Biochem. J. (1980) **190**, 505–512
Printed in Great Britain

Androgenic regulation of messenger RNA in rat epididymis

Angela D'AGOSTINO,* Roy JONES, Roger WHITE* and Malcolm G. PARKER*
Agricultural Research Council Institute of Animal Physiology, 307 Huntingdon Road, Cambridge CB3 0JQ, U.K.

(*Received 8 February 1980*)

1. The regulation by testosterone of mRNA complexity and mRNA activity was investigated in rat caput and cauda epididymidis. 2. The sequence complexity of cytoplasmic poly(A)-containing RNA from normal rats was determined by homologous hybridization with radiolabelled complementary DNA probes by using RNA in excess. Computer analysis of results suggested that hybridization could best be described by curves composed of two components distinguished by their relative abundance. Thus caput-epididymidal RNA consists of approx. 260 moderately abundant and 16400 scarce sequences, whereas cauda-epididymidal RNA consists of approx. 124 moderately abundant and 13400 scarce sequences. Judging by heterologous-hybridization reactions, castration did not result in appreciable alterations in either sequence complexity or the relative abundance of the two classes of poly(A)-containing RNA. 3. To investigate if individual mRNA sequences were regulated by androgens, mRNA was translated in a cell-free system derived from reticulocyte lysate. Since most of the translation products had a different mobility on sodium dodecyl sulphate/polyacrylamide gels from the authentic proteins synthesized in tissue minces, antibodies were used to identify specific translation products. Antibodies to the two related major proteins (mol.wt. 18500 and 19000) secreted by the caput epididymidis and whose synthesis is stimulated by testosterone both precipitated a single translation product of mol.wt. 21000. That this polypeptide was a precursor to the secreted proteins was suggested by the fact that the addition of microsomal membranes isolated from dog pancreas resulted in the appearance of a polypeptide of mol. wt. 19000. 4. Translation of RNA from the caput epididymidis of rats of different hormonal status showed that mRNA activity for the 21000-dalton polypeptide declined after castration, but could be restored by treating rats with testosterone. 5. It is concluded that testosterone stimulates the synthesis of a major protein secreted by the caput epididymidis by regulating its mRNA activity.

We have previously described the hormonal regulation of protein synthesis in the rat epididymis (Jones *et al.*, 1980). It was found that the synthesis of four proteins (mol.wts. 18500, 19000, 32000 and approx. 80000) in the caput epididymidis and that of one protein (mol.wt. 47000) in the cauda epididymidis were regulated preferentially by testosterone, whereas the synthesis of a protein (mol.wt. 23000) in the initial-segment region was dependent on factors present in testicular fluid. These proteins are major components of the epididymal luminal plasma and may be involved in the acquisition of motility, fertilizing capacity and storage of spermatozoa in the epididymis (Bedford, 1966; Orgebin-Crist, 1969; Jones & Glover, 1975; Fournier-Delpech *et al.*, 1977; Acott & Hoskins, 1978; Lea *et al.*, 1978).

Most evidence to date suggests that androgens control protein synthesis in target tissues mainly by regulating the cellular mRNA concentration (Parker, 1978). In the rat ventral prostate, testosterone increases preferentially the amount of mRNA coding for the major secretory proteins (Parker *et al.*, 1978; Parker & Scrace, 1979), whereas in the seminal vesicles testosterone exerts a more generalized response by stimulating total

Abbreviations used: cDNA, complementary DNA; SDS, sodium dodecyl sulphate.

* Present address: Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

0306-3283/80/090505-08$01.50/1

protein synthesis (Higgins *et al.*, 1976; Higgins & Burchell, 1978). The situation in other rat sex-accessory tissues is not known, and hence it was decided to investigate the effect of testosterone on mRNA activity in the caput and cauda epididymidis, two regions of the gland in which the synthesis of specific proteins is stimulated by androgens (Jones *et al.*, 1980).

The present paper describes the analysis of epididymal mRNA sequences from rats of different hormonal status, by using cDNA probes to investigate mRNA complexity and a cell-free translation system derived from reticulocyte lysate to measure mRNA activity. Analysis of mRNA complexity is particularly useful when a significant fraction of the mRNA is altered in its abundance after hormonal stimulation (Parker *et al.*, 1978), whereas the analysis of translation products by radioautography allows the identification of individual mRNA species whose concentration is altered by the hormone.

Materials and methods

Animals

Male Wistar rats (300–350g) were lightly anaesthetized with diethyl ether, the testes removed and the epididymides returned to the scrotum. Testosterone propionate ('Androject'; Intervet Laboratories Ltd., Viking House, Bar Hill, Cambridge, U.K.) was injected subcutaneously in the flank region at a dose of 1 mg/rat per day.

