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Reduction of Prostatic Binding Protein-Messenger Ribonucleic Acid Sequences in Rat Prostate by Castration†

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ABSTRACT: Messenger RNA coding for the three subunits of prostatic binding protein was isolated from polysomal RNA of rat ventral prostate by oligo(dT)-cellulose affinity chromatography and purified by repeated sedimentations through sucrose gradients under denaturing conditions. The purified mRNA migrated as a 9S peak in sucrose gradient centrifugation and hybridized with its cDNA within 2 log R_0t units. In a cell-free reticulocyte lysate system, the mRNA directed the synthesis of three polypeptides of 12 000, 9000, and 8000 daltons. These translation products were identified as the subunits of prostatic binding protein by immunoreaction with antibodies to this protein. Quantitation of prostatic binding protein-mRNA sequences in normal and castrated rats by hybridization with the cDNA probe showed that 3-day castration reduced the prostatic binding protein-mRNA sequences to less than 2% of the normal level. Similar hybridization was performed by using the cDNA to determine the level of prostatic binding protein coding sequences in polysomal poly(A) RNA following castration. The results showed a first-order rate constant of 3.92×10^{-2} h^{-1} for reduction of prostatic binding protein-mRNA sequences in polysomes. The period of castration required to reduce the level of these sequences to 50% of the normal level was calculated to be 17.6 h.

Testosterone regulates the levels of several mRNAs in rat prostate coding for androgen-specific proteins (Heyns & DeMoor, 1977; Parker et al., 1978). Among these, the only known protein is the prostatic binding protein (PBP),[1] which is probably the same protein as the α-protein (Fang & Liao, 1971; Chen et al., 1979), the estramustine binding protein (Forsgren et al., 1979), and prostatein (Lea et al., 1977, 1979). PBP is the most abundant protein synthesized and secreted by rat ventral prostate (Heyns et al., 1977). It consists of three subunits with molecular weights of 13 000, 11 000, and 8000 (Peeters et al., 1980). It is encoded by mRNAs in the abundant class of prostatic poly(A) RNA regulated by androgens (Parker & Scrace, 1978). Three-day castration results in a dramatic decrease in PBP-mRNA as well as cytosolic PBP (Heyns et al., 1977; Hiremath et al., 1981). In vitro translation of rat prostatic poly(A) RNA and immunoprecipitation of the translational product by anti-PBP serum have shown that synthesis of PBP-mRNA occurs early following androgen treatment, detectable 1 h after injection of testosterone to castrated rat (Hiremath et al., 1981).

In the present work, we have examined the androgen regulation of PBP-mRNA sequences in rat prostate by quantitation of PBP-coding sequences following androgen withdrawal. To this end, we have purified the PBP-mRNA from polysomal poly(A) RNA of normal rat prostate and synthesized cDNA complementary to the purified PBP-mRNA to determine the level of PBP-mRNA sequences by RNA excess hybridization. The results are reported herein.

Materials and Methods

Materials. Male Sprague-Dawley rats (300 g) were purchased from Harlan-Sprague Dawley, Madison, WI. Guan-

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[1] Abbreviations used: PBP, prostatic binding protein; TKM, 50 mM Tris-HCl, pH 7.9, 25 mM KCl, and 5 mM $MgCl_2$; EDTA, ethylenediaminetetraacetate; $NaDodSO_4$, sodium dodecyl sulfate; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid.

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idine hydrochloride, urea, and sucrose, all of ultrapure grade, were purchased from Schwarz/Mann, Inc., Spring Valley, NY. Oligo(dT)$_{12-18}$, oligo(dT)–cellulose (type 7), and S1 nuclease were from P-L Biochemicals, Milwaukee, WI. [^{3}H]dCTP and L-[^{35}S]methionine were from New England Nuclear, Boston, MA. Unlabeled deoxyribonucleoside 5′-triphosphates, steroid, diethyl pyrocarbonate, and cyanogen bromide were obtained from Sigma Chemical Co., St. Louis, MO. Avian myeloblastosis virus RNA-dependent DNA polymerase was a gift from Dr. J. Beard through the National Cancer Institute. Goat anti-rabbit IgG was purchased from Calbiochem-Behring Corp., San Diego, CA. Macaloid was obtained from Baroid Division, National Lead Co., Houston, TX. All apparatus were rinsed with double glass-distilled water containing 0.1% diethyl pyrocarbonate and autoclaved before use.

