Dependence on androgens of the specific DNA-binding activity of rat ventral-prostate non-histone chromosomal proteins

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(Received 16 June 1980/Accepted 26 August 1980)

Interactions between rat prostate non-histone chromosomal proteins and DNA were studied by using a nitrocellulose-filter-binding technique to monitor the formation of DNA–protein complexes. The total binding activity of the non-histones, as measured by binding of proteins to a trace quantity of labelled DNA, displays no preference for rat DNA relative to *Escherichia coli* DNA. Sequestration of non-specific binding proteins by preincubation with unlabelled bacterial DNA enables detection of a fraction of rat prostate non-histones that binds preferentially to labelled rat DNA relative to labelled *E. coli* DNA. After castration of adult male rats, both total and specific binding activities decrease. Administration of 5 α -dihydrotestosterone to castrated rats stimulates both total and specific DNA-binding activities of prostate non-histones; specific binding is stimulated to a greater extent than total DNA binding, indicating that the specific binding proteins constitute a larger fraction of the non-histone proteins in the presence of androgens. The specific DNA-binding activity is dependent on the dose of steroid administered.

It is now generally accepted that steroid hormones modulate cellular function in their target tissues via interaction with the genetic apparatus (Schrader & O'Malley, 1978; Liao *et al.*, 1979). On entering the cell, hormone binds to a steroid-specific receptor protein present in the cytosol of target tissues. After translocation to the nucleus, steroid–receptor complexes interact with chromatin, and stimulation of RNA synthesis is observed. RNA sequences whose synthesis is stimulated by steroids include rRNA and mRNA species; the latter code for various cellular proteins as well as for steroid-induced secretory proteins. The particular protein(s) induced depends on the steroid and the target tissue, e.g. androgens stimulate the synthesis of prostatic binding protein in the rat prostate (Heyns *et al.*, 1977; Parker *et al.*, 1978). This regulation has been shown to occur at the level of RNA transcription (Parker & Scrace, 1978). In addition to stimulating the secretory function of their target tissues, steroids are also responsible for maintaining the normal cellular complement of the tissue. Hence, in the case of the prostate, castration is followed by atrophy of the tissue, and cell proliferation occurs in atrophied tissues in response to androgen administration until the normal cellular complement has been restored (Lesser & Bruchovsky, 1974). Administration of androgens to animals whose prostate already con-

tains the normal cellular complement stimulates synthesis of RNA and protein, but not DNA replication or cell proliferation.

Relatively little is known about the molecular events by which the interaction of steroid–receptor complexes with chromatin leads to qualitative and quantitative changes in RNA sequences present in target tissues. In bacteria, proteins that bind to specific DNA sequences play a central role in the regulation of certain genes (Goldberger, 1979), and in eukaryotes chromosomal proteins (histones and non-histones) are believed to regulate differential gene activity. Relatively abundant nuclear proteins, such as histones and high-mobility-group proteins, are associated with structural features of chromatin such as nucleosomes, and are responsible in part for structural differences between transcriptionally active and transcriptionally inactive chromatin (Davie & Candido, 1978; Weisbrod & Weintraub, 1979). However, the ubiquity and lack of complexity of these protein fractions make them poor candidates for the primary regulators of genetic activity. The non-histones, on the other hand, are a very complex group of proteins whose tissue specificity and changes in composition in response to gene activation are properties expected of regulatory elements (Allfrey, 1974). Furthermore, some of the non-histones show specificity in their interaction with

DNA (Lesser & Comings, 1978; Sevall *et al.*, 1978).

In order to determine the possible role of non-histone proteins in androgen action in the rat prostate at the genetic level, we have examined the DNA-binding properties of these proteins in tissues undergoing atrophy after castration, and in tissues in which proliferation or secretory function is being stimulated by hormone.

Materials and methods

Animals

Adult male Sprague-Dawley rats (350–400 g) were bred at the University of Calgary or the University of Alberta and maintained on a diet of Wayne laboratory chow and water *ad libitum*. Castration was performed via the scrotal route under methoxyflurane anaesthesia. Animals were injected subcutaneously with 5 α -dihydrotestosterone in about 0.75 ml of an aqueous medium containing (v/v) 10% ethanol + 10% polyoxyethylene sorbitan monopalmitate (Tween 40; Sigma Chemical Co., St. Louis, MO, U.S.A.). At the appropriate time the rats were killed by decapitation and prostates quickly removed, stripped of connective tissue, placed on ice and weighed; 12–24 prostates, depending on their size, were pooled for each experiment.