Materials

Chemicals were of the highest purity available and supplied by either Sigma (London) Chemical Co. or BDH, both of Poole, Dorset, U.K. L-[^{35}S]-Methionine (900–1200Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., proteinase K from Merck, 61 Darmstadt, W. Germany, and oligo(dT)–cellulose from Collaborative Research (Waltham, MA, U.S.A.). Rabbit reticulocyte lysate was obtained from Dr. T. Hunt, Department of Biochemistry, Tennis Court Road, Cambridge, U.K., and dog pancreatic membranes were from Dr. M. Owen, Imperial Cancer Research Fund, Lincoln's Inn Fields, London. *Staphyloccus aureus* was kindly supplied by Dr. D. Critchley, Department of Biochemistry, University of Leicester, Leicester, U.K.

Isolation of total RNA and poly(A)-containing RNA

Caput and cauda regions of the rat epididymis were dissected free of fat and connective tissue under a ×10 stereo-microscope, minced and washed five times in sterile phosphate-buffered saline, pH7.4, containing bentonite (2mg/ml) and heparin (1mg/ml) to remove spermatozoa. When wash fluids were free of spermatozoa the tissue was homogenized in 10vol. of 0.1M-NaCl, pH7.5, containing 0.02M-EDTA, bentonite (2mg/ml) and heparin (1mg/ml). After centrifuging for 10min at 1000*g* at 4°C, the supernatant was made 0.5% with SDS and extracted with an equal volume of phenol/chloroform (1:1, v/v) at room temperature (Penman *et al.*, 1968). The aqueous phase was incubated with proteinase K (20μg/ml) at 37°C for 15min, extracted twice with phenol/chloroform and precipitated with ethanol at −20°C. Nucleic acids were dissolved in 3M-sodium acetate/5mM-EDTA, pH6.0, left overnight at −20°C, and centrifuged at 25000*g* for 10min at 4°C. The RNA pellet was redissolved in 0.1M-sodium acetate, pH7.0, and precipitated with 2vol. of ethanol at −20°C. The poly(A)-containing RNA was separated by chromatography on an oligo(dT)–cellulose column (Aviv & Leder, 1972), precipitated with ethanol and stored at −70°C in distilled water.

Cell-free protein synthesis

Total RNA or poly(A)-containing RNA was translated in a cell-free system derived from reticulocyte lysate that had been treated with micrococcal nuclease as described by Pelham & Jackson (1976). Incorporation of [^{35}S]methionine into protein was measured after treatment of 2μl portions of translation-system products with 0.3M-NaOH for 30min at 37°C by precipitation with 5% (w/v) trichloroacetic acid. In some cases, microsomal membranes derived from dog pancreas (Garoff *et al.*, 1978) were included in the translation system. The samples were then adjusted to contain 1% SDS, 1% β-mercaptoethanol and 62.5mM-Tris/HCl, pH7.2, and portions containing 20000–40000c.p.m. were applied to 15% (w/v) polyacrylamide gels containing SDS (Jones *et al.*, 1980). Labelled proteins were detected by fluorography (Bonner & Laskey, 1974).

Whole-cell protein synthesis

Incorporation of [^{35}S]methionine into protein was performed as described by Jones *et al.* (1980). Briefly, freshly minced tissue was washed in sterile phosphate-buffered saline to remove spermatozoa and luminal secretions, and 30mg portions were incubated for 2h at 33°C in 1ml of Dulbecco's modified Eagle's medium (without methionine) (Dulbecco & Freeman, 1959) containing 15μCi of L-[^{35}S]methionine. The tissue was homogenized, centrifuged at 1000*g* for 15min and labelled proteins in the post-nuclear supernatant were separated by electrophoresis on SDS/polyacrylamide slab gels (Jones *et al.*, 1980) and identified by fluorography (Bonner & Laskey, 1974).

Preparation of antisera

Epididymal androgen-dependent proteins with

mol.wts. 18500 and 19000 were purified from epididymal secretions as described by Jones *et al.* (1980). Portions (10 and 100μg) of each purified protein were emulsified in 1ml of sterile phosphate-buffered saline, pH7.4, and 1ml of Freund's Complete Adjuvant and injected subcutaneously at multiple sites into female New Zealand White rabbits. A 5ml sample of blood serum was collected at the time of the first immunization. After 3 weeks each animal was given a booster injection of 10μg or 100μg of protein, and the presence of antibodies in the serum was monitored at weekly intervals thereafter on Ouchterlony double-diffusion agar gels (Crowle, 1961). When a good antibody titre was obtained (4–6 weeks after the first injection), animals were bled out by cardiac puncture and serum was stored at −20°C.

