Isolation and characterisation of genes for androgen-responsive secretory proteins of rat seminal vesicles

Charles McDonald, Lindsay Williams, Pamela McTurk*, Frances Fuller, Elizabeth McIntosh and Stephen Higgins[†]

Department of Biochemistry, University of Leeds, Leeds LS2 9JT, and *Department of Biochemistry, Leicester University, Leicester LE1 7RH, UK

Received 10 January 1983; Accepted 28 January 1983

ABSTRACT

Under the influence of testosterone, rat seminal vesicles synthesise large amounts of a tissue specific protein, S. Recombinant λ clones have been isolated containing overlapping sequences covering a 27.5 kilo base region of the rat genome within which the gene for protein S is located. Recombinant plasmids bearing cDNA sequences for protein S were constructed in pBR328. One (pcS2) contains a 690 nucleotide insert and is probably full length. Detailed restriction maps of the S-gene are presented and the structure was confirmed by analysis of R-loops and heteroduplexes. The S-gene covers a 2 kbp region of the genome and consists of a 5' intron (490 bp) separating a leading exon (120 bp) containing the 5' untranslated region from a central exon (310 bp) containing most of the coding sequence and part of the 3' untranslated region. A larger intron (1100 bp) lies within the 3' untranslated region. The cloned gene is representative of the native gene but the S gene may be heterogeneous. Using pcS2, the hormonal control of S-specific mRNA was examined and a pronounced differential response to testosterone was observed.

INTRODUCTION

Androgens are essential for structural and functional integrity of male sex accessory tissues (1). We are using the seminal vesicles of the male rat as a model system to explain the molecular details of testosterone action. Previous studies (2-5) have shown that testosterone promotes the synthesis of large amounts of two seminal vesicle secretory proteins (proteins S and F) by regulating the steady-state levels of their messenger RNAs.

The precise manner in which testosterone regulates mRNA levels could be more readily investigated if androgen-responsive genes were isolated and characterised. Recombinant plasmids containing cDNA sequences homologous to mRNAs for proteins S and F have been constructed (4,6,7) and the nucleotide sequence determined for one S-specific cDNA clone (4). We now report the successful isolation and characterisation of both cDNA and genomic clones for seminal vesicle proteins.

MATERIALS AND METHODS

Materials

Adult male Sprague-Dawley rats were used, castrated and induced with testosterone phenylpropionate as before (2,3,8). Sources of most chemicals have been reported (2,3,8). Restriction enzymes and other DNA modifying enzymes were from BRL, New England Biolabs, Cambridge Biotechnology or Boehringer. The following were generous gifts: reverse transcriptase (J.W. Beard), Pst I (A. Hawkins), *E. coli* WJB654 (W.J. Brammar) and LE392 (M.G. Parker), plasmids pBR-328 (F. Bolivar) and pSV2 (S.E. Harris), the partial Hae III rat 'gene bank' (T. Sargent, L. Jagodzinski & J. Bonner). Recombinant DNA work described was carried out under conditions advised by the U.K. Genetic Manipulation Advisory Group.

Isolation and Identification of Genomic Clones

A partial Hae III rat 'gene bank' constructed in λ phage Charon 4A with liver DNA from a single Sprague-Dawley rat (9) was screened (10) in *E. coli* LE-392 using total [32 P]cDNA (3), selecting clones with strong hybridisation 'signals'. After purification the clones were distinguished by using the recombinant DNA, attached to DBM-paper (11), to affinity purify seminal vesicle mRNA (12) for translation in a wheat germ lysate with [35 S]methionine (2). Eco RI fragments of selected recombinants were inserted into the Eco RI site of pBR328 (13) and the recombinant plasmids used to transform competent *E. coli* WJB654 (14).