Isolation of chromosomal proteins

Nuclei were purified by the method of Bruchovsky *et al.* (1975). All procedures were performed at 0–4°C. The nuclei were washed once in 0.2 M-MgCl₂/50 mM-Tris/HCl containing soya-bean trypsin inhibitor (1 μ g/ml; Sigma Chemical Co.) (pH 7.5) and twice in 0.15 M-NaCl/0.2 M-MgCl₂/10 mM Tris/HCl (pH 7.5), with centrifugation each time in a Sorvall SS-34 rotor for 10 min at 4°C and 1000 g (r_{av} 10.8 cm). The nuclear pellet was resuspended in 5 ml of 0.5 M-Na₂HPO₄/0.5 M-NaH₂PO₄, pH 6.8, by homogenization in a ground-glass homogenizer and sonicated in an ice bath at intensity 4 for four 10 s pulses (50% duty cycle) interspersed with cooling periods of 30 s, by using a Heat Systems-Ultrasonics Inc. (Plainview, NY, U.S.A.) model W-350 sonifier equipped with a micro-tip. To the nuclear suspension was added slowly with continuous stirring 100 ml of 2 M-NaCl/1 M-sodium phosphate buffer, pH 6.8, followed by centrifugation in a Sorvall SS-34 rotor for 20 min at 4°C and 31 000 g (r_{av} 10.8 cm). To the supernatant was added phenylmethanesulphonyl fluoride (Sigma Chemical Co.) to a final concentration of 1 mM (17.5 mg of phenylmethanesulphonyl fluoride in 0.5 ml of ethanol) and 36 g of ultra-pure urea (Schwarz/Mann; Becton-Dickinson, Mississauga, Ontario, Canada) to give a final urea concentration of 5 M. The DNA content of the nuclear lysate was

estimated from its A_{260} , by using $A_{1\text{cm}}^{1\%}$ 250. The nuclear lysate was fractionated by hydroxyapatite column chromatography as described by MacGillivray *et al.* (1972), except that the non-histone fraction was eluted in 2 M-NaCl/5 M-urea/30 mM-sodium phosphate buffer, pH 6.8, instead of 2 M-NaCl/5 M-urea/50 mM-sodium phosphate buffer, pH 6.8, to minimize contamination of the non-histone fraction by nucleic acids. Before use in binding experiments, the protein fractions were dialysed against 0.2 M-NaCl/0.1 M-EDTA/10 mM-Tris/HCl (pH 7.5) and centrifuged in a Sorvall SS-34 rotor for 10 min at 4°C and 17 000 g (r_{av} 10.8 cm). Protein concentrations were determined by a dye-binding assay (Spector, 1978) and nucleic acid contamination was estimated by measurement of A_{280}/A_{260} (Warburg & Christian, 1941). Protein fractions were routinely analysed by electrophoresis in linear 10–20%-polyacrylamide gradient gels in the presence of sodium dodecyl sulphate, essentially as described by Comings & Harris (1975, 1976).

Isolation of DNA

Escherichia coli strain K12 was grown in Medium 9 (Miller, 1972) supplemented with 0.2% glucose and thiamin (1 μ g/ml). To label DNA, exponentially growing cells were inoculated into the above medium containing 1 mM-adenosine (Boyce & Setlow, 1962) and [³H]thymidine (5 μ Ci/ml; 40–60 Ci/mmol; New England Nuclear, Lachine, Quebec, Canada), and incubation was continued for 5–6 h.

Unlabelled rat DNA was prepared from rat liver, spleen and kidney. Labelled rat DNA was isolated from XC rat sarcoma cells (CCL 165; American Type Culture Collection, Rockville, MD, U.S.A.) grown for 24 h in Eagle's minimal essential medium with Earle's salts (Grand Island Biological Co., Santa Clara, CA, U.S.A.) supplemented with 5% (v/v) foetal calf serum (GIBCO), streptomycin (100 μ g/ml), penicillin (100 i.u./ml) and [³H]thymidine (2 μ Ci/ml).

