Isolation of mouse THP gene promoter and demonstration of its kidney-specific activity in transgenic mice

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Zhu, Xinhua, Jin Cheng, Jing Gao, Herbert Lepor, Zhong-Ting Zhang, Joanne Pak, and Xue-Ru Wu. Isolation of mouse THP gene promoter and demonstration of its kidney-specific activity in transgenic mice. Am J Physiol Renal Physiol 282: F608–F617, 2002; 10.1152/ajprenal.00297.2001.—Tamm-Horsfall protein (THP), the most abundant urinary protein synthesized by the kidney epithelial cells, is believed to play important and diverse roles in the urinary system, including renal water balance, immunosuppression, urinary stone formation, and inhibition of bacterial adhesion. In the present study, we describe the isolation of a 9.3-kb, 5′-region of the mouse THP gene and show the highly conserved nature of its proximal 589-bp, 5′-flanking sequence with that in rats, cattle, and humans. We also demonstrate using the transgenic mouse approach that a 3.0-kb, proximal 5′-flanking sequence is sufficient to drive the kidney-specific expression of a heterologous reporter gene. Within the kidney, transgene expression was confined to the renal tubules that endogenously expressed the THP protein, which suggests specific transgene activity in the thick ascending limb of Henle and early distal convoluted tubules. Our results establish the kidney- and nephron-segment-specific expression of the mouse THP gene. The availability of the mouse THP gene promoter that functions in vivo should facilitate additional studies of the molecular mechanisms of kidney-specific gene regulation and should provide new molecular tools for better understanding renal physiology and disease through nephron-specific gene targeting.

Tamm-Horsfall protein; expression; thick ascending limb of Henle’s loop; reporter gene

TAMM-HORSFALL PROTEIN (THP), also named uromodulin, is an 85- to 95-kDa glycoprotein that is synthesized by the kidney epithelial cells of all placental mammals (19, 20). Within the kidney, THP has been localized by immunohistochemistry and in situ hybridization to the thick ascending limb of Henle’s loop (TALH) and the early distal convoluted tubules (2, 3, 17, 27, 40). At these locations, THP is believed to be membrane-anchored via its COOH-terminal glycosphingolipidylsitol linkage, but the protein can be released into the urine by the action of phospholipases or proteases (7, 9, 15, 28, 34). The released THP constitutes the most abundant protein in normal human urine with a daily excretion rate of 50–200 mg (19, 20).

Because of its abundance, species conservation, and unique nephron association, THP is believed to play critical roles in urinary physiology. First, there has been tremendous interest surrounding the role of THP in immunoregulation, because THP binds avidly to recombinant interleukin (IL)-1, IL-2, tumor necrosis factor (TNF), complement 1q, and immunoglobulins and inhibits lectin- and IL-induced T-cell activation (15, 33, 51, 55). Such inhibitory activity is largely attributed to the oligosaccharid moieties of THP (8, 37, 43). Because the kidney is the main site of IL catabolism, it has been suggested (15, 20) that THP might act as a potent immunosuppressant. Second, THP has been found to be involved in regulating urinary stone formation, although it is controversial whether it promotes or inhibits the stone formation (13). Nevertheless, some in vitro studies indicate that purified THP is capable of inhibiting the growth of calcium oxalate crystals (14, 52). Consistent with this, patients who are prone to forming renal stones have appreciably lower urinary THP levels than healthy controls (5, 10, 11, 35). Third, via its high mannose residues, THP can bind to type-1 fimbriated Escherichia coli, which is the most common pathogen to cause urinary tract infection (26, 32). We have recently shown that THP at a physiological concentration can effectively block type-1 fimbriated E. coli from binding to uroplakins, the latter of which are putative urothelial receptors (25, 41, 53). These data indicate that THP can serve as a potentially defensive factor in the urinary tract against uropathogenic E. coli. Fourth, it has been suggested that the gel-forming capability of THP within the TALH may contribute to the water impermeability of this nephron segment (20). Finally, molecular cloning and sequencing of THP has revealed several domains that are shared by many important molecules. Thus the NH2-terminal region of THP contains four epidermal growth factor-like domains that are present in...
epidermal growth factor precursor, LDL receptor, thrombomodulin, and tissue plasminogen activator (28). THP also contains at the COOH-terminal region a ZP domain that is found in zona pellucida proteins ZP-2 and ZP-3, betaglycan, and pancreatic protein GP-2 (16, 30). Although currently unclear, it is likely that the epidermal growth factor and ZP domains may play a functional role in renal physiology.

