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Androgen regulated expression of a spermine binding protein gene in mouse ventral prostate

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ABSTRACT

A full length cDNA (MP25) encoding the major mouse prostatic secretory glycoprotein (p25), whose expression is androgen dependent, has been cloned and characterised. Steady-state levels of mRNA are decreased approximately 100-fold after 3 days castration but are restored progressively over 4 days with testosterone treatment. The secreted glycoprotein appears to be a spermine binding protein since the nucleotide and predicted amino acid sequence of MP25 shares extensive homology with a spermine binding protein (SBP) found in rat ventral prostate. Genomic clones indicate that there is a single gene for SBP which consists of 4 exons, the first of which is only 11bp in length. The second exon encodes the signal peptide, the third contains a portion of the spermine binding protein unique to the mouse and the largest exon encodes the bulk of the secreted protein.

INTRODUCTION

Steroid hormones are known to influence cellular development and functional differentiation by alterations in the expression of specific genes, primarily at the level of transcription. It has been proposed that such steroid hormone gene regulation is mediated via specific DNA sequences termed hormone response elements, which once activated by the binding of steroid hormone-receptor complexes can function as transcriptional enhancers (1-3). The glucocorticoid response element of the mouse mammary tumour virus (MMTV) LTR has been well characterised, both in vivo by gene transfer studies (4-7) and in vitro by binding studies with purified glucocorticoid receptor complexes(8,9). In addition to glucocorticoids, transcription from the MMTV promoter is activated by progestins (10) and androgens (11,12), effects which are mediated through the same hormone response element.

We are investigating the mechanisms whereby androgens regulate the expression of specific cellular genes in the male sex accessory tissues. Within the C3(1) rat prostate gene which encodes a subunit of the prostate steroid binding protein (13-15) two regions, one upstream of the promoter and one within the first intron, bind purified androgen receptor

(16,17). In transfection experiments the upstream region, however, fails to function as an androgen response element (18).

In view of the contrast between the effects of androgens on the MMTV and rat prostate C3(1) promoters in gene transfer experiments (18) we initiated a study to investigate genes in the mouse ventral prostate whose expression was stimulated by testosterone but which are not related to the rat prostate genes. We have identified two secretory glycoproteins, p12 and p25 in mouse prostate whose synthesis is dependent upon androgens (19). In this paper we extend our analysis of androgen-dependent gene expression in this tissue by describing the isolation and characterisation of cDNA and genomic clones which encode p25 and which share extensive homology with an androgen-regulated spermine binding protein found in the rat ventral prostate.

MATERIALS AND METHODS

Animals

Tissues were obtained from young adult male mice (C57/BT) and rats (Sprague-Dawley) maintained in "conventionalised" conditions at the Imperial Cancer Research Fund. Androgens were withdrawn by castration and where indicated testosterone (Androject) was injected subcutaneously in arachis oil in the flank region at a dose of 5 µg/g of body weight.

DNA clones

Poly(A+) RNAs were used as templates for the synthesis of double-stranded cDNAs by reverse transcriptase (Northumbria Biologicals Ltd.) primed by oligo-dT following the method of Gubler and Hoffman (20). Oligo-dC tails were added to mouse ventral prostate cDNA using terminal transferase (PL-Biochemicals) and annealed with oligo-dG tailed pUCK8, a derivative of pUC8 (21) in which the BamH1 site has been replaced with a Kpn1 linker. The method of Hanahan (22) was then used to transform *E. coli* DH1 cells. Colony filter hybridisations were carried out using procedures as described by Maniatis *et al.* (23). A genomic library was constructed in λ EMBL 4 (24) using mouse seminal vesicle DNA which had been partially cleaved with Sau3A and size selected on sucrose gradients. Genomic clones were isolated using a full length cDNA clone (MP25) as probe (Figure 1).

Appropriate cDNA and genomic restriction fragments were subcloned into M13 mp18 and mp19 to facilitate nucleotide sequence analysis by the dideoxy chain termination method of Sanger (25) using [α^{35}S]-ATP (400 Ci/mmol, Amersham International plc). Nucleotide and predicted

amino acid sequence data were compared with the EMBL, Doolittle and Los Almos databases using the local optimal alignment procedure of Wilbur and Lipman (26). The mouse β-actin cDNA was a gift from Dr. K. Willison (Chester Beatty Research Institute, London).

Plasmid manipulations

Standard methods for the isolation and manipulation of plasmid DNAs were as described by Maniatis et al. (23).