Construction and Identification of cDNA Plasmids

Poly(A)-rich RNA from seminal vesicles of normal rats (2,3) was reverse transcribed using [3 H]dCTP (3). Double stranded cDNA was then synthesised (15) adding ~20 dCMP residues in the 'tailing' reactions with terminal transferase. Plasmid pBR328 was restricted with Pst I and tailed with ~20 dGMP residues (15). Plasmid and cDNA (4:1 molar ratio) were annealed and used to transform competent *E. coli* WJB654 (14). Recombinants (amp^S, tet^R, cm^R) were screened (16,17) using nick-translated (18) E2 insert from pgS7E2 which contains the S-gene (Results). About 40-50% of the recombinants contained cDNA_S. Plasmid DNA was purified (19) from cultures amplified with spectinomycin (300 μ g/ml).

Restriction Enzyme Mapping

Rat liver DNA was extracted (20) and purified through CsCl gradients. DNA samples were digested with restriction enzymes using the Manufacturer's conditions and separated by electrophoresis on agarose or polyacrylamide gels (21). After transfer of DNA to nitrocellulose (22) hybridisation was carried out with nick-translated [32 P]probes (18) or total [32 P]cDNA (3) including

dextran sulphate (23) when testing cell DNA blots. RNA was denatured, electrophoresed in the presence of 4 mM methyl mercuric hydroxide (Alfa) (24) and transferred to DE8-paper (11). Hybridisation with [^{32}P]cDNA_S was as described (23). Kinetics of hybridisation of nick-translated cDNA_S to excess RNA were followed using S₁ nuclease (3).

Electron Microscopy of Nucleic Acids

Heteroduplexes (λ gS7 and pcS2) were formed essentially as described (25). The hyperphase (0.1 ml) contained 30ng DNA and 10 μ g cytochrome c and the hypophase was deionised H₂O. Homoduplexes (pcS2) were observed as described (26). R-loops formed between pgS7E2 and poly(A)-rich RNA were observed (27) using a urea-formamide hyperphase and deionised H₂O hypophase. Grids were processed (26) and hybrids visualised in a Jeol 100CX microscope (accelerating voltage 60 KV, magnification 26000X). Size standards used were pBR322 and single-strand M13 with the 482bp 'snapback' of pBR328 (28) as internal marker for heteroduplexes. Measurements ($\pm$ 50bp) were taken from prints using a Kontron digitizing tablet and computer.

RESULTS

Isolation and Identification of Genomic Clones

Twenty recombinant λ phage clones giving strong hybridisation 'signals' with [^{32}P]cDNA were isolated and purified from among 500,000 clones screened. Those containing genes coding for seminal vesicle proteins S and F were identified by affinity purification of mRNA. Seven of the clones isolated (λ gS2, λ gS3, λ gS5, λ gS6, λ gS7, λ gS12 and λ gS13) are complementary to mRNA sequences for polypeptides having mobilities identical to protein S on SDS-polyacrylamide gels, while two clones (λ gF9 and λ gF20) appear to code for protein F (representative results, Fig.1). Further studies established the usefulness of some of these genomic clones for investigating the structure and organisation of androgen-responsive genes. This paper describes the structure of the S-gene and its response to androgens.

Preliminary Restriction Analysis of S-Recombinants

DNA from the seven S-specific λ recombinants was digested with restriction enzymes (Fig.2) recognising 6-nucleotide sequences and whose sites are known in the vector (29). Restriction fragments carrying the S-gene were identified by hybridisation to [^{32}P]cDNA. In each case the recombinants could be divided into three groups (λ gS2 and λ gS3; λ gS5, λ gS6, λ gS12 and λ gS13; λ gS7) based on their restriction and hybridisation patterns. Sufficient common features could be recognised for these different groups to be regarded as containing

Fig.1. Identification of Genomic Clones

Seminal vesicle mRNA was affinity purified using recombinant genomic DNA and was translated in a wheat germ system with [35 S]methionine. Translation products were separated on SDS-PAGE and detected by fluorography. Translation of mRNA homologous to λ gF9 (lane 1), λ g8 (lane 2), λ gS2 (lane 3) and salmon testis DNA (control, lane 4). Translation of water (lane 5), wheatgerm tRNA used to precipitate mRNA (lane 6) and seminal vesicle poly(A)-rich RNA (1 μ g, lane 7; shorter exposure 7a). S and F indicate positions of translation products of mRNA_S and mRNA_F respectively.

over-lapping segments of a 27.5 kbp region of the rat genome encompassing the S-gene rather than totally unrelated regions or major rearrangements of the S-gene. Comparison of the restriction patterns for the enzymes allowed us to construct a map aligning the recombinant groups (Fig.2). When S-specific cDNA (cDNA_S) was available (see below), we confirmed that all seven recombinants contain an S-gene. Furthermore, comparison of hybridisation patterns for total cDNA and cDNA_S were identical suggesting that the λ recombinants contain no genes other than the S-gene that are expressed to any significant extent in seminal vesicles of normal rats.

