activity of rat ventral prostate chromatin from pressure-disrupted nuclei was assayed with excess *Micrococcus lysodeikticus* RNA polymerase, no consistent difference was observed between chromatin isolated from 3-day castrates and that from castrates treated with testosterone propionate for 3 days (Liao and Lin, 1967). Compared to the endogenous RNA polymerase activity of intact nuclei, chromatin template activity was about ten times greater, nearest neighbor frequency analysis of the newly synthesized RNA differed markedly, and binding of actinomycin D was increased. These results suggested that the bacterial enzyme utilized template sites that were not transcribed by the endogenous RNA polymerase of isolated nuclei.

Mangan and coworkers compared the template activity of isolated prostate nuclei and prostate chromatin (Mangan *et al.*, 1968). The template activity for *Escherichia coli* polymerase of prostate nuclei from testosterone-treated rats exceeded the activity of nuclei from castrates, but chromatin template activity exhibited no consistent differences. Mainwaring *et al.* (1971) observed that 4 hr after administration of androgens, prostate chromatin, assayed under conditions of low ionic strength, exhibited an increase in template activity of about 10%.

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The reasons for the marked increase in total incorporation and loss in fidelity of transcription when chromatin serves as a template for bacterial RNA polymerases are not established. There is uncertainty concerning the significance of qualitative or quantitative hormone-induced changes in the template activity of mammalian chromatin determined by bacterial RNA polymerases. To examine these problems, we compared the effects of androgens on the chemical composition, some physical properties, and the template activities of rat ventral prostate chromatins prepared by two different methods, the first employing intentional shearing, the second avoiding it.

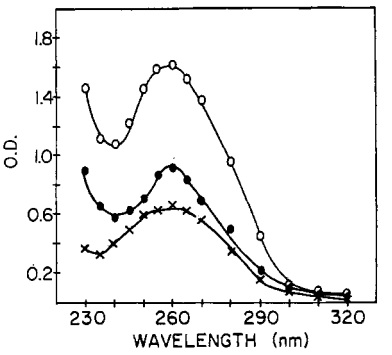

FIGURE 1: Ultraviolet absorption spectra of minimally sheared and sheared chromatin and its deproteinized DNA from rat ventral prostate. Unsheared chromatin, in 0.4 mM Tris, pH 8.0, and sheared chromatin or DNA in 0.01 M Tris, pH 8.0, were scanned in a Gilford Model 2400 spectrophotometer: (O) minimally sheared chromatin; (●) sheared chromatin; (×) deproteinized sheared chromatin.

Experimental Section

Materials. Chemicals were reagent grade from several commercial sources and biochemicals and enzymes were purchased from Sigma, Calbiochem, or Worthington, and radioisotopes from New England Nuclear. Reagents for polyacrylamide gel electrophoresis were obtained from Eastman Kodak. *E. coli* B RNA polymerase was purchased from Miles Laboratories and actinomycin D was a gift from Merck and Co. MN polygram plates were purchased from Mackerey Nagel & Co.

Male Wistar rats, 3–5 months old, were maintained on Purina laboratory chow and tap water *ad libitum*. Castration was performed *via* the scrotal route, using ether anaesthesia. Hormone-treated castrates were injected subcutaneously with 8 mg of testosterone propionate in 1 ml of olive oil either daily for 3 days (CT_{72}) or 4 hr before sacrifice (CT_4). Normals (N) and untreated castrates (C) received an equal amount of the vehicle.

Preparation of Chromatin. Animals were killed by cervical dislocation and Triton X-100-washed prostate nuclei were isolated as described (Anderson *et al.*, 1970). Two methods of preparing chromatin were used. (All operations were performed at 4°.)

METHOD A (Bonner *et al.*, 1968). Purified nuclei were washed with a solution of 0.15 M NaCl, suspended in 0.01 M Tris solution, pH 8.0, and dialyzed overnight against this buffer. Nuclei were then "sheared" for 2.5 min in a Sorvall Omnimixer at a setting of 5, stirred for 30 min in the cold, and centrifuged at 10,000*g* for 30 min. Material remaining in the supernatant was termed "soluble" chromatin.

METHOD B (Shaw and Huang, 1970). Nuclei, resuspended in 0.075 M NaCl–0.024 M EDTA solution, pH 8.0, were centrifuged at 7700*g* for 15 min. This was repeated three times. The lysed residue was washed twice in 0.05 M Tris solution, twice each in solutions of 0.01 M Tris, 0.002 M Tris, and finally 0.0004 M Tris, all at pH 8.0. Chromatin was gently stirred overnight in 0.0004 M Tris solution and "solubilized" with five strokes in a hand homogenizer. This material was termed "minimally sheared"chromatin.

