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Prostate Epithelial Expression of a Novel Androgen Target Gene

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ABSTRACT: To better understand the role of androgens in prostate development and disease it is important to characterize androgen-regulated genes in the prostate. Using suppression subtractive hybridization between congenitally androgen-deficient (*hpg*) and androgen-replaced *hpg* mouse prostates, we have cloned a novel *a*ndrogen *u*p-regulated gene from *m*ouse *p*rostate (AUMP). The messenger RNA sequence of AUMP consists of 805 nucleotides with an open reading frame of 408 base pairs. In non-*hpg* mice with normal androgen levels, AUMP is selectively expressed in the prostate, as shown by reverse transcriptase-polymerase chain reaction and Northern blot analysis of 9 organs. Depletion of androgens via castration of mature mice resulted in loss of AUMP expression, whereas testosterone replacement restored it. Tissue in situ hybridization localized AUMP expression to the luminal epithelial cells of the androgen-sufficient prostate. Database searches indicate that AUMP codes for a novel protein that shares approximately 65% similarity and 35% identity to palmitoyl protein thioesterase of human, rat, mouse, and bovine. A motif for protein-transport protein, which promotes translocation as well as integration of secretory proteins into membrane, is also present. Further efforts will be made to obtain the human homologue of AUMP that will enable evaluation of its role in normal and diseased human prostate.

Key words: *Hpg,* up-regulation, subtractive hybridization, differential expression, in situ hybridization.

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Androgens are essential for various aspects of prostatic growth and maintenance, including cell proliferation (Isaacs et al, 1992), differentiation (Bonkhoff and Remberger, 1996), and apoptosis (English et al, 1985; Kyprianou and Isaacs, 1988; Isaacs et al, 1992). Patients castrated before puberty or with genetic diseases that impair androgen action or production do not develop benign prostatic hyperplasia or prostate cancer (McConnell, 1995). Androgens are thus involved both in normal development of prostate and in subsequent prostate diseases in humans. Although the prostate is a common site of neoplasia in men, the genetic basis of prostate development and its maintenance is not well understood. Because androgen action is mediated through its target genes, it is necessary to characterize androgen-regulated genes to understand the mechanisms underlying normal prostate physiology or disease.

The choice of paradigm to identify androgen-regulated genes requires consideration, because genes that mediate androgen action are likely to be cell type specific and temporally regulated. Using individual cell lines was considered unsuitable because each cell type in the prostate is a potential target for androgen action. Although androgen-regulated genes from the prostate can be identified from mice undergoing androgen withdrawal, replacement, or both, these animals have had exposure to androgens until castration. As such, genes mediating androgen action at an early stage of prostate development may be missed. We therefore decided to utilize the *hpg* mouse (Cattanach et al, 1977) to identify androgen-regulated genes in the prostate. The *hpg* mouse has a deletion (>33.5 kilobases) in the gonadotropin-releasing hormone gene (Mason et al, 1986), resulting in a substantial decrease in blood gonadotropins and immature sex accessory organs, including the prostate. Using a subtractive hybridization approach between androgen-deficient and androgen-replaced prostate glands, we have identified a novel gene, AUMP (ie, *a*ndrogen *u*p-regulated gene in the *m*ouse *p*rostate) whose expression is up-regulated by androgens in the course of growth from rudimentary to mature prostate, and which codes for a palmitoyl protein thioesterase (PPT)-like protein.

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Materials and Methods

Animals

The *hpg* mouse colony was maintained at the University of Sydney Animal House. They were bred from fertile heterozygotes obtained originally from F1 hybrids of 2 inbred strains, C3H/HeH and 101/H. Mice were genotyped by a polymerase chain

reaction (PCR) on proteinase-K digests of tail snips as described previously (Singh et al, 1995).

Tissue Preparation

Prostates from homozygous *hpg* mice with (n = 12) or without (n = 35) testosterone treatment and from non-*hpg* mice with (n = 20) or without (n = 12) orchidectomy were excised using a micro-dissection microscope, washed in cold phosphate-buffered saline (PBS) and snap-frozen in liquid nitrogen before being transferred to a −70°C freezer. Testosterone was administered as described previously (Singh and Handelsman, 1996). Briefly, a silastic tube (1.47 mm inner diameter and 1 cm in length) filled with crystalline testosterone (Sigma, Sydney, NSW, Australia) was implanted in homozygous *hpg* mice subdermally for 14 days before ventral prostates were removed. Orchidectomy was performed on 8-week-old mice via scrotal incision 14 days prior to prostate tissue harvesting. All procedures were performed under anesthesia and were approved by the University of Sydney's Animal Care and Ethics Committee.

RNA Isolation and Reverse Transcription

Pooled prostate glands (40–70 mg) were homogenized in 1 mL of TRI Reagent (Sigma) using a pellet pestle (Kontes, Vineland, NJ). Total RNA was isolated following the TRI Reagent protocol. RNA concentration was determined by absorbance at 260 nm using a UV spectrophotometer (Pharmacia Biotech, Uppsala, Sweden). The quality of total RNA was assessed by formaldehyde-agarose gel electrophoresis. Poly(A)$^+$ RNA was isolated from total RNA using oligo(dT) attached magnetic beads (DYNAL, Oslo, Norway). Poly(A)$^+$ RNA (250 ng) was reverse transcribed to first-strand complementary DNA (cDNA) using 200 U/μL of Moloney murine leukemia virus reverse transcriptase in the presence of a modified oligo(dT) primer provided by the SMART PCR cDNA synthesis kit (Clontech, Palo Alto, Calif). The single-stranded cDNA was then used for suppressive subtractive hybridization, PCR, and virtual Northern analysis as described below.

