Hormonal Regulation of Protein Synthesis in Rat Ventral Prostate

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Synopsis

To elucidate details of hormonal regulation mechanism in rat ventral prostate, synthesis of RNAs that contain polyadenylate sequences, polyribosomal sedimentation profile, ribosomal capacity of ^3H -Met-tRNA binding, ribosomal initiation factor activity and the rate of polypeptide chain elongation were examined in the ventral prostate from control and castrated animals. The following results were obtained; 1) The concentration of RNAs that contain polyadenylate sequences in cytoplasm of castrated rats was markedly decreased, while the content in nuclear fraction was not influenced significantly by castration. 2) Polyribosomes disappeared quite rapidly after castration and were completely restored 48 hr after testosterone administration. 3) Ribosomal binding capacity with ^3H -Met-tRNA and ribosomal initiation factor activity were also reduced significantly. Testosterone treatment of castrated animals completely abolished the effect of castration on ribosomal binding capacity with ^3H -Met-tRNA and initiation factor activity. 4) A relatively small effect of castration on the relative rate of polypeptide chain elongation in rat ventral prostates was observed. These results may indicate that the regulation of protein synthesis exerted by testosterone in rat ventral prostates is not restricted at the step of the transcription of mRNA.

The effect of androgen on the protein synthesis in male accessory sex organs has mostly been attributed to the formation of new mRNAs and ribosomes (see a review by Williams-Ashman and Shimazaki, 1969). However, there has been considerable evidence to suggest that the protein synthesis is regulated at the posttranscriptional level (Thompson *et al.*, 1970; Tomkins *et al.*, 1972). It has also been claimed by several investigators that the rate determining step in the protein synthesis occurs

during the initiation phase of the translation (Fleck *et al.*, 1965; Stirewalt *et al.*, 1967; Vaughan *et al.*, 1971; Van Venrooji *et al.*, 1972; Pain, 1973).

To elucidate details of hormonal regulation mechanisms of protein synthesis in one of the androgen target tissues, we compared the rate of synthesis of RNAs that contain polyadenylate sequences (poly A-RNA), polyribosomal sedimentation profile, ribosomal capacity of ^3H -Met-tRNA binding, ribosomal initiation factor (IF) activity and the rate of polypeptide chain elongation in ventral prostates from castrated rats with the above rate in control rats. In some experiments, the effect of testosterone administration to castrated animals was also examined. The results obtained will be described as follows.

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Abbreviations used; Met-tRNA; Methionine accepting transfer ribonucleic acid, ^3H -Met-tRNA; tRNA which was charged with ^3H -methionine, poly A; polynucleotide of adenylate, poly U; a synthetic polynucleotide of ulidylate, poly AUG; a synthetic polynucleotide of adenylate, uridylate and guanylate (1:1:1).

Materials and Methods

Animals

Male Wistar rats weighing 300–350 g were used. They were fed *ad libitum* with balanced diet (Clea CA-1, Nippon Clea K.K. Tokyo) and kept in a room of constant temperature (25°C) and humidity (60%) with 7 a.m.–7 p.m. day-light cycle. Castration was performed via scrotal route under ether anesthesia. Sham-operations were performed in the same manner except that testes were not removed. For replacement therapy of castrated animals, 2 mg of testosterone propionate dissolved in 0.1 ml of cotton seed oil were injected subcutaneously to each animal once every 24 hrs and in most cases they were sacrificed for experiments 12 hrs after the last hormone administration.

Determination of poly A-RNA in nuclear and cytoplasmic fractions

The method used for the determination of poly A-RNA was essentially the same as described by Sheldon *et al.* (1972). 1.2 mCi of ^{32}P -orthophosphate were injected to each rat intraperitoneally and animals were sacrificed 40 min postinjection. Approximately 2 g of the tissue (from 6 control rats or from 8 castrated rats) were pooled and homogenized with 7 volumes of ice-cold medium containing 250 mM sucrose, 25 mM KCl, 5 mM MgCl_2 and 50 mM Tris-HCl (pH 7.6). The homogenate was centrifuged at $700 \times g$ to separate the nuclear fraction from cytoplasmic one. RNA was extracted from these two fractions by the SDS-phenol method of Lee *et al.* (1971) with minor modifications. 200 μg of poly U (Boehringer, Mannheim, Germany) were dissolved in distilled water and applied onto fiberglass filter (Whatman GF/C, 2.4 cm), dried and irradiated with UV-Lamp as described by Sheldon *et al.* (1972). Extracted RNA in which the relative concentration of poly A-RNA is to be determined was dissolved in a medium containing 10 mM Tris-HCl (pH 7.5) and 120 mM NaCl and applied onto a poly U filter. The filtration was performed by light suction at the rate of 1.0 ml/min, followed by washing the filter with 30 ml of the buffer and 10 ml of 5% trichloroacetic acid. The radioactivity retained on the dried filter was counted in a liquid scintillation spectrometer. Almost linear relationship was observed between the amount of the radioactivity retained on the poly U filter and the amount of input RNA within a limited range of input RNA. Three different amounts of RNA was applied to the filter for each assay and an average was taken as the rate of poly A-RNA concentration.

