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A tissue-specific promoter that can drive a foreign gene to express in the suprabasal urothelial cells of transgenic mice

(uroplakins/integral membrane protein/asymmetrical unit membrane/urothelial heterogeneity/bladder diseases)

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ABSTRACT **Uroplakins are a group of integral membrane proteins that are synthesized as the major differentiation products of urothelium. The luminal portions of these proteins form 12-nm protein particles arranged in a two-dimensional crystalline array. The expression of uroplakin genes is bladder specific and differentiation dependent; little is known, however, about their molecular regulation. Here we describe the cloning of mouse uroplakin II gene and demonstrate, in transgenic mouse experiments, that a 3.6-kb 5′-flanking sequence of this gene can drive a bacterial *lacZ* (reporter) gene to express in the suprabasal cell layers of the urothelium. The transgene was not expressed in any tested (nonurothelial) epithelia and other tissues (except hypothalamus). These results suggest that most of the cis elements that confer the bladder-specific and differentiation-dependent expression of mouse uroplakin II gene must reside in the 3.6-kb sequence. The availability of a promoter capable of delivering a foreign molecule to the differentiated cell layers of bladder epithelium opens avenues for studying normal and pathological urothelial differentiation in transgenic mice.**

Uroplakins (UPs) are a group of integral membrane proteins that have recently been identified as the major proteins of urothelial plaques (1, 2). These plaques, first described by Porter and Bonneville (3), cover a large portion of the apical surface of mammalian urothelium (4–6). They are thought to play a crucial role as a permeability barrier (7) and/or as a physical stabilizer of the urothelial apical surface (6). When viewed in cross section, the outer leaflet of the plaque is almost twice as thick as the inner one (hence the term "asymmetrical unit membrane" or AUM). Negative staining of urothelial plaques established that the thickened outer leaflet consists of crystalline arrays of 12-nm protein particles, each of which can be further resolved by image enhancement techniques into six inner and six outer domains (8, 9). Three integral membrane proteins of 47, 27, and 15 kDa were found to be the major proteins of purified bovine urothelial plaques (1, 2). Ultrastructural localization showed that these proteins are all AUM-associated *in situ*. These results established UPI (27 kDa), UPII (15 kDa), and UPIII (47 kDa) as the major protein subunits of urothelial plaques (1, 2). Immunohistochemical survey of various bovine tissues established that these UPs are urothelium specific and represent excellent markers for an advanced stage of urothelial differentiation (1, 2).

The primary structures of UPs have recently been elucidated by cDNA cloning. The results revealed the existence of two closely related UPI isoforms (the 27-kDa UPIa and the 28-kDa UPIb) (10). Hydropathy algorithm and proteolytic digestion data suggest that UPIa and UPIb span the lipid bilayer four times, with a major and a minor hydrophilic loop both extending into the luminal space (10). The 15-kDa UPII (11) and the 47-kDa UPIII (12) both span the membrane once, each having a main luminal N-terminal domain and a small cytoplasmic C-terminal domain (0 and ≈5 kDa for UPII and UPIII, respectively). Finally, the mRNAs of all four known UPs have recently been shown to be bladder specific, suggesting that the expression of UP genes is transcriptionally regulated (10–12).

In this paper, we describe the cloning of mouse UPII gene,* which is a single-copy gene (13). We show here that the expression of this mouse gene, like its bovine counterpart, is bladder specific. We also show, by transgenic mouse techniques, that a 3.6-kb 5′-flanking sequence of this mouse UPII gene can drive a *lacZ* reporter gene to be expressed efficiently in the suprabasal cell layers of the urothelium, in a pattern similar to that of the endogenous UPII gene. The transgene product was not detected in skin, cornea, esophagus, intestine, stomach, and a number of other tissues (with the exception of hypothalamus), thus establishing that many of the cis elements that define the bladder specificity and differentiation dependence of the UPII gene must reside in this 3.6-kb sequence. These data establish the mouse UPII gene as an excellent model system for studying molecular events during urothelial differentiation.

MATERIALS AND METHODS

Characterization of the Mouse UPII Gene. A bovine UPII cDNA (11) was used as a probe to screen a mouse EMBL3-SP6/T7 genomic library (Clontech). Two overlapping clones (G1 and G2) were isolated, and they were sequenced by the dideoxynucleotide termination method. The transcriptional initiation site was determined by sequencing three clones of 5′-RACE (rapid amplication of cDNA ends) products of mouse bladder cDNA.

