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IDENTIFICATION OF TWO cDNA CLONES CODING FOR ANDROGEN-DEPENDENT POLYPEPTIDES IN RAT VENTRAL PROSTATE

Y. H. Xu, O. D. Mpanias and T. Y. Wang

Division of Cell and Molecular Biology
Department of Biological Sciences
State University of New York at Buffalo
Buffalo, New York 14260

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SUMMARY: cDNA clones were obtained by transformation of E. coli χ1776 with pBR322 containing insert of ds cDNA synthesized from total rat prostate poly(A)RNA. Two prostate-specific cDNA clones were isolated by colony hybridization and identified by message selection/translation as encoding polypeptides of M_r: 13,500 and 9,300. Hybridization of poly(A)RNA from normal and castrated rat prostates to the cloned cDNAs indicated that the levels of mRNAs coding for M_r: 13,500 and 9,300 polypeptides are regulated by testosterone.

Testosterone, or its active metabolite, dihydrotestosterone, has been shown to stimulate protein synthesis in androgen responsive organs (1). In rat prostate, there are four abundant androgen regulated polypeptides: three of which are associated in the prostatic binding protein (PBP), while the fourth is present as a 20,000 dalton glycoprotein (2 and unpublished results). Since androgens act by regulating gene activity (3), this steroid induced gene expression is an important clue in the understanding of androgen action. In this study, we have investigated androgen-altered transcription in rat prostate by cloning cDNA synthesized from prostate poly(A)RNA and used the cDNA to identify the coding polypeptides. We report here the isolation of two cDNA clones coding for prostate-specific polypeptides. The cloned cDNAs were used as probes in hybridization experiments to determine androgen-dependence of the selected messages.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats of 300 g body weight were used in this study. Bilateral orchidectomy was performed under ether anesthesia. Ventral

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prostates were removed from rats castrated for three days or from normal rats as indicated.

Construction of hybrid plasmid. Synthesis of cDNA from prostate poly(A)RNA and of ds cDNA by AMV reverse transcriptase, insertion of dC-tailed ds cDNA into Pst 1 site of dG-tailed pBR322, transformation of E. coli strain χ1776 by the recombinant plasmid, screening of clones, and selection of Amp^S-Tet^r transformants, were carried out according to Berger et al. (4).

Colony hybridization selection. Selection of clones containing androgen-dependent sequences was carried out by the in situ hybridization technique of Grunstein and Hogness (5). To select androgen-dependent and prostate-specific clones, ^{32}P-labeled cDNAs were synthesized from poly(A)RNAs of normal rat prostate and spleen and castrated rat prostate. The difference in the hybridization observed between the normal and castrated prostate cDNA probes represents the cloned ds cDNA sequences which are complementary to the androgen regulated prostate mRNAs. The spleen cDNA probe served as a second control for prostate specificity. Two cDNA clones, #92 and #119, were selected and shown to have no sequence homology to each other.

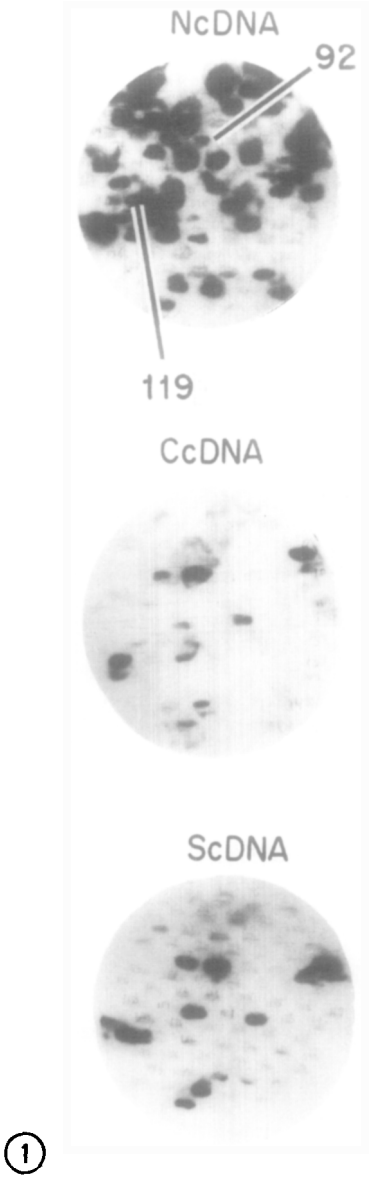
Selection and translation of mRNA. Binding of the DNA on nitrocellulose circles, hybridization with prostate poly(A)RNA from normal rat, and elution of the selected mRNA were done as described by Ricciardi et al. (6). The cell-free protein synthesis system and procedure of Held et al. (7) were used for in vitro translation of the selected mRNA. The translation products were analyzed by electrophoresis on 12% polyacrylamide slab gel (8). A Kodak SB x-ray film was used for the auto-radiography. Immunoreaction of translation products with antisera raised against PBP was carried out as described elsewhere (9).