Under normal conditions, the kidney synthesizes large amounts of THP. This implies that the reduced synthesis of this protein may cause or reflect renal dysfunction. Indeed, urinary THP reduction has been associated with certain pathological conditions, including acute tubular necrosis, diabetic nephropathy, hyperprostaglandin E syndrome, and active lupus nephritis (4, 23, 39, 45, 47). Although THP has been frequently used as an indicator for renal tubular function (46, 57), the mechanisms by which THP gene expression are regulated have not been studied.

In this paper, we describe the isolation and sequencing of the 5′-region of the mouse THP gene and characterization of the gene structure. We show that, like its human counterpart, mouse THP expression is highly kidney specific. In addition, we have generated transgenic mice that harbor a 3.0-kb, 5′-flanking region of the mouse THP gene and an enhanced green fluorescence reporter gene and have demonstrated that the 3.0-kb sequence contains all the necessary elements to direct kidney-specific expression of the reporter gene. Within the kidney, transgene expression is colocalized with the endogenous THP, thus establishing that transgene expression is restricted to the TALH. The availability of a kidney- and segment-specific gene promoter opens new avenues for studying the molecular mechanisms of kidney-specific gene regulation, renal physiology, and disease.

METHODS

Extraction of total RNA and PCR. For the determination of THP gene expression in mice, total RNA was extracted from various tissues using a total RNA extraction kit (Promega). Total RNA (2 μg) was reverse-transcribed and PCR amplified using one pair of primers corresponding to mouse THP cDNA (sense: 5′-AGGGCTTTACAGGGATGTTG-3′; anti-sense: 5′-GATTGACTCAGGGGCTGCTGT-3′) (30). PCR was performed as follows: 94°C for 5 min, 55°C for 30 s, 72°C for 1 min for 35 cycles; and 94°C for 5 min, 55°C for 30 s, 72°C for 8 min for the last cycle. Amplification with glycerol-aldehyde-6-phosphate dehydrogenase gene primers was used as a normalization control.

Isolation of genomic clones containing the mouse THP gene. A mouse genomic library constructed with bacterial artificial chromosome (BAC) as a vector and 129/SvJ mouse genomic DNA as inserts (average insert size, 50–240 kb; Incyte Genomics) was mass screened with PCR using oligonucleotide primers designed according to mouse THP cDNA (sense: 5′-AGGGCTTTACAGGGGATGTTG-3′; anti-sense: 5′-GATTGACTCAGGGGCTGCTGT-3′). Positive clones identified in the initial screen were verified with a second round of PCR using nested primers (sense: 5′-GCCTCAAGGGCCCAGTG-GAAAG-3′; antisense: 5′-GCACAGTGGTCGCTCCAGTGT-3′). For the identification of the 5′-portion of the THP gene encompassing the 5′-flanking region, the large genomic clones from PCR screening were subjected to restriction digestion followed by Southern blotting using three different cDNA probes located at the 5′-end, middle, and 3′-end of the mouse THP cDNA. Restriction fragments that reacted with the 5′-end probe but not the middle or 3′-end probes were cloned, as these most likely contained the 5′-coding region as well as the upstream region. These fragments were cloned into pBluescript (Stratagene) and fully sequenced, and the genomic structure was delineated by restriction digestion and comparison with the existing mouse THP cDNA sequence.

Construction of the THP-enhanced green fluorescent protein chimeric gene and expression in transgenic mice. One of the two isolated genomic clones (C2, Fig. 2A) was used as a template for PCR amplification using a sense primer located at −3.0 kb (5′-GGGCCCCCAAGAGATCAAAGTCTCCT-3′) in relation to the first base of exon 1 and an anti-sense primer ending at the ninth base of exon 1 (5′-GGGCCCTGGTTC-CAGTCAACAAGTAAG-3′). The A of the first ATG, although noncoding in endogenous THP, was mutated to C to avoid potential translation interference with the initiation codon of the reporter gene. The ends of each primer were supplemented with an Apal restriction sequence to facilitate cloning. After the 3.0-kb PCR product was subcloned into the TA cloning vector (Invitrogen) and its authenticity was verified by DNA sequencing, it was retrieved by Apal digestion and cloned into the same site of the pEGFP vector (Clontech). Restriction digestion and DNA sequencing of the fusion-gene junction were carried out to verify the correct orientation. The 4.0-kb THP-enhanced green fluorescent protein (EGFP) chimeric gene was then excised en bloc by KpnI/AflIII digestion, gel-purified, and microinjected into fertilized eggs of FVB/N inbred mice for transgenic mouse production according to established protocols (6).