Treatment of Animals. Rats were castrated by bilateral orchidectomy for 12, 24, 48, and 72 h. The animals were killed by decapitation and the ventral prostates taken directly in 8 M guanidine hydrochloride solution for isolation of total RNA. For preparation of polysomes, the tissue was minced and suspended in TKM containing 1 mg/mL Macaloid. The unoperated (normal) animals were treated in a similar manner.

Isolation of Total RNA. RNA was isolated from prostate or polysomes by a modified procedure (Toole et al., 1979) of Cox (1968). The tissue or polysomes were suspended in 10 volumes of 8 M guanidine hydrochloride in buffer A (50 mM Tris-HCl, pH 7.5, and 10 mM EDTA), and the mixture was homogenized in an omnimixer for 2 min. The homogenate was centrifuged for 5 min at 3000*g*. The RNA in the supernatant was precipitated by addition of 0.5 volume of ethanol, and the solution was allowed to stand at −20 °C for 1 h. The precipitate was collected by centrifugation at 10000*g* for 10 min, suspended in 6 M guanidine hydrochloride in buffer A, and reprecipitated with ethanol. This step was repeated twice more. The precipitate was then suspended in distilled water and precipitated 4 times by ethanol, each time by addition of 0.1 volume of 2 M sodium acetate, pH 5.5, and 2 volumes of ethanol, followed by standing at −20 °C for 1 h. The final precipitate was freeze-dried.

Isolation of Polysomal RNA. The minced tissue was suspended in TKM buffer containing 1 mg/mL Macaloid and homogenized with a motor-driven Teflon homogenizer. The homogenate was centrifuged at 6000*g* for 10 min. The postnuclear fraction was centrifuged at 27000*g* for 15 min, from which the supernatant was centrifuged at 135000*g* for 90 min. The pelleted polysomes were suspended in guanidine-HCl–buffer A, and RNA was isolated as described above.

Isolation of Polyadenylated RNA. The poly(A) RNA was isolated essentially as described by Aviv & Leder (1972). Total RNA was suspended in buffer B (10 mM Tris-HCl, pH 7.5, 0.1% NaDodSO$_4$, and 1 mM EDTA) containing 0.4 M KCl and passed through an oligo(dT)–cellulose column previously equilibrated with 0.4 M KCl–buffer B. The unbound RNA was washed through with the same buffer, and the bound poly(A) RNA was eluted with buffer B containing no KCl. The poly(A) RNA was precipitated by the addition of 0.1 volume of 2 M sodium acetate, pH 5.5, and 2 volumes of ethanol, followed by standing at −20 °C overnight. The yield of poly(A) RNA was about 5–7% of the totoal RNA.

Sucrose-Gradient Centrifugation. The lyophilized poly(A) RNA sample was dissolved directly in buffer C (10 mM Tris-HCl, pH 7.5, 0.5% NaDodSO$_4$, 6 M urea, and 10 mM EDTA), incubated for 1 min at 70 °C, and applied on 11.6 mL of 5–20% sucrose gradients in buffer C. Centrifugation was carried out in a Spinco SW41 rotor at 38000 rpm for time periods as indicated in the figure legends. The gradients were fractionated by using an ISCO gradient fractionator.

Cell-Free Translation. Protein synthesis in vitro was performed by using the reticulocyte lysate system of New England Nuclear. About 0.5 μg of template RNA was used in a total reaction volume of 25 μL. Total radioactivity incorporated was determined as described by Bollum (1968). For the analysis of the translation products, the reaction mixture was made to 0.065 M Tris-HCl, pH 6.8, 1% NaDodSO$_4$, and 5% β-mercaptoethanol, heated in a boiling water bath for 3 min, and analyzed by NaDodSO$_4$–polyacrylamide gel electrophoresis (Laemmli, 1970). The gel was stained and, after destaining, treated with Enhancer (New England Nuclear) for 1 h. It was dried and autoradiographed by using Kodak BB-1 X-ray film.