Suppression Subtractive Hybridization

Double-stranded cDNA was generated using primers provided by the SMART PCR cDNA synthesis kit (Clontech) after optimizing cycle numbers to ensure that the cDNA remained in the exponential phase of amplification. The resultant cDNA was subjected to column purification following the protocol provided by the SMART PCR cDNA synthesis kit. Suppression subtractive hybridization (SSH) was conducted as described previously (Diatchenko et al, 1996) using a PCR-Select cDNA subtraction kit (Clontech). The tester was cDNA derived from testosterone-replaced *hpg* prostate, and the driver was from *hpg* prostate. Both underwent *Rsa*I digestion, but only the tester cDNA was ligated with adaptors. After 2 rounds of hybridization between excess driver and ligated tester cDNA, PCR amplification was carried out using primers based on adaptor sequences.

Differential Screening

The PCR products were subcloned into pGEM T-Easy Vector (Promega, Sydney, Australia), and the resultant constructs were used to transform *Escherichia coli*. White colonies were randomly selected and cultured for 4–5 hours at 37°C in 96-well microtiter plates containing 200 μL of Luria Bertani medium supplemented with ampicillin (Sigma) (100 μg/mL). PCR products of LB culture were denatured and dot-blotted onto 2 sets of identical nylon membranes (Hybond, Amersham, Buckinghamshire, United Kingdom) and fixed by UV irradiation (Stratagene, La Jolla, Calif). The 2 sets of membranes were hybridized overnight at 72°C to radiolabeled *hpg* and the testosterone-replaced *hpg* probe, respectively. The washing condition included low (2× saline-sodium citrate [SSC] and 0.5% sodium dodecyl sulfate [SDS]; 4 times for 20 minutes) and high (0.2× SSC and 0.5% SDS; twice for 20 minutes) stringency washes at 68°C. The hybridization signals on the membranes were detected with a PhosphorImager (Cyclone; Packard, Mount Waverly, Australia) after overnight exposure. To decrease the number of false positive clones being selected for plasmid purification and sequencing, all positive clones identified were dot-blotted in duplicate on 2 sets of fresh membranes, and underwent a second round of hybridization using the same probes and conditions. Insert sequences of the truly differentially expressed clones were determined by single direction sequencing.

Reverse Transcription and PCR

Total RNA (1 μg) extracted from various normal and *hpg* mouse organs were treated for elimination of DNA with 1 unit of DNase I (Gibco BRL, Melbourne, Australia) and reverse transcribed to cDNA using Superscript II RNase H-reverse transcriptase (Gibco BRL). Both oligo(dT) and random hexamer primers were included in final concentrations of 20 ng/mL and 1 ng/mL, respectively. For PCR, the single-stranded cDNA was amplified using AUMP forward 5′-GCGGGGACATTTGTTGGTAT-3′ and reverse 5′–GGACAGAGAAAGAAAGCGGCTA-3′ primers. PCR amplification was performed using 0.4 mM primers, 1′ PCR buffer (Perkin Elmer), 0.4 mM dNTPs, and 0.02 U/mL of *Taq* DNA polymerase. Samples were denatured at 95°C for 1 minute, followed by 25 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds. Mouse β-actin primers (forward, 5′-CCTAAGGCCAACCGTGAA-3′; reverse, 5′-AACCGCTCGTTGCCAATA-3′) were used to monitor equality of loading.

Virtual and Classical Northern Analysis

Virtual Northern analysis was performed to examine the transcript size using the SMART cDNA synthesis kit (Clontech). A total of 200 ng of double-stranded cDNA obtained following 17–20 cycles of PCR was electrophoresed on 1% agarose gel containing 10 mg/mL of ethidium bromide for 1 hour at 90 V and transferred onto a positively charged Hybond N nylon membrane and covalently bound using a UV cross-linker. The probe was the PCR product of AUMP. The PCR product was purified from a 1% low-melting-point agarose gel with the GeneClean kit (BIO 101, Vista, Calif) and labeled with [α-^{32}P]dCTP. Labeled probe was added to a hybridization solution (ExpressHyb, Clontech) to give a concentration of 1×10^6 cpm/mL and hybridized with the membrane at 68°C for 12–16 hours. The washing conditions were 2× SSC, 0.05% SDS (4 times for 20 minutes) followed by 0.1× SSC and 0.1% SDS (twice for 20 minutes) at 50°C. The same membrane after stripping was hybridized with mouse β-

actin as a control for loading. For classical Northern analysis, 20 μg of total RNA from each of 8 normal mouse organs was electrophoresed for 2.5 hours at 55 V on a formaldehyde (pH 7) denaturing gel as described previously (Margan et al, 2000). Denatured total RNA was transferred to a nylon membrane. The probe, hybridization, and wash conditions were the same as those used for virtual Northern analysis.