DNA was purified as described by Lesser & Comings (1978), except that shearing was accomplished by sonication in an ice bath at intensity 2 for four 10 s pulses (50% duty cycle) interspersed with cooling periods of 30 s by using a Heat Systems-Ultrasonics model W-350 sonifier equipped with a micro-tip. Unlabelled DNA was sonicated at a concentration of 1 mg/ml and labelled DNA at 50 μ g/ml. DNA fragment size was determined by agarose-gel electrophoresis (Loening, 1969), with as standards endonuclease-*Eco*RI-digested phage- λ DNA and endonuclease-*Hae*III-digested phage-PM2 DNA (Bethesda Research Laboratories, Rockville, MD, U.S.A.). Radioactively labelled DNA fragments ranged in size from 500 to 3500 base-pairs, with a peak size of 1300 base pairs, and unlabelled DNA fragments ranged in size from 12 000 to

25 000 base pairs. This size difference reflects the different DNA concentrations during sonication.

Filter-binding assays

Filter-binding assays were performed essentially as described by Lesser & Comings (1978). Assay mixtures for measurement of total DNA-binding activity contained 0.1 μg of ^3H -labelled *E. coli* or ^3H -labelled rat DNA (42 000 d.p.m.), 20 μg of bovine serum albumin (Sigma Chemical Co.) and various quantities of non-histone proteins in 0.2 ml of buffer containing 0.2 M-NaCl/0.1 mM-EDTA/3 mM-MgCl₂/1 mM-dithiothreitol/10 mM-Tris/HCl (pH 7.5). Incubation was for 1 h at 0°C. For measurement of specific DNA-binding activity, 5 μg of unlabelled *E. coli* DNA, 20 μg of bovine serum albumin and various quantities of non-histone proteins were incubated in 0.18 ml of buffer containing 0.2 M-NaCl/0.1 mM-EDTA/3 mM-MgCl₂/1 mM-dithiothreitol/10 mM-Tris/HCl (pH 7.5) for 1 h at 0°C. Then 0.1 μg of ^3H -labelled *E. coli* or ^3H -labelled rat DNA (42 000 d.p.m.) in 20 μl of the same buffer was added and incubation continued for a further 1 h. At the appropriate time, samples were diluted to 1 ml with 0.2 M-NaCl/0.1 mM-EDTA/3 mM-MgCl₂/1 mM-dithiothreitol/10 mM-Tris/HCl (pH 7.5), applied to a 25 mm nitrocellulose filter (pore size 0.45 μm ; Millipore Corp., Bedford, MA, U.S.A.) that had been pre-soaked in the same buffer, and filtered at a rate of 1 ml/20–30 s. The filters were washed three times with 1 ml portions of 0.2 M-NaCl/0.1 mM-EDTA/3 mM-MgCl₂/1 mM-dithiothreitol/10 mM-Tris/HCl (pH 7.5), solubilized with 1 ml of methoxyethanol and counted for radioactivity in an LS-9000 scintillation counter (Beckman Instruments, Fullerton, CA, U.S.A.) with as scintillant 10 ml of Aquasol II (New England Nuclear). The amount of DNA bound to the filters in the absence of protein was less than 0.2% of input, and this background binding was subtracted.

Results

Determination of DNA binding

Fig. 1 illustrates the DNA-binding properties of rat prostate non-histone proteins. When proteins are allowed to bind directly to a trace quantity of labelled DNA, retention of DNA by nitrocellulose filters is the same regardless of the source of DNA (Fig. 1, open symbols). This indicates that the majority of the DNA-binding activity of the non-histone proteins is of a non-specific nature. This type of binding activity will be referred to in subsequent Figures as total binding activity, measured as ng of DNA bound/ μg of protein. When proteins are incubated with unlabelled *E. coli* DNA before addition of a trace quantity of labelled DNA, retention of labelled rat DNA is significantly greater

Fig. 1. Binding of ^3H -labelled DNA from rat and *E. coli* by rat prostate non-histones

Non-histones were prepared from prostates of rats that had been castrated for 1 day, followed by treatment for 6 days with daily doses of 400 μg of 5 α -dihydrotestosterone/100 g body wt. The indicated quantities of protein were incubated for 1 h at 0°C with 0.1 μg of ^3H -labelled DNA from rat (□) or *E. coli* (○), and retention of DNA by nitrocellulose filters was measured as described in the Materials and methods section. Alternatively, protein was incubated for 1 h at 0°C with 5 μg of unlabelled *E. coli* DNA and then for a further 1 h after addition of 0.1 μg of ^3H -labelled rat DNA (■) or of 0.1 μg of ^3H -labelled *E. coli* DNA (●), or protein was incubated with 5 μg of unlabelled rat DNA followed by addition of 0.1 μg of ^3H -labelled rat DNA (▲); retention of ^3H -labelled DNA by nitrocellulose filters was measured as described in the Materials and methods section. Each point represents the mean of duplicate determinations.