Isolation of E. coli RNA Polymerase and Chromatin Template Assay. E. coli K-12-leucine and *E. coli* K-12 were used for the isolation of DNA-dependent RNA polymerase (RNA nucleotidyl-transferase, EC 2.7.7.6). RNA polymerase was isolated from 50 g of *E. coli* K-12 or *E. coli* K-12-Leu by the method of Burgess (1969). The enzyme corresponded to fraction 4 and had a A_{280}/A_{260} of greater than 1.6. Chromatin template activity for RNA synthesis was assayed essentially as described by Bonner *et al.* (1968) with 1.0 μCi of either [^{3}H]-GTP (5.66 Ci/mmol) or [^{3}H]UTP (21 Ci/mmol) as the labeled substrate. Acid-insoluble material was collected on Millipore filters (HA 0.45 μ) and washed with 5% trichloroacetic acid. The filters were dried and radioactivity determined.

Sucrose Density Gradient Centrifugation. "Sheared" chromatin (method A) was layered over a linear 5–30% sucrose gradient containing 0.01 M Tris, pH 8.0. Gradients were centrifuged for 2 hr at 240,000*g* in an SW 50.1 rotor, ten-drop fractions were collected from the bottom of the tubes, and the A_{260} was measured.

"Minimally sheared" chromatin (method B) was layered over a linear 0.25–2.8 M sucrose gradient containing 0.0004 M Tris, pH 8.0, and centrifuged in an SW 50.1 rotor for 30 min at 64,000*g*. The tubes were punctured from the bottom; 20-drop fractions were collected and read at 260 nm.

Other Procedures. Ribonuclease or deoxyribonuclease activity was assayed by incubating ^{14}C-labeled rat liver RNA (50,000 cpm/mg) or DNA (8000 cpm/mg) with chromatin in the reaction mixture (without the labeled ribonucleoside triphosphate and RNA polymerase) for 30 min at 37° and determining the acid-soluble radioactivity. Ribonucleoside triphosphatase activity was measured at 37° in the reaction mixture used to assay template activity, without RNA polymerase. The amounts of GTP, GDP, and GMP were determined by thin layer chromatography on MN polygram plates.

DNA was measured by the method of Burton (1956) as modified by Shatkin (1969) with calf thymus DNA as standard, and RNA was determined by ultraviolet absorption of the acid-soluble material remaining after the incubation of chromatin in 0.3 N NaOH for 2 hr at 37° (1.0 A_{260} = 32 μg/ml). Protein was measured by the method of Lowry *et al.* (1951), as modified by Shatkin (1969), using bovine serum albumin as the standard. Histones were extracted from chromatin by stirring aliquots for 30 min in cold 0.4 N H_2SO_4. The pellet remaining after the extraction of histones was dissolved in 0.3 N NaOH, and the nonhistone protein determined.

Electrophoresis of acid-extracted histones from ventral prostate chromatin on 15% polyacrylamide gels, containing 2.5 M urea, was carried out according to the method of Panyim and Chalkley (1969).

Millipore filters, containing washed radioactive RNA and sections of thin layer chromatograms of ribonucleoside triphosphatase assays, were placed in glass scintillation vials with 10 ml of the solution described by Buhler (1962). Samples from ribonuclease and deoxyribonuclease determinations were counted in a Triton-toluene scintillation mixture (Patterson and Green, 1965). Radioactivity was measured in a

TABLE I: Composition of Soluble and Pellet Fractions of Sheared Chromatin and of *Minimally Sheared* N, C, CT_4, and CT_{72} Chromatins.

	RNA/DNA	Total Protein/DNA	Histone/DNA	Nonhistone/DNA
		Sheared Chromatin		
Fraction				
Soluble	0.048 (5)[a]	2.5 ± 0.16 (6)	1.50 (3)	0.87 (3)
Pellet	0.054 (3)	2.17 (3)		
		Minimally Sheared Chromatin		
Chromatin				
N	0.090 (6)	1.79 ± 0.10 (7)	1.09 ± 0.08 (7)	0.70 ± 0.03 (7)
C	0.055 (3)	1.50 ± 0.06 (5)	0.98 ± 0.05 (5)	0.52 ± 0.04 (5)
CT_{72}	0.081 (3)	1.89 ± 0.11 (5)	1.06 ± 0.06 (5)	0.83 ± 0.12 (5)
CT_4	0.063	1.53	1.00	0.53
	0.060	1.65	0.98	0.67
		1.72	1.05	0.67
Av	0.062 (2)	1.63 (3)	1.01 (3)	0.62 (3)

[a] Numbers in parentheses are number of experiments.