In Situ Hybridization

Frozen sections (8 μm) were cut using a cryostat (Shandon, Cheshire, United Kingdom) mounted on charged SuperFrost (Menzel-Glaser, Germany) slides and fixed in 4% paraformaldehyde in PBS (4°C) for 10 minutes followed by 3 washes in 1× PBS and one in 2× SSC at room temperature. The slides were then placed in a solution of proteinase K (2 μg/mL) for 7 minutes at 37°C, then in 0.1 M glycine for 5 minutes at 37°C, followed by two washes in 2× SSC at room temperature. Slides covered with prehybridization solution (50% deionized formamide, 6× SSC, 5× Denhardt solution, 0.1 mg/mL salmon sperm DNA (Gibco BRL), 10% dextran sulfate, 5 mg/mL sodium pyrophosphate, 0.5% SDS, and 1 mM levamisole) were incubated for 30 minutes at 55°C in an Omnislide thermal cycler (Hybaid, Ashford, Middlesex, United Kingdom). All components of the prehybridization solution were obtained from Sigma, Sydney, Australia, unless stated otherwise. The hybridization solution, consisting of the prehybridization solution and digoxigenin (Roche) (DIG)-labeled sense or antisense probe was then applied to the slides, covered with parafilm to avoid drying, and incubated at 55°C for 19 hours. After hybridization, slides were washed with 2× SSC, 0.2× SSC, and 0.1× SSC at 55°C for 15 minutes each. The last rinse was in 0.1× SSC at room temperature. For detection of hybridization signals, the slides were first blocked in 2% goat serum (Sigma) in Tris-buffered saline (100 mM Tris pH 7.4 and 150 mM NaCl) containing 0.1% Triton X-100 for 30 minutes followed by 30 minutes in 2% blocking solution (Roche, Castle Hill, Australia). The slides were then incubated in a 1:250 dilution of anti-DIG antibody-alkaline phosphatase conjugate (Roche) in blocking buffer for 2 hours at room temperature. After washing in Tris-buffered saline, the hybridization signal was developed overnight in a dark chamber using 4-nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate (Roche) in Tris-buffered saline (100 mM Tris, 100 mM NaCl, and 50 mM $MgCl_2$ pH 9.5) at room temperature. The sense and antisense probes were prepared by conducting an asymmetric PCR (Paine et al, 1995; Gibbins et al, 1999) for 50 cycles using 250 ng of template AUMP cDNA and 20 pmol of AUMP forward (5′-CCTGAACCATCCCAAATGT-3′) or reverse (5′-AGAGAAAGAAAGCGGCTATGT-3′) primers in the presence of 5 μL of 10× DIG-labeled dUTP (Roche).

5′-Genomic DNA Walking

Genome walking was performed with the Mouse Genome Walker kit (Clontech) following the manufacturer's protocol. Four mouse genomic DNA libraries were provided with the kit. Each library was amplified using the adaptor primer and AUMP-specific primer, 5′-GTTATTTGCCATGATTCTAGCTGT-3′. To improve specificity, a nested PCR was performed using the nested adaptor primer and AUMP-specific primer, 5′-AGGACCTAGTGACCTGGCATCA-3′. Nested PCR products were subcloned into pGEM T-Easy Vector, and recombinant plasmids were purified using the GeniePrep DNA Isolation kit (Ambion, Thebarton, Australia) and sequenced unidirectionally.

Bioinformatics

Sequence homology searches, open reading frame (ORF) determination, and isoelectric point (pI) calculation were performed using Bionavigator (http://www.eBioinformatics.com).

Results

Identification of AUMP in Prostate

Suppression subtractive hybridization was conducted between cDNA derived from congenital androgen-deficient *hpg* and testosterone-replaced *hpg* mouse prostates. After screening 672 clones, 8 differentially expressed genes were identified. Of these, 3 sequences were shown to preferentially hybridize with testosterone-replaced *hpg* but not with *hpg* probe. One of the sequences that showed a high degree of redundancy in the sequenced clones was novel and therefore selected for this study. Alignment of sequences from 6 clones resulted in the assembly of the cDNA sequence of 805 base pairs (bp). Sequencing revealed no match with any existing gene with annotation. A further search of the draft human genome database, using AUMP cDNA and the predicted protein sequence as the queries, found no significant match with any human sequence. This gene was thus assigned the name AUMP, for *a*ndrogen *u*p-regulated gene identified from the *m*ouse *p*rostate.

To obtain the genomic DNA sequence upstream of the AUMP transcription start site, 5′ genome walking was performed using AUMP gene-specific primers, and adaptor primers were used to ligate genomic DNA digests. A 457-bp genomic DNA fragment was obtained after 2 rounds of genome walking. No androgen response element was found within the region. The only steroid-binding site identified was for glucocorticoid receptor at position −430 (Figure 1B).

Prediction of AUMP function

The longest ORF of AUMP was defined as 408 bp (Figure 1A). The initiation codon (ATG) is positioned at nt 94 and the stop codon at nt 499, encoding for a protein of 135 amino acids (Figure 1C). The predicted protein has a molecular weight of 16 kd with a pI of 7.4. Amino acids 48 to 119 were found to share approximately 65% similarity and 35% identity to PPT of human (SwissProt P50897), rat (P45479), mouse (O88531), and bovine (P45478) (Figure 2), as determined by the BlockSearcher program, with e values of <0.015. A motif for protein-transport protein, SecY/Sec61-α subunit (P38379), was present at amino acid positions 38–78.