(4–5-fold) relative to retention of labelled *E. coli* DNA; this difference is greatly decreased when preincubation is with unlabelled rat DNA rather than unlabelled *E. coli* DNA (Fig. 1, closed symbols). Hence preferential binding of a fraction of rat prostate non-histones to rat DNA can be observed under conditions where the non-specific binding proteins are sequestered by binding to an excess of bacterial DNA. This type of binding activity will be referred to in subsequent Figures as specific binding activity, measured as the increase in

retention of labelled rat DNA relative to retention of labelled *E. coli* DNA in the presence of an excess of unlabelled *E. coli* DNA.

Effect of castration on non-histone-DNA interactions

Removal of the endogenous supply of androgens by castration of an adult male rat is followed by a shutdown of biosynthetic activities in the prostate and autolysis of cells, resulting in atrophy of the tissue. This is illustrated in Fig. 2(a) by the decline in the wet weight and DNA content of the prostate; by 7 days after castration, these parameters have decreased to about 20% of the normal values. Accompanying the atrophic response is a decline in the DNA-binding activity of the prostate non-histones (Fig. 2b), indicating that this activity is androgen-dependent. Both total and specific binding activity decrease in parallel, such that by 7 days after castration the activities are about 30% of normal. Since the DNA-binding activities are ex-

pressed per μg of protein, the decline in activity reflects a decrease in the concentration of DNA-binding proteins and is not simply a reflection of the decrease in content of non-histones in the prostate after castration.

Effect of androgen-replacement therapy on non-histone-DNA interactions

In order to determine whether the decrease in DNA-binding activity of the prostate non-histones after castration can be reversed by injection of androgens, rats that had been castrated for various periods of time were injected daily with $400\mu\text{g}$ of 5α -dihydrotestosterone/100 g body wt. This is the dose of hormone that has been shown to produce a maximal rate of DNA synthesis and cell proliferation in 7-day-castrated animals (Lesser & Bruchovsky, 1973).

At 1 day after castration, there has been no significant decrease in either the wet weight or DNA content of the prostate, indicating that the atrophic process has not yet begun. When 1-day-castrated

Fig. 2. Effect of castration on prostatic weight and DNA content, and the DNA-binding activity of prostate non-histones

Prostates were removed from normal rats or from rats at the indicated times after castration. (a) The tissue was weighed (O), and DNA content was estimated from the A_{260} of the purified nuclear lysate as described in the Materials and methods section (●). (b) Non-histones were isolated as described in the Materials and methods section. Total DNA-binding activity (□) and specific DNA-binding activity (■) were measured as described in Fig. 1.

Fig. 3. Effect of androgen administration to 1-day-castrated rats on prostatic weight and DNA content, and the DNA-binding activity of prostate non-histones

Rats that had been castrated 1 day previously were injected daily with $400\mu\text{g}$ of 5α -dihydrotestosterone/100 g body wt. and prostates were removed after the indicated period of treatment. (a) The tissue was weighed (O), and DNA content was estimated from the A_{260} of the purified nuclear lysate, as described in the Materials and methods section (●). (b) Non-histones were isolated as described in the Materials and methods section. Total DNA-binding activity (□) and specific DNA-binding activity (■) were measured as described in Fig. 1.

animals are treated with steroid, there is no increase in DNA content above the normal value (Fig. 3a). Hence proliferation is not stimulated in this tissue; this is in agreement with previous studies measuring rates of DNA synthesis (Lesser & Bruchovsky, 1974). The wet weight of the prostate, on the other hand, is stimulated to increase, until by day 4 of treatment it has reached twice the normal wet weight (Fig. 3a). Therefore the response elicited in 1-day-castrated rats is a hypertrophic response involving stimulation of secretory activity but not cell proliferation. Measurements of the DNA-binding activities of the prostate non-histones during treatment are shown in Fig. 3(b). Total DNA binding is not stimulated during the first 2 days of treatment, then increases to 3–4 times the normal value. Specific DNA-binding activity has returned to the normal value within 24 h of the onset of treatment, and increases further to 12–13 times the normal value by day 6 of treatment. These results indicate that the DNA-binding activity of rat prostate non-histones is indeed stimulated by androgens, and furthermore that the specifically binding component of the non-histones becomes a larger fraction of the total DNA-binding proteins.