Other methods. Nick translation of cloned DNA was done by the procedure of Maniatis et al. (10), and Northern hybridization, Alwine et al. (11). Dot hybridization was performed according to White and Bancroft (12).

RESULTS AND DISCUSSION

Fig. 1 shows the colony hybridization of selected Amp^S transformants with [^{32}P]cDNA probes synthesized from prostate poly(A)RNAs of normal (NcDNA) and 3-day castrated (CcDNA) rats and from rat spleen poly(A)RNA (ScDNA). Clones #92 and #119, which were present in NcDNA, were feebly visible in CcDNA and undetectable in ScDNA and, therefore, contained inserts complementary to androgen-dependent, prostate-specific mRNAs. A Northern analysis of the cloned cDNAs is shown in Fig. 2.

Messages of the two cDNA clones were selected from normal rat prostate poly(A)RNA and translated in vitro as described under Materials and Methods. Fig. 3 shows electrophoretic patterns of the translation products of the two selected mRNAs. The molecular weight of the polypeptide coded for by cloned cDNA #92 was determined to be 13,500, and that by clone #119, 9,300.



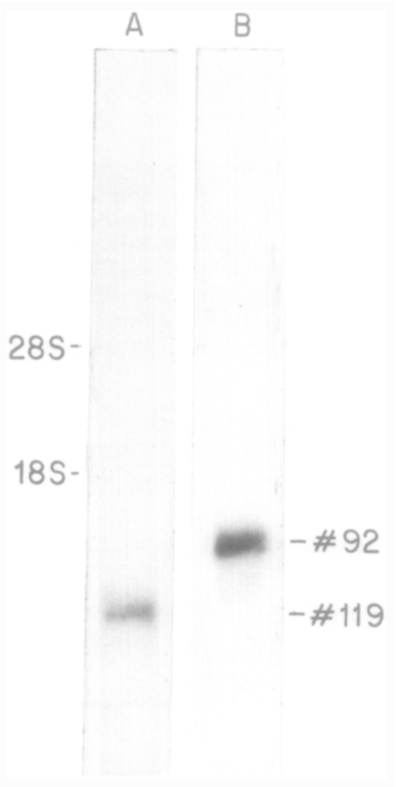


Figure 1. Colony hybridization of the Amp^S transformants. The clones were plated on nitrocellulose filters, grown at 37° C. overnight on agar plates and the colonies were lysed in situ. The DNA was denatured, fixed, and hybridized to 50 x 10^6 cpm of [^{32}P]cDNA synthesized from normal (NcDNA) and castrated (CcDNA) rat prostates and spleen (ScDNA).

Figure 2. Northern hybridization of normal prostatic poly(A)RNA to plasmids #119 (A) and #92 (B). 28S and 18S indicate positions of ribosomal RNA markers.

Since the molecular weight of the translation product of PBP-C1 subunit mRNA is 9,000, it was necessary to determine whether or not clone #119 coded polypeptide was different from PBP-C1. Accordingly, the translation product coded for by clone #119 was assessed by immunoreaction with antiPBP-C1.

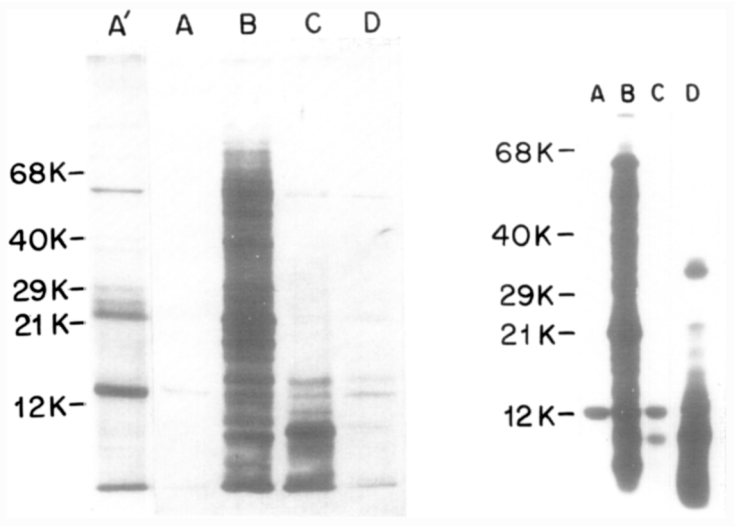


Figure 3. Translation in vitro of mRNAs selected by hybridization of total poly(A)RNA to isolated plasmid DNAs. Left diagram: A, minus mRNA; B, mouse liver poly(A)RNA; C, rat prostate poly(A)RNA; D, selected #92 mRNA; A', same as A, except that the film was exposed 4 times longer to reveal the background bands. Right diagram: A, minus mRNA; B, mouse liver poly(A)RNA; C, selected #119 mRNA; D, rat prostate poly(A)RNA.