A

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   1  GATCACCCAG TAGGGTTGTG GTCACATGTG TCCTGTAAGG CATTCTGATT
  51  GCCAGCCAGG GGTGGAAACA TTTGTTGGTA TCACTGAGCT AAGATGGTGT
 101  TACCTCGATC CCTGTGGCTG CTGTCTGTCT GTCTTCTGTC CTGGTGTTGT
 151  GATGCCAGGT CACTAGGTCC TCAAGATCTA CAGCTAGAAT CATGGCAAAT
 201  AACTTTTGTG TTCTTGCATC TGAAAGGAGA AACAATAAGT ATGGATGACA
 251  TTGAAGTGAT GTTTCAAATG AATAAAGCTG GAGCTTATGT CCCATCTGTT
 301  GAGACTGAGA AGGCTGTGAT GGAGGATGTG GATAACAAAG CCTTGTATGT
 351  CAGCTCACAT GCATTTAAGA TTCTCCCTAA GCAGCCTAAG TTGAGACATG
 401  GCCACACTGA TTTTAAATTT CCTGAACCAT CCCAAATGTG GAAGGTATTG
 451  ATGAACGATG ACTCTACCCG CTACAAATTG AAATTTTGGC AAAGAACTTG
 501  AGAGAGTCCT GAAGAAACTT GTCCCAATAT GGGATATTCC AGGGAATGGT
 551  CATAAGACAA ACCCAAGGCT TGTGTAAACA TTGTGAAGAC AGCAATACTT
 601  TGCATGCATT TTGAACTATG AAGACCCAAC AAAACTGAAA AAGAACAACC
 651  CTGCTTACAA AGTAGAGTTT AGTCTCCTGC TACTTAACAT AGCCGCTTTC
 701  TTTCTCTGTC CTCATTTGGG AAAATTTCCT AATCTGTAAG ACAGGCATGC
 751  TCACTTAGTG TAAGCTCTTC TCAGGTTCTC TGAAATAAAC TTAACTGCCC
 801  TTTGTAAAA
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B

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-457  ATGCTTTATG AAAGAGTTTA AAGGATTAAA GGCCCTGACG AGGATTAAAA
-407  AAATCCACCA GGAAAATCAA CAGAGTCAAA CTAACCTGGA TCCTTGGCAC
-357  CTCTCAGAGA CTGAGCAAAT CAAAGATCAT ATGTGAGCTT GGAGTGGGGC
-307  CCTGGCACAT AGGGCTGCAT TTTCTGGCTG CAGTGGGAGA GGCTGAACAT
-257  AACCCTGAAG AGATTTGATG ACCCATGATG GGGGAAATAC AATGTAGCAT
-207  ACTCTGAACA GATAAGGGTA GTAGGGATGG GGAGGTAATC TTTGAGGGGG
-157  AAGTTAGAGT GGTGACAGTG TTTATGATGT AAATCCATTA AAATAAAATG
-107  GGAACCTTTA ATGTTGTTTA AAGAACAAAA GAGTACTTTA TGTTCTGTTG
 -57  TAAAAACAAA ATAGATACAT GTCAGTAATG ATATTGCTGC AGGGAACTCT
  -7  CAGACTG
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C

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   1  MVLPRSLWLL SVCLLSWCCD ARSLGPQDLQ LESWQITFVF LHLKGETISM
  51  DDIEVMFQMN KAGAYVPSVE TEKAVMEDVD NKALYVSSHA FKILPKQPKL
 101  RHGHTDFKFP EPSQMWKVLM NDDSTRYKLK FWQRT
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Figure 1. **(A)** Nucleotide and predicted protein sequence of the AUMP gene. Boldface letters indicate the ORF (nt 94–501). The in-frame stop codon at the 5′ untranslated region is underlined (nt 85). The poly(A)$^+$ signal is underlined (nt 784) and the tail is at nt 806. The entire sequence has been deposited in GenBank (AF319955). **(B)** 5′ Flanking region of AUMP (AY037887). **(C)** Predicted peptide sequence of AUMP gene.

Androgen Regulation of AUMP Expression

To evaluate the AUMP expression level in prostate under various androgen conditions, RT-PCR was conducted on poly(A)$^+$ RNA derived from *hpg*, testosterone-replaced *hpg*, castrate non-*hpg*, and intact non-*hpg* mouse prostates. AUMP expression was strong in testosterone-replaced *hpg* and normal mouse prostate, but was absent in untreated *hpg* as well as castrate mouse prostate (Figure 3A).