By day 3 after castration, the wet weight and DNA content of the prostate have decreased to 70 and 50%, respectively, of the normal values. When 3-day-castrated animals are treated with steroid, the DNA content increases, until by day 4 of treatment it has returned to the normal value; continued administration of androgens does not result in any further increase in DNA content (Fig. 4a). Thus a transient proliferative response is induced in 3-day-castrated animals until the normal cellular complement of the prostate has been restored, and then proliferation ceases; this is in agreement with previous studies measuring rates of DNA synthesis (Lesser & Bruchovsky, 1974). The wet weight increases throughout the course of treatment, reaching a value 50% greater than normal by day 6 (Fig. 4a), indicating that the tissue continues its hypertrophic response to androgens even after proliferation has ceased. Total DNA-binding activity of prostate non-histones (Fig. 4b) has returned to the normal value within 24 h of treatment. By day 3, this activity is 3 times the normal value, and no further increase is observed. Specific DNA-binding activity is also stimulated within 24 h, and continues to increase to 11–12 times the normal value by day 6. These results indicate that the DNA-binding activity of rat prostate non-histones is stimulated during a proliferative response induced by androgens. The magnitude of the stimulation is similar to that observed during the hypertrophic response occurring in 1-day-castrated animals, and stimulation of DNA-binding activity is maintained during the period when the proliferative response has shut off.

Fig. 4. Effect of androgen administration to 3-day-castrated rats on prostatic weight and DNA content, and the DNA-binding activity of prostate non-histones

Rats that had been castrated 3 days previously were injected daily with 400 μg of 5 α -dihydrotestosterone/100 g body wt. and prostates were removed after the indicated period of treatment. (a) The tissue was weighed (○), and DNA content was estimated from the A_{260} of the purified nuclear lysate, as described in the Materials and methods section (●). The normal values of wet weight and DNA content are represented by ——. (b) Non-histones were isolated as described in the Materials and methods section. Total DNA-binding activity (□) and specific DNA-binding activity (■) were measured as described in Fig. 1.

Again, specific binding activity is stimulated to a greater extent than total binding activity, indicating that the specifically binding component is a larger fraction of the DNA-binding non-histone proteins.

When 5 α -dihydrotestosterone is administered to 7-day-castrated animals, in which extensive atrophy of the prostate has occurred, a proliferative response is induced, as in 3-day-castrated animals. DNA-binding activity of the prostate non-histones is stimulated to the same extent as in 3-day-castrated animals, and specific binding is stimulated to a greater extent relative to total DNA binding (B. H. Lesser & N. L. Elliot, unpublished work).

The dependence of the DNA-binding activity of the prostate non-histones on the dose of steroid administered was examined by treating 1-day-castrated animals for 4 days with various doses of hormone. Fig. 5 demonstrates that the specific DNA-binding activity is linearly dependent on the dose of androgen up to a dose of 10 μg/100 g body

Fig. 5. Effect of dose of androgen on prostatic DNA content and the specific DNA-binding activity of prostate non-histones

Rats that had been castrated 1 day previously were injected daily for 4 days with the indicated dose of 5α -dihydrotestosterone. Prostates were removed and DNA content was estimated from the A_{260} of the purified nuclear lysate, as described in the Materials and methods section (●). Non-histones were isolated as described in the Materials and methods section, and specific DNA-binding activity was measured as described in Fig. 1 (■).

wt. Higher doses do not further stimulate specific binding activity. A similar dependence of total DNA binding on dose of hormone is observed (B. H. Lesser & N. L. Elliot, unpublished work). Fig. 5 also demonstrates that a dose of $10\mu\text{g}/100\text{g}$ body wt. is the minimal dose required to maintain the DNA content of the prostate at the normal value. Below this dose, partial atrophy of the tissue occurs, whereas higher doses do not stimulate the DNA content to increase above normal. Hence the DNA-binding activity of the prostate non-histones is dependent on the dose of androgen administered, and stimulation of this activity by various doses of hormone correlates with the ability of steroid to prevent atrophy of the prostate.