RNA analysis

Total cellular RNA was isolated by the guanidinium thiocyanate extraction procedure (27). RNA was separated by elecrophoresis in agarose gels containing 2.2M formaldehyde (28) and transferred to nitrocellulose paper for Northern analysis (29). Filters were prehybridised for 4 h at 60°C in 50% formamide, 50mM sodium phosphate buffer (pH 6.5), 0.8M NaCl, 1mM EDTA, 0.1% SDS, 2.5 x Denhardt's, 250 µg/ml denatured salmon sperm DNA and 500 µg/ml yeast RNA and hybridised for 16 h at 60°C in the same buffer containing 1.5 x 10^6 dpm/ml of [^{32}P]-labelled RNA probe (30). Filters were then washed at 65°C for four, 30 min periods in 50mM NaCl, 10mM sodium phosphate buffer (pH 6.3), 1mM EDTA, 0.1% SDS and exposed to autoradiography with XAR-5 film (Kodak). Probes were subsequently removed by boiling filters for 20 min in 0.1% SDS, 1mM EDTA.

The method used for primer extension was essentially that described by Green et al. (31). A 20bp oligonucleotide primer (5'TTGTGGTACAATCTGGCACT 3') corresponding to nucleotides 45-65 was end-labelled with (γ ^{32}P) ATP (3,000 Ci/mmol, Amersham International plc) using polynucleotide kinase (Pharmacia) and purified on a 20% acrylamide gel. Labelled primer (5 x 10^4 dpm at 2 x 10^6 dpm per pmole) was annealed to 10 µg of total RNA in 40 mM PIPES pH 6.4, 40mM NaCl and 10% formamide at 85°C for 2 min and 42°C overnight. After ethanol precipitation the annealed primer was extended using 10 units of reverse transcriptase (NBL Enzymes Ltd.) in 50mmM Tris-Cl, pH 8.3, 40mM KCl, 8mM $MgCl_2$, 0.5mM dNTPs and 0.4mM DTT at 42°C for 40 min. The RNA was then digested with RNase-T1 (Cal-Biochem) at 1 µg/ml and RNase-A (Sigma) at 20 µg/ml for 30 min at room temperature After phenol:chloroform extraction and ethanol precipitation the samples were electrophoresed on a standard 6% sequencing gel.

In vitro transcription and translation

MP25 specific mRNA was obtained by the in vitro transcription of the MP25 cDNA using SP6 polymerase (32). This mRNA was translated in a

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                                                    AGAGAGCACAGAGCGTCACTGAAATCTTACCATTGTGGCATCTAAGTGCC  50

     RsaI
AGATTGTACCACAAACCCTCAGGAGCC ATG CTG CTG TTG CTG ACT TTG GCT TTC CTG GCC 110
                            MET Leu Leu Leu Leu Thr Leu Ala Phe Leu Ala
                            1                                       10

AGT CCC ACA TGC AGA GCC CAG AAT GTC CTG GGG AAT GCT GCT GGC AAG TAT TTC 164
Ser Pro Thr Cys Arg Ala Gln Asn Val Leu Gly Asn Ala Ala Gly Lys Tyr Phe
                                20

TAC GTT CAA GGT GAA GAT CAG GGG CAA CTC AAG GGC ATG CGG ATC TTC TTA AGT 218
Tyr Val Gln Gly Glu Asp Gln Gly Gln Leu Lys Gly MET Arg Ile Phe Leu Ser
30                                      40
                                     PstI
GTT TTC AAG TTC ATC AAG GGC TTC CAG CTG|CAG TTT GGC AGT AAC TGG ACT GAT 272
Val Phe Lys Phe Ile Lys Gly Phe Gln Leu Gln Phe Gly Ser Asn Trp Thr Asp
        50                                      60          (CHO)

GTC TAT GGA ACC CGG TCA GAT AAC TTT ATA GAC TTC CTT CTG GAG GAC GGA GAG 326
Val Tyr Gly Thr Arg Ser Asp Asn Phe Met Asp Phe Leu Leu Glu Asp Gly Glu
                70                                      80

CAC GTG ATA AAG GTA GAG GGC AGT GCA GTG ATC TGC CTG ACT TCC CTG ACC TTC 400
His Val Met Lys Val Glu Gly Ser Ala Val Ile Cys Leu Thr Ser Leu Thr Phe
                        90                                      100

ACT ACA AAC AAG GGG CGT GTG GCC ACC TTT GGT GTT AGA AGG GGT CGA TAC TTC 434
Thr Thr Asn Lys Gly Arg Val Ala Thr Phe Gly Val Arg Arg Gly Arg Tyr Phe
                                110

AGT GAC ACT GGA GGT TCA GAC AAG CAT CTA GTG ACT GTC AAT GGA ATG CAT GCA 488
Ser Asp Thr Gly Gly Ser Asp Lys His Leu Val Thr Val Asn Gly MET His Ala
120                                     130

CCG GGC CTC TGC GTC AGA GGG ATT GGC TTC AAA TGG GGA AAT ATC AAT GCT AAT 542
Pro Gly Leu Cys Val Arg Gly Ile Gly Phe Lys Trp Gly Asn Ile Asn Ala Asn
        140                                     150

GGC AAT GAC CAT TAT AAT AAC AAG GAA GAT AAG GCT GAC AAC AAA GAT GCT GAC 596
Gly Asn Asp His Tyr Asn Asn Lys Glu Asp Lys Ala Asp Asn Lys Asp Ala Asp
                160                                     170