Immunoelectrophoresis on 1.5% (w/v) agar gels in 0.025M-veronal buffer, pH8.6, revealed that antisera from all rabbits immunized with the 18500-dalton protein cross-reacted with the 19000-dalton protein, and vice versa, suggesting that the two proteins have many antigenic determinants in common. However, antisera were specific to these proteins, as they did not react with other proteins in the epididymal secretion or with blood serum.

Immunological identification of caput androgen-dependent proteins

Immunoprecipitation of the caput proteins of mol.wts. 18500 and 19000 was carried out by the procedure of Kessler (1975). Briefly, epididymal secretions of ^{35}S-labelled proteins were incubated with 100μl of the appropriate serum for 1h at room temperature. Then 100μl of a 10% (w/v) suspension of formaldehyde-treated *Staphylococcus aureus* was added and incubated for a further 15min at room temperature. The mixture was centrifuged at 1000*g* for 10min, washed twice in a buffer containing 150mM-NaCl, 5mM-EDTA, 50mM-Tris and 0.02% NaN_3, pH7.4, and heated at 100°C in 50μl of 62.5mM-Tris/HCl, pH7.4, containing 1% sodium dodecyl sulphate and 1% β-mercaptoethanol before electrophoresis.

Synthesis of cDNA and hybridization to poly(A)-containing RNA

The synthesis of [^{3}H]cDNA to poly(A)-containing RNA isolated from caput and cauda epididymidis was carried out as described previously for the ventral prostrate (Parker & Scrace, 1978). Hybridization of [^{3}H]cDNA was performed in RNA excess as described by Parker & Mainwaring (1977) by using S1 nuclease to monitor the extent of hybridization. RNA concentrations were calculated from A_{260} measurements by assuming that one A_{260} unit/ml was equal to 40μg of RNA. The possibility that RNA samples were contaminated to varying degrees with rRNA was checked by assaying poly(A) content, which in all cases was 3–4%, irrespective of the hormonal status of the rats from which the RNA was isolated. Nevertheless it is likely that there is some contamination of RNA samples with rRNA. Since it was fractionated twice on oligo(dT)–cellulose, contamination would probably be less than 20%, and this would lead to an overestimate in the complexity of the poly(A)-containing RNA by up to 20%. Poly(A) content was assayed with [^{3}H]poly(U) by the method of Bishop *et al.* (1974).

The hybridization data were analysed by Dr. D. Herries (Department of Biochemistry, University of Leeds), using a computer program designed to fit curves of up to four components. The choice between different numbers of components was made by comparing the sums of squares of deviations in each case. The coefficients of multiple correlation were 0.997 (two components), 0.998 (three components) and 0.997 (four components) for caput poly(A)-containing RNA and 0.993 (two or three components) for cauda RNA. The curves were therefore fitted to two components, there being little improvement with more components.

Results and discussion

Effect of androgens on mRNA complexity in caput and cauda epididymidis

The effect of androgens on cellular contents of mRNA can be determined by measuring the mRNA complexity in tissues of different hormonal status (Parker *et al.*, 1978). First, the complexity of poly(A)-containing RNA from the caput (Fig. 1*a*) and cauda (Fig. 1*b*) epididymidis of normal rats was measured by using cDNA species in homologous hybridization reactions, and then these 'normal' cDNA species were hybridized in heterologous reactions with RNA from castrated rats (Fig. 2). The saturation values of the hybridization reactions indicate the extent of overlapping sequences, and the kinetics of the reactions indicate the relative abundance of these sequences. Hybridization was carried out in RNA excess so that the rate of hybridization is determined both by the sequence complexity of the RNA and by the relative concentrations or abundance of the different sequences.