Packard TriCarb Model 3380 liquid scintillation spectrometer.

Results

Composition of Prostate Chromatins. Prostate chromatin, prepared by either procedure and scanned from 230 to 320 nm, exhibited similar absorption profiles (Figure 1). The low absorption at 320 nm (320/260 = 0.04) is considered to be characteristic of highly purified chromatin that is neither contaminated with cytoplasmic proteins nor aggregated. Incubation with RNase and Pronase reduced the strong absorption at 230 nm, in large part due to chromosomal proteins.

In "sheared" preparations (method A), 30% of the DNA remained "solubilized" after centrifugation at 10,000*g* for 30 min (Table I). DNA accounted for 27.2%, RNA for 1.4%, and protein for 71.4% of the total mass. Histones comprised 58% of the total protein. Except for a 10% reduction in protein, similar values were found for the material remaining in the pellet.

"Minimally sheared" chromatin, prepared by a procedure in which intentional shearing was minimized (method B), differed in composition. DNA comprised 34.8%, RNA 3.3%, and protein 61.9% of the chromatin mass. Histones represented 61% of the total chromosomal protein and the histone to DNA ratio was close to 1. Three days of castration reduced the RNA content 39%, while treatment with testosterone propionate maintained it at 90% of normal. Following castration, the nonhistone protein content fell to 74% of normal but after 3 days of hormone replacement, it increased 19%, compared with N. Histone contents of these chromatins were similar. Within 4 hr of administering testosterone propionate, the chemical composition of chromatin from 3-day castrates returned toward normal. Nonhistone protein and RNA contents were 27 and 22% greater, respectively, in CT_4 than C chromatin. This is similar to the rapid increases in protein and RNA of uterine chromatin after brief exposure of castrated rats to 17β-estradiol (Teng and Hamilton, 1969).

The distribution of histones after polyacrylamide gel electrophoresis has been used as a criterion of the purity and extent of degradation of chromatin preparations (Spelsberg

TABLE II: Effect of Androgen on the Histone Composition of Rat Ventral Prostate Chromatin.

Hormonal Status	Histone Fraction (% of Total Histones)[a]			
	F_1	F_1'[b]	F_3, F_{2b}, F_{2a2}	F_{2a1}
N	9.3	4.3	58.6	26.4
C	9.5	5.3	58.5	25.5
CT_{72}	12.5	6.4	50.6	28.7

[a] % of total histones was determined from the area under the absorption peaks of scanned polyacrylamide gels; average of three determinations. [b] F_1', the protein situated between histones F_1 and F_3 (see Figure 2).

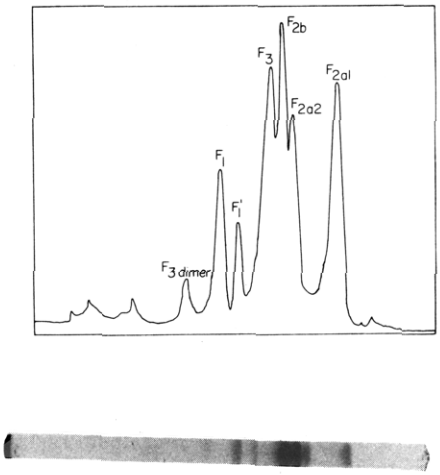

FIGURE 2: Polyacrylamide gel electrophoresis pattern of histones extracted from minimally sheared rat ventral prostate chromatin. Histone (24 μg), extracted in 0.4 N H_2SO_4 from prostate chromatin isolated from normal rats, was electrophoresed, and the absorption profile obtained by scanning the destained gel at 600 nm.