Preparation of PBP and Anti-PBP. PBP was isolated according to the procedure described by Heyns et al. (1978). The purified protein gave three bands on the NaDodSO$_4$–polyacrylamide gel electrophoresis system of Laemmli with calculated molecular weights of 12000, 10000, and 8000. For preparation of PBP antiserum, about 3 mL of the protein solution (A_{280} = 0.86) was emulsified with an equal volume of Freund's adjuvant (Difco) and administered to two female New Zealand rabbits by intradermal injections at multiple sites. The rabbits were bled from an ear vein 6 weeks later. The antiserum was found to be specific to PBP as checked by Ouchterlony double immunodiffusion and by Laurell rocket immunoelectrophoresis. In order to avoid nonspecific precipitation, anti-PBP was purified by affinity chromatography using PBP immobilized on cyanogen bromide activated Sepharose 4B (March et al., 1974) as described by Shapiro et al. (1974).

Immunoprecipitation. Cell-free protein synthesis was done as described previously. At the end of the protein synthesis reaction, an equal volume of buffer D (50 mM potassium phosphate, pH 7.5, 1% deoxycholate, and 1% Triton X-100) was added to the reaction mixture. To this mixture was further added 10 μL of the purified anti-PBP. The mixture was incubated for 60 min at 37 °C. The antigen–antibody complex was precipitated by addition of an excess of goat anti-rabbit IgG and incubated at 37 °C for 2 h. The mixture was then centrifuged through 1 M sucrose in buffer D, and the immunoprecipitate was washed with buffer D. For polyacrylamide gel electrophoretic analysis, the immunoprecipitate was suspended in 0.068 M Tris-HCl, pH 6.8, containing 1% NaDodSO$_4$ and 5% β-mercaptoethanol, and heated in a boiling water bath for 10 min before being subjected to electrophoresis. Radioactivity of the immunoprecipitate was determined by incubation in 0.7 mL of NCS tissue solubilizer (Amersham-Searle) at 37 °C for 3–4 h and counting in 10 mL of toluene scintillation cocktail in a Beckman LS 80 scintillation spectrometer.

[^{3}H]cDNA Synthesis. Complementary DNA to purified PBP-mRNA using [^{3}H]dCTP as the labeled substrate was synthesized according to Toole et al. (1979). The reaction mixture contained 50 mM Tris-HCl, pH 8.3, 6 mM $MgCl_2$, 12 mM β-mercaptoethanol, 60 μM [^{3}H]dCTP, 240 μM each of dATP, dCTP, and dTTP, 10 μg/mL oligo(dT)$_{12-18}$, 40 μg/mL actinomycin D, 50 units/mL avian myeloblastosis virus reverse transcriptase, and 1 μg of the purified mRNA. The reaction mixture, in a final volume of 30 μL, was incubated at 45 °C for 30 min, and the synthesized [^{3}H]cDNA was isolated by chromatography on a Sephadex SP-50 column (Toole et al., 1979) equilibrated with 0.01 M sodium acetate,