In view of the complexity of the results, we have fitted the data points to curves consisting of two, three or four components and compared the sums of squares of deviations in each case. For both caput and cauda epididymidis the data were fitted to curves with two components (see the Materials and methods section). The observed $R_0t_{\frac{1}{2}}$ values for each component have been corrected to allow for the fact that they are not pure and their hybridization is influenced by the other components (Table 1). The

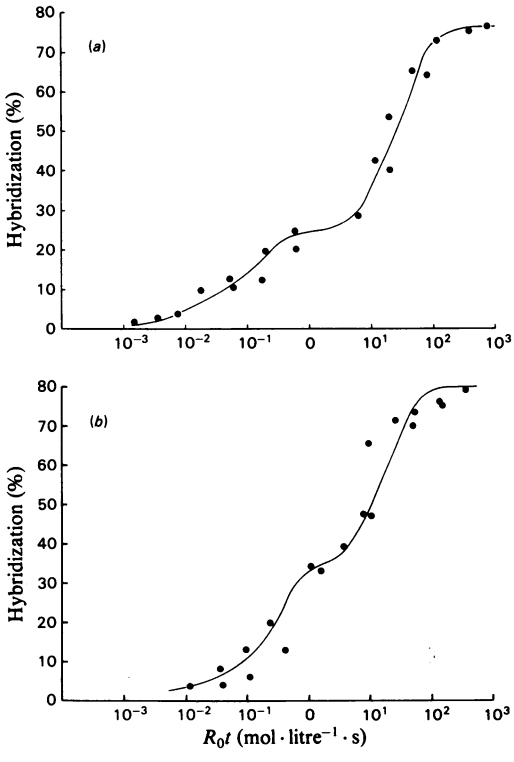

Fig. 1. *Homologous hybridization of poly(A)-containing RNA from caput and cauda epididymidis to complementary DNA*

Total poly(A)-containing RNA samples from the caput epididymidis (*a*) and cauda epididymidis (*b*) of normal rats were hybridized in RNA excess with their respective cDNA species. The extent of hybridization was determined by resistance to S1 nuclease, and line fitting was by computer analysis. R_0t values are products of RNA concentration (mol of nucleotide·litre^{-1}) and time (s). The RNA concentrations used were from 60μg/ml and 500μg/ml.

sequence complexity of each component was then calculated by comparing its corrected $R_0t_{\frac{1}{2}}$ value with the $R_0t_{\frac{1}{2}}$ value (5×10^{-4}mol·litre^{-1}) for globin ($\alpha+\beta$) mRNA, which consists of about 1320 nucleotides (Bishop *et al.*, 1974). From a knowledge of RNA/DNA ratios in the tissue and the fraction of poly(A)-containing RNA in the total RNA, it is possible to calculate approximate numbers of copies per cell for each mRNA species, assuming that the mRNA is uniformly distributed among cells.

The data indicate that in normal rats the caput epididymidis contains approx. 260 moderately abundant sequences and 16000 scarce sequences, whereas the cauda epididymidis contains approx. 120 moderately abundant and 13000 scarce sequences (Table 1). Distributions similar to these have been reported for a wide variety of tissues from different organisms (Lewin, 1975; Hastie & Bishop, 1976), but, in general, tissues secreting large amounts of protein contain a class of more highly abundant poly(A)-containing RNA species that code for such proteins. Other sex-accessory glands such as the rat ventral prostate and seminal vesicle and the chick oviduct each contain a class of mRNA consisting of one to five different sequences, present in more than 50000 copies per cell, that codes for specific secretory proteins (Axel *et al.*, 1976; Monahan *et al.*, 1976; Higgins *et al.*, 1978; Parker & Scrace, 1979). Thus the average concentration of the most abundant mRNA in these tissues is about 100-fold higher than the 200–300 copies per cell found in the epididymis.

The presence of a high concentration of protein in epididymal secretion (30–60mg/ml), despite the absence of highly abundant mRNA, may be explained as follows. The proteins are heterogeneous and therefore must be coded for by a large number of different mRNA species, each present at a moderate concentration. In addition, some of the proteins, such as androgen-binding protein, originate

Table 1. *Sequence complexity of poly(A)-containing RNA from rat caput and cauda epididymidis*

Hybridization data from Fig. 1 were analysed by computer. $R_0t_{\frac{1}{2}}$ values are corrected for the fact that each class is not a pure component and were obtained by dividing the observed $R_0t_{\frac{1}{2}}$ value by the fraction of RNA represented by that class. Nucleotide complexity was calculated by comparing the corrected $R_0t_{\frac{1}{2}}$ values with that of rabbit globin mRNA of 1320 nucleotides ($R_0t_{\frac{1}{2}} = 5 \times 10^{-4}$mol·s·litre^{-1}) and assuming that the number-average nucleotide length of epididymal mRNA was 1400 nucleotides. Number of RNA copies per cell was calculated from the RNA/DNA ratio by assuming that there was 6.5 pg of DNA per cell (Sober, 1970).