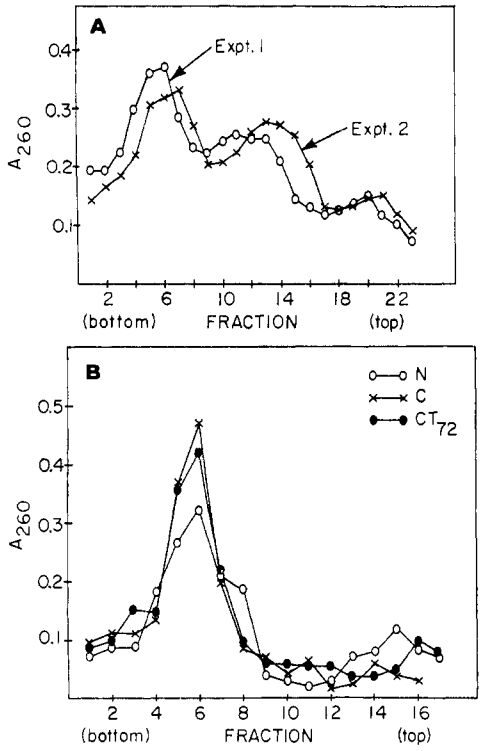

FIGURE 3: Sucrose density gradient sedimentation of sheared and minimally sheared ventral prostate chromatin from N, C, and CT_{72} rats. (A) Sheared chromatin: 250 μg of chromatin DNA, in 0.8 ml of 0.01 M Tris, pH 8.0, was layered onto a 5–30% linear sucrose gradient, containing the same buffer, and centrifuged at 240,000g for 2 hr. (B) Minimally sheared chromatin: 40 μg of chromatin (as DNA) in 0.4 ml of 0.4 mM Tris, pH 8.0, was layered onto a 0.25–2.8 M linear sucrose gradient, containing 0.4 mM Tris, pH 8.0, and centrifuged at 64,000g for 30 min.

et al., 1971). When acid-extracted histones from prostate chromatin were electrophoresed, the five major histone fractions were observed (Table II, Figure 2). Histone bands from all chromatin preparations were sharp and distinct, indicating negligible proteolysis, and there was little evidence of contamination by H_2SO_4-soluble nonhistone proteins. In addition, a sixth band (F′), migrating between F_1 and the F_3 monomer, was seen consistently. This band is probably not due to proteolytic degradation of F_1 histone by a histone protease, as described by Bartley and Chalkley (1970) or Garrels *et al.* (1972), since proteolysis of F_1 histone produces a broad smear rather than a sharp band. Therefore, this protein may be similar to the histone found by Panyim and Chalkley (1969) in nonreplicating tissues of the calf. Williams-Ashman and Shimazaki (1967) and Chung and Coffey (1971) reported a decrease in the F_1 histone content of prostate nuclei after long-term castration, but we observed no significant quantitative or qualitative changes in the histones after 3 days of castration.

Physical Properties of Prostate Chromatins. Sucrose density gradient studies of sheared and minimally sheared chromatin differed (Figure 3). Sheared chromatin (5–30% gradient) included three major components, comprising 50, 35, and 15% of the total uv-absorbing material. Minimally sheared chromatin, analyzed on a 0.25–2.8 M sucrose density gradient, separated into a major high density fraction (85%) and a minor lower density species (15%). In these experiments no consistent differences in the distribution of these species between N, C, and CT_{72} chromatins were observed.

TABLE III: Analytical Ultracentrifugation Analysis of Sheared N and Minimally Sheared N, C, CT_{72}, and CT_4 Chromatins.

Age of Chromatin	Size of Components	% Total Material
	Sheared Chromatin[a]	
<1 week	>100 S[b]	15–50
	52–60 S	18–30
	32–34 S	10–30
	<5000 mol wt	22–25
>1 week	95–108 S	15–20
	17–20 S	33–43
	<5000 mol wt	37–52

Expt No.	Chromatin	% Total Material >200 S[c]	Value of Resolved Component (S)
	Minimally Sheared Chromatin		
1	C	25	56 (75)[d]
	CT_{72}	75	55 (25)
2	N	50	31 (50)
	C	35	34 (65)
	CT_{72}	67	42 (33)
3	N	56	25 (44)
	C	68	47 (32)
	CT_{72}	59	22 (41)
4	N	69	57 (31)
	C	66	55 (34)
	CT_{72}	72	59 (28)
5	N	46	31 (54)
	C	55	30 (45)
	CT_4	45	27 (55)
6	N	78	43 (22)
	C	79	60 (21)
	CT_4	78	51 (22)

[a] Results from three experiments. [b] s_{20}, 0.01 M Tris, pH 8.0. [c] s_{20}, 0.4 mM Tris, pH 8.0. [d] % of total material. Approximately 1.0 A_{260} unit of sample was examined by velocity sedimentation analysis in a Spinco analytical ultracentrifuge equipped with ultraviolet optics and an automatic differentiator.

Analytical ultracentrifugation studies are presented in Table III. In three experiments, sheared chromatin contained variable amounts of a large component of >100S and 50–60S and 33S fractions. Chalkley and Jensen (1968) have shown that the sedimentation values of sheared chromatin can be ascribed to nonspecific aggregation of chromatin components. About 25% of the total uv-absorbing material did not sediment at 60,000 rpm and had an estimated mol wt of 5000 or less. With continued storage of chromatin, a general shift to lower molecular weight material occurred, accompanied by an increase in the nonsedimentable fraction. In contrast, minimally sheared chromatin exhibited two components; the larger one, sedimenting at very low speed, was in excess of 200 S, while a smaller fraction had values from 22 to 59 S. Generally, the fraction in excess of 200 S comprised over 50% of the total material. The distribution of large and small fractions was not affected by prior hormone treatment. In view of the ease with which intentionally sheared chromatin has been shown to aggregate, the nature