AAC AAA GAT GCT GAC AAC AAA GAT GAT GGA GAT GAG GAT GAT GAT GGA AAC GAT 650
Asn Lys Asp Ala Asp Asn Lys Asp Asp Gly Asp Glu Asp Asp Asp Gly Asn Asp
                        180                                     190

GAT GAT GAC CAG AAA GAT GAA AGC TGAGTAGCATGCTCAGAGCTCAGTCCTGCATCTCCACAT 713
Asp Asp Asp Gln Lys Asp Glu Ser
                        199

GGCAGCGCCTCCTCTCAGCCAAGCTCTCCCACTGGAGGAAGTAATATACCAGGAGACTCCAGGCTGGGGGG 784

AGCAAAGCCCAAATGCCTTTTATCTCTCTCTGTCTAATAAACTATGTGCATTCT..Poly(A)         838
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Figure 1.
Nucleotide sequence and predicted amino acid sequence of MP25. The numbers on the right-hand side refer to the nucleotide sequence. Numbering of amino acid residues are given below the sequence starting from the first ATG. A putative signal peptide at the amino terminus (amino acid residues 1-18) and a polyadenylation signal (AATAAA) at nucleotide 820-825 is underlined. A potential N-linked carbohydrate attachment site (CHO) at amino acid residues 62-64 and pertinent restriction enzyme sites are also indicated.

methionine dependent wheat-germ in vitro translation system (Amersham International plc) following the suppliers instructions. The isolation of mouse ventral prostate proteins, immunoprecipitation with prostate specific antiserum and SDS-polyacrylamide gel analysis were as described previously (19).

RESULTS

Isolation and nucleotide sequence analysis of MP25 cDNA

We have previously identified two secretory proteins (p12 and p25) in mouse ventral prostate whose synthesis is dependent upon testicular androgens (19). In order to isolate cDNA clones corresponding to these proteins, poly(A+) mRNA from mouse ventral prostate was used to generate a cDNA library which was differentially hybridised with cDNA probes derived from prostatic RNA of normal and 3 day castrate mice. Of 24 differentially hybridising clones identified, 19 were related as defined by cross-hybridisation analysis on Southern blots (data not shown). The relative abundance of these clones in the library (19 in 7,000) suggests that their corresponding mRNA represents approximately 0.3% of the ventral prostate poly A+ mRNA.

The four longest cDNA clones were selected for further analysis. The nucleotide sequence of one such clone, designated MP25, which is full length (see below) is shown in Figure 1. The sequence encodes an open reading frame of 199 amino acids where the ATG at nucleotides 78-80 represents the first methionine residue. MP25 has 5′ and 3′ untranslated regions of 77 and 164 nucleotides respectively and a consensus polyadenylation signal (AATAAA) (33) is located at nucleotides 820-825 which is 14 nucleotides upstream of the poly(A) tail. The amino terminal portion of the MP25 ORF is rich in hydrophobic residues, a feature common to the signal peptides of most secretory proteins (34). Although the amino-terminal sequence of the mature protein encoded by MP25 has not been directly determined, on the basis of (a) the (-3,-1) rule and weight matrix analysis (35) and (b) the exon-intron structure of the MP25 gene (see below) the signal peptide cleavage site is likely to be between residues 18 (Gln) and 19 (Asn). Analysis of the sequence data reveals a potential N-linked carbohydrate attachment (glycosylation) site (Asn-X-Thr) at amino acid residues 62-64. The protein encoded by MP25 is rich in acidic residues such that of 36 amino acids at the carboxy terminus, there is a total of 20 aspartic and glutamic acid residues.

Androgen induction of MP25 mRNA in ventral prostate

The effect of androgens on the steady-state level of MP25 mRNA in ventral prostate was analysed by Northern blotting and primer extension A Northern blot of total cellular RNA from ventral prostate of mice of defined androgen status, that is, normals, 3 day castrates and mice subjected to testosterone therapy after 3 days castration, was probed

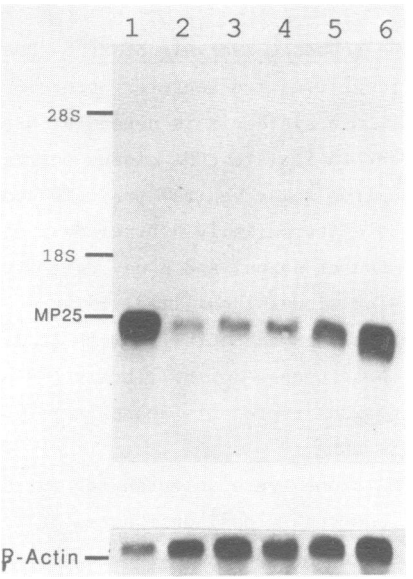

Figure 2.
Effect of androgens on MP25 expression in ventral prostate. Total ventral prostate RNA (10 µg) was analysed by Northern blot hybridisation. RNA was isolated from normal mice (Lane 1), and mice after 3 days castration (Lane 2), 3 days castration and 4 h testosterone treatment (Lane 3), 3 days castration and 16 h testosterone treatment (Lane 4), 3 days castration and 48 h testosterone treatment (Lane 5) and 3 days castration and 96 h testosterone treatment (Lane 6). The nitrocellulose filter was hybridised with an antisense RNA probe corresponding to the entire MP25 cDNA (upper panel) and subsequently with an antisense probe for mouse β-actin (lower panel). Filters were exposed to X-ray film for 4 h without intensifying screens. The migration of 28S and 18S ribosomal RNAs are indicated on the left.