Region	Component	Fraction (%)	$R_0t_{\frac{1}{2}}$(mol·s·litre^{-1})	Complexity (sequence)	No. of RNA copies/cell
Caput	Moderately abundant	20.3 ± 1.7	6.4×10^{-2}	260 ± 70	230
	Scarce	50.2 ± 1.7	1.6×10^{1}	16400 ± 1600	8
Cauda	Moderately abundant	30.5 ± 3.1	2.0×10^{-1}	124 ± 20	335
	Scarce	44.7 ± 2.7	1.5×10^{1}	13400 ± 3800	5

from the testis rather than from the epididymis. Finally, it is also possible that the mRNA species are translated highly efficiently in the epididymides and that the proteins themselves are cleared from the lumen of different tissues at different rates.

After castration, there was a decline in the amount of RNA in both the caput and the cauda epididymidis (R. White, unpublished work), but the total complexity and the relative proportions of the two classes of poly(A)-containing RNA appear unchanged. As shown in Fig. 2, both the extent and kinetics of hybridization of the homologous reactions (dashed line) and heterologous reactions (data points) are similar, suggesting that RNA in castrated rats resembles that in normal rats both qualitatively and quantitatively. However, a major limitation with this approach is that alterations in the abundance of individual mRNA species, which account for, say less than 5% of the total poly(A)-containing RNA, could easily be overlooked. Since the most abundant epididymal mRNA species probably represent no more than 1–2% of the total, because several hundred different species comprise 20–30% of the total poly(A)-containing RNA (Table 1), the detection of alterations in the concentration of these mRNA species requires a more sensitive approach.

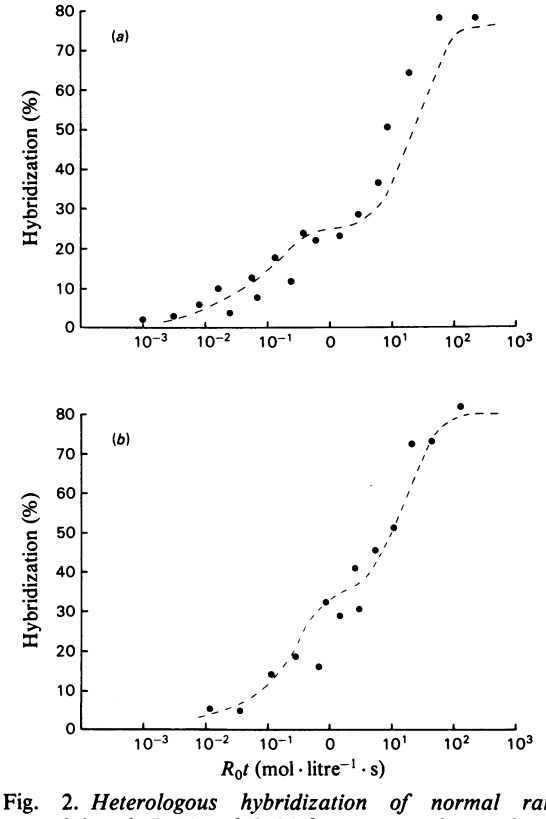

Fig. 2. *Heterologous hybridization of normal rat epididymal cDNA with RNA from castrated animals*
Total poly(A)-containing RNA samples from the caput (*a*) and cauda epididymidis (*b*) of rats that had been castrated 8 days previously were hybridized with their respective cDNA derived from 'normal' RNA. The RNA concentrations used were 60 μg/ml and 500 μg/ml respectively. The dashed line indicated the computer-fitted curve of the data from Fig. 1 (homologous hybridization).

Translation of epididymal mRNA in vitro

Individual mRNA species can be quantified by translating them in a cell-free system and analysing the amounts of radiolabelled translation products on an SDS/polyacrylamide gel. Preliminary experiments using a reticulocyte-lysate cell-free system indicated that the translation products from both caput RNA and cauda RNA (Fig. 3) had different mobilities from those proteins synthesized in tissue minces. The most likely explanation for this observation is that the most predominant proteins syn-