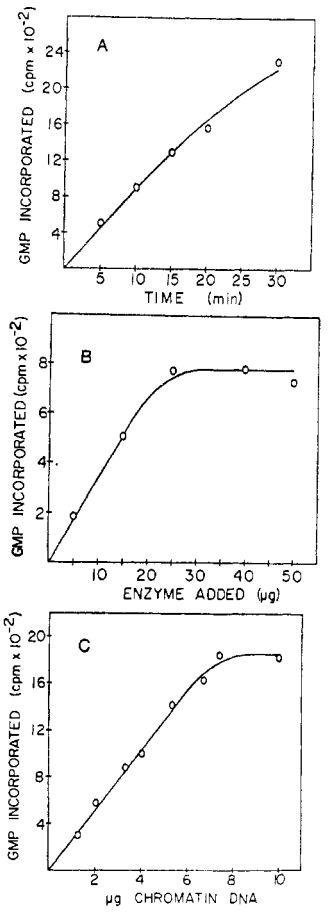

FIGURE 4: Template activity of minimally sheared rat ventral prostate chromatin. (A) Chromatin (5.4 μg) (DNA) was incubated with 40 μg of *E. coli* K-12-Leu RNA polymerase. Other conditions were described. (B) Dependence of incorporation upon increasing amounts of chromatin DNA; 40 μg of RNA polymerase was incubated with increasing amounts of chromatin, expressed as DNA. (C) A constant amount of chromatin (5.4 μg of DNA) was incubated with increasing amounts of RNA polymerase.

of the structures produced with either method of preparation is not certain.

Template Activity of Prostate Chromatins. Soluble chromatin prepared by both methods provided a template for *E. coli* RNA polymerase (Table IV). The reaction required all four ribonucleoside triphosphates and was inhibited by actinomycin D. No significant endogenous RNA polymerase activity was detected. Soluble sheared chromatin was 22% as active as the same amount of calf thymus DNA. Because of the greater heterogeneity of sheared preparations, in most experiments minimally sheared chromatin was used. Incorporation was proportional to time for 20 min (Figure 4A) and saturation of chromatin by polymerase (Figure 4B) or of polymerase by chromatin (Figure 4C) occurred at an enzyme:DNA ratio of 5.5:1.

The effects of castration and hormone replacement on chromatin template activity are presented in Table V. In

TABLE IV: Properties of RNA Synthesis Templated by Sheared Rat Ventral Prostate Chromatin.

Assay	Template	GMP Incorp (cpm)
Complete[a]	Calf thymus DNA	7865
	Soluble chromatin	1760
	Pellet chromatin	335
− RNA polymerase	Soluble chromatin	32
− RNA polymerase	Pellet chromatin	27
+ 5 μg of actinomycin D	Soluble chromatin	137
− ATP	Soluble chromatin	141

[a] Complete reaction mixture (0.25 ml/tube) contained 1 μg of DNA and 4 μg of *E. coli B* RNA polymerase (Miles).

TABLE V: Effect of *in Vivo* Testosterone Propionate on *in Vitro* Template Activity of Rat Ventral Prostate Chromatin from Sheared N, C, and CT_{72}, and Minimally Sheared N, C, CT_{72}, and CT_4 Chromatins.[a]

	Expt No.	Hormonal Status	Amount Chromatin DNA Assayed (μg)	Incorp. (cpm)
		Sheared		
Soluble fraction	1	N	1	690
		C	1	1,964 (285)
		CT_{72}	1	727 (105)
Pellet fraction	1	C	1	523
		CT_{72}	1	264
Soluble fraction	2	N	8	9,492
		C	8	15,024 (158)
		CT_{72}	8	6,834 (72)
	3	N	1	1,602
		C	1	2,853 (178)
		CT_{72}	1	1,307 (82)
		Minimally Sheared		
	4	N	6.7	2,388
		C	6.7	5,618 (234)
		CT_{72}	6.7	2,040 (86)
	5	C	11.4	411
		CT_{72}	11.4	168
	6	N	14.4	446
		C	14.4	585 (131)
		CT_{72}	14.4	531 (119)
	7	N	12.7	533
		C	12.7	639 (119)
		CT_{72}	12.7	520 (99)
	8	N	10	620
		C	10	1,020 (165)
		CT_4	10	1,940 (314)
	9	N	10	620
		C	10	1,080 (174)
		CT_4	10	1,870 (302)
	10	N	10	1,620
		C	10	2,940 (181)
		CT_4	10	5,000 (310)

[a] Experiments 1–3 were carried out in the presence of 4 μg of *E. coli* B RNA polymerase (Miles). The remaining experiments were performed with 20–40 μg of *E. coli* K-12 RNA polymerase. Numbers in parentheses are % of incorporation compared with prostate chromatin from normal rats.