Ribosomal sedimentation profiles

The homogenate of the ventral prostate prepared as described above was fractionated by the method of Wettstein *et al.* (1963) and polysomal pellets were precipitated from the postmitochondrial fraction through a discontinuous sucrose gradient of 0.5 and 2.0 M after addition of sodium deoxycholate at the final concentration of 0.5%. The pellets were re-suspended in the medium used for the homogenization and layered on a continuous sucrose gradient (0.3 M to 1.0 M). After centrifugation in a Hitachi RPS 25 A rotor at 25,000 rpm for 2 hr, each 1 ml fraction was taken from the top of the gradient and UV-absorption at 260 nm was determined.

Preparation of ribosomes, initiation factor (IF) fraction and ^3H -Met-tRNA

Ventral prostates obtained from 15 intact control, 12 hormone-treated and 20 castrated rats were pooled by each group and homogenized in 2 volumes of the homogenizing medium. Ribosomes were separated according to the method described by Schreier and Staehelin (1973a) through a cushion of 2 M sucrose. Ribosomal pellets were suspended in 1.2 ml of a medium containing 50 mM Tris-HCl (pH 7.6), 10 mM MgCl_2 , 100 mM NH_4Cl and 4 mM mercaptoethanol. An aliquot of this suspension was used for the determination of ^3H -Met-tRNA binding capacity and another aliquot was used for the preparation of IF fraction.

The IF fraction was prepared essentially according to the method described by Schreier and Staehelin (1973a). To the suspension of ribosomes, 4 M KCl solution was slowly added to make the final concentration of 0.5 M with respect to the total salt concentration and stirred for 60 min at 0°C. Ribosomes were removed from this suspension by centrifugation at $250,000 \times g$ for 100 min and the supernatant was dialysed overnight against a large volume of medium which consisted of 10% glycerol, 20 mM Tris-HCl (pH 7.6), 0.5 mM EDTA and 4 mM mercaptoethanol. An aliquot of this dialysate was used for the determination of IF activity. Rat liver IF was prepared by almost the same method but the ribosomal salt-wash fraction was further fractionated by $(\text{NH}_4)_2\text{SO}_4$, the precipitate obtained between 35% and 80% saturation was dialyzed and used for the experiment.

tRNA was prepared from rat livers by the method of Zubay (1962) and charged with ^3H -methionine (specific activity 3 Ci/mmol, The Radiochemical Center, Amersham, England) by the method of Hoagland *et al.* (1958) using the rat liver pH 5 enzyme. The specific activity was 1,700 cpm/pmol.

Assay of ribosomal ^3H -Met-tRNA binding capacity and IF activity

The incubation mixture for the determination of

ribosomal capacity to bind ^3H -Met-tRNA contained 50 mM Tris-HCl (pH 7.6), 100 mM NH_4Cl , 3 mM MgCl_2 , 4 mM mercaptoethanol, 2 mM GTP (Boehringer, Mannheim, Germany), 16 pmoles of ^3H -Met-tRNA (27,200 cpm) and 70–90 μg of ribosomes (as protein) in a final volume of 0.25 ml. Incubations were carried out at 37°C for 20 min. After incubation, the reaction mixture was diluted with 3 ml of ice-cold buffer solution containing 50 mM Tris-HCl (pH 7.6), 100 mM NH_4Cl , 7 mM MgCl_2 and 4 mM mercaptoethanol and filtered through a Millipore filter (HA 0.45 μ , 25 mm). The filter was washed with 6 ml of the diluting buffer solution and the radioactivity retained on the dried filter was counted in a liquid scintillation spectrometer. Under these incubation conditions, the reaction proceeds with incubation time up to 40 min but the rate of the reaction showed a tendency of leveling-off after 25 min. The almost linear relationship was observed between the amount of ^3H -Met-tRNA retained on the filter and the amount of ribosomes added to the incubation mixture up to 400 μg (as protein).