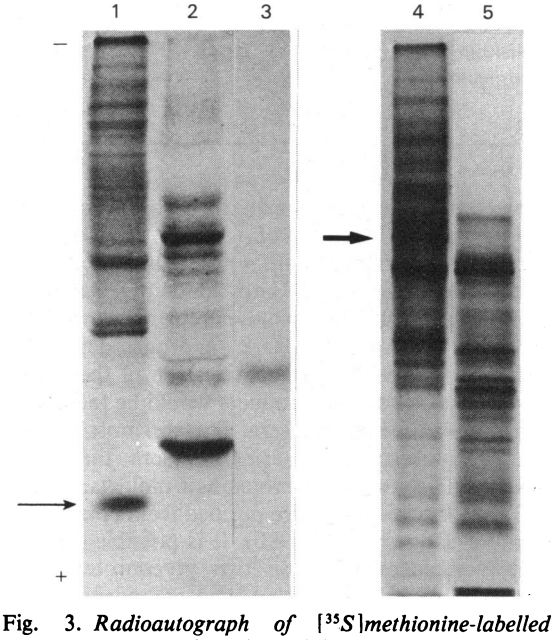

Fig. 3. *Radioautograph of [^{35}S]methionine-labelled caput- and cauda-epididymal proteins*
Labelled proteins that were synthesized in caput (track 1) and cauda (track 4) tissue minces and translation products that were obtained by incubating caput (track 2) and cauda (track 5) poly(A)-containing RNA in a reticulocyte cell-free systems were separated on SDS/polyacrylamide gels and detected by fluorography. Track 3 is the reticulocyte-lysate control. Arrows indicate tissue proteins whose synthesis is stimulated by androgens.

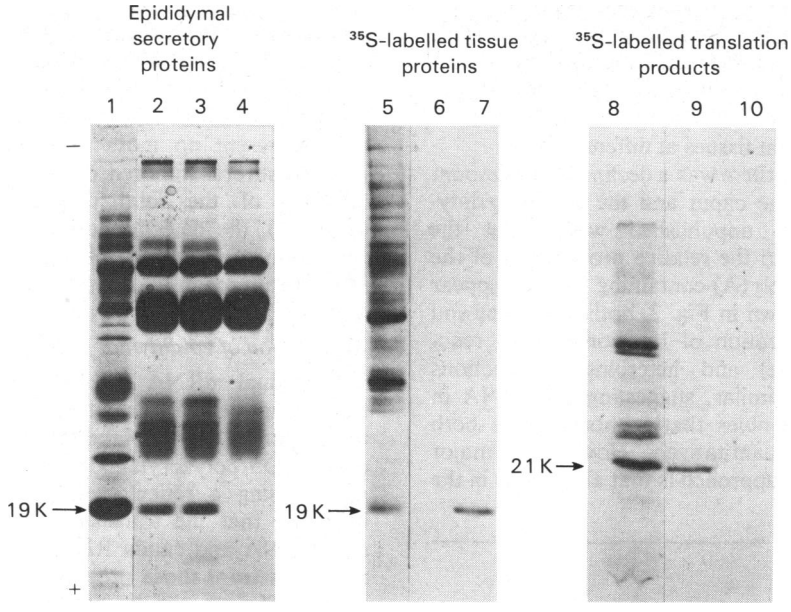

Fig. 4. *Immunoprecipitation of epididymal protein with specific antisera*
Proteins in epididymal secretion (tracks 1–4), ^{35}S-labelled proteins synthesized in caput-epididymal tissue minces (tracks 5–7) and [^{35}S]methionine-labelled translation products of caput-epididymal mRNA (tracks 8–10) were immunoprecipitated with antiserum against a glycoprotein of mol.wt. 18500 (tracks 2, 7 and 9), pre-immune serum (tracks 4, 6 and 10) and an antiserum against a glycoprotein of mol.wt. 19000 (track 3). The proteins were separated by SDS/polyacrylamide-gel electrophoresis and either stained with Coomassie Brilliant Blue (tracks 1–4) or identified by fluorography (tracks 5–10). '19K' and '21K' denote proteins of mol.wts. 19000 and 21000 respectively.

thesized by the epididymis are both glycosylated and secreted (Jones *et al.*, 1980); therefore the translation products are probably precursors incompletely processed into authentic secretory proteins. Among these epididymal proteins, we have identified several that are androgen-dependent (Jones *et al.*, 1980); the identification of these proteins in the translation products would be facilitated by the use of specific antisera. To date, most success has been obtained by using antisera that were raised against two glycoproteins of mol.wts. 18500 and 19000, which we have purified from epididymal secretion (Jones *et al.*, 1980). It is possible that the protein moieties of these two glycoproteins are related, because their amino acid compositions are similar (Jones *et al.*, 1980) and antisera to each cross-react (Fig. 4). By using *Staphylococcus aureus* to precipitate antibody–antigen complexes (Kessler, 1975), it is clear that there was no quantitative difference in the ability of each antiserum to precipitate either protein, whereas preimmune serum was ineffective (Fig. 4, tracks 1–4). Furthermore, antisera precipitated a single [^{35}S]methionine-labelled polypeptide of mol.wt. 19000 that was synthesized in tissue minces (Fig. 4, tracks 5–7) and a single translation product of mol.wt. 21000 that was synthesized in a reticulocyte cell-free system (Fig. 4, tracks 8–10). We presume that this translation product is a precursor of the secreted proteins, which themselves are modified forms of the same polypeptide. Addition of microsomal membranes derived from dog pancreas to the cell-free system results in the appearance of a new translation product of mol.wt. 19000 and concomitant loss of most of the 21000-mol.wt. translation product (Fig. 5). Presumably such a loss of 2000 daltons is due to the cleavage of a signal peptide (Blobel & Dobberstein, 1975). Differences in carbohydrate content and other modifications of the 18500- and 19000-mol.wt. proteins require investigation.