Expression of a Fusion Gene (*UPII–lacZ*) in Transgenic Mice. A 6-kb *Xho* I DNA fragment of the G1 genomic clone (Fig. 1*a*) was subcloned in pGEM7Z and then restriction-cut to yield a 3.6-kb DNA fragment of G1 clone (extending from the *Xho* I site at −3.6 kb to the *Bam*HI site at +42 bp relative to the transcription initiation site) and inserted into the *Sma* I site of a *lacZ* vector [placF; a gift from Jacques Peschon, Immunex, Seattle (14, 15)] to generate pUPII-LacZ (Fig. 4). The 7.1-kb fusion gene was excised by using *Kpn* I and *Hin*dIII, gel-purified, and microinjected into fertilized mouse eggs (from F_1 hybrids of C57BL/6J × DBA2), which were implanted into CD-1 foster mothers (courtesy of the E. Newcomb, Transgenic Mouse Facility of New York University Medical Center). The *lacZ* transgene was identified by Southern blot analysis of tail DNA. Positive founder animals were back-crossed with (C57BL/6J × DBA2) F_1 hybrids to generate hemizygous animals that were used for studying transgene expression.

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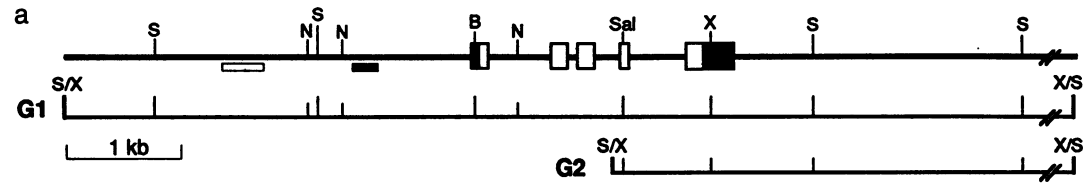
Abbreviations: UP, uroplakin; AUM, asymmetrical unit membrane.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. U14421).

RESULTS

Structural Organization of the Mouse UPII Gene. Using a bovine UPII cDNA as a probe, we isolated an ≈16-kb mouse genomic clone (G1) which contained an ≈2.5-kb transcribed region that was flanked by ≈3.6 kb and ≈10 kb of 5′ and 3′ sequences, respectively (Fig. 1*a*). Alignment of the coding sequence with the UPII cDNA sequences of cattle (11) and mouse (unpublished data), which were highly homologous to each other, allowed us to define the exon/intron junctions of four introns (Fig. 1*b*). 5′-RACE (17) experiments using mouse bladder mRNA as a template established that the transcription initiation site of the UPII gene was located at 60 bp 5′ upstream of the translation initiation codon and 27 bp downstream of a putative TATA box. The 5′ upstream region contained an *Alu*-like B1 repetitive sequence (−830 bp) and a $(CA)_n$ stretch (≈−2.1 kb). Finally, a polyadenylylation signal (AATAAA) resided ≈230 bp downstream of the translation stop codon (Fig. 1).

Mouse UPII Gene Is Tissue Specific and Differentiation Dependent. To determine the tissue specificity of the mouse