Clone λ gS7 was chosen for further detailed study, preparatory to investigating testosterone action, since it contains substantial nucleotide sequences on either side of a complete S-gene whose orientation has been determined (see later). To simplify further mapping the 3.8 kbp Eco RI fragment (E2; Fig.2) from λ gS7 carrying the S gene was subcloned into the Eco RI site of plasmid pBR328 (13) to produce the recombinant plasmid pgS7E2.

To map unambiguously the limits of the S gene in λ gS7 and to determine the position and extent of intervening sequences, cDNA_S clones were constructed.

Fig.2. Restriction Enzyme Maps of S-genes and cDNA_S

Maps were constructed on the basis of restriction enzyme analysis and blotting. Genomic clones (upper part) are aligned with respect to common restriction sites and areas occupied by S-gene (bold blocks, with vector shown as thin lines). In the lower part, the Eco RI fragment (E2) of λgS7 bearing the S-gene is expanded and its map compared with those of cDNA_S clones pcS2 and pSV2. Solid blocks are exons. Enzymes: A, Ava I; B, Bam HI; B1, Bgl I; B2, Bgl II; E, Eco RI; H, Hind III; K, Kpn I; P, Pvu II; S, Sac I; Sp, Sph I; X, Xba I. E2 and cDNA_S contain no sites for Cla I, Eco RI, Pst I or Xho I. A site for Bgl I predicted from the sequence (4) to lie in the 3' exon does not exist.

Construction and Isolation of cDNA_S Clones

Complementary DNA synthesised from mRNA isolated from seminal vesicles of normal rats was cloned into the Pst I site in pBR328 (13) using a method calculated to preserve the 5' ends of the cDNA (15). Nearly half the recombinants isolated contained sequences positive for the E2 fragment of pgS7E2 and those with the largest Pst I insert were selected. Four clones (pcS1, pcS2, pcS3 and pcS4; Fig.3) possess cDNA inserts larger than the Pst I insert of another cDNA_S plasmid (pSV2) that is known from its nucleotide sequence to lack the codons for the three N-terminal amino acids of protein S and all the 5' untranslated region (4).

Using both Pst I and Hind III, pcS1-4 were shown to contain more extensive 5' sequences than in pSV2. Thus from pSV2 two cDNA Pst-Hind fragments are produced that can be unambiguously identified from the nucleotide sequence (4)

Fig.3. Analysis of cDNA_s Clones

DNA from cDNA_s clones was digested with Pst I (lanes 1-5) or Pst I plus Hind III (lanes 6-10), electrophoresed in agarose, transferred to nitrocellulose and hybridised to nick-translated E2 insert from pgS7E2. A Hae III digest of ϕ X174RF DNA was used for sizing. Lanes contain pcS1 (1,7), pcS2 (2,8), pcS3 (3,9), pcS4 (4,10) and pSV2 (5,6).

as arising from the 3' ($\sim$ 400 bp) and 5' ($\sim$ 225 bp) ends (Fig.3). Clones pcS1-4 also contain Pst-Hind fragments identical in size to the 3' fragment of pSV2 but their other Pst-Hind fragment is larger than the 5' portion of pSV2 (Fig.3) showing that their 5' ends are more extensive. This has been confirmed by detailed mapping and electron microscopy (see later). The clone with the largest cDNA_s insert and the most extensive 5' sequence (pcS2) was used in the rest of this study. Restriction sites suitable for characterising pcS2 were predicted (30) from the sequence of pSV2 (4). In each case, the predictions were substantiated and additionally confirmed in pSV2 (Fig.2). The maps for the cDNA inserts in the two plasmids are identical apart from an extended 5' nucleotide sequence in pcS2, confirming the earlier conclusions using Pst I and Hind III (Fig.3).