with [^{32}P]-labelled MP25 antisense RNA probe (Figure 2). Messenger RNA of 800-900bp is observed in prostate RNA from normal mice. After 3 days castration the steady state level of MP25 mRNA is reduced to approximately 1% of that in normal mice. Re-administration of testosterone to 3 day castrates results in increased levels of MP25 RNA, detectable within 16 h and reaching normal levels after 96 h of testosterone therapy. Castration for periods longer than 3 days resulted in a gradual decrease in MP25 mRNA levels in ventral prostate, but after 8 days castration MP25 mRNA was still readily detectable (data not shown). A control experiment shows that the concentration of β-actin mRNA in the ventral prostate is not induced by testosterone (Figure 2,

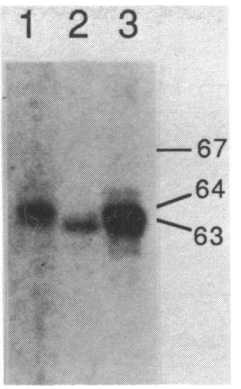

Figure 3.
Primer extension analysis of the 5′ end of MP25 mRNA.
Total ventral prostate RNA (10 µg) from (Lane 1) normal mice, (Lane 2) 3-day castrates and (Lane 3) 3 day castrates treated with testosterone for 96 h was annealed with a 20bp oligonucleotide primer (Materials and Methods) and extended with reverse transcriptase. The major extension products of 63bp and 64bp and the position of DNA molecular eight markers are indicated.

lower panel) but the amount of RNA from normal mice appears to have been under-loaded.

The 5′ end of MP25 mRNA was analysed by primer extension (Figure 3). Primer extension products were detected in RNA from normal, castrated and testosterone treated mice, but the signal intensity of the extension products in the castrate samples was much reduced, confirming the androgenic regulation of this mRNA. Products of 64bp and 63bp were detected in RNA from normal and testosterone treated mice which confirms that the MP25 cDNA represents the entire length of its corresponding cellular mRNA. In RNA from castrated animals the 64bp extension product is not apparent whereas the extension product of 63bp is still detectable.

MP25 encodes the major prostatic secretory glycoprotein (p25)

Several lines of evidence support the assumption that the MP25 cDNA corresponds to the previously identified major secretory glycoprotein of mouse ventral prostate (19). Both are regulated by testicular androgens and the predicted protein size deduced from the cDNA is in agreement with the size of authentic p25. The most convincing evidence, however, comes from the *in vitro* translation of mRNA derived from the MP25 cDNA which generates a polypeptide that is immunoprecipitated by a p25 specific