The assay of IF activity was carried out by essentially the same method described by Dubnoff and Maitra (1971). The salt-washed ribosomes used in this assay were separated from rat livers and incubated in a complete protein-synthesizing system described by Schreier and Staehelin (1973a) at 35°C for 50 min. After the incubation, the mixture was chilled and 4 M KCl was added to bring the salt concentration to 0.5 M. The ribosomes were recovered by centrifugation at $150,000 \times g$ for 60 min after stirring the mixture for 60 min at 0°C and then suspended in a medium containing 20 mM Tris-HCl (pH 7.6), 10 mM MgCl_2 , 30 mM NH_4Cl , 10 mM mercaptoethanol and 0.5 mM EDTA. The suspension was passed through a column of Sephadex G-100 (1 cm $\times$ 50 cm) equilibrated with the suspending medium and the ribosomes eluted at the excluded volume were collected. These ribosomes could be stored at 0°C for at least 6 weeks without any detectable loss of binding activity. The reaction mixture contained 50 mM Tris-HCl (pH 7.8), 100 mM NH_4Cl , 4 mM MgCl_2 , 4 mM mercaptoethanol, 2 mM GTP, 60 μg of salt-washed ribosomes (as protein), initiation factor fraction, 6 μg of poly AUG (PL Biochemicals Inc., Milwaukee, Wis., U.S.A.) and 8 pmoles of ^3H -Met-tRNA (13,600 cpm) in a final volume of 0.3 ml. Incubations were carried out at 37°C for 10 min. ^3H -Met-tRNA bound to ribosomes was separated by a Millipore filter as described in the preceding section. One unit of IF was defined tentatively as the amount of IF which provokes binding of 1 pmole of ^3H -Met-tRNA to salt-washed ribosomes under the incubation conditions described above. Typical reaction curves against increasing amounts of IF are presented in

Fig. 1. Effect of various amounts of the ribosomal salt-wash fraction isolated from ribosomes of the ventral prostate of control and castrated rats on the ^3H -Met-tRNA binding to rat liver salt-washed ribosomes in the presence of poly AUG and GTP.

Fig. 1. The almost linear relationship was obtained between ^3H -Met-tRNA retained on the filter and the amount of the IF fraction added within a limited range of protein concentrations.

Determination of relative rate of polypeptide elongation

To assess the relative rate of polypeptide elongation in the ventral prostate from control and castrated rats, the ratio method described by Palmiter (1972) was adopted. 20 μCi of ^{14}C -L-Leucine (300 mCi/mole, The Radiochemical Centre, Amersham, England) was injected intraperitoneally into one rat and 100 μCi of 4,5- ^3H -L-leucine (32 Ci/mole, Daiichi Pure Chemical Co., Tokyo) was injected to another one. Exactly 10 min later, both animals were sacrificed. The tissues obtained from each animal received ^3H - or ^{14}C -leucine were combined and the homogenate was prepared as described. The post-mitochondrial supernatant prepared after centrifugation at $12,000 \times g$ for 20 min was recentrifuged at $105,000 \times g$ for 60 min to separate microsomes and cytosol. The microsomal fraction were resuspended in the homogenizing medium and spun down again by centrifugation. Proteins in each fraction was precipitated by adding trichloroacetic acid to a final concentration of 5%. The precipitates were washed twice with ice-cold 5% trichloroacetic acid and dissolved in 1 ml of NCS (Amersham/Searle, Arlington Heights, Ill, U.S.A.) for the estimation of radioactivity of ^3H and ^{14}C separately. The ratio of ^3H to ^{14}C radio-

activity in the supernatant proteins (Sr) and in the microsomal proteins (Pr) was calculated. The ratio of Sr/Pr is an estimate of the difference in the relative rate of elongation in the two tissues.

Other analytical methods

Protein and RNA were determined by the method of Lowry *et al.* (1951) and Mejbaum (1939), respectively. The radioactivity of ^3H , ^{14}C and ^{32}P was counted in a Tri-Carb liquid scintillation spectrometer model 3380. 10 ml of toluene containing 0.4% DPO and 0.01% POPOP was used for the determination of radioactivity. The radioactivity in the trichloroacetic acid precipitate was determined after dissolving in NCS. An automatic external standardization was applied to quenching correction in each sample.

Results and Discussion

Poly A-RNA in the nuclear and the cytoplasmic fraction of the ventral prostate from control and castrated rats

It is known that poly A sequences are a

Table 1. Content of RNAs that contains polyadenylate sequences in fractions of cytoplasm and nuclei of ventral prostate from control and castrated rats.