The amount of 5' untranslated sequence in pcS2 has been calculated using Pst I, Hind III and Sac I. Thus Pst I plus Sac I cut cDNA_s of pSV2 into two similar-sized fragments ($\sim$ 310 bp) but pcS2 produced one Pst-Sac fragment of $\sim$ 310 bp and another larger fragment from the 5' end. Hind III should reduce the size of the 5' Pst-Sac fragment by 76 bp since there is a Hind III site

exactly this distance from the Sac I site towards the 5' end in both pcS2 and pSV2 (4) (Fig.2). After removal of this 76 bp fragment by Hind III, the remainder of the 5' Pst-Sac fragment of pcS2 migrated with the 3' Pst-Sac fragment (~310 bp) (not shown) suggesting that pcS2 contains the 9 nucleotides for the 3 codons missing from pSV2 and about 60-65 nucleotides from the 5' untranslated region. A 5' sequence of this size is typical of many genes so it is likely that pcS2 contains a complete cDNA_S sequence.

Detailed Organisation of S-gene

Genomic clones. Further detailed mapping of the S gene region in λgS7 was undertaken using plasmid pgS7E2, locating sequences homologous to cDNA_S by hybridisation to the Pst insert of pcS2 (Fig.2).

Orientation of the S-gene in pgS7E2 was determined from the relative positions of Pvu II and Sac I sites. In cDNA_S the single Sac I site is 67 bp on the 5' side of the single Pvu II site (Fig. 2 & Ref.4). In the E2 fragment of pgS7E2 there are two Pvu II sites but only one is surrounded by sequences hybridising to cDNA_S (Fig.2). The position of this Pvu II site relative to the Sac I site in the E2 insert established the orientation of the S-gene in pgS7E2 (Fig.2). This has been confirmed by other experiments (see later) and further mapping (summarised in Fig.2). Comparison of the maps of pgS7E2 and λgS7 established the orientation of the S-gene in the λ recombinants (Fig.2).

Intervening Sequences. The S-gene contains at least two intervening sequences (introns). One lies towards the 3' end and was first observed in triple digests of pgS7E2 with Bam HI, Eco RI and Hind III, where a cDNA-negative Bam-Hind fragment mapped between two fragments carrying cDNA_S sequences (Fig. 2). Further identification and sizing came from experiments with Pvu II and Sph I. The cDNA sequence of pcS2 contains only one site for each of these enzymes with the Sph I site 67 bp on the 3' side of the Pvu II site (confirmed by the sequence of pSV2; ref.4). Although the E2 sequence of pgS7E2 contains two sites for each of these enzymes only one of each is surrounded by cDNA_S-positive regions (Fig.2). This genomic Pvu-Sph sequence, which should correspond to that in pcS2 (67 bp), contains about 1170 bp so ~1100 bp must be in an intervening sequence. Additional evidence for this intervening sequence comes from the existence in this area of sites for Bam HI, Hind III and Kpn I all of which are absent from the corresponding region of pcS2 and pSV2 (Fig.2).

The 3' end of the S-gene has been located accurately. From the Sph I site to the site of attachment of the 3' polyadenylated sequence in both pcS2 and pSV2 is 131 bp (Fig.2 & Ref.4). From the Sph I site to a 3' Ava I site, which is not in cDNA_S and marks the 3' limit of cDNA_S-positive restriction sites in

E2, lies a sequence of 160 bp (Fig.2). So the 3' end of the S-gene must lie within 29 bp of the Ava I site.

A further intervening sequence lies in the 5' region where there are sites for Bgl I and Xba I both of which are absent from pcS2 but are surrounded by sequences hybridising to pcS2 (Fig.2). Using mapping techniques, the exact size of the 5' intron and the precise location of the 5' end of the gene could not be determined. Nevertheless the gene does not extend beyond the Ava I site located 110 bp from the 5' Eco RI site of pgS7E2 since the 5' Eco-Ava sequence (Fig.2) does not hybridise to [³²P]cDNA.