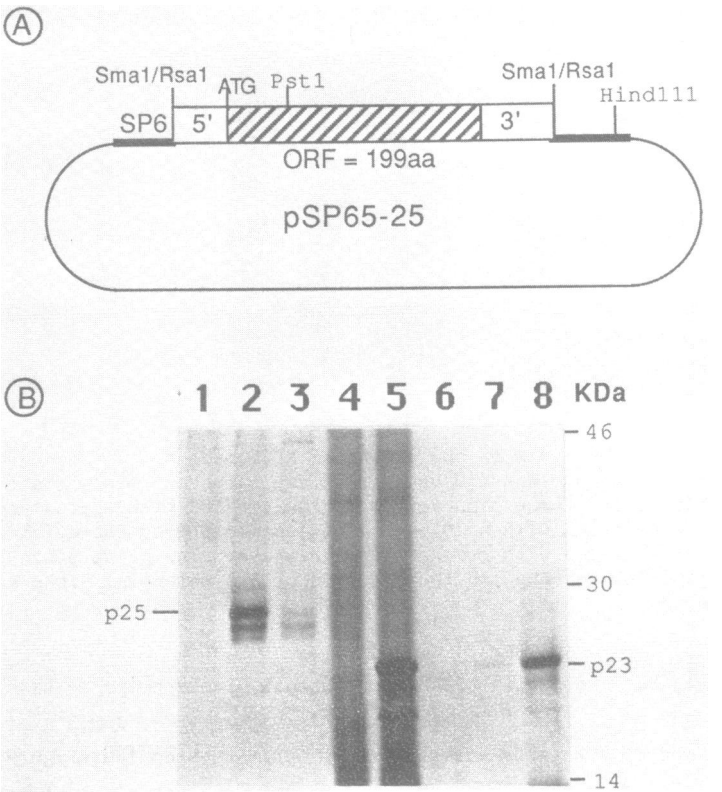

Figure 4.
Identity of MP25 and mouse prostatic p25.
A. Structure of pSP65-25 used for in vitro synthesis of MP25 mRNA. The open reading frame of MP25 is indicated by the hatched area and the untranslated regions by the unshaded areas. The SP6 promoter and polylinker is indicated by the thick line and plasmid sequences are shown as a thin line. The ATG and pertinent restriction enzyme sites are indicated.
B $_{35}$ Autoradiograph of a SDS-polyacrylamide gel (15%) of the following [^{35}S] methionine labelled protein samples, (Lane 1) normal ventral prostate proteins, immunoprecipitated with pre-immune serum, (Lane 2) normal ventral prostate proteins immunoprecipitated with p25 specific immune serum, (Lane 3) ventral prostate proteins from 3-day castrates, immunoprecipitated with p25 specific immune serum, (Lanes 4-8) wheat germ in vitro translation products with, (Lane 4) no exogenous RNA added, (Lane 5) 100 ng MP25 specific mRNA added, (Lane 6) 100 ng MP25 specific mRNA added, immunoprecipitated with pre-immune serum, (Lane 7) 10 ng MP25 specific mRNA, immunoprecipitated with p25 specific antiserum, (Lane 8) 100 ng MP25 specific mRA, immunoprecipitated with p25 specific antiserum. The positions of standard molecular weight markers (KDa) are indicated.

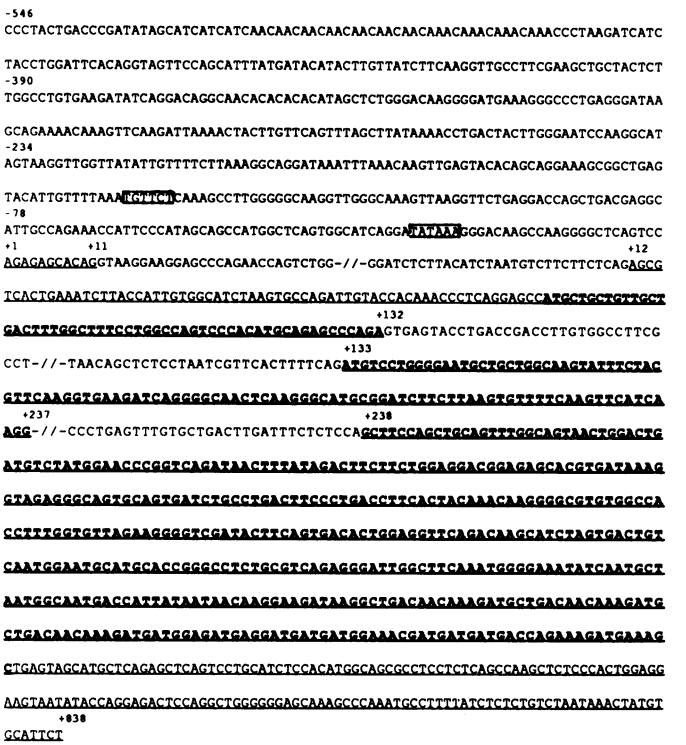

Figure 5.
Sequence analysis of the MP25 gene.
The promoter region and intron sequences are in regular type. The 5′ and 3′ untranslated regions of the MP25 cDNA are in underlined regular type, while the open reading frame is in underlined bold type. The promoter sequence extends to -546, where +1 is the mRNA CAP site. The nucleotide positions of the intervening sequences with respect to the cDNA are indicated at the exon-intron boundaries. -||- indicates gaps. Sequences of interest are boxed; see text for details.

antiserum (Figure 4). The complete open reading frame of MP25 was subcloned as a RsaI fragment into the SmaI site of pSP65 generating pSP65-25 so that the ATG of the open reading frame is 40 nucleotides from the SP6 promoter (Figure 4A). The SP65-25 plasmid was linearised at the Hind III site, 3′ to the open reading frame and MP25 RNA was transcribed in vitro using SP6 polymerase. In vitro translation of this mRNA in a wheat germ system resulted in a major polypeptide species of approximately 23 KDa (Figure 4B, Lane 5). This protein was immunoprecipitated with polyclonal antisera raised to purified mouse

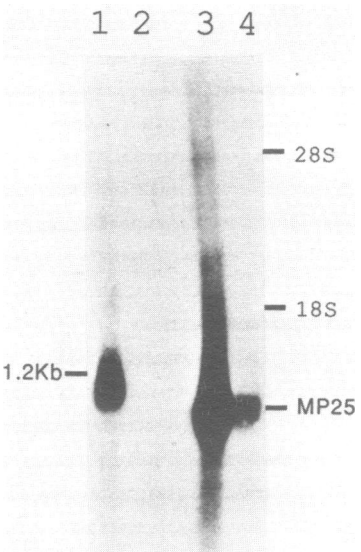

Figure 6.
Cross hybridisation of MP25 with an androgen dependent mRNA of rat ventral prostate.
Total cellular RNA (10 µg) from the ventral prostates of (Lane 1) normal rats, (Lane 2) rats castrated for 3 days, (Lane 3) normal mice, (Lane 4) mice castrated for 3 days, was electrophoresed on a denaturing gel and transferred to nitrocellulose. The filter was subsequently hybridised with an antisense RNA probe corresponding to MP25 and washed at high stringency (Materials and Methods). The filter was exposed for 8 h without intensifying screens.