b

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                                -1232  GAGCTCAGGTCCTATCGAGTTCACCTAGCTGAGACACCCACGCCCCTGCAGCCACTTTGCAG
-1170  TGACAAGCCTGAGTCTCAGGTTCTGCATCTATAAAAACGAGTAGCCTTTCAGGAGGGCATGCAGAGCCCCCTGGCCAGCGTCTAGAGGAG
-1080  AGGTGACTGAGTGGGGCCATGTCACTCGTCCATGGCTGGAGAACCTCCATCAGTCTCCCAGTTAGCCTGGGGCAGGAGAGAACCAGAGGA
 -990  GCTGTGGCTGCTGATTGGATGATTTACGTACCCAATCTGTTGTCCCAGGCATCGAACCCCAGAGCGACCTGCACACATGCCACCGCTGCC
 -900  CCGCCCTCCACCTCCTCTGCTCCTGGTTACAGGATTGTTTTGTCTTGAAGGGTTTTGTTGTTGCTACTTTTTGCTTTGTTTTTTCTTTTT
 -810  TAACATAAGGTTTCTCTGTGTAGCCCTAGCTGTCCTGGAACTCACTCTGTAGACCAGGCTGGCCTCAAACTCAGAAATCCACCTTCCTCC
 -720  CAAGTGCTGGGATTAAAGGCATTCGCACCATCGCCCAGCCCCCGGTCTTGTTTCCTAAGGTTTTCCTGCTTTACTCGCTACCCGTTGCAC
 -630  AACCGCTTGCTGTCCAAGTCTGTTTGTATCTACTCCACCGCCCACTAGCCTTGCTGGACTGGACCTACGTTTACCTGGAAGCCTTCACTA
 -540  ACTTCCCTTGTCTCCACCTTCTGGAGAAATCTGAAGGCTCACACTGATACCCTCCGCTTCTCCCAGAGTCGCAGTTTCTTAGGCCTCAGT
 -450  TAAATACCAGAATTGGATCTCAGGCTCTGCTATCCCCACCCTACCTAACCAACCCCCTCCTCTCCCATCCTTACTAGCCAAAGCCCTTTC
 -360  AACCCTTGGGGCTTTTCCTACACCTACACACCAGGGCAATTTTAGAACTCATGGCTCTCCTAGAAAACGCCTACCTCCTTGGAGACTGAC
 -270  CCTCTACAGTCCAGGAGGCAGACACTCAGACAGAGGAACTCTGTCCTTCAGTCGCGGGAGTTCCAGAAAGAGCCATACTCCCCTGCAGAG
 -180  CTAACTAAGCTGCCAGGACCCAGCCAGAGCATCCCCCTTTAGCCGAGGGCCAGCTCCCCAGAATGAAAAACCTGTCTGGGGCCCCTCCCT
  -90  GAGGCTACAGTCGCCAAGGGGCAAGTTGGACTGGATTCCCAGCAGCCCCTCCCACTCCGAGACAAAATCAGCTACCCTGGGGCAGGCCTC
    1  ATTGGCCCCAGGAAACCCCAGCCTGTCAGCACCTGTTCCAGGATCCAGTCCCAGCGCAGTATGGCATCCACACTGCCTGTCCAGACCTTG
                                                          M  A  S  T  L  P  V  Q  T  L
   91  CCCCTGATCCTGATTCTGCTGGCTGTCCTGGCTCCGGGGACTGCAGGTCTCTATTGCTGGTGGGTGCGAGGAGGGTTTCAGAGCGCTAGA
       P  L  I  L  I  L  L  A  V  L  A  P  G  T  A  D↳1
  181  CAGGGAACATTGTCTCCCCAGGGCTCTCAAGGACAGGAATGTTGGTCTAGCTGGTTGGGGTTGAGAGTTACTAGTGGTAGGAATCAGGTG
  271  ACAAATTCCTGGGCTTCTTCCCAGATCCAGGAGTCAAGAAATTTGGGTAAGTGTCCAAGGTTTGTGTGAGTTGGGCGAGACTGGGGACTG
  361  ACTGGGTGCCATGGTCTAGTTTGGGTCGGTAGGGCTATCTGGCTCCCAACAGCGCGGCGTACCCACCATCTGCAGATCAAGCCTGCCATC
  451  TGGTGGTCAGATCCACACGCTCCTCTTCTGTCTCTGCACCCTTAGCAATGACCACCCACCCACCCCGCCAGCTCTGAGTTAAGAGGGGGC
  541  TAACTCCTGAGTTCCCTCTCGGCTCCCTAACAGACTTCAACATCTCAAGCCTCTCTGGTCTGCTGTCTCCGGCGCTAACAGAAAGCCTGT
                                    1↳  F  N  I  S  S  L  S  G  L  L  S  P  A  L  T  E  S  L  L
  631  TAATTGCCTTGCCCCCATGTCACCTCACGGGAGGTAATGCCACATTGATGGTCCGGAGAGCCAACGACAGCAAAGGTAGACCTCCCTTGT
         I  A  L  P  P  C  H  L  T  G  G  N  A  T  L  M  V  R  R  A  N  D  S  K  V↳2
  721  ACCCATTTATTCTACTCGTCGTAACCCCTCTTAACGATACCCAAGAGCTGCCCGTTCTACAAGAGTGGACGCTAGAATCTGATCTTGCCT
  811  TTCACTCCTATTTCCCCTCAGTGGTTAAGTCAGACTTTGTGGTGCCTCCATGTCGCGGGCGCAGGGAGCTTGTGAGCGTGGTGGACAGTG
                  2↳  V  K  S  D  F  V  V  P  P  C  R  G  R  R  E  L  V  S  V  V  D  S  G
  901  GGTCTGGCTACACCGTCACAAGGCTCAGCGCATATCAGGTGACAAACCTAACACCAGGAACCAAATACTAGTAGGTACCGATGGACACCT
         S  G  Y  T  V  T  R  L  S  A  Y  Q  V  T  N  L  T  P  G  T  K  Y  Y  L↳3
  991  GTGGAGGTGGGATGGCAAAAAAGGGAAGTGGAGGTCCCGTGAGGGTGGGGAAGTGCCGGGAAGCATGAGTTAGAGAGGGCACAGCTAAAG
 1081  GGTAGGAAATGTGAACCTGGACCCCAGGAGGGCCCAGATGGGACACATAGCTAGAAGGTGGAGGCTGGAACCCCTCCTCCCGAGTGCCAG
 1171  ATACGTACAACCTCTGCTTTCTCTCAACTCCGCCTCTAAAGCATATCCTACCGAGTACAGAAGGGGACGTCGACCGAGTCCAGTCCAGAG
                                    3↳  I  S  Y  R  V  Q  K  G  T  S  T  E  S  S  P  E