Electron Microscopy of S-gene

DNA-RNA hybrids and heteroduplexes were observed by electron microscopy. Fig. 4(A) shows a structure typical of hybrids between pgS7E2 and seminal vesicle poly(A)-rich RNA and is entirely consistent with, and thus substantiates, the exon-intron arrangement deduced from mapping experiments. As predicted, pgS7E2 linearised with Pst I (8.7 kbp) contains a 2 kbp S-gene with 3 exons and 2 introns whose positions and sizes correspond with the map. Heteroduplexes (Fig.4B) between pcS2 and λ gS7 are also consistent with the R-loops and confirm the map. Since the orientation of cDNA_S in pcS2 is known relative to the plasmid sequence forming the 'snapback' in pBR328 (28), heteroduplexes also confirmed the orientation of the S-gene in λ gS7 and thus in pgS7E2. Hence we are able to identify the poly(A)-tail of mRNA_S in R-loops (Fig.4A). The similarities of R-loop and heteroduplex analyses confirm that the cDNA_S sequence of pcS2 (homoduplexes of pcS2 give a size of 690 nt) is probably full length and therefore the 5' end of the gene is within ~50bp of the position shown in Fig.2. Analysis of over 50 R-loop structures (accuracy ~50 bp) shows the S-gene to comprise a 111 bp leading 5' exon, a 330 bp central exon and 104 bp 3' exon. The intron contains 490 bp and the 3' intron contains 1100bp.

Identification of S-gene in Cell DNA and Existence of Related Genes

The S-genes present in the seven λ gS recombinants and studied most extensively in λ gS7 and pgS7E2 may not be representative of the natural S gene if rearrangements have occurred during the cloning procedures or if they are only individual examples of a larger family of related genes.

If cell DNA contains the same S-gene as the genomic clones, it should lie within a 3.8 kbp Eco RI fragment as in pgS7E2. Hybridisation of an Eco RI digest of male liver DNA with cDNA_S from pcS2 revealed a single cDNA_S-positive fragment (Fig.5). The absence of other cDNA_S-positive Eco RI fragments in liver DNA is consistent with there being one or only a few copies of the S-gene and this is further supported by autoradiographs of Southern transfers (Fig.5)

Fig.4. Electron Microscopy of S-gene

(A), R-loops formed between pgS7E2 (linearised with Pst I) and seminal vesicle poly(A)-rich RNA. (B), heteroduplex formed between λgS7 and pcS2 (linearised with Eco RI). Insets show interpretation of structures (solid lines, DNA; broken lines, mRNA; plasmid, p; λ DNA, λ) with orientation of S-gene and positions of introns and exons. Note 482 bp 'snap back' (28) in pcS2 (arrowed).

where the 'signals' from 10 pg of the E2 fragment of pgS7E2 and from 30 μg of restricted liver DNA were similar, this being the ratio expected for a gene present once or only a few times per haploid genome. However the S-gene in

Fig.5. Analysis of S-gene in Rat Genome

DNA (30 μ g) from male rat livers was digested with Eco RI, separated on a 0.8% agarose gel, transferred to nitrocellulose and hybridised to [32 P]cDNA_S from pcS2 (lane 1). Also included are 30 μ g undigested liver DNA (lane 2) and 10 μ g E2 fragment of pgS7E2 (lane 3). Size scale (kbp) was obtained from Hind III digest of λ DNA.

liver DNA appears to lie on an Eco RI fragment of about 4.0 kbp rather than 3.8 kbp as in λ gS7 and pgS7E2 (Fig.5). This difference may be more apparent than real since [32 P]-labelled Hind III fragments of λ DNA migrate less rapidly in the presence of large amounts of cell DNA (not shown). Nevertheless it is possible that the S-gene of liver DNA differs from that in E2 by an additional $\sim$ 200 bp.

In fact detailed mapping of the seven λ gS clones has revealed differences in the Bam-Bam fragment containing the 3' exon of the S-gene (Fig.2). In λ gS2, λ gS3 and λ gS7 this sequence is 1.7 kbp but in λ gS5, λ gS6, λ gS12 and λ gS13 it is $\sim$ 200 bp larger. This extra sequence could lie between the 3' end of the gene and the Bam HI site immediately downstream or in the intron at the 3' end of the gene giving rise to at least two versions of the S-gene.