prostatic secretory p25 (Figure 4B, Lanes 7-8) but was not immunoprecipitated with pre-immune serum (Figure 4B, Lane 6). The size difference between the immunoprecipitated *in vitro* translation product and p25 in normal prostate (compare Lanes 2 and 7, Figure 4B) arises as a result of the inability of the wheat germ *in vitro* translation system to remove the signal peptide and to glycosylate the primary translation product.

Structure of the mouse SBP gene

Overlapping genomic clones, λMP25-1 and λMP25-2, were isolated from a mouse genomic library using MP25 cDNA as probe. The nucleotide sequence of the promoter region to -546 and the exon-intron boundaries with respect to the cDNA is illustrated in Figure 5. The first exon of the gene is located within the 5′ untranslated region and is 11bp in length. The second exon from +12 to +132 represents the remainder of the 5′

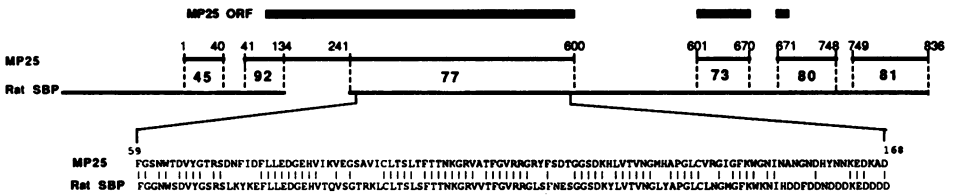

Figure 7.
Homology of MP25 with rat prostatic spermine binding protein.
The MP25 open reading frame is shown as a thick line. The cDNAs for MP25 and rat SBP have been aligned and are shown as thin lines, gaps have been introduced to maximise the alignment. The percentage homology at the nucleotide sequence level is indicated in the boxes, above which are shown the nucleotide positions for the MP25 cDNA. The homology at the amino acid level between residues 59-168 in MP25 and the rat SBP is illustrated below, with the amino acids given in single letter code. The sequence of the rat SBP cDNA was obtained from Chang et al. (40).

untranslated region and the coding sequence for the presumptive signal peptide. Exon 3 is from +133 to +237 while the remainder of the open reading frame and the 3′ untranslated region is encoded within the fourth exon from nucleotides +238 to +838. Within the upstream sequence there are several interesting features to note. There is a "TATA" box homology (36) located 24 nucleotides upstream of the mRNA CAP site and a TGTTCT motif at -136 which forms part of a consensus sequence required for the binding of steroid receptor complexes to hormone response elements (37-39).

MP25 is homologous to a rat ventral prostate spermine binding protein

We have investigated the tissue distribution of MP25 mRNA in the mouse by Northern blot analysis and have found that expression is ventral prostate specific, with no detectable MP25 transcripts being observed in other sex accessory tissues (seminal vesicle and coagulating gland) or in liver, spleen, kidney and salivary gland (data not shown). MP25 did not cross-hybridise with RNA from human benign prostatic hyperplasia tissue but when rat ventral prostate RNA was analysed by Northern blotting a mRNA species of approximately 1.2 Kb was detected. This mRNA is also dependent upon testicular androgens for its expression but differs from MP25 mRNA in that it is not detectable after 3 days castration (Figure 6). MP25 is highly homologous with a secretory prostatic spermine binding protein (SBP) identified in the rat (Figure 7) (40) and shares no homology with any other sequences in the nucleotide and protein data bases. At the nucleotide sequence level, the greatest degree of homology

is 92% between nucleotides 41-134 in MP25, which corresponds to the signal peptide and a portion of the 5′ untranslated region. Within the MP25 open reading frame, the nucleotides between 241-600 and 601-670 are 77% and 73% homologous with the rat SBP cDNA. The 3′ untranslated regions are approximately 80% homologous. A region within the MP25 sequence between nucleotides 134-241, which is not found in the rat SBP cDNA corresponds to the third exon of the MP25 gene. When the predicted amino acid sequences of the two proteins are compared the greatest degree of homology is apparent in the central portion of the protein where the amino acid homology is 64% over a 110 amino acid stretch (Figure 7). Despite the extremely high nucleotide sequence homology (92%) within the region of the amino terminus of the open reading frame, the two proteins apparently share no homology in this region. We have found, however, that by inserting an extra nucleotide in the rat SBP sequence near the 5′ end, the resulting frameshift means that the predicted open reading frames then align exactly at the amino terminus with respect to the first ATG and furthermore, the mouse SBP signal peptide is then 83% homologous with a potential signal peptide at the amino terminus of rat SBP.