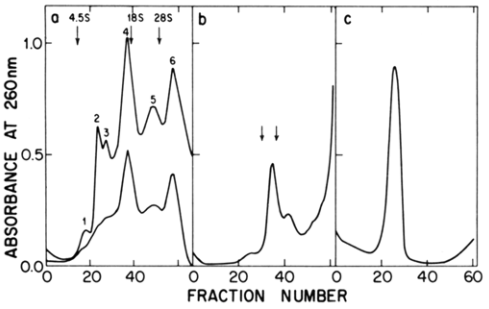

FIGURE 1: Purification of PBP-mRNA. (a) Sucrose-gradient centrifugation of polysomal poly(A) RNA from rat prostate. Prostatic polysomal poly(A) RNA prepared by oligo(dT)–cellulose affinity chromatography was dissolved in 0.25 mL of buffer C, incubated at 70 °C for 1 min, and applied on 11.6 mL of 5–20% linear sucrose gradients in buffer C. The centrifugation was carried out with a Spinco SW41 rotor at 38 000 rpm for 24 h. Sedimentation is from left to right. Upper curve, poly(A) RNA (100 μg) from normal rat prostate; lower curve, poly(A) RNA (50 μg) from 2-day castrated rat prostate. (b) Normal rat prostate polysomal poly(A) RNA was centrifuged through sucrose gradients as in (a) but for 36 h. The fraction indicated between arrows was collected from six runs and pooled, and RNA was precipitated with ethanol. (c) The precipitated RNA from (b) was dissolved in buffer C and subjected to sucrose-gradient centrifugation as in (a).

pH 5.0, containing 0.3 M NaCl and 0.2% $NaDodSO_4$. The [^{3}H]cDNA was precipitated by ethanol as described previously, with λ DNA as carrier (10 μg/mL), and washed twice at −20 °C with 70% ethanol containing 10 mM Tris-HCl, pH 7.4, and 50 mM NaCl. The [^{3}H]cDNA had an average chain length of 350 nucleotides as determined by alkaline sucrose gradient centrifugation (Imiazumi et al., 1973) and had a specific activity of about 10^7 cpm/μg.

RNA–cDNA Hybridization. Nucleic acid hybridization was performed essentially as described by Hastie et al. (1979). The reaction was carried out in 53 μL containing 20 mM Hepes buffer, pH 7.0, 0.36 M NaCl, and 1 mM EDTA at 70 °C in a small plastic vial overlaid with mineral oil. Aliquots of 3 μL were withdrawn at varying time intervals and diluted to 0.35 mL with cold distilled water. To each mixture were added 100 μL of 5-time concentrated S1 nuclease buffer (100 mM sodium acetate, pH 5.0, 250 mM NaCl, and 3 mM $ZnSO_4$) and 25 μg/mL sheared and denatured calf thymus DNA, and the volume was adjusted to 500 μL. Two equal aliquots were taken out, to one was added 50 units of S1 nuclease, and both were incubated at 37 °C for 1 h. After addition of 100 μL of 5 mg/mL bovine serum albumin, the mixtures were made 5% in trichloroacetic acid and let stand on ice for 5–10 min. The precipitate formed was collected on GF/C (Whatman) glass fiber filters and washed with 5% trichloroacetic acid. The filters were dried, treated with 0.4 mL of NCS tissue solubilizer, and counted in 10 mL of toluene scintillation cocktail. Hybridization was expressed as the percentage of undigested control [^{3}H]cDNA.

Results

Isolation of PBP-mRNA. Polysomal poly(A) RNA isolated from normal and castrated rat prostates was initially analyzed by sucrose-gradient centrifugation to determine androgen-regulated changes in prostatic mRNA. The sedimentation profiles are shown in Figure 1a in which six well-defined peaks may be discerned. Androgen deprivation resulted in a decrease in fraction 2 and a slightly reduced fraction5, while the relative amounts of other gradient fractions were not affected by changes in the androgenic state.

Selected peak fractions from the sedimentation pattern of normal poly(A) RNA gradients were collected to identify the