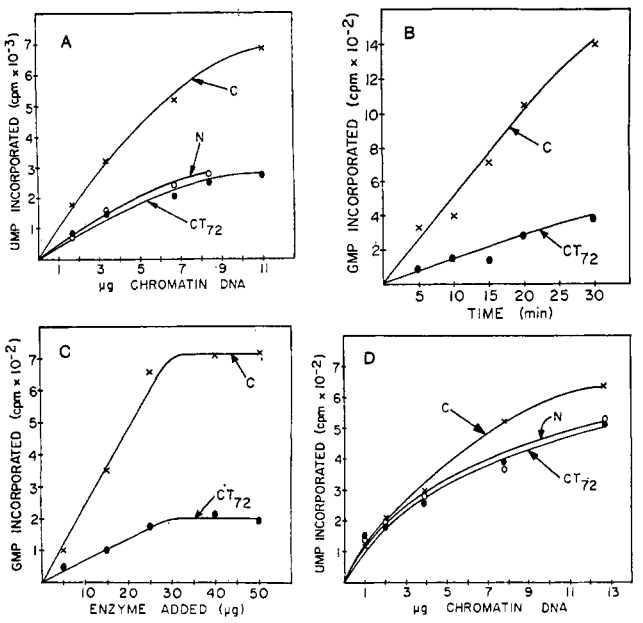

FIGURE 5: Template activities of minimally sheared N, C, and CT_{72} chromatins: (A and D) incubation in the presence of 20 μg of *E. coli* K-12 RNA polymerase; (B and C) 5.3 μg of DNA was incubated with 40 μg of *E. coli* K-12-Leu RNA polymerase.

all comparisons of N, C, and CT_{72} chromatin, independent of the method of preparation, the amount of DNA, or the time of incubation, C chromatin characteristically exhibited the greatest template activity (Figure 5).

Using sheared chromatin, differences of 100% or more between C and N or CT_{72} chromatins were observed. This was also true in about 75% of the experiments employing nonsheared chromatin. In some experiments (4 and 5 of Table V, corresponding to 1 and 2 of Table III), a much more active C template correlated with a larger amount of less rapidly sedimenting material. In other experiments, comparable amounts of this fraction were associated with from 20 to 100% greater template activity in C than in N chromatin. The reasons for these differences are not known.

Unexpectedly, the template activity of CT_4 chromatin was 80% greater compared with C chromatin (Table V, Figure 6). In these experiments, N chromatin consistently exhibited half the activity of C chromatin. Increased template activity was accompanied by a 28% increase in nonhistone protein content. Within a given experiment, the amount of the fraction resolved by analytical ultracentrifugation and the estimated sedimentation values did not differ markedly.

In order to examine several possible artifacts that might account for these results, prostate chromatins from N, C, CT_{72}, and CT_4 chromatins were incubated in the standard mixture, without labeled nucleoside triphosphate and polymerase, with either 500 μg of [^{14}C]RNA or 300 μg of [^{14}C]-DNA from rat liver. All chromatins exhibited negligible ability to degrade the labeled nucleic acids (not shown). However, the presence of endonucleases which could "nick" the DNA, producing artificial initiation sites, or degrade template-bound RNA, causing its release, is not excluded. Apparent differences in the template activity of C and CT_{72} chromatins might have been due to the presence of ribonucleoside triphosphatases. If labeled GTP was the only triphosphate present, C chromatin converted 32% of it to GDP, compared with a 64% conversion by CT_{72} chromatin. When equal amounts of prostate chromatin from C and CT_{72}

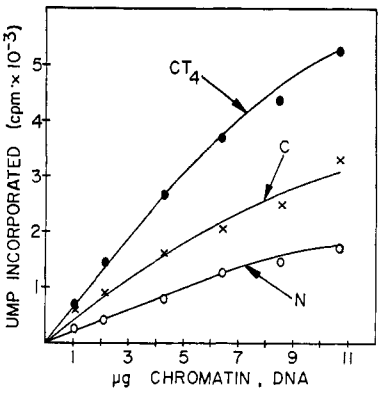

FIGURE 6: Template capacity of minimally sheared N, C, and CT_4 rat ventral prostate chromatin. Chromatin was incubated with 40 μg of *E. coli* K-12 RNA polymerase.

rats were incubated with all four triphosphates, there was negligible degradation of the labeled substrate. Triphosphatase activity was also masked by a tenfold excess of nonradioactive GTP. Thus, because of the large excess of substrate, nucleoside triphosphatase activity is not a factor in the results of the template assay.