Virtual Northern assay was used to examine the AUMP transcript size under various androgen conditions. The reason for conducting virtual rather than a classical Northern assay was the insufficient amount of RNA generated from *hpg* mouse prostates. The weight of *hpg* prostate (which is about 8% of intact non-*hpg* mice) would necessitate using a large number of mice for prostate collection. As evident in Figure 3B, AUMP transcript size in testosterone-replaced *hpg* mouse prostate was the same as that in non-*hpg* normal prostates. Equally important was the finding that AUMP transcript size determined by virtual and classical Northern analysis was consistent (around 800 bp).

```
            54                                                  100
PPT_Mouse    SMGVIKK MVEKEIPGIY VLSLEIGKNM MEDVENSFFL NVNVQVNMVC
  PPT_Rat    SMGSIKK MVEKEIPGIY VLSLEIGKNM VEDVENSFFL NVNLQVGMAC
PPT_Bovin    SMGAIKK MVEKKIPGIH VLSLEIGKTL REDVENSFFL NVNSQVTTVC
PPT_Human    SMGAIKK MVEKKIPGIY VLSLEIGKTL MEDVENSFFL NVNSQVTTVC
Consensus    SMG-IKK MVEK-IPGI- VLSLEIGK-- -EDVENSFFL NVN-QV---C
 AUMP-pep    SMddIev MfqmnkaGaY VpSvEteKav MEDVdNkaly .VsSh...Af

            101
PPT_Mouse    QILEKDPKLQ QGYNAIGFSQ GGQFLRAV
  PPT_Rat    QILEKDPKLQ HGYNAIGFSQ GGQFLRAV
PPT_Bovin    QILAKDPKLQ QGYNAMGFSQ GGQFLRAV
PPT_Human    QALAKDPKLQ QGYNAMGFSQ GGQFLRAV
Consensus    QIL-KDPKLQ -GYNA-GFSQ GGQFLRAV
 AUMP-pep    kILpKqPKLr HGhtdfkFpe psQmwkvl
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Figure 2. Alignment of AUMP amino acid sequence (48–119) with mouse, rat, bovine, and human PPT (54–128). For AUMP, an uppercase letter indicates a match with one or more PPTs, and a lower case letter represents no match.

Tissue and Cellular Distribution of AUMP

The tissue expression profile of AUMP was studied by RT-PCR and classical Northern analysis on total RNA derived from 9 organs (cerebral cortex, cerebellum, heart, kidney, liver, lung, prostate, testis, and spleen) in normal mice. Both analyses showed exclusive expression of AUMP in the prostate (Figure 4A and B). A search of the mouse expressed sequencing tag (EST) database revealed that no sequences had a significant match with AUMP except those derived from male bladder. Similarly, RT-PCR analysis of various *hpg* mouse organs showed no AUMP signal in any of the 9 organs (data not shown). In situ hybridization was used to characterize the cellular distribution of AUMP mRNA in mouse prostate. In testosterone-replaced *hpg* and intact non-*hpg* mouse prostates, a strong hybridization signal with AUMP antisense probe was observed in the cytoplasm of luminal epithelial cells. Although it appears that AUMP signal may also be present in basal cells, further studies using specific basal cell markers would be required to confirm this. No AUMP signal was detected in any cells of the stromal compartment of the prostate. Hybridization signal with AUMP antisense probe was undetectable in *hpg* and castrate mouse prostates (Figure 5).