Southern blot analysis of mouse-tail DNA. Transgene-bearing founder animals and their germ-line transmission to offspring were determined by Southern blotting analysis. Briefly, mouse-tail genomic DNA was extracted using proteinase K and a salt-precipitation method. The purified DNA was then digested with HindIII, electrophoresed, transferred onto a nylon membrane, and hybridized with a 520-bp, 32P-labeled BamHI/AatI fragment of the 5′-upstream sequence of the mouse THP gene, which would allow the detection of both endogenous and transgene fragments.

Northern blot analysis. For the assessment of transgene expression on a messenger RNA level, Northern blotting was performed using various tissues from a transgenic line that harbored the THP-EGFP transgene. Total RNA was extracted, resolved by agarose gel electrophoresis, transferred onto a nylon membrane, and hybridized with a 520-bp, 32P-labeled BamHI/AatI restriction fragment of the EGFP gene. After autoradiography, the probe was stripped by boiling the membrane in a high-stringency buffer and rehybridized with a mouse β-actin probe.

Fluorescence microscopy. Freshly dissected mouse tissues were fixed in Zamboni’s fixative (2% paraformaldehyde and 15% picric acid prepared in phosphate-buffered saline) for 2 h, embedded in optimum cutting temperature (OCT) embedding medium, frozen in liquid nitrogen, and sectioned using a cryostat into 5-μm-thick sections. The sectioned tissues were either directly examined by fluorescence microscopy for the expression of green fluorescent protein or were stained with a polyclonal antibody against THP after a 1:100 dilution (Biodesign International) followed by a rhodamine-conjugated secondary antibody.
RESULTS

THP is kidney specific in mice. Although previous immunohistochemical staining and Northern blotting could detect THP only in the kidney (3, 28), more sensitive assays had not been performed to verify these observations. We extracted total RNA from multiple mouse tissues and carried out RT-PCR using oligonucleotide primers specific for mouse THP cDNA (30). A 440-bp product that matched the predicted length was amplified from the mouse-kidney RNA preparation but not from that of the urinary bladder, liver, esophagus, spleen, skin, stomach, small intestine, large intestine, lungs, heart, testes, brain, skeletal muscle, thymus, forestomach, and seminal vesicles (Fig. 1A). This result firmly established the kidney specificity of THP gene expression in mice.

Isolation and characterization of the 5′-upstream region of the mouse THP gene. The remarkable tissue specificity of THP prompted us to isolate its regulatory sequence for further study of the molecular mechanisms of its gene expression. A pair of oligonucleotide primers was synthesized according to the previously published mouse THP cDNA and was used to mass screen a 129/SVJ-mouse genomic DNA library constructed with BAC vectors (see METHODS). The primary screening yielded two positive clones, each of which harbored a 60- to 70-kb insert. The identity of both clones was verified by a secondary screening using nested primers. One of two positive clones was chosen for further characterization. Because of the large insert size, this clone was digested with multiple restriction enzymes and probed with three different cDNA fragments located in the 5′-end, middle, and 3′-end of the mouse THP cDNA (data not shown). A 9.3-kb KpnI fragment and an 8.0-kb ApaI fragment hybridized strongly with the 5′-end probe but not the middle and 3′-end probes, which suggests that these fragments contained the 5′-portion of the coding region as well as a 5′-flanking sequence. These two fragments (C1 and C2; Fig. 2A) were subcloned and completely sequenced (GenBank accession no. AF420599).