Tissues from	Percent of input RNA retained on Poly U filter	
	Nuclear	Cytoplasmic
Control	$0.16 \pm 0.02^*$	$0.19 \pm 0.02^*$
Sham-operated (48 hr)	0.20, 0.16	0.21, 0.26
Castrated (48 hr)	0.14, 0.15	0.06, 0.04

* Values expressed as mean $\pm$ standard error of 7 independent experiments.

characteristic of all mRNA's in animal cells with the exception of histone mRNA (see a review by Williamson, 1973). Therefore the rate of binding of RNA fractions to immobilized poly U seems to be a feasible method to assess the content of mRNA in tissue extracts. As shown in Table 1, the content of poly A-RNA was markedly reduced in the cytoplasmic fraction from castrated animals, while no significant difference was observed in the poly A-RNA content of nuclear fractions from the tissue of the intact, sham-operated and castrated animals. The incorporation of ^{32}P per gram of wet tissue in cytoplasm of castrated animals was approximately 60% of that of control animals (Table 2), therefore, the concentration of poly A-RNA in the castrated cytoplasm was calculated to be approximately 1/6 of that of the control animals. These results may indicate that the processing of transcribed precursor mRNA, such as polyadenylation, cleavage to smaller molecules and transportation from nuclei to cytoplasm (Scherrer, 1963; Nikolaev *et al.*, 1973; Schum and Webb, 1972) seems to be also influenced by castration.

Changes in the ribosomal sedimentation profile after castration and testosterone treatment

Castration caused a rapid reduction in polyribosomal fraction; the effect was already evident 2 hr after castration and almost back ground level of 260 nm absorbtion in polyribosomal zone after 24 hr or later (Fig. 2). Injection of testosterone propionate into

Table 2. Incorporation of ^{32}P into RNA in nuclear and cytoplasmic fractions of rat ventral prostates.

	Nuclear	Cytoplasm
Control	9.08×10^6 cpm/g tissue*	3.83×10^6 cpm/g tissue
	1.83×10^6 cpm/mg RNA	1.24×10^6 cpm/mg RNA
Castrated (48 hr)	4.69×10^6 cpm/g tissue	2.39×10^6 cpm/g tissue
	1.78×10^6 cpm/mg RNA	1.41×10^6 cpm/mg RNA

* Values are the average of 2 independent experiments.

Each animal received 1.2 mCi of ^{32}P -orthophosphate and sacrificed 40 min later. For other details of the experimental conditions, see the test.

Fig. 2. Changes in the ribosomal sedimentation profile of the rat ventral prostate after castration.

castrated animals (24 hr) induced a relatively rapid reformation of polyribosomes but 48 hr were necessary for the complete restoration of the profile (Fig. 3).

³H-Met-tRNA binding to and IF activity in ribosomes from control, castrated and castrated testosterone-treated animals

³H-Met-tRNA binding capacity of ribosomes isolates from the ventral prostate of castrated rats (48 hr) was significantly reduced and this was partly restored by supplementing the IF fraction isolated from rat liver (Table

Fig. 3. Changes in the ribosomal sedimentation profile of the ventral prostate from castrated rats after administration of testosterone.

3). Administration of testosterone propionate to castrated animals (48 hr) completely abolished the effect of castration. The ratio of the rate of binding in the presence of IF to that in the absence of IF was significantly higher in castrated ribosomes than in control and this might indicate that the castration caused a decrease in the ribosomal IF content of the tissue. However, in the present experiment, tRNA used for charging with ³H-methionine was not fractionated, then the tRNA_M^{met} must also be labelled. Therefore, observed radioactivity retained on the filter would possibly

Table 3. Effect of castration and testosterone-treatment on the binding activity of ribosomes from rat ventral prostates to ^3H -Met-tRNA.

Animals	^3H -Met-tRNA bound (pmoles/ mg ribosomal protein)		
	-IF	+IF*	+IF/-IF
Intact control	14.37	23.57	1.64
"	16.14	23.35	1.45
Sham-operated (48 hr)	14.08	21.92	1.56
Castrated (48 hr)	5.89	16.73	2.81
"	3.82	12.61	3.61
Castrated and	18.06	21.07	1.17
testosterone-treated	15.58	21.84	1.40

* 136 μg of IF isolated from rat liver ribosomes were added. This amount of IF bound 0.041 pmole of ^3H -Met-tRNA and this was subtracted from the data of IF-added experiments. For other details of experimental animals and incubation conditions, see the text.

Table 4. IF content in ribosomes of the ventral prostate from intact, castrated and testosterone-treated rats.