Unincubated chromatin and chromatin which had been incubated at 37° for 10 min were compared by sucrose gradient centrifugation (Figure 7). Incubation did not cause a significant increase in the amount of low-density material, indicating that chromatin was not being grossly degraded.

Discussion

Androgens rapidly stimulate rat ventral prostrate nuclear RNA synthesis. This could be due to a more efficient utilization of template previously transcribed at a reduced rate. In addition, qualitatively similar but previously inactive template may become engaged in RNA synthesis. Whether products from qualitatively "new" template also participate is not known. Studies of chromatin template activity may help

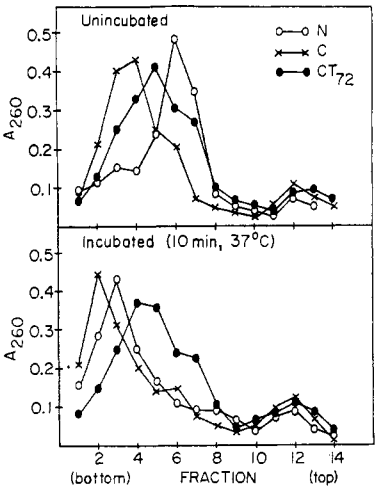

FIGURE 7: Sucrose density gradient sedimentation before and after incubation of minimally sheared N, C, and CT_{72} rat ventral prostate chromatin. Samples, containing 45 μg of chromatin (as DNA), in 0.4 ml of 0.4 mM Tris, pH 8.0, incubated or not at 37° for 10 min, were layered onto a 0.25–2.8 M linear sucrose gradient, containing 0.4 mM Tris, pH 8.0. After centrifugation at 64,000*g* for 30 min, samples were collected and absorption at 260 nm determined.

to define these relationships, which are central to the question of how androgens increase transcription.

Chromatin prepared by extensive shearing of lysed nuclei was less satisfactory, since only 30% of the DNA was solubilized and the material was very heterogeneous. One of these species may have been particularly active as a template for RNA synthesis. By controlled shearing and low-speed centrifugation, Duerksen and McCarthy (1971) were able to obtain a separation of condensed mouse and crab chromatin, containing the satellite DNA sequences from the diffuse chromatin.

In contrast, prostate chromatin prepared by gentle stirring retained properties that were perhaps more chracteristic of native chromatin. This minimally sheared chromatin was shown by analytical ultracentrifugation and sucrose gradient sedimentation to contain two major components. When separated on sucrose density gradients, the less dense component represented about 12–15% of the total uv-absorbing material. Whether such fractions are nonspecific aggregates, or have some structural or functional significance (*i.e.*, euchromatin, low density material; heterochromatin, high density fraction), as suggested by the work of Dolbeare and Koenig (1970), is under study. After controlled sonication of liver nuclei, these workers observed a similar separation of chromatin by sucrose gradient centrifugation. "Solubilizing" prostate chromatin by gentle stirring may also prove to separate it into structurally and functionally distinct components.

As early as 4 hr after administration of testosterone propionate to rats castrated for 3 days, RNA and nonhistone protein contents increased 22 and 27%, respectively, representing half the increases for CT_{72} chromatin. Similar rapid changes in the macromolecular composition of uterine chromatin follow the administration of 17β-estradiol to ovariectomized rats (Barker and Warren, 1966; Teng and Hamilton, 1969). The histone content of prostate chromatin was not markedly altered by 3 days of hormone withdrawal or replacement. A fall in the histone to DNA ratio after 7 days of castration, due almost entirely to reduction of the F_1 fraction, has been observed (Chung and Coffey, 1971; Williams-Ashman and Shimazaki, 1967). This may only occur subsequent to a decline in total prostate DNA content, which becomes apparent after 48–72 hr of castration.

The template activity of prostate chromatin from C rats was greater than that of N or CT_{72} chromatin. Chromatin from castrates contained less nonhistone protein. Although the template activity of some chromatins and DNA–polypeptide complexes has been shown to be proportional to the fractional content of free DNA (Shih and Bonner, 1970), a simple analogy with these experimental situations fails, for CT_4 chromatin, containing 27% more protein, exhibited 78% greater template activity than C chromatin. None of these results correlated closely with the amount of small molecular weight material, estimated by analytical ultracentrifugation, nor did differences in activities of nucleases, ribonucleoside triphosphatases, or histone proteases appear to account for them. Decreased protein content might confer less resistance to shearing, leading to "nicking" of the DNA and the formation of artifactual initiation sites for RNA synthesis. Structural and biochemical similarities between chromatins from control and experimental animals should be greater the less time between hormone treatment and sacrifice of the animal, C chromatin may be a suitable control only for CT_4 chromatin. Early androgen-induced changes in chromatin template activity might be obscured in comparisons between C and N or CT_{72} chromatins, which have not undergone regression.