Discussion

This is the first study to use the *hpg* mouse to identify androgen target genes in the prostate. The *hpg* mouse is congenitally androgen deficient due to a large deletion in the gonadotropin-releasing hormone gene (Mason et al, 1986), resulting in reduced gonadotropin levels and immature reproductive and sex accessory organs, including prostate. However, the immature prostate is inherently normal and capable of undergoing development and full restoration of weight and histology after androgen replacement (Singh and Handelsman, 1999). Because the nature of androgen target genes in prostate is unknown, two approaches—expression array and subtractive hybrid-

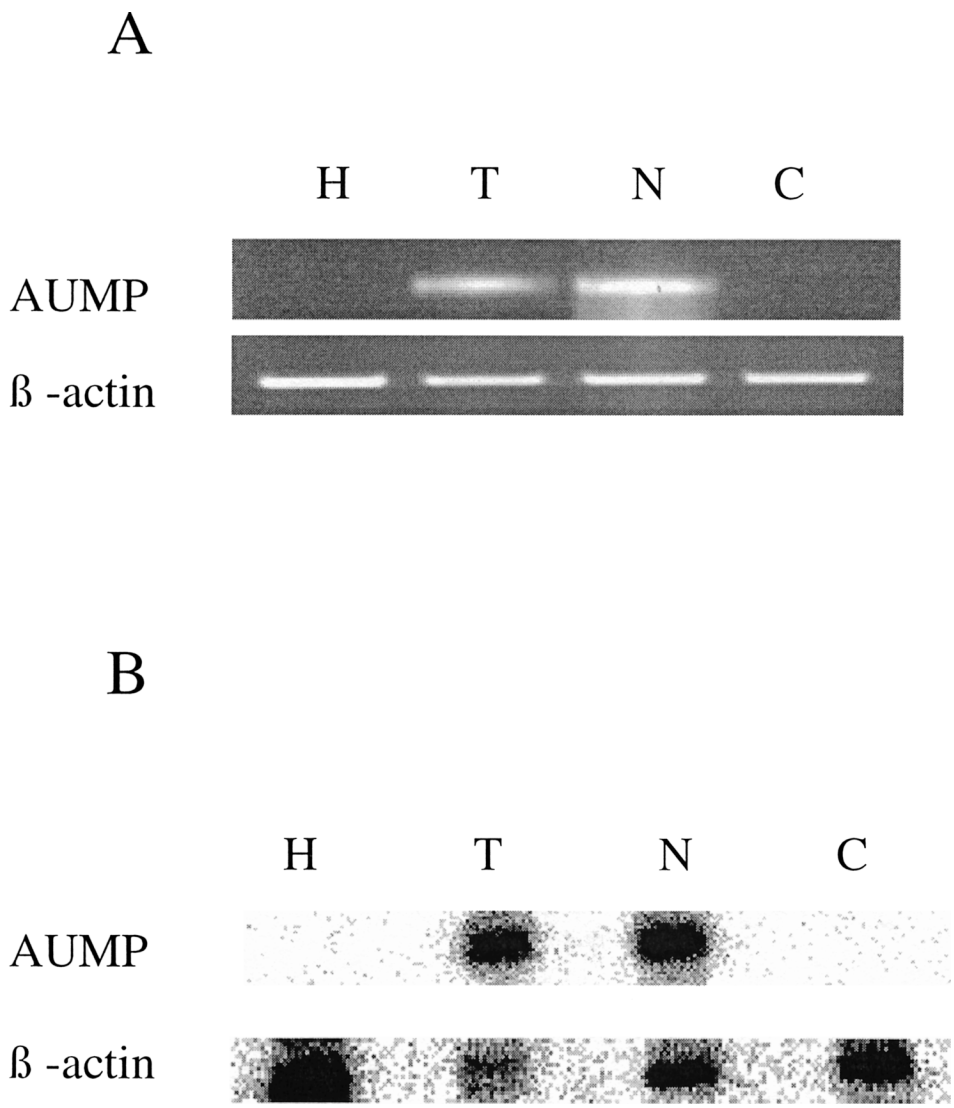


Figure 3. Differential expression of AUMP in ventral prostates among *hpg* (H), testosterone-replaced *hpg* (T), intact non-*hpg* (N), and castrate non-*hpg* (C) mice. **(A)** RT-PCR with AUMP gene-specific primers and with mouse β-actin primers as control for loading. **(B)** Virtual Northern analysis of cDNA with radiolabeled AUMP and mouse β-actin probe.

ization—were considered. Mouse cDNA arrays were unavailable until recently, but the number of genes on the mouse array is still limited. Suppression subtractive hybridization avoids physical separation of hybridized from unhybridized molecules. It also enriches for rare messenger RNA (mRNA; Diatchenko et al, 1996). By using subtractive hybridization between prostatic cDNA derived from *hpg* mice treated with or without testosterone, we have now identified a novel androgen up-regulated gene, AUMP, which codes for a PPT-like protein. It also shows a motif for Sec61-α, which is a major component of the endoplasmic reticulum (ER) translocation site, that promotes translocation as well as integration of secretory proteins into the ER membrane (Greenfield and High, 1999; Oliver et al, 1995).

The mRNA sequence of AUMP consists of 805 nucleotides with an ORF of 408 bp. Alignment of AUMP sequences from 6 clones resulted in the assembly of the cDNA sequence of 805 bp. This was considered to be the full length for the following reasons. First, some AUMP clones showed the presence of a GGG sequence at the 5′ end of mRNA that is generated by SMART technology. The addition of dG by reverse transcriptase occurs only after it has reached the 5′ end of mRNA. Other AUMP clones contained both poly(A)$^+$ signal and tail. Second, the assembled transcript was consistent with the size of the transcript determined by Northern analysis as described below. Last, during preparation of this manuscript, a full-length enriched male mouse bladder cDNA of 768 bp was submitted to GenBank (accession number