Animals	Units/mg RSW*	Units/mg Ribosomal Protein
Intact Control	7.97	2.11
"	8.63	2.56
Sham-operated (48 hr)	7.33	2.02
Castrated (48 hr)	5.18	1.20
"	4.55	0.93
Castrated and	8.84	2.31
testosterone-treated	8.26	2.19

* Per mg protein in ribosomal salt-wash fraction. For experimental conditions, see the text.

be a sum of the ^3H -Met-tRNA in the initiation complex and ^3H -methionine incorporated into peptide chains. To further clarify the role of the initiation in the hormonal regulation of protein synthesis, IF activity in ribosomes obtained from the ventral prostate of castrated rats was compared with that from control and castrated, testosterone-treated animals. As indicated in Table 4, IF activity in ribosomes from the tissue of castrated animals (48 hr) was significantly lower either on the

basis of protein in the ribosomal salt-wash fraction or of protein in ribosomes. Again, the diminished IF activity was completely restored by the replacement therapy of castrated animals. However, it was noticed in preliminary experiments that the affinity between ribosomes and IF did not seem to be very strong, therefore, a possibility that the decreased ^3H -Met-tRNA binding capacity and low IF activity observed in ribosomes of castrated rats were caused merely by the decreased affinity of ribosomes with IF which resulted in the liberation of IF during the procedures for preparation of ribosomes, can not be excluded.

From the results obtained, it is suggested that IF is involved in the hormonal regulation of protein synthesis in a male accessory sex organ. A work made by Gambino *et al.* (1973) also suggests the important role of ribosomal salt-wash fraction in the protein synthesis in which they observed significant differences in acrylamide gel pattern as well as in physiological properties of ribosomal salt-wash fraction between unfertilized and fertilized sea urchin eggs as early as 2 min after fertilization.

In the present study, IF activity only from ribosomes has been examined but it has been reported that proteins required for the initiation of protein synthesis also exist in the cytosol of animal cells in addition to being associated with ribosomes (Fuhr *et al.*, 1969; Sampson and Borghetti, 1972; Schreier and Staehelin, 1973b). The relationships between IF associated with ribosomes and IF associated with cytosol in the regulation of protein synthesis have to be examined.

Effect of castration on the relative rate of polypeptide elongation

The relative rate of polypeptide chain elongation was examined by the method of Palmiter (1972) in the tissue of control and castrated animals. The method used seems to allow a rough estimation of the difference in the relative rate of elongation between the tissues from two experimental animals. The results obtained

Table 5. Effect of castration on the relative rate of polypeptide chain elongation in the rat ventral prostate.

Isotope administered		Sr/Pr*
³ H-Leucine	¹⁴ C-Leucine	
Control	Control	1.05
Sham-operated	"	0.97
Castrated (48 hr)	"	0.77
"	"	0.72
"	"	0.75

* Sr signifies the ratio of ³H/¹⁴C in the cytosol fraction and Pr signifies the same ratio in the polyribosomal fraction. Sr/Pr is an estimate of the difference in the relative rates of elongation in the tissues obtained from two animals.

are summarized in Table 5. The value of 1.05 obtained from two control animals seems to validate the method employed. 23–28% reduction of the relative elongation was noticed in castrated animals. Sham-operation did not seem to cause any influences.

General Remarks

In the oviduct of laying hens, the rate of protein synthesis after estrogen stimulation increased 7-fold (Palmiter *et al.*, 1970), while only 2-to 3-fold increase in the translatable mRNA was observed (Palmiter, 1972). From these results, it has been suggested that the 7-fold increase in protein synthesis may stem from an increase in the rate of polypeptide chain initiation, chain elongation or elevated mRNA concentration (Palmiter, 1972). In the present study, it was found that the poly A-RNA content in cytoplasm was significantly reduced in castrated animals, while the poly A-RNA content in nuclear fraction was almost similar in two experimental animals. These may indicate that castration influences the transportation of mRNA from nuclei to cytoplasm and also possibly the processing of precursor mRNA in addition to the transcription. Furthermore, the rate of ³H-Met-tRNA bind-

ing and IF content in the tissue of castrated animals were significantly lower than that of control animals, while the effect of castration on the peptide chain elongation was relatively small. From these observations, it may be safely concluded that in rat ventral prostates, as in oviducts of laying hen, the hormonal regulation of protein synthesis is not restricted at the step of the transcription of translatable mRNA but the site of hormonal regulation is rather widely distributed over several steps of protein biosynthesis. The control mechanisms by hormone in each process of protein biosynthesis still remain to be clarified.

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