Discussion

It is well established that steroid-receptor complexes enter the cell nucleus and interact with chromatin, resulting in stimulation of RNA polymerase activity and increased RNA synthesis (Schrader & O'Malley, 1978; Liao *et al.*, 1979). Although the molecular events by which these processes occur are poorly understood, the non-histone chromosomal proteins appear to play an important role. There is considerable evidence

indicating that the non-histone proteins play a key role in defining chromatin acceptor sites for steroid-receptor complexes (Thrall *et al.*, 1978; Tymoczko *et al.*, 1978), and the synthesis and phosphorylation of prostate non-histones is stimulated by androgens (Ahmed & Wilson, 1978). Despite strong indications that protein-DNA interactions are important in regulation of transcriptional activity, only one study on such interactions in the rat prostate has been performed. Using DNA-cellulose chromatography, Rennie *et al.* (1975) and Mainwaring *et al.* (1976) demonstrated that androgens stimulate the synthesis of prostate proteins with high affinity for DNA; these proteins were shown to possess DNA-unwinding activity. These proteins appeared to be distinct from histones, but they could not be conclusively identified as non-histone chromosomal proteins, since they were isolated from unfractionated tissue rather than from purified nuclei. In the present study, purified non-histone chromosomal proteins are examined with respect to their DNA-binding properties. By using a different technique to measure DNA-protein interactions, the stimulation of total DNA-binding activity by androgens is confirmed. Furthermore, some of the prostate non-histones are shown to bind preferentially to rat DNA relative to bacterial DNA, and the activity of these specific binding proteins is regulated by androgens.

The nitrocellulose-filter-binding assay provides a rapid and convenient technique for the measurement of DNA-protein interactions. The technique is based on the ability of nitrocellulose filters to retain proteins and protein-DNA complexes, but not uncomplexed DNA. The DNA species employed in these experiments have been treated with the single-strand-specific nuclease S_1 (Lesser & Comings, 1978), and therefore only binding to native DNA is being examined. Fig. 1 shows that total DNA-binding activity can be measured by an assay in which proteins are allowed to bind directly to labelled DNA. Under these conditions, rat prostate non-histones display no preference for binding to rat DNA relative to *E. coli* DNA. Specificity of binding is, however, observable if non-specific binding proteins are sequestered by binding to unlabelled *E. coli* DNA before addition of a trace quantity of labelled DNA. Under these conditions, binding of labelled *E. coli* DNA is decreased to very low values, whereas labelled rat DNA is bound to a 4–5-fold greater extent (Fig. 1). This preferential binding of labelled rat DNA is greatly decreased if pre-incubation is with unlabelled rat DNA. These differences are significant, since duplicate determinations vary by less than 10%, and the differences are reproducible in independent experiments. Similar experiments with rat prostate histones, which are considered to be non-specific with respect to

DNA binding except for a generalized preference for DNA rich in adenine and thymine (Hnilica, 1972), reveal a small preference for binding to rat DNA (30% greater than binding to *E. coli* DNA; B. H. Lesser & N. L. Elliot, unpublished work), but this preference is much less than the 4–5-fold greater binding of rat DNA exhibited by the non-histones and may simply reflect the higher adenine + thymine content of rat DNA (Szybalski, 1968). This is the first demonstration that non-histone proteins isolated from a steroid target tissue display a preference for binding to homologous DNA relative to a bacterial DNA.

Non-histone proteins prepared by the procedure that we have used are contaminated with a small amount of RNA (MacGillivray *et al.*, 1972). In some preparations, RNA contamination in excess of 2% by weight was observed to inhibit retention of DNA by nitrocellulose filters, presumably by competition for binding to protein; these preparations were discarded. We have modified the procedure so as to decrease RNA contamination to less than 1.5% by weight. In order to ensure that even this small amount of RNA is not influencing DNA–protein interactions, a sample of non-histones was digested with ribonuclease A; no effect on DNA-binding activity was observed. DNA-binding activity was completely eliminated by digestion with Pronase (B. H. Lesser & N. L. Elliot, unpublished work).