DISCUSSION

We have isolated and sequenced a full length cDNA (MP25) from mouse ventral prostate which corresponds to a mRNA whose expression is dependent upon testicular androgens. This cDNA encodes p25, the major secretory glycoprotein of ventral prostate, as judged by immunoprecipitation of cell-free translation products with a specific antiserum. Sequence analysis reveals that MP25 shares extensive homology at the nucleotide and amino acid level with a secretory spermine binding protein of rat ventral prostate. Whilst we have not directly demonstrated the ability of p25 to bind polyamines including spermine, the degree of homology between p25 and rat SBP is such that it is reasonable to conclude that they are functionally equivalent proteins. The biochemical and immunochemical properties of rat SBP have been studied in some detail (41,42) but its precise physiological function in prostatic secretion is unclear. It is known, however, that prostatic fluid is rich in polyamines (43) including spermine and the SBP may serve therefore as a "carrier" for these molecules in the seminal fluid. Both mouse and rat SBPs are rich in acidic amino acid residues, particularly at the carboxy terminus which could be involved in

polyamine binding, but in any event may be a feature relevant to its physiological role.

The kinetics of induction of androgen responsive mRNAs varies widely, ranging from rapid responses detectable within a few hours, e.g. rat prostatic C3 polypeptide (44) and mouse mammary tumour virus (45), to very slow responses occurring over days such as β-glucoronidase (46,47). The mRNA for spermine binding protein resembles that for ornithine decarboxylase in the mouse kidney (48) by increasing about 2-3 fold within 16 h and continuing progessively over several days. Another mouse prostate mRNA, specific for a secretory protease inhibitor (49) differs from SBP mRNA by being undetectable in castrated animals and by increasing much more rapidly after testosterone treatment The variability in induction kinetics between androgen regulated genes may reflect the different levels at which steroids exert their effects, be it transcriptional or post-transcriptional..

Primer extension analysis indicates differences in the 5′ end of RNA transcripts in normal and testosterone treated mice versus castrated mice. The possibility of two genes with different CAP sites that are regulated differently by androgen has been ruled out by our analysis of genomic DNA (unpublished data) which indicate the existence of a single gene. While the primer extension data may be artefactual, it is conceivable that there is one SBP gene whose two major transcripts of differing size arise through variability in CAP site usage and testosterone may play a role in the mechanism generating such micro-heterogeneity.

Analysis of the structural gene for the mouse SBP with respect to the protein coding sequence reveals that the exon structure corresponds to potential functional domains within the protein. The 5′ untranslated region is encoded within two exons, the first of which is 11bp and the second of which also encodes a presumptive signal peptide. Exon 3 encodes a protein domain which is not present in the rat SBP and probably arose by an insertion event after the evolutionary divergence of rat and mouse. The fourth exon, encodes the major portion of the protein which contains the highly acidic region at the carboxy terminus. In addition to the TATA box homology and the TGTTCT hormone response element consensus sequence, the nucleotide sequence of the SBP gene reveals several other features of interest. Within the 5′ flanking region of the gene there are multiple direct repeats of 5′-CAA-3′ (nucleotides -518 to

-498) and of 5'-CAAA-3' (nucleotides -497 to -482). Furthermore, within the open reading frame of the SBP there are two copies of a direct repeat 5'-GACAACAAAGATGCT-3'and also a third copy with one mis-match (nucleotides -579 to -623) which encodes a repetitive pentapeptide motif in the protein structure. The biological relevance of these repeated structures is not known.

We are currently analysing the mouse SBP promoter sequences in some detail and using gene transfer as an approach to help is the elucidation of the mechanisms by which androgens exert their effects on gene expression in the mouse prostate gland.

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REFERENCES

1. Jensen, E.V., Suzuki, T., Kawashima, T., Stumpf, W.E., Jungblut, P.W. and DeSombre, E.R. (1968) Proc. Natl. Acad. Sci. USA 59, 632-638.
2. Yamamoto, K.R. (1985) Ann. Rev. Genet. 19, 209-252.
3. Parker, M.G. (1986) Cancer Surv. 5, 625-633.
4. Buetti, E. and Diggelmann, H. (1983) EMBO J. 2, 1423-1429.
5. Chandler, V.L., Mater, B.A. and Yamamoto, K.R. (1983) Cell 33, 489-499.
6. Hynes, N., van Ooyen, A.J.J., Kennedy, N., Herrlich, P., Ponta, H. and Groner, B. (1983) Proc. Natl. Acad. Sci. USA 80, 3637-3641.
7. Ponta, H., Kennedy, N., Skroch, P., Hynes, N.E. and Groner, B. (1985) Proc. Natl. Acad. Sci. USA 82, 1020-1024.
8. Payvar, F., DeFranco, D., Firestone, G.L., Edgar, B., Wrange, O., Okret, S., Gustafson, J.-A., Yamamoto, K.R. (1983) Cell 35, 381-392.
9. Scheidereit, C. and Beato, M. (1984) Proc. Natl. Acad. Sci. USA 81, 3029-3033.
10. Cato, A.C.B., Miksicek, R., Schutz, G., Arnemann, J. and Beato, M. (1986) EMBO J. 5, 2237-2240.
11. Darbre, P., Page, M. and King, R.J.B. (1986) Mol. Cell. Biol. 6, 2847-2854.
12. Cato, A.C.B., Henderson, D. and Ponta, H. (1987) EMBO J. 6, 363-368.
13. Parker, M.G., Scrace, G.T. and Mainwaring, W.I.P. (1978) Biochem. J. 170, 115-121.
14. Hurst, H.C., and Parker, M.G. (1983) EMBO J. 2, 769-774.
15. Parker, M.G., White, R., Hurst, H., Needham, M. and Tilly, R. (1983) J. Biol. Chem. 258, 12-15.
16. Perry, S.T., Viskochil, D.M., Ho, K.-C., Fong, K., Stafford, D.W., Wilson, E.M. and French, F.S. (1985) In Bruchovsky, N.,