Response of the S-gene to Androgens

Using pcS2, the response of the S-gene(s) to androgens can be investigated. Total RNA from rats of different androgen status was separated on agarose gels containing the denaturant, methyl mercuric hydroxide, transferred to DBM-paper and hybridised to cDNA_S from pcS2 (Fig.6A). The results show that mRNA_S is virtually undetectable in seminal vesicle RNA from rats castrated six weeks before but treatment with testosterone *in vivo* results in appearance of large amounts of mRNA_S whose size ($\sim$ 650 nt) is expected from the cDNA_S sequence (Fig.

Fig.6. Androgenic Regulation of $mRNA_S$

In (A), a 2.5% agarose gel containing methyl mercuric hydroxide was used to separate seminal vesicle poly(A)-rich RNA (10 μ g; lanes 1- 4), total RNA (20 μ g; lanes 5 and 6) and poly(A)-minus RNA (20 μ g; lanes 7 and 8). RNA was transferred to DE8-paper and probed with [32 P]cDNA_S from pcS2. RNA was from: rats castrated 2 weeks (lane 3) and then given testosterone 4 days (lane 1); castrated 6 weeks (lanes 4,5,7) and then given testosterone 4 day (lanes 2,6, 8). Size of $mRNA_S$ (~650 nt) was estimated from mobilities of rat rRNAs.

In (B), [32 P]cDNA_S (5 x 10 dpm/ml; 10 ng/ml) from pcS2 was hybridised with total seminal vesicle RNA (10-1000 μ g/ml) from rats: normal (●), castrated 6 weeks (▲) or castrated 6 weeks then given testosterone 4 days (○) and the extent of hybridisation was measured with S_1 nuclease.

2 & Ref.4). These changes in $mRNA_S$ abundance were quantified by measuring the kinetics of hybridisation of nick-translated [32 P]cDNA_S from pcS2 in the presence of excess total RNA (Fig.6B). Comparison of $R_0t_{1/2}$ values (R_0t for 50% hybridisation) leads to estimations of the number of $mRNA_S$ copies per epithelial cell (Table 1). Six weeks after castration less than 0.5% of the $mRNA_S$ remains but treatment with testosterone for 4 days increases the concentration of $mRNA_S$ to about 30% of normal.

The use of total RNA cannot distinguish differential changes (selective towards $mRNA_S$) from generalised effects involving the total mRNA population. However, analysis of poly(A)-rich RNA ($mRNA$ fraction) shows that testosterone does exert a pronounced differential effect, and furthermore that the concentration of $mRNA_S$ is negligible even two weeks after castration (Fig.6A).

Table 1. Effects of Testosterone on mRNA_S in Seminal Vesicle Epithelial Cells

Androgen Status ^a	R _O r _{1/2} ^b	mRNA _S ^c (fraction)	RNA/DNA ^d (ratio)	Epithelial cells (fraction) ^d	mRNA _S /epithelial cell ^e	
					(fg)	(copies)
Normal	0.1	1.7 x 10 ⁻³	3.4	0.70	65	203,000
Castrated	7.9	2.2 x 10 ⁻⁵	0.7	0.40	0.3	920
Induced	0.32	5.4 x 10 ⁻⁴	2.8	0.65	19	58,000

- a. Rats were castrated 6 weeks and induced for 4 days with testosterone.
b. R_Ot_{1/2} from Fig.6B.
c. Determined using R_Ot_{1/2} = 3 x 10⁻⁴ for purified mRNA_S (4); value for normal RNA in this Table is similar to Ref.4.
d. Data from (8).
e. Assumes 8 pg DNA/cell, all mRNA_S in epithelium and mRNA_S 650 nt.