 1261  ACTCCCATGTCCACGCTTCCTCGTTAAGTAAAATGCCCGTCTCTCACACTTCCCTAAGCTCCGACTTTTTTCTCCTAGAGCAAGTTAGCT
       T  P  M  S  T  L  P  R↳4
 1351  AAACTGTTTCCCGAGTGCTCAGTCGCACACACACCCCCTCCCCAACCCCCCAGTATTTGGTATGGCCCCTCCTGTCCTGTTCAATCATCT
 1441  CTGCACTAGAGGTTCCTTGTGCAGAGGGATGATGTCCTCCTTGGTGGCTCCTAAGTGTTGCTGTGAGGGGGGTCTATGTTTGCTTGACTG
 1531  GTTGGCTGGATGACCAGTTGAACTGATGCTGGAGGCTACTGGATGGCTGGGCTAATGCTGTGAACCACAGGAGCTACCTAGGAACCCCTT
 1621  CAACTCACAGAGGTTCCCCCATCTTCTTCTGACAGGAAAAAACATGGAGTCTATTGGGTTAGGAATGGCCCGGACAGGAGGGATGGTGGT
                                    4↳  K  N  M  E  S  I  G  L  G  M  A  R  T  G  G  M  V  V
 1711  CATCACAGTGCTGCTGTCTGTGGCCATGTTCCTGTTGGTCGTGGGTCTTATTGTTGCCCTGCACTGGGATGCCCGCAAATGAAAAGGGCT
         I  T  V  L  L  S  V  A  M  F  L  L  V  V  G  L  I  V  A  L  H  W  D  A  R  K  *
 1801  CTCCTGCATCCCAGGCTCCTCCAAGAAGTCCAGCCTGCCTCCTGCCAGGCTGTAGTCACTGGCTTCTCAGTGGCTTTTCTTCCCTCTCCC
 1891  CGCCCCCTCCTCGAGTCCACTCCTGACAGTGCCCCCTCCCTGCTCCCTGTCTCACCTTGCAGCACTCCCTGCTAGCCCCACTGCAATCCT
 1981  GCCAACACTGATTTATCTCTTAACTGTACTTAATTCTCACAATAAAGGCTGACCCACGTAGTATGTCTCATCTCCGACCATGTCTATGTG
 2071  AGTCACCCCTTTAGCTGGTCCCCTTATGCACATATCAAAACTACCAATGTCAAGGTCACGTGCATGTCATTTTCTCTATCCCAAACCCCA
 2161  AGGGTGACTTTTACCAGGAGGGAGGCAAGCAGAGGCAGAGATAATGAAGCCTCAAGCCCAGACTAGGGAAGCCCTCCAAGCCCCAGACCT
 2251  AGGGCTTGGGTTTTGCATCCTGCACTCAGTAGATACCCAAGCAGGAGTCTAGTTGGGCAGGGGGTAGAAGCTGGATCACCATGTGAGCCT
 2341  GACTGGGAAGCTGACAGAACTAGGGAAGAACTAGAGAAAACACAAACAGGGCAGGCCCTCCAGCCCTGGGTGAAGAACATGCTAAACGGT
 2431  TCTAGACCCCTAGAGCCGAGGTGGACGGAAGCTCCTGGAAGGGGGAGGGGGGGACACAACATAGGTAAACAGGCAGTGGCACCCTCGTCC
 2521  ATTTTTAAAATATAGTTTTGTTCTATAAAAGTTTTATTTATTTATTTATTTGCTTGTTTTTATTTGTTTGTTTGTTTTCCAGAGCTGAGG
 2611  CAAAAACCCAGGACCTTGAGCTTGCTAGGCAAGTGCTCTACCACTGAGCTAAATCCCCAACCCCTGTTTTTGTTTTTTTGAAGCAGGGTT
 2701  TCTCTGTGTAGCTCTGGCTGTCCTAGAGCTC  2731
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FIG. 1. Organization and nucleotide sequence of mouse UPII genomic DNA. (*a*) Exon–intron organization of mouse UPII gene. The open and filled thick boxes denote the coding sequence and noncoding sequences, respectively, of the five exons. The open and filled thin boxes represent a $(CA)_n$ dinucleotide repeat region and an *Alu*-like murine B1 repeat, respectively. G1 and G2 designate two independent and partially overlapping genomic clones. The restriction sites are *Sac* I (S), *Nco* I (N), *Bam*HI (B), *Sal* I (Sal), and *Xho* I (X). Several (potential) transcription factor binding sites, including one Sp1, three NF-κB, and four AP2 motifs, reside between the transcription initiation site and the B1 repetitive sequence, which can potentially be involved in transcriptional insulation (16). (*b*) Nucleotide sequence of a 4-kb *Sac* I fragment of mouse UPII gene. A reversed B1 repetitive sequence (in the 5′ upstream region) and a potential polyadenylylation site (AATAAA; in the 3′ untranslated region) are underlined and double-underlined, respectively. The wavy arrow denotes the transcriptional initiation site. Broken arrows marked 1 to 4 denote the intron/exron junctions of the four introns. The predicted first amino acid residue of mature UPII protein sequence is marked with an asterisk. The preceding domain contains a pre and a pro sequence of 25 and 59 amino acid residues, respectively (11).