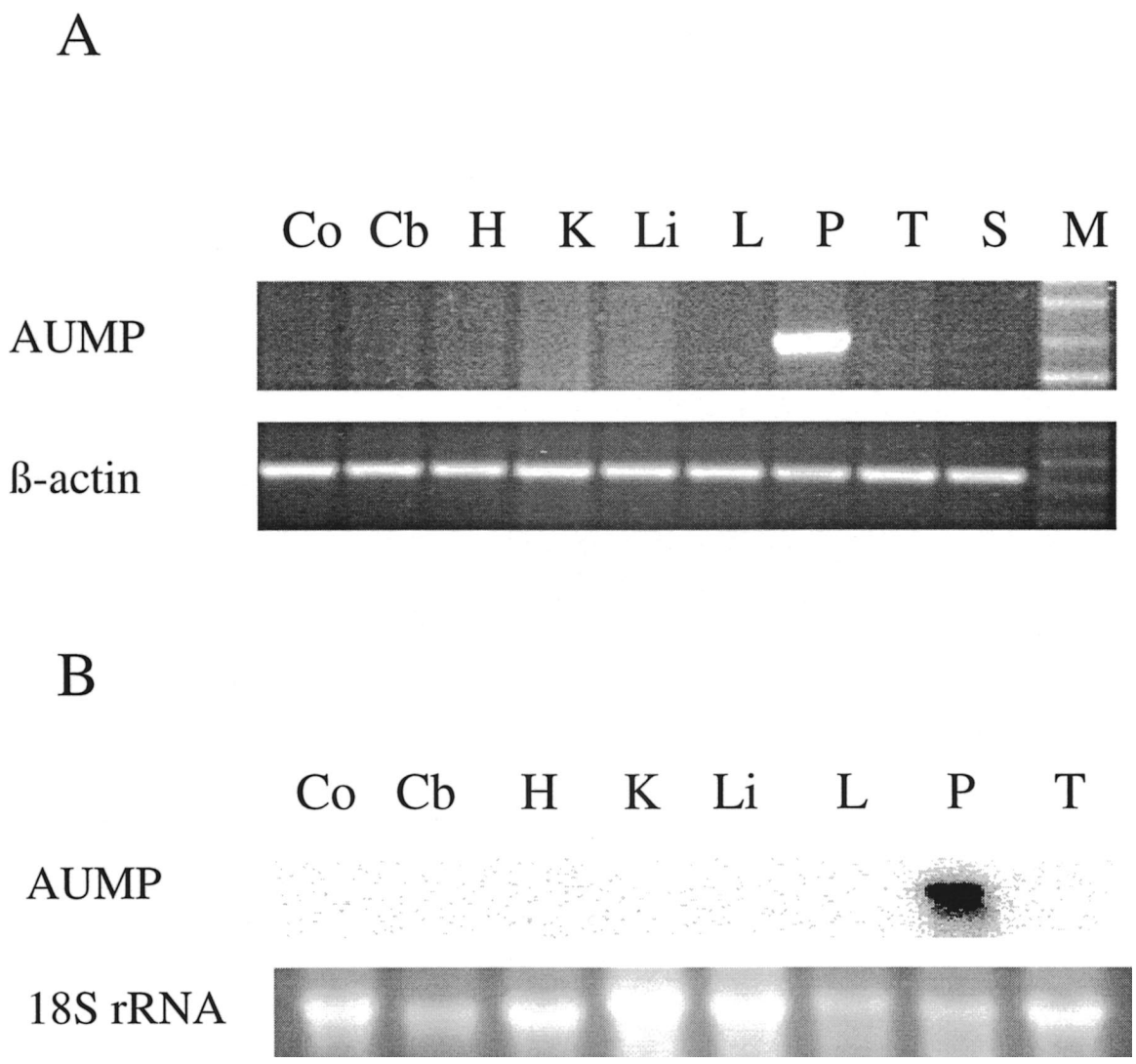
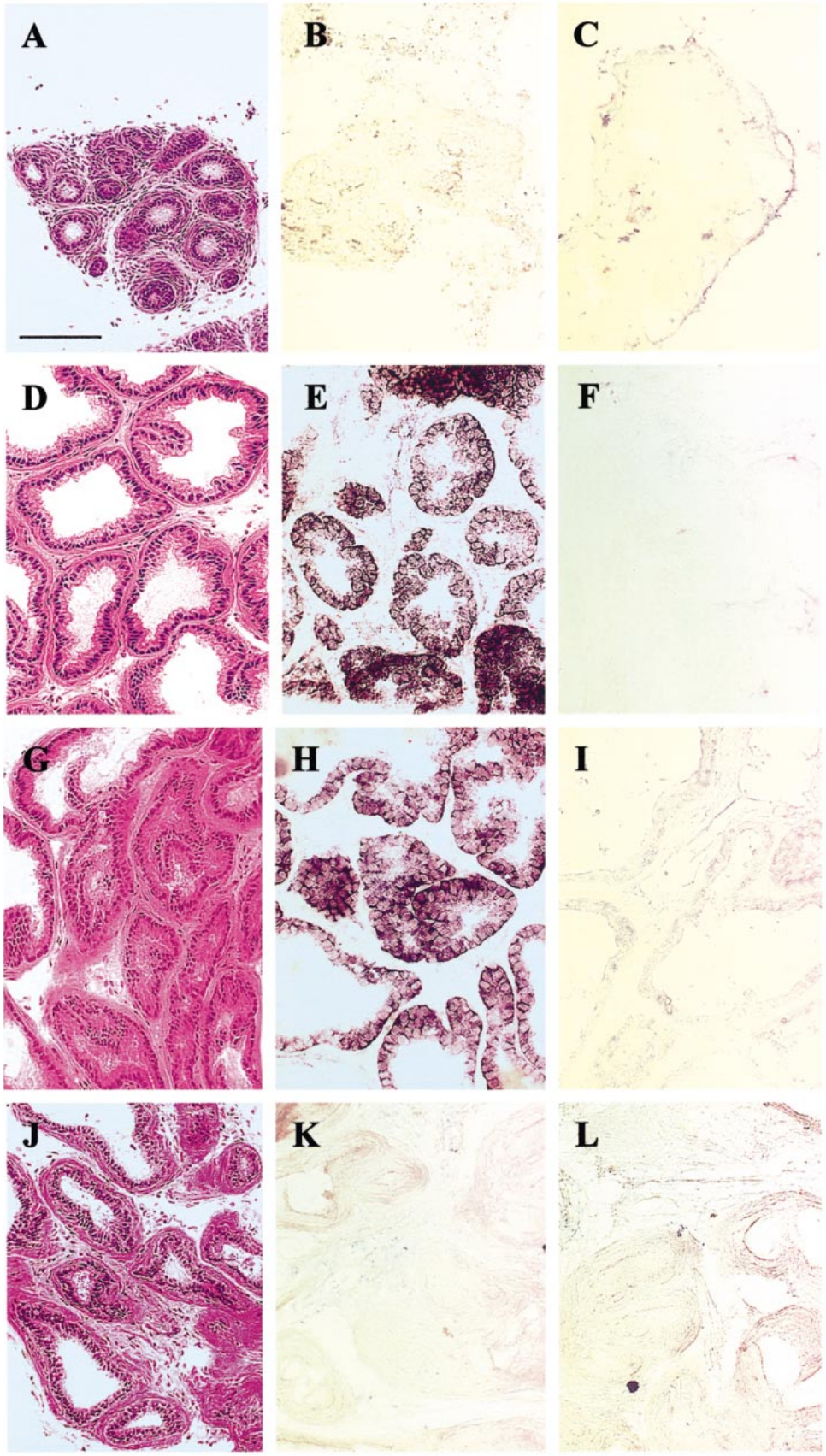


Figure 4. Tissue distribution of AUMP in various mouse organs—cerebral cortex (Co), cerebellum (Cb), heart (H), kidney (K), liver (Li), lung (Lu), prostate (P), testis (T), and spleen (S). **(A)** RT-PCR of total RNA (1 μg) from 9 mouse organs using AUMP primers and with mouse β-actin primers as the control for loading. **(B)** Classical Northern analysis of total RNA (20 μg) from 8 mouse organs with radiolabeled AUMP (upper panel). Ribosomal RNA was used as a control for loading (lower panel).

AK020534). Mouse bladder cDNA has 99% identity to AUMP cDNA except for the missing 35 bp at its 5′ UTR.

AUMP is clearly up-regulated by androgens because its transcripts are present only in testosterone-replaced *hpg* or intact non-*hpg* mouse prostates, but they are absent in untreated *hpg* and castrate mouse prostates. In addition, this androgen-induced AUMP expression is highly selective. Among the 9 mouse organs examined, AUMP is expressed only in the prostate. Searching the mouse EST database revealed that the only cDNAs showing a significant match with AUMP were from the male mouse bladder cDNA library. Although it is likely that some cell lineages in the bladder may express AUMP, the possibility that these transcripts are of prostate origin, arising from contamination of the bladder with adjacent prostate tissue, cannot be excluded. A high level of expression restricted to the prostate gland indicates that AUMP may have important roles related to prostate physiology. Although it is clear that AUMP is an androgen target gene, it is unknown whether it is under direct or indirect regulation by androgens, because we were unable to locate any androgen response element within the 457 bp in the 5′ flanking region of AUMP. Considering the potential of selectively driving gene expression to the androgen-sufficient prostate, further study is required to clone the promoter responsible for AUMP expression.

When studying gene expression in the prostate, it is important to consider the changes in the proportion of different cell types following androgen depletion or repletion. Morphometric studies have shown that androgen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ablation can result in a major reduction of glandular epithelial cells (DeKlerk and Coffey, 1978), leading to an altered ratio of epithelial to stromal cells in the prostate (English et al, 1985; Montpetit et al, 1986; Isaacs et al, 1992). As a result of the changes in cellular composition, any gene that is localized in the epithelium may appear to be down-regulated after androgen ablation due to a decrease in epithelial cell numbers and not due to a decrease in expression level. Techniques such as