DISCUSSION

The gene coding for seminal vesicle protein S occupies 2 kbp of the rat genome with no other gene expressed to any significant extent in seminal vesicles of normal animals nearer than 10 kbp and 15 kbp in the 5' and 3' directions respectively. The structure of the S-gene, established by restriction mapping and electron microscopy, is similar to those of many protein-coding genes of eukaryotes (31). A 5' intron (~490 bp) separates a leading exon (~120 bp) containing the 5' untranslated region and nucleotides coding for probably all 21 amino acids of the hydrophobic 'signal' peptide from the central exon. This ~310 bp sequence contains the remainder of the coding sequence plus the first part of the 3' untranslated region (214 bp, ref.4) and is separated from the rest of that region by the 3' intron (~1100 bp). The 3' end of the gene is within 29 bp of a downstream Ava I site. Using restriction mapping and electron microscopy the 5' transcriptional start point can only be established within ~50bp but lies on the 3' side of the Ava I site upstream from the gene (Fig.2).

We have sequenced a 200 bp Hind-Hind fragment that contains parts of both the central exon and the 3' intron. Comparison of the sequence with that of pSV2 (4) places the exon-(3') intron junction 43 bp into the 3' untranslated region. Furthermore, the exact correspondence of the coding sequence with that of pSV2 (4) and the derived amino acid sequence with that of protein S (4, 32) suggests that λ gS7 contains a functional androgen-responsive S-gene

rather than a 'pseudo-gene' related to the S-gene in the same ways as, for instance, the X and Y genes are related to the ovalbumin gene of chicken oviduct (33). Nonetheless liver may contain more than one version of the S-gene differing by ~200 bp in the 3' region. Heterogeneity of androgen-responsive genes has been reported in other systems (34, 35). We are completely unable to explain a report (7) that liver DNA, also from Sprague-Dawley rats, contains S-genes located on two Eco RI fragments (5.2 and 4.5 kbp). However, the limited information given concerning the cDNA_S probe used does not seem compatible with the structure and sequence of cDNA_S in both pcS2 and pSV2 (4).

Use of cDNA_S has allowed us to determine specifically the amount of mRNA_S in seminal vesicle epithelial cells and to confirm that testosterone greatly increases the amount of mRNA_S available for synthesis of protein S. The changes observed in this study are intermediate between those reported by others (4,5). By examining poly(A)-rich RNA we have shown that testosterone regulates mRNA_S in a markedly differential fashion, confirming the results of a detailed examination of protein synthesis (36) and substantiating the inferences of others (4,5). S-specific mRNA does not appear to be unusually stable, as others have suggested (4,5), since mRNA_S is greatly reduced even two weeks after castration. Our previous proposal (1-3) that testosterone acts generally (not selectively) was based on indirect measurements which are clearly insufficiently definitive.

Whether changes in mRNA_S result from hormonal regulation of S-gene transcription or of stability and processing of RNA sequences is not known but precedents exist for both (37-39).

ACKNOWLEDGEMENTS

We thank Judith Wilson for technical help, Malcolm Parker and Jeff Williams for helpful advice, Bill Brammar, Alistair Hawkins and Annie McMorris for invaluable assistance and hospitality, Chris Boyd for computing. Financial support was provided by the Medical Research Council (Project Grant G8109670).

⁺To whom correspondence should be sent.

REFERENCES

1. Higgins, S.J. and Parker, M.G. (1980) *Biochem. Actions of Hormones* 7, 287-309
2. Higgins, S.J. and Burchell, J.M. (1978) *Biochem. J.* 174, 543-551
3. Higgins, S.J., Burchell, J.M., Parker, M.G. and Herries, D.G. (1978) *Eur. J. Biochem.* 91, 327-334
4. Mansson, P-E., Sugino, A. and Harris, S.E. (1981) *Nucleic Acids Res.* 9, 935-946
5. Ostrowski, M.C., Kistler, M.K. and Kistler, W.S. (1982) *Biochemistry* 21,