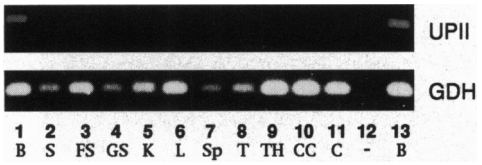

FIG. 2. Tissue distribution of UPII mRNA as assayed by RT–PCR. Poly(A)$^+$ mRNAs (0.3–0.4 μg) from mouse bladder (lanes 1 and 13), skin (2), forestomach (3), glandular stomach (4), kidney (without renal pelvis) (5), liver (6), spleen (7), testis (8), and thalamus/hypothalamus (9), cerebral cortex (10), and cerebellum (11) regions of the brain were reverse-transcribed, and amplified with either UPII-specific primers (*Upper*; 266 bp) or glyceraldehyde-3-phosphate dehydrogenase (GDH)-specific primers (*Lower*, as an internal control for comparison; 130 bp). The PCR products were then electrophoresed on a 1.3% agarose gel and stained with ethidium bromide. Lane 12 serves as a negative control (no cDNA template). Note that the 266-bp UPII product was detected in abundance in bladder, but not in any other tested tissues, including the hypothalamus.

UPII gene, we prepared mRNAs from various mouse tissues and performed the reverse transcription–polymerase chain reaction (RT–PCR) assay. A large amount of UPII product of expected size (266 bp) was generated from the bladder, but not from skin, forestomach, glandular stomach, kidney, liver, spleen, testis, or the hypothalamus/thalamus, cortex and cerebellum of the brain (Fig. 2). This result indicated that the UPII gene of the mouse, like that of cattle (11), was bladder specific.

We have previously prepared a rabbit antiserum against a synthetic peptide corresponding to the amino acid sequence ELVSVVDSGSG (positions 1–11) of mature bovine UPII (11). This antiserum recognized the 15-kDa bovine UPII (Fig. 3*a*), which by immunofluorescence staining was localized to the superficial cell layers of bovine urothelium (Fig. 3*b*). As expected, this antiserum cross-reacted well with mouse UPII, which is closely related to bovine UPII and contains an identical epitope (Fig. 1*b*; ref. 18)—although the mouse UPII migrated slightly slower (apparent molecular mass 17 kDa) during SDS/PAGE than its bovine counterpart (molecular mass 15 kDa; Fig. 3*a*). Immunofluorescent staining of frozen sections of mouse bladders showed that the UPII was associated with all the suprabasal cell layers (Fig. 3*b*), suggesting that the onset of UPII gene expression in mouse was earlier than that in cattle.

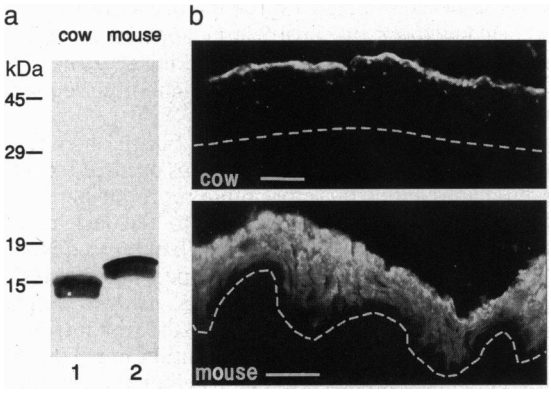

FIG. 3. Differentiation-dependent expression of bovine and murine UPII. (*a*) Immunoblot of total bovine (lane 1) and murine (lane 2) AUM proteins, using a rabbit antiserum raised against a UPII-specific synthetic peptide. Note that bovine UPII migrates slightly faster than the murine protein during SDS/PAGE. (*b*) Indirect immunofluorescence staining of bovine and murine bladder epithelia. Frozen sections of bladders were stained by indirect immunofluorescence using an affinity-purified UPII-specific antiserum. The dashed lines represent epithelial–stromal junctions. No staining was observed with a preimmune serum or with an antiserum preabsorbed with a UPII synthetic peptide. Note that the staining in cow is restricted to the superficial umbrella cells, but the staining is extended to all the suprabasal cell layers in murine urothelium. (Bars, 50 μm.)