in situ hybridization are therefore essential to demonstrate alteration in the expression level of a gene. In the present study, in situ hybridization has localized AUMP expression to the epithelial cytoplasm of the luminal cells in androgen-sufficient mouse prostates. AUMP expression was absent in androgen-depleted prostates even though epithelial cells are maintained in *hpg* as well as 14-day castrated mice. Thus, the lack of mRNA in androgen-depleted prostates was not due to a reduction in epithelial cell numbers, but to an absence of AUMP expression. Our results, therefore, demonstrate that AUMP gene expression in the prostate is both epithelial-specific as well as androgen-regulated. Based on subcellular location of PPT and SecY/Sec61-α, it is likely that AUMP may be localized to lysosome or ER.

The presence of a PPT-like gene in the prostate and its functional significance are unclear at present. PPT is a lysosomal enzyme (Verkruyse and Hofmann, 1996) that is involved in deacylation of palmitoylated proteins. It was first isolated from bovine brain (Camp and Hofmann, 1993) and subsequently shown to be present in several tissues in rats (Camp et al, 1994), mice (Salonen et al, 1998), and humans (Schriner et al, 1996). Palmitoylation is believed to promote membrane localization of proteins and to mediate protein-protein interactions at the plasma membrane (Camp and Hofmann, 1993). Deficiency of PPT has been shown to cause a neurodegenerative disease in infants (Vesa et al, 1995). Recent evidence indicates that it is developmentally regulated in rat brain (Suopanki et al, 1999) and may have a protective role against apoptosis in human neuroblastoma cells (Cho and Dawson, 2000). However, we have not come across any studies reporting androgen regulation of PPT. Induction of AUMP by testosterone and its down-regulation by castration suggests that it may have a role in suppression of apoptosis.

Searching the draft human genome database has so far revealed no matching human sequence. Because the overall ontogeny of rodent prostate is very similar to that of humans, further efforts will be made to obtain the human homologue of AUMP that will enable evaluation of its role in normal and diseased prostate.

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Figure 5. Comparative in situ hybridization results across prostates in *hpg* (top row), testosterone-replaced *hpg* (2nd row), non-*hpg* (3rd row), and castrate non-*hpg* mice (last row). The first column in each row **(A, D, G, J)** shows sections stained with hematoxylin and eosin, the second column **(B, E, H, K)** shows staining with DIG-labeled AUMP antisense probe, and the third column **(C, F, I, L)** shows staining with AUMP sense probe. Scale bar = 100 μm.

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