- 3525-3529
6. Kistler, M.K., Taylor, R.E., Kandala, J.C. and Kistler, W.S. (1981) *Biochem. Biophys. Res. Commun.* 99, 1161-1166
 7. Abrescia, P., Guardiola, J., Felsani, A. and Metafora, S. (1982) *Nucleic Acids Res.* 10, 1159-1174
 8. Higgins, S.J., Burchell, J.M. and Mainwaring, W.I.P. (1976) *Biochem. J.* 160, 43-48
 9. Sargent, T.D., Wu, J.R., Sala-Trepat, J.M., Wallace, R.B., Reyes, A. and Bonner, J. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3256-3260
 10. Benton, W.D. and Davis, R.W. (1977) *Science* 196, 180-182
 11. Alwine, J.C., Kemp, D.J., Parker, B.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.M. (1979) *Methods in Enzymol.* 68, 220-242
 12. Parker, M.G., White, R. and Williams, J.G. (1980) *J. Biol. Chem.* 255, 6996-7001
 13. Soberon, X., Covarrubias, L. and Bolivar, F. (1980) *Gene* 9, 287-305
 14. Cohen, S.N., Chang, A.C.Y. and Hsu, L. (1973) *Proc. Natl. Acad. Sci. USA* 70, 3240-3244
 15. Land, H., Grez, M., Hauser, H., Lindenmaier, W. and Schütz, G. (1981) *Nucleic Acids Res.* 9, 2251-2266
 16. Grunstein, M. and Hogness, D.S. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3961-3965
 17. Williams, J.G. and Lloyd, M.M. (1979) *J. Molec. Biol.* 129, 19-35
 18. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) *J. Molec. Biol.* 113, 237-251
 19. Birnboim, H.C. and Doily, J. (1979) *Nucleic Acids Res.* 7, 1513-1523
 20. Jeffreys, A.J. and Flavell, R.A. (1977) *Cell* 12, 429-439
 21. McDonnell, M.W., Simon, M.N. and Studier, F.W. (1977) *J. Molec. Biol.* 110, 119-146
 22. Southern, E.M. (1975) *J. Molec. Biol.* 98, 503-517
 23. Wahl, G.M., Stern, M. and Stark, G.R. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3683-3687
 24. Thomas, P.A. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5201-5205
 25. Durham, D.M., Perrin, F., Gannon, F. and Palmiter, R.D. (1980) *Proc. Natl. Acad. Sci. USA* 77, 6511-6515
 26. Davis, R.W., Simon, M.M. and Davison, N. (1971) *Methods in Enzymol.* 21, 413-428
 27. Schafer, M.P., Boyd, C.D., Tolstoshev, P. and Crystall, R.G. (1980) *Nucleic Acids Res.* 8, 2241-2253
 28. Prentki, P., Karch, F., Lida, S. and Meyer, J. (1981) *Gene* 14, 289-299
 29. de Wet, J.R., Daniels, D.L., Schroeder, J.L., Williams, B.G., Denniston-Thompson, K., Moore, D.D. and Blattner, F.R. (1980) *J. Virol.* 33, 401-410
 30. Staden, R. (1977) *Nucleic Acids Res.* 4, 4037-4051
 31. Naora, H. and Deacon, N.J. (1982) *Proc. Natl. Acad. Sci. USA* 79, 6196-6200
 32. Pan, Y.E., Silverberg, A.B., Harris, S.E. and Li, S.S.L. (1980) *Int. J. Pept. Protein Res.* 16, 143-146
 33. Royal, A., Garapin, A., Cami, B., Perrin, F., Mandel, J.L., Lemeur, M., Breggère, F., Gannon, F., Lepennec, J.P., Chambon, P. and Kourilsky, P. (1979) *Nature (London)* 279, 125-132
 34. Parker, M., Needham, M., White, R., Hurst, H. and Page, M. (1982) *Nucleic Acids Res.* 10, 5121-5132
 35. Clissold, P.M. and Bishop, J.O. (1982) *Gene* 18, 211-230
 36. Higgins, S.J. and Fuller, F.M. (1981) *Molec. Cell. Endocr.* 24, 85-101
 37. Page, M.J. and Parker, M.G. (1982) *Molec. Cell. Endocrinol.* 27, 343-355
 38. McKnight, G.S. and Palmiter, R.D. (1979) *J. Biol. Chem.* 254, 9050-9058
 39. Swaneck, G.E., Nordstrom, J.L., Kreuzaler, F., Tsai, M.J. and O'Malley, B.W. (1979) *Proc. Natl. Acad. Sci. USA* 76, 1049-1053