Chapdelaine, A. and Neumann, F. (eds), Regulation of Androgen Action, Congressdruck R. Bruckner, West Berlin, pp.167-173.
17. Rushmere, N.K., Parker, M.G. and Davies, P. (1987) Mol. Cell. Endocrinol. 51, 259-265.
18. Parker, M.G., Webb, P., Needham, M., White, R. and Ham, J. (1987) J. Cell. Biochem. in press.
19. Mills, J.S., Needham, M. and Parker, M.G. (1987) Mol. Cell. Endocrinol. in press.
20. Gubler, U. and Hoffman, B.J. (1983) Gene 25, 263-269.
21. Viera, J. and Messing, J (1982) Gene 19, 259-268.
22. Hanahan, D. (1983) J. Mol. Biol. 166, 557-580.
23. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
24. Frischauf, A., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol. 170, 827-842.
25. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
26. Wilbur, W.J. and Lipman, D.J. (1983) Proc. Natl. Acad. Sci. USA 80, 726-730.
27. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
28. Lehrach, H., Diamond, D., Wozney, J.M. and Boedtler, H. (1977) Biochemistry 16, 4743-4751
29. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
30. Melton, D.A. Krieg, P.A., Rebagliati, M.R.,Maniatis, T., Zinn, K. and Green, M.R. (1984) Nucleic Acids Res. 12. 7035-7056.
31. Green, S., Walter, P., Kumar, V., Krust, A., Bornert, J.-M., Argos, P. and Chambon, P.(1986) Nature 320, 134-139.
32. Krieg, P.A.and Melton, D.A.(1984) Nucleic Acids Res. 12, 7057-7070.
33. Proudfoot, N.J. and Brownlee, G.G. (1976) Nature 263, 211-214.
34. Blobel, G and Dobberstein, B. (1975) J. Cell Biol. 67, 835-851.
35. von Heijne, G. (1986) Nucleic Acids Res. 11, 4683-4690.
36. Breathnach, R. and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349-383.
37. Scheideret, C., Geisse, S., Westphal, H.M. and Beato, M. (1983) Nature 304, 749-752.
38. Karin, M., Haslinger, A., Holtgreve, A., Richards, R.I., Krauter, P., Westphal, H.M. and Beato, M. (1984) Nature 308, 513-519.
39. Von der Ahe, D., Naich, S., Scheideret, C., Renkawitz, R., Schutz, G. and Beato, M. (1985) Nature 313, 706-709.
40. Chang, C., Saltzman, A.G., Hiipakka, R.A., Huang, I.-Y. and Liao, S. (1987) J. Biol. Chem. 262, 2826-2831.
41. Mezzetti, G., Loor, R. and Liao, S. (1979) Biochem. J. 184, 431-440.
42. Hiipakka, R.A., Chen, C., Schilling, K., Oberhauser, A., Saltzman, A. and Liao, S. (1984) Biochem. J. 218, 563-571.
43. Pegg, A.E., Lockwood, D.H. and Williams-Ashman, H.G. (1970) Biochem. J. 117, 17-31.
44. Parker, M.G., White, R. and Williams, J.G. (1980) J. Biol. Chem. 255, 6996-7001.
45. Darbre, P.D., Moriarty, A., Curtis, S.A. and King, R.J.B. (1985) J. Steroid Biochem. 23, 379-384.
46. Catterall, J.F. and Leary, S.L. (1983) Biochemistry 22, 6049-605.
47. Watson, G., Felder, M., Rabinow, L., Moore, K., Labarca, C.,

Tietze, C., van der Molen, G., Bracey, L., Brabant, M., Cai, J. and Paigen, K. (1985) Gene 36, 15-25.
48. Berger, F.G., Loose, D., Meisner, H. and Watson, G. (1986) Biochemistry 25, 1170-1175.
49. Mills, J.S., Needham, M. and Parker, M.G. (1987) in preparation.