Increased binding of the prostate cytosol–dihydrotestosterone receptor–protein complex to prostate chromatin is one of the early effects of androgen administration (Liao and Fang, 1969). It is doubtful whether a bacterial enzyme would "recognize" this or other putative regulatory factors of mammalian orgin. Liao and Lin (1967) have already shown that the qualitative nature and quantitative amount of chromatin-primed RNA differed markedly from the product formed by endogenous nuclear RNA polymerase under similar conditions of low ionic strength. That bacterial RNA polymerase would exhibit the fidelity of transcription of mammalian enzymes, with their differing intranuclear localization, individual ion requirements, differential response to inhibitors, and functional specialization for the synthesis of discrete RNA products is unlikely.

Steroid hormones have been shown to increase the chromatin template activity of rat uterus, skeletal muscle, liver, and chick oviduct (Barker and Warren, 1966; Breuer and Florini, 1966; Dahmus and Bonner, 1965; O'Malley *et al.*, 1969). However, transcription of mammalian chromatin by bacterial RNA polymerases chiefly seems to represent a means of detecting the appearance of sites which become available for transcription, many of which may not be utilized *in vivo*. That this is indeed the case has been suggested by comparing transcription by bacterial and mammalian polymerases (Butterworth *et al.*, 1971; Keshgegian and Furth, 1972). Although bacterial RNA polymerases transcribe repetitive DNA sequences, the sites at which they bind and initiate are different from those of mammalian nucleoplasmic RNA polymerases.

The early changes in CT_4 chromatin structure implied by the massive uptake of nonhistone proteins and 80% increase in template activity could be necessary for previously in accessible regions of the genome to become available for transcription. The extent to which these regions are utilized *in vivo* is not known, but it may be that shortly after exposure to androgens, qualitatively "new" prostate template becomes available for transcription by endogenous mammalian RNA polymerases.

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Different Biological Properties of the Two Constituent Peptide Chains of Ricin, a Toxic Protein Inhibiting Protein Synthesis†

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ABSTRACT: The toxic protein ricin was purified to homogeneity and its constituent peptide chains were isolated. The purification involved extraction from castor beans and chromatography on a CM-52 column and subsequently on a Sepharose-4B column. The LD_{50} dose in mice was 0.2 μg. The isolated toxin had only a slight hemagglutinating activity. After reduction of the toxin with β-mercaptoethanol in the presence of galactose the two constituent chains were isolated by chromatography on a DE-52 and a CM-52 column. The smaller peptide chain (the A chain) strongly inhibited protein synthesis in a cell-free system from rabbit reticulocytes, whereas the larger peptide (the B chain) lacked this ability. Human erythrocytes pretreated with intact ricin or with B chain were agglutinated by an antiserum directed specifically against ricin, whereas erythrocytes pretreated with A chain were not agglutinated under the same conditions. Only the B chain was bound to a Sepharose column, a binding which could be abolished by the presence of galactose. The results indicate that the toxic action of ricin is associated with the A chain and that the B chain functions as a carrier moiety which binds the toxin to the cell surface, a binding which probably involves galactose-containing receptors.

Ricin, a highly toxic protein present in castor beans, inhibits protein synthesis in a cell-free system even when it is present in extremely small concentrations (Olsnes and Pihl, 1972a). As previously reported ricin consists of two peptide chains held together by disulfide bonds (Olsnes and Pihl, 1972b; Olsnes, 1972). Recently, we have shown that after treatment with β-mercaptoethanol the ability of ricin to inhibit protein synthesis in a cell-free system is increased 50- to 100-fold, whereas its toxic effect in living animals and in cells in culture is virtually abolished (Olsnes and Pihl, 1972c), suggesting that the two chains have different functions. Similar findings were obtained with abrin, a toxic protein present in the seeds of *Abrus precatorius L.*, an entirely different plant (Olsnes and Pihl, 1972c,d).

In the present paper we have described the purification of ricin to homogeneity, the separation of its constituent peptide chains, and the study of their properties. Evidence is presented that, as in the case of abrin, the smaller peptide chain inhibits protein synthesis in a cell-free system, whereas the larger chain appears to bind the toxin to the cell surface.

Materials and Methods

Materials. Castor beans (the seeds of *Ricinus communis L.*)

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