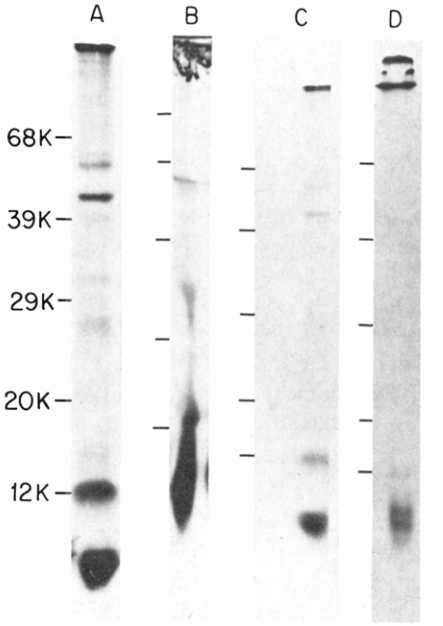

FIGURE 2: In vitro translation of mRNA. (A) Sucrose-gradient fraction 2 shown in Figure 1a was precipitated, washed, and translated in a reticulocyte lysate system as described under Materials and Methods. The translation product was treated with 1% $NaDodSO_4$ containing 5% mercaptoethanol and 0.068 M Tris-HCl, pH 6.8, heated in a boiling water bath for 3 min, and subjected to $NaDodSO_4$ electrophoresis (Laemmli, 1970) on 12.5% acrylamide gels. (B) Cell-free protein synthesis directed by sucrose-gradient RNA fraction 2 was carried out as in (A). The reaction mixture was subjected to immunoprecipitation with purified anti-PBP, and the precipitate was processed as described under Materials and Methods. The immunoprecipitate was treated with 1% $NaDodSO_4$ containing 5% mercaptoethanol and 0.068 M Tris-HCl, pH 6.8, heated in a boiling water bath for 10 min, and analyzed by $NaDodSO_4$ electrophoresis on a 15% acrylamide gel. The lines correspond to markers as in (A). (C) In vitro protein synthesis in a reticulocyte lysate system directed by the purified mRNA shown in Figure 1c was performed as in (A). The product was subjected to $NaDodSO_4$ electrophoresis on a 12.5% acrylamide gel. (D) The reaction product shown in (C) was immunoprecipitated, and the immunoprecipitate was subjected to $NaDodSO_4$ electrophoresis on a 15% acrylamide gel.

mRNA activities of six RNA fractions, and RNA was precipitated with ethanol as described under Materials and Methods. The pellet was washed twice with 70% ethanol containing 10 mM Tris-HCl, pH 7.4, and 50 mM NaCl and used for translation in a reticulocyte lysate system. Of the six RNA gradient fractions, fractions 5 and 6 coded for high molecular weight polypeptides of >44 000, and fractions 2 and 3, mainly low molecular weight polypeptides <20 000. Fraction 4 showed translated polypeptides with a range of sizes. Fraction 1, owing to its low concentration, manifested only feeble messenger activity and coded for an 8000-dalton polypeptide. Since the subunits of PBP are of low molecular weight (Heyns et al., 1979), only the translation products of fractions 2 (9 S) and 3 (12 S) were of concern. Three major polypeptides of 12 000, 9000, and 8000 daltons were encoded by fraction 2, as shown in Figure 2A. Fraction 3 coded for an additional polypeptide of 20 000 daltons. The molecular weights of the three small polypeptides encoded by fraction 2 correspond closely to those of the subunits of PBP. For determination of whether these were the PBP subunits, each of the above polysomal poly(A) RNA gradient fractions was translated in the cell-free system, the translated products were tested by immunoprecipitation with anti-PBP, and the immunoprecipitates were analyzed by $NaDodSO_4$ electrophoresis on 15% polyacrylamide gel. The results, shown in Figure 2B, showed that the low molecular weight polypeptides were im-

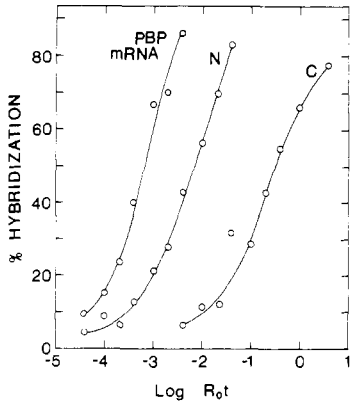

FIGURE 3: RNA excess hybridization of [^{3}H]DNA complementary to purified PBP-mRNA. Purified PBP-mRNA and total poly(A) RNA from normal (N) and 3-day castrated (C) rat prostates were each hybridized with [^{3}H]cDNA (40 000 cpm) as described under Materials and Methods.

munoprecipitated by PBP-specific antibodies and were present only in the translation products of fraction 2 (and fraction 3, data not shown).