In addition to demonstrating for the first time the existence of specific DNA-binding non-histone proteins in a steroid target tissue, these experiments also show that the DNA-binding activity of these proteins is hormone-dependent. After castration, decreased activity compared with normal rats is observed; this difference is significant by day 7 after castration (Fig. 2). When 1-day-castrated animals are treated with 5 α -dihydrotestosterone, increased activity is observed within 24 h of the onset of treatment, and this increase is significant by day 3 of treatment (Fig. 3). Total DNA-binding activity is increased as well as specific DNA-binding activity, but to a lesser extent, such that the specific binding activity becomes a larger fraction of the total. For example, in 1-day-castrated animals, the amount of DNA bound in a specific manner is 4% of the total amount of DNA bound by a given quantity of protein, whereas after 6 days of steroid injections this fraction has increased to 13%. Similar results are observed when 3-day-castrated animals are treated with androgen (Fig. 4), with specific DNA-binding activity being stimulated more rapidly. DNA-binding activity is elevated to comparable values during both proliferative periods (the first 4 days of treatment in 3-day-castrated animals) and non-proliferative periods (1-day-castrated animals and days 4–6 of treatment in 3-day-castrated animals). Examination of the prostate non-histones

by electrophoretic techniques during these courses of treatment (B. H. Lesser & N. L. Elliot, unpublished work) reveals variations in the profile of proteins present under various hormonal conditions, as observed previously (Kadohama & Anderson, 1976), but the electrophoretic profiles of non-histones are very complex, and no clear correlation between variations in specific DNA-binding activity and the presence of particular bands can be discerned. The variations in specific DNA-binding activity observed in these experiments are not due to changes in histone contamination, since histones are not detectable in the non-histone fractions by electrophoretic techniques at extents that would be high enough to affect specific DNA-binding activity (Lesser & Comings, 1978). The possibility that nucleases present in the non-histone preparations might nick or cleave DNA during incubation of non-histone proteins with DNA and affect retention of DNA or protein–DNA complexes by nitrocellulose filters cannot be eliminated. However, specific DNA-binding activity is measured by comparing retention of rat DNA and *E. coli* DNA, and nucleases would be expected to affect both DNA species equally.

Stimulation of specific DNA-binding activity is dependent on the dose of steroid. Maximal stimulation is observed with a daily dose of 10 μ g of 5 α -dihydrotestosterone/100 g body wt.; the normal rate of testosterone production in humans is 10 μ g/100 g body wt. per day (Lipsett, 1975). Assuming that the normal rate of production in the rat is similar, this indicates that physiological doses of androgen are capable of stimulating specific DNA-binding activity. A steroid dose of 10 μ g/100 g body wt. is also the minimum dose that maintains the DNA content of the prostate at the normal value after castration (Fig. 5). This is considerably less than the dose of 400 μ g of 5 α -dihydrotestosterone/100 g body wt. per day that elicits the maximal proliferative response (Lesser & Bruchovsky, 1973), but is in the dose range that stimulates the appearance of a prostate-specific isoenzyme of acid phosphatase (Rennie *et al.*, 1978). Hence the specific DNA-binding non-histone proteins may be involved in the appearance of hormone-specific proteins and only indirectly in cell proliferation. In this respect, these proteins are distinct from the androgen-dependent DNA-unwinding proteins (Mainwaring *et al.*, 1976), which are associated with DNA replication and mitosis.

Although further studies will be necessary to establish that the specific DNA-binding activity of the prostate non-histones demonstrated in these experiments is directly involved in hormonal stimulation of genetic activity, it is reasonable to speculate on two ways in which these types of proteins might play a role. Firstly, since there is evidence that

non-histones participate in defining chromatin-acceptor sites for steroid-receptor complexes (Thrall *et al.*, 1978), it is logical to postulate that the fraction of non-histones functioning in this way is the fraction that binds to specific DNA sequences. Thus the hormone-receptor complexes would be directed to specific sites in the nucleus. Secondly, it is not clear whether steroid-receptor complexes interact with chromatin sites coding for hormone-stimulated proteins and stimulate transcription directly, or whether interaction is with distal sites to stimulate the synthesis of intermediate regulatory elements, which then stimulate transcription of hormone-regulated genes (Schrader & O'Malley, 1978; Thrall *et al.*, 1978). There is some evidence, obtained by using inhibitors of protein synthesis, that protein synthesis is required for the appearance of steroid-stimulated mRNA molecules (McKnight, 1978; Chen & Feigelson, 1979). If intermediate regulatory elements are indeed involved in steroid-hormone action, then this might be another likely role for non-histone proteins that bind to specific DNA sequences.

We thank Dr. C. H. Vrba for assistance with animal procedures and Dr. R. D. MacDonald for assistance with tissue-culture procedures. This work was supported by the Medical Research Council of Canada.

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