Having established the identity of the 21000-mol.wt. translation product, we next compared its androgen-dependence in the cell-free system with that in tissue minces. Fig. 6 shows that the protein is synthesized in normal tissue (track 1), decreases after castration (track 2), but can be restored by testosterone treatment (track 3). Comparison of translation products of poly(A)-containing RNA isolated from normal rats (track 4), 8-day-castrated rats (tracks 5 and 6) and castrated rats treated

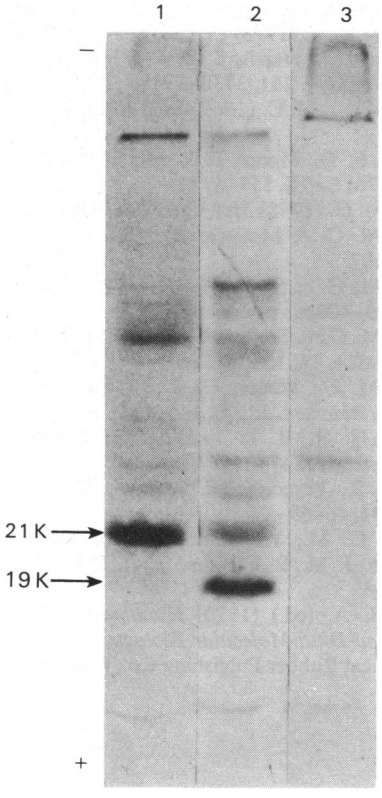

Fig. 5. *Effect of microsomal membranes on cell-free translation of caput-epididymal mRNA*

Caput-epididymal mRNA (track 1), caput-epididymal mRNA plus microsomal membranes (track 2) and microsomal membranes alone (track 3) were translated in a cell-free system derived from reticulocyte lysate. The 21K (21000-mol.wt.) translation product corresponds to that immunoprecipitated with specific antisera (Fig. 4) and the 19K (19000-mol.wt.) translation product co-migrates with the 19K androgen-dependent protein synthesized in caput-epididymal tissue minces (Fig. 6).

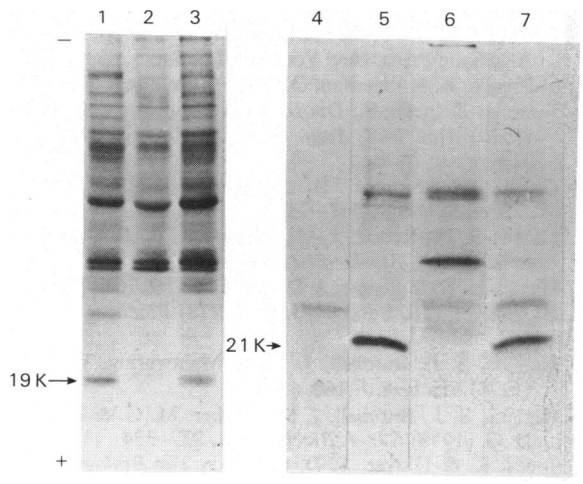

Fig. 6. *Effect of androgens on protein synthesis in caput epididymidis*

[^{35}S]Methionine-labelled proteins (40000c.p.m.) that had been synthesized in tissue minces (tracks 1–3) and in a reticulocyte cell-free system (tracks 4–7) were separated by electrophoresis on SDS/polyacrylamide gels and identified by fluorography. Samples loaded on the gels were from normal rats (tracks 1 and 5), rats castrated 8 days before (tracks 2 and 6) and 8-day-castrated rats treated with testosterone for 4 days (tracks 3 and 7). Track 4 is the reticulocyte-lysate control. The arrows indicate the position of the 19K (19000-mol.wt.) androgen-dependent proteins secreted by the caput epididymidis and the 21K (21000-mol.wt.) primary translation product.

with testosterone for 4 days (track 7) indicates that the translation product of mol.wt. 21000 decreases appreciably after castration, but can be restored by testosterone. In view of these results we conclude that androgens stimulate the synthesis of the 19000-mol.wt. secretory proteins by controlling the cellular amounts of their mRNA.