For purification of the PBP-mRNA from fraction 2, batches of about 150 μg of polysomal poly(A) RNA were centrifuged through 5–20% sucrose gradients in buffer C at 38 000 rpm in a Spinco SW41 rotor for 36 h. This effectively pelleted the larger species of poly(A) RNA and separated fractions 1, 2, and 3 from the other fractions, as shown in Figure 1b. Fraction 2 was collected, precipitated with ehtanol, and dissolved in buffer C. The sample was recycled on 5–20% linear sucrose gradients in buffer C at 38 000 rpm for 24 h. A sedimentation profile of the purified fraction 2 as a single peak is shown in Figure 1c, estimated to be of 9 S.

Purity of the PBP-mRNA. About 0.2 μg of the purified mRNA was translated in the reticulocyte lysate system. The translation products were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis, and the autoradiograph is shown in Figure 2C, which shows the association of radioactivity with the three migrating polypeptides of molecular weights of 12 000, 9000, and 8000. The translation products were subjected to immunoprecipitation using the PBP-specific antibodies to ascertain the relationship of these polypeptides with PBP. The immunoprecipitate was then analyzed by $NaDodSO_4$ electrophoresis on a 15% acrylamide gel. The autoradiograph in Figure 2D shows that these polypeptides encoded by this mRNA fraction were the subunits of PBP.

Quantitation of the immunoprecipitable and total radioactivities incorporated in the in vitro protein synthesis reaction, determined as described under Materials and Methods, showed that more than 95% of the incorporated radioactivities was precipitated by the specific antibodies to PBP.

Complementary DNA directed by the purified PBP-mRNA was synthesized in an avian myeloblastosis virus reverse transcriptase reaction to further characterize the purified PBP-mRNA. Analysis of the PBP-cDNA in alkaline sucrose gradients showed a broad sedimentation pattern with a peak of 3.7 S, estimated to be about 350 nucleotides in length. RNA excess hybridization of the PBP-mRNA with [^{3}H]-cDNA was performed as described under Materials and Methods, and the results are shown in Figure 3. The hybridization was nearly complete within 2 log R_0t units, and the $R_0t_{1/2}$ of the PBP-mRNA was 5.6×10^{-4} mol·s/L.

Effect of Castration on the Levels of PBP-mRNA Sequences in Total and Polysomal Poly(A) RNA. To ascertain androgen regulation of PBP-mRNA, we used hybridization with [^{3}H]cDNA to quantitate the PBP-mRNA sequences in

Table I: Levels of PBP-mRNA Sequences in Polysomal RNA of Rat Prostate

period of castration (h)	polysomal RNA $R_0t_{1/2}$	polysomal RNA % of normal (P)
0	5.01×10^{-3}	100
12	7.94×10^{-3}	63.1
24	1.26×10^{-2}	39.8
48	5.62×10^{-2}	8.9
72	2.5×10^{-1}	2.0

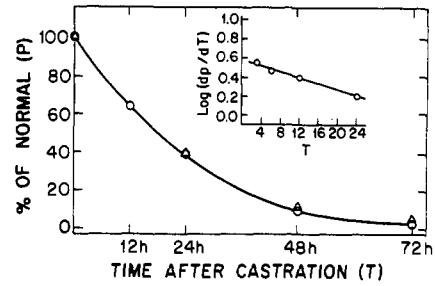

FIGURE 4: Effect of castration on the reduction of PBP-coding sequences in polysomal poly(A) RNA. The curve for polysomal poly(A) RNA (○) was plotted from the hybridization data shown in Table I. From this, the slope dP/dT, was calculated at different time points (T), and the insert shows a plot of log (dP/dT) vs. T. The symbols used are the same. The nuclear androgen receptor levels (△) were calculated from the data of Davies et al. (1979).

total prostatic poly(A) RNA from normal and castrated rats, as shown in Figure 3. The data showed that about 10% of total prostatic poly(A) RNA is represented by PBP-mRNA sequences in normal rat. Castration for 3 days resulted in reduction of these sequences to 0.17% of total sequences, a difference of almost 2 orders of magnitude.