Analysis of the UPII Promoter Activity in Transgenic Mice. To study the molecular mechanisms underlying the tissue specificity of the mouse UPII gene, we tested, in transgenic mice, the expression of a chimeric gene in which a *lacZ* reporter gene was under the regulation of a 3.6-kb 5′-flanking sequence of the mouse UPII gene (Fig. 4*b*). The DNA construct was injected into fertilized mouse eggs for transgenic mouse production. Southern blot analyses of the tail DNAs showed that the transgene was integrated into the genomes of 4 of 25 mice. Three of these animals transmitted the reporter gene into their progeny. Southern blot analyses established that the genomic DNAs of these three transgenic lines, TG1, TG2, and TG3, contained roughly 40, 6, and 30 copies, respectively, of the reporter gene per diploid genome (Fig. 4*c*). Probing the same Southern blot with the *lacZ* sequence showed that the transgenes of all three lines were in tandem repeats and were integrated into independent sites (data not shown).

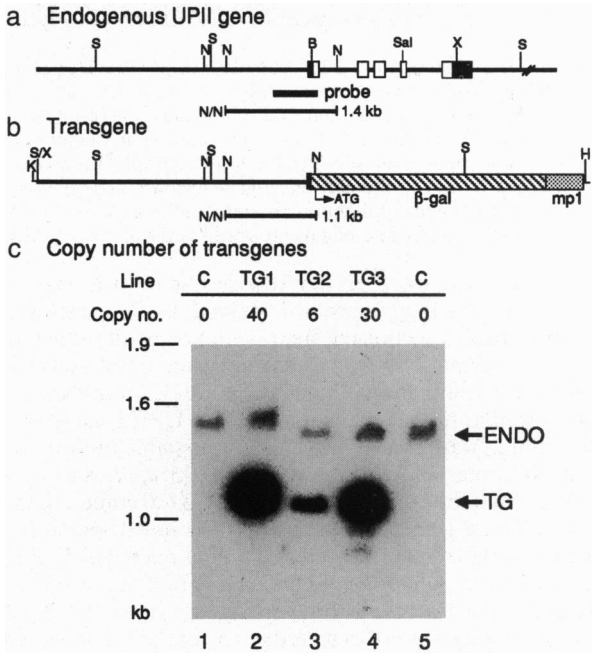

FIG. 4. Construction and quantitation of the transgene. (*a*) Restriction map (abbreviations as in Fig. 1) of the endogenous murine UPII gene. A 500-bp PCR fragment (thick bar) was used as a probe which detects a 1.4-kb *Nco* I fragment of the endogenous UPII genome but a shorter 1.1-kb *Nco* I fragment of the transgene. (*b*) Restriction map of the transgene. A 3.6-kb 5′-flanking sequence of the UPII gene was inserted into an *Escherichia coli* β-galactosidase (β-gal)-encoding placF vector. In this expression vector, a sequence containing a part of exon 1 and all of intron 1 and exon 2 of the mouse protamine-1 gene (mp1) was placed at the 3′ end of the β-gal (or *lacZ*) gene to provide an exon/intron splicing site and a polyadenylylation signal (14). This chimeric gene was cut out from the vector, gel-purified, and microinjected into mouse eggs. (*c*) Determining the copy number of the transgene. Ten micrograms of genomic DNAs isolated from the tails of five founder mice was digested with *Nco* I, electrophoresed, and blotted onto a nylon filter. The filter was then hybridized with a ^{32}P-labeled 500-bp probe (*a*), washed, and autoradiographed. The intensities of the 1.1-kb band (transgene, TG) and the 1.4-kb band (endogenous UPII gene, ENDO) were quantitated by using a PhosphorImager (Molecular Dynamics). The ratio of the TG/ENDO intensities yielded the copy number of the transgene in three transgenic lines (TG1, TG2, and TG3). Two founder lines (labeled C) negative for the β-galactosidase DNA were included as a negative control.