Thus, despite our inability to detect differential stimulation of specific mRNA species by androgens by measuring the complexity of poly(A)-containing RNA in normal and castrated rats, cell-free translation studies do indicate that androgens stimulate at least one mRNA species within the caput epididymidis. In regulating specific protein synthesis via mRNA availability, the action of testosterone in the epididymis resembles the situation in the rat ventral prostate (Parker & Mainwaring, 1977; Parker & Scrace, 1979) and for most other steroid hormones (Harris *et al.*, 1975; McKnight, 1978; Ringold *et al.*, 1975; Palmiter *et al.*, 1976).

A. D'A, on leave from the Institute of Histology and General Embryology, University of Rome, Italy, is a recipient of a long-term fellowship from EMBO.

References

Acott, T. S. & Hoskins, D. D. (1978) *J. Biol. Chem.* **253**, 6744–6750

Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408–1412

Axel, R., Feigelson, P. & Schutz, G. (1976) *Cell* **7**, 247–254

Bedford, J. M. (1966) *J. Exp. Zool.* **163**, 319–329

Bishop, J. O., Rosbash, M. & Evans, D. (1974) *J. Mol. Biol.* **85**, 78–86

Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851

Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88

Crowle, A. J. (1961) *Immunodiffusion*, pp. 37–93, Academic Press, New York and London
Dulbecco, R. & Freeman, G. (1959) *Virology* **8**, 396–397
Fournier-Delpech, S., Danzo, B. & Orgebin-Crist, M. C. (1977) *Ann. Biol. Anim. Biochim. Biophys.* **17**, 207–213
Garoff, H., Simons, K. & Dobberstein, B. (1978) *J. Mol. Biol.* **124**, 587–600
Harris, S. E., Rosen, J. M., Means, A. R. & O'Malley, B. W. (1975) *Biochemistry* **14**, 2072–2081
Hastie, N. D. & Bishop, J. O. (1976) *Cell* **9**, 761–774
Higgins, S. J. & Burchell, J. M. (1978) *Biochem. J.* **174**, 543–551
Higgins, S. J., Burchell, J. M. & Mainwaring, W. I. P. (1976) *Biochem. J.* **160**, 43–48
Higgins, S. J., Burchell, J. M., Parker, M. G. & Herries, D. G. (1978) *Eur. J. Biochem.* **91**, 327–334
Jones, R. & Glover, T. D. (1975) in *The Biology of the Male Gamete* (Duckett, J. G. & Racey, P. A., eds.), pp. 367–384, Academic Press, London
Jones, R., Brown, C. R., Von Glos, K. T. & Parker, M. G. (1980) *Biochem. J.* **188**, 667–676
Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624
Lea, O. A., Petrusz, P. & French, F. S. (1978) *Int. J. Androl. Suppl. 2*, 592–607
Lewin, B. (1975) *Cell* **4**, 77–93
McKnight, G. S. (1978) *Cell* **14**, 403–413
Monahan, J. J., Harris, S. E. & O'Malley, B. W. (1976) *J. Biol. Chem.* **251**, 3738–3748
Orgebin-Crist, M. C. (1969) *Biol. Reprod. Suppl.* **1**, 155–175
Palmiter, R. D., Moore, P. B., Mulvihill, E. R. & Emtage, S. (1976) *Cell* **8**, 557–572
Parker, M. G. (1978) *Mol. Cell. Endocrinol.* **10**, 119–133
Parker, M. G. & Mainwaring, W. I. P. (1977) *Cell* **12**, 401–407
Parker, M. G. & Scrace, G. T. (1978) *Eur. J. Biochem.* **85**, 399–406
Parker, M. G. & Scrace, G. T. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1580–1584
Parker, M. G., Scrace, G. T. & Mainwaring, W. I. P. (1978) *Biochem. J.* **170**, 115–121
Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256
Penman, S., Vesco, C. & Penman, M. (1968) *J. Mol. Biol.* **34**, 46–69
Ringold, G. M., Yamamoto, K. R., Tomkins, G. M., Bishop, J. M. & Varmus, H. E. (1975) *Cell* **6**, 299–305
Sober, H. A. (ed.) (1970) *Handbook of Biochemistry, Selected Data Molecular Biology*, 2nd edn., p. H-112, Chemical Rubber Publishing Co., Cleveland, OH