Since the total prostatic poly(A) RNA included both nuclear and polysomal RNAs, it was not clear whether the reduced PBP-mRNA sequences were matured mRNA. For determination of this, poly(A) RNA was isolated from polysomes of unoperated (normal) rats and rats castrated for 12, 24, 48, and 72 h. These polysomal poly(A) RNA preparations were hybridized to [^{3}H]cDNA as described under Materials and Methods. The $R_0t_{1/2}$ values obtained are summarized in Table I. Taking the number of PBP-mRNA sequences in normal prostate as 100%, we calculated the percentage (P) of PBP-mRNA sequences remaining at the end of each period following castration (T). Figure 4 shows a plot of P vs. T. From this curve, the slope (dP/dT) at different time castration periods was calculated, and the relationship between log (dP/dT) vs. T, shown in the insert, yielded a straight line, indicating that the reduction of PBP-mRNA sequences by castration in a polysomal fraction was a simple first-order process. The castration period required to reduce the PBP-mRNA sequences to half of the normal prostate level was estimated to be 17.6 h. The calculated rate constant for these decreases following castration was 3.94×10^{-2} h^{-1}.

Discussion

Isolation of PBP-mRNA by sucrose-gradient centrifugation of rat prostatic poly(A) RNA has recently been reported by Peeters et al. (1980). The biological purity of this PBP-mRNA, determined by immunoprecipitation, is 75%. In the present work, recycling sucrose-gradient centrifugation under denaturing conditions has been used for further purification of the PBP-mRNA. Immunoprecipitation of the mRNA-directed cell-free translation products showed that more than 95% of incorporated [^{35}S]methionine was immunoprecipitable

by anti-PBP. However, since much of the label did not enter the gel, the purity of the mRNA may be significantly less than 95%. Hybridization reaction of the RNA with complementary DNA showed a rapid annealing reaction occurring within 2 log units, indicating a relatively homogeneous population of the PBP-mRNA sequences.

When centrifuged through sucrose gradients, the purified PBP-mRNA appeared as a single sedimentation peak with an apex at 9 S, corresponding to about 600 nucleotides, or a molecular weight of 2×10^5. These values compare well with those of Peeters et al. (1980), who estimate the PBP-mRNAs to contain 660–760 nucleotides (molecular weight 2.2×10^5–2.5×10^5), including the 5′-capping leader sequence and the 90-nucleotide poly(A) tail. Parker et al. (1980) have determined the size of the mRNAs of the three PBP subunits and found them to be 930, 640, and 550 nucleotides.

The estimated molecular weights of the PBP subunits of the PBP-mRNA-directed translation polypeptides are somewhat at variance with the 13 000, 11 000, and 8000 values reported by Peeters et al. (1980), although the PBP preparation we used to raise the antisera was prepared by the same procedure as theirs (Heyns et al., 1979). This discrepancy could be due to different in vitro protein synthesizing systems employed or may be attributed to the $NaDodSO_4$–polyacrylamide gel electrophoresis system for the analysis of the subunits. In a 15% $NaDodSO_4$–acrylamide gel, a system used by Peeters et al. (1980) as well as in the present work, migration of polypeptides of molecular weights of <12 000 (cytochrome *c*) is not completely resolved, consequently resulting in the variances in the calculated molecular weights of these polypeptides.

Heyns et al. (1977) have shown that 4-day castration results in a marked decrease in PBP, accompanied by a corresponding decrease of PBP-mRNA activity. Using cloned complementary DNA, Parker et al. (1980) have shown that the 10 000-dalton polypeptide mRNA copies per cell in rat prostate decrease to about 1% of that of the normal rat after castration for 3 days. These decreases are partially restored (Heyns et al., 1977; Parker & Scrace, 1978, 1979) as early as 2 h after androgen replacement (Hiremath et al., 1981). Consistent with these observations, the level of PBP-mRNA sequences in castrate prostate, calculated from the hybridization data, was reduced to 1.7% of the normal value.

It is interesting to note that Davies et al. (1979) have determined the cellular complements of both cytoplasmic and nuclear androgen–receptor complexes following castration. The rate of reduction of nuclear receptor levels, plotted from their data, coincides with that of polysomal PBP-coding sequences following castration (Figure 4). This is consistent with the results that regulation of transcription of PBP genes is one of the androgen actions in rat ventral prostate.

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