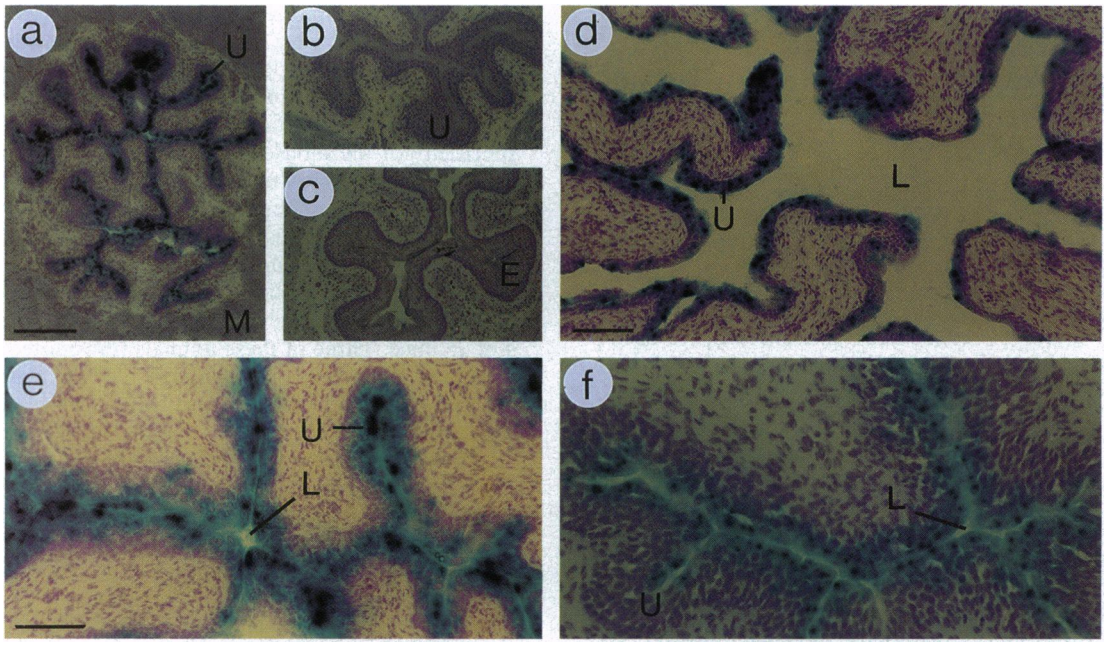

FIG. 5. Expression of the β-galactosidase (*lacZ*) reporter gene in transgenic mice. The β-galactosidase activity was visualized (as blue staining) by incubating frozen tissue sections with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (19). The bladder epithelium of transgenic mice was intensely stained (*a*), but not that of a sibling nontransgenic mouse (*b*). Other epithelial tissues of the transgenic mouse, including esophagus (*c*), were not stained. As shown in *d* and *e*, the staining in bladder epithelium is confined to suprabasal cell layers, where the endogenous UPII gene is expressed (compare with Fig. 3*b*). Photomicrograph *f* shows that the urothelium of the trigone area is much thicker than that covering the bladder wall and that in this epithelium the β-galactosidase activity was present only in the uppermost three or four cell layers. U, E, L, and M denote urothelium, esophageal epithelium, lumen, and muscularis, respectively. [Bar in *a* equals 160 μm (*b* and *c* are the same magnification); bar in *d* equals 80 μm; and bar in *e* equals 40 μm (*f* is the same magnification).]

To determine whether the reporter gene was expressed properly in these animals, we stained for β-galactosidase activity in frozen sections of their bladders. In all three mouse lines, the enzymatic activity was seen in the suprabasal cells of the bladder epithelium (Fig. 5 *a*, *d*, and *e*), in an expression pattern similar to that of the endogenous UPII gene (Fig. 3*b*). The staining was intense in the TG1 line (which has incorporated 40 copies of the transgene; Fig. 5 *a*, *d*, and *e*) and moderate in lines TG2 (6 copies) and TG3 (30 copies; data not shown). The β-galactosidase activity was not detected in the bladder epithelium of the control sibling mice which did not inherit the transgene (Fig. 5*b*), indicating that the reaction was transgene specific. In all three transgenic lines, the β-galactosidase activity was not detected in any of the (nonurothelial) stratified epithelia tested, including those of the skin, tongue, cornea, esophagus (Fig. 5*c*), and forestomach. The reporter gene product was also not detectable in all other epithelia tested, including those of the liver, lung, glandular stomach, small and large intestine, uterus, and testis; or in mesenchymal tissues, including fibroblasts, endothelial cells, spleen, and various muscle cells (data not shown). Ectopic expression was detected only in hypothalamus (data not shown), a tissue where the *lacZ* gene driven by some other epithelial-specific promoters also expressed artificially (see *Discussion*).

Epithelia closely related to the epithelium of the bladder are known to cover other areas of the urinary tract, including the renal pelvis of the kidney, the ureter, and the urethra. All these epithelia (known collectively as urothelium) share the common feature of elaborating AUM plaques as their terminal differentiation products. As expected, the reporter gene was expressed in all these urothelia (data not shown). Unexpectedly, however, we found that the urothelium of the trigone region of the bladder (7–10 cell layers) to be thicker than that of the bladder wall (4–5 layers). The expression of the transgene in this thick trigone urothelium was limited to the uppermost 3–4 cell layers (Fig. 5*f*).

DISCUSSION

A Tissue-Restricted Promoter. Our results indicate that a 3.6-kb 5′-flanking sequence of mouse UPII gene can drive, in transgenic mice, a *lacZ* reporter gene to express β-galactosidase in the upper cell layers of the bladder epithelium (Fig. 5). This transgene was not expressed in all the nonurothelial epithelia that we have tested (Fig. 5 and data not shown), indicating a high degree of tissue specificity. This conclusion was based on an analysis of three independent transgenic lines. Thus the observed selective expression of the transgene in the upper cell layers of the urothelium appears to reflect the promoter activity of the tested 5′-flanking sequence (Figs. 1 and 4*a*) rather than the effect of the neighboring sequences of the transgene integration sites.