Previous work (9) has shown that antiPBP immunoreacts equally efficiently with PBP subunits and the oligomeric protein. AntiPBP was therefore also included in the test. The results in Table I indicate that the 9,300 dalton polypeptide was not related to PBP or its subunits.

To verify that the two selected messages were androgen-dependent, the cloned cDNAs was labeled with [^{32}P]and prostate poly(A)RNAs prepared from normal and castrated rats were hybridized to the [^{32}P]cDNA probes. The results are shown in Fig. 4. In both cases, the concentrations of prostatic mRNAs complementary to the cloned cDNAs were decreased in castrated poly(A)RNA. Semiquantitation of the mRNA levels, determined by counting radioactivities of the dots and shown in the accompanying curves, shows that castration reduced the levels of both mRNAs by about 40%.

Although the cytosolic proteins of rat ventral prostate are predominantly PBP and the 20,000 dalton glycoprotein, both of which account for about 50% of the proteins synthesized in the rat ventral prostate (2, 13), translation in vitro of total prostate poly(A)RNA revealed the presence of several other androgen-dependent polypeptides (8, 13). The strong colony hybridiza-

TABLE I - Immuno-Nonreactivity of 9,300 Dalton Polypeptide to AntiPBP

Antibody	mRNA Directed Translation	% Immunoprecipitation*
AntiPBP-C1	#119 mRNA	0.3
	Total prostatic poly(A)RNA	10.8
AntiPBP	#119 mRNA	0.
	Total prostatic poly(A)RNA	24.6

*Immunoprecipitation is expressed as the percentage of total incorporated radioactivity ([^{35}S]methionine) that was present in the immunoprecipitate. Control assays without added mRNA were run in parallel and the background counts were subtracted from the total incorporated counts and the immunoprecipitated counts.

tion of the two clones and a significantly stained 13,500 dalton band among the translation products of prostate poly(A)RNA (8) indicate that the two coded polypeptides are moderately abundant proteins in rat prostate. Because of the similarity in molecular weight to PBP-C1, the 9,300 dalton polypeptide would generally escape detection among the translated polypeptides of prostate

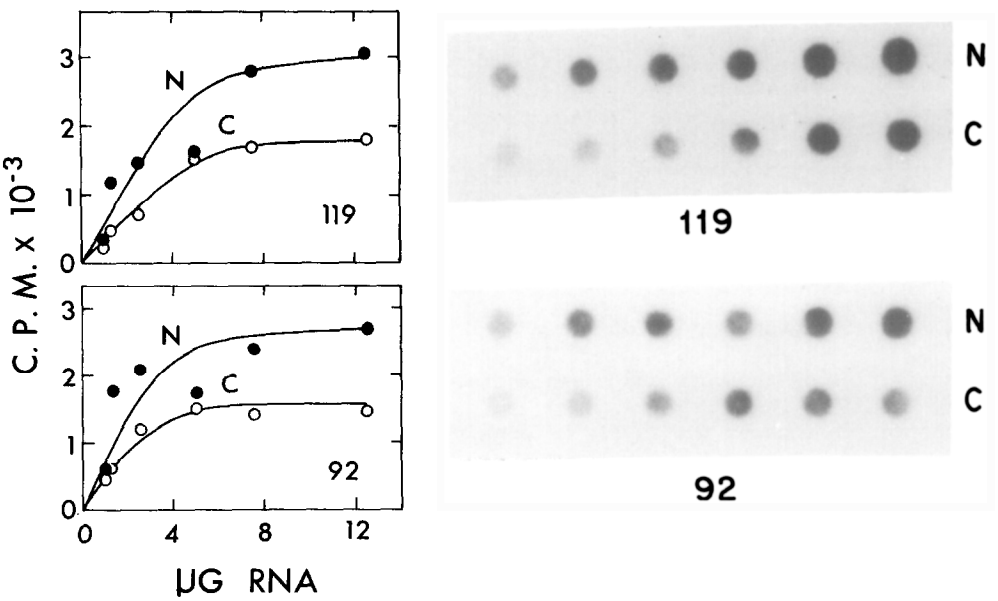


Figure 4. Dot hybridization of prostate poly(A)RNAs from normal (N) and castrated (C) rats to cloned cDNAs #119 and #92. The curves represent radioactivity counts of the dots in relation to concentrations of poly(A)RNA.

poly(A)RNA. The identification of the two cloned sequences should facilitate future isolation of these two androgen-dependent prostate proteins.

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