The *lacZ* transgene is also expressed in the hypothalamus region of the brain (data not shown); this occurred in all three transgenic lines and is therefore quite reproducible. This may represent an ectopic expression due to the lack of certain regulatory elements in the 3.6-kb 5′-flanking sequence that was used in our experiments. This idea can be tested by repeating the transgenic experiments using a longer 5′-flanking sequence, in conjunction with the first intron, which in some genes have been shown to contain important tissue-specific enhancers (20, 21), to see whether the ectopic brain expression can be abolished. It is also possible that the sequence of the bacterial *lacZ* gene is not entirely "neutral" (22). In this regard, it is interesting to note that Byrne and Fuchs (19) also found ectopic brain expression of *lacZ* gene driven by a 6000-bp 5′-flanking region of a keratinocyte-specific keratin (K5) gene, raising the possibility that the *lacZ* gene *per se* contributed to this expression.

Onset of UPII Gene Expression Is Species Dependent. Although UPII is clearly differentiation related in both cow and mouse bladder epithelia, the onset of its expression is different in these two species. In the cow, UPII appears to be associated with only the very superficial (umbrella) cells (Fig.

3*b*; ref. 2). The lack of UPII staining in the lower cell layers does not appear to be due to the masking of a single epitope (of amino acid residues 1–11 of mature UPII), because similar superficial cell staining was produced by a polyclonal antiserum raised against intact UPII, as well as by several polyclonal antisera against intact UPI and UPIII (data not shown). In contrast, UPs can be detected in almost all suprabasal cells of mouse urothelium (Fig. 3*b*). These immunofluorescence data are consistent with electron microscopy data showing that the onset of AUM formation is earlier in the bladder epithelium of the mouse than in cattle (refs. 1 and 23; F. X. Liang, X.-R. Wu, J.-H.L., T.-T.S., M. X. Ding, and Z. H. Zhai, unpublished results).

The amino acid sequences of cow and mouse UPII are highly similar, sharing 84 of their 100 amino acid residues (18). One difference, however, is that mouse UPII contains a potential N-glycosylation site [amino acid residue 24 (Fig. 1*b*; ref. 18)] which is absent from cow UPII. Glycosylation of this site, if it occurs, can explain why the mouse UPII migrates slower (with an apparent molecular mass of 17 kDa) than its cow counterpart (15 kDa; Fig. 3*a*).

Although it is generally assumed that the urothelia of different regions of the urinary tract are the same, our results indicate that the urothelium covering the trigone area is slightly thicker than that of the bladder wall and that the transgene is expressed only in the uppermost cell layers of this trigone urothelium (Fig. 5*f*). Similar results were obtained with transgenic mice of both sexes. This result raises the possibility of urothelial heterogeneity. This issue is potentially important because a large portion of human bladder cancer, which occurs more frequently in males than females, is associated with the trigone area (24). Moreover, interstitial cystitis (the "painful bladder syndrome"), which affects predominantly women, spares the trigone (25). More data are needed to define this heterogeneity and to determine whether it reflects intrinsic (epithelial) divergence or different external (mesenchymal) modulation (26, 27) or a combination of both.

Implications and Concluding Remarks. Despite the fact that urothelium has many interesting properties and is ranked high as a site of carcinoma formation, relatively little attention has been paid in the past to the biochemistry of urothelial differentiation. Our demonstration that UP genes are bladder specific and that a 3.6-kb 5′-flanking sequence of this gene can duplicate this tissue specificity to a large extent has made it now possible to further dissect the cis elements that are involved in regulating this process. This would also enable us to examine the possible existence of urothelium-specific transcriptional factors. The availability of LacZ-tagged urothelium of the transgenic mice described here can be useful for transplantation studies, for tracing the cell lineage, and for possible identification of urothelial stem cells (28, 29). Our results also have major implications for the AUM function. Although many biological functions have been ascribed to AUMs—including the possibility that they serve as a permeability barrier (7), as a physical stabilizer of the apical surface (6), and as a retrievable membrane system that contributes to the reversible regulation of apical surface area (3, 30)—they remain largely speculative. It should now be possible to use the 3.6-kb 5′-flanking sequence of the mouse UPII gene to drive the urothelial expression of various mutated or truncated UP molecules and to see whether some of these can specifically perturb the AUM structure. The ensuing physiological and pathological changes of the urothelium can provide important clues to AUM function (cf. ref. 31). These experiments, as well as those designed to test the effects of additional flanking and intragenic DNA sequences on the promoter activity, will undoubtedly yield important new information on the molecular regulation of urothelial growth, differentiation, and diseases.

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