Comparison of the sequence of the genomic clones with that of the published mouse THP cDNA revealed that the longer genomic clone (C1) contained the first two exons and the partial exon 3 intervened by the first two introns, and that the shorter clone (C2) had the less-distal 5′-flanking sequence and terminated within the second intron (Fig. 2A). A BLAST nucleotide search of GenBank using the coding regions of the isolated mouse THP gene fragment as a query yielded two highly homologous sequences, both of which represented the mouse THP cDNA clones. The first (GenBank accession no. BC012973) showed 100% identity (641/641 nucleotides), and the second (GenBank accession no. L33406), which showed 97% identity (713/732 nucleotides), was the previously published mouse THP cDNA (30). Because the majority of sequence discrepancies between our genomic sequence and the second THP cDNA clone resided in the third position of a codon, they were most likely due to the polymorphisms between the mouse strains that were previously used for cDNA library screening and those presently used for genomic DNA screening.

The 5′-flanking region contained a proximal TATA box that was 30 bp before the first exon (designated as +1) and a canonical CCAAT enhancer binding site at −209 bp (Fig. 2B). In the more distal regions resided three stretches of CA(46) repeat and an Alu-Sx repetitive sequence. A computer-assisted sequence search of the 5′-flanking region of the mouse THP gene against the Web-based TRANSFEC database and FindPatterns of SeqWeb 1.2 revealed a number of consensus-recognition sequences for known transcription factors. These included two activator protein-2 (AP-2) sites arranged in tandem neighboring the TATA box; one AP-1 site at −779 bp; two GATA-1 sites at −527 and −870 bp, respectively; two hepatocyte nuclear factor-5 (HNF-5) sites at −419 and −635 bp, respectively; one keratinocyt enhancer binding site at −421 bp; one AML-1a site at −952 bp; and five heat shock transcription factor (HSF) binding sites scattered throughout the proximal region (Fig. 2B). It is noteworthy that most of these sites were located downstream of the repetitive sequences and are thus potentially important for regulating THP gene transcription (see DISCUSSION). Finally, sequence alignment of the 5′-flanking regions of THP genes from mice, rats, cattle, and hu-
mans (56) revealed a high degree of cross-species conservation (Fig. 3). Mouse and rat sequences were 90% identical, those from humans and cattle were 75% identical, and those from mice and humans were 66% identical. In addition, there existed several highly conserved regions that could be important for kidney-specific and nephron-segment-specific gene expression (Fig. 3).

In vivo activity of the 5‘-flanking region of the mouse THP gene. To test whether the 5‘-flanking region of the mouse THP gene can confer kidney specificity, we

Fig. 2. Organization and nucleotide sequence of the 5‘-region of the mouse THP gene. A: C1 (9.3 kb) and C2 (8.0 kb) represent the two overlapping genomic clones isolated from a bacterial artificial chromosome mouse genomic library. Comparison of genomic DNA sequence with that of mouse THP cDNA reveals the existence in the 3‘-portion of the genomic clones of exons 1 and 2 (solid bars), introns 1 and 2 (thick lines connecting the exons), and partial exon 3. Upstream of the coding region is a 7.0-kb, 5‘-flanking sequence (distal portion is truncated with double slashes for presentation). Restriction sites: K, KpnI; A, ApaI; P, PstI; X, XbaI; and S, SpeI. B: nucleotide sequence (GenBank accession no. AF420599) of the proximal 5‘-flanking region (2.0 kb) is shown with exon (bold letters) and abbreviated intron sequences (between the slashes in lower-case letters). Start of exon 1; arrow, translation start site. Consensus recognition sequences that are potentially important for transcription-factor binding are boxed and include a proximal TATA box, a CCAAT box, binding sites for activator protein-2 (AP-2; 2 sites), heat shock transcription factor (5 sites), AP-1, GATA-1 (2 sites), hepatocyte nuclear factor-5 (2 sites), keratinocyte enhancer (1 site), and AML-1a (1 site). Mouse CA(46) (double underline) and Alu-Sx (single underline) repetitive sequences are also indicated.
linked a 3.0-kb THP 5′-flanking sequence to a downstream EGFP reporter gene (Fig. 4A). This chimeric construct was microinjected into one-cell mouse embryos of the inbred FVB/N strain for transgenic mouse production. Southern blotting of the live-born animals identified three founder animals, all of which transmitted the transgenes to their offspring (Fig. 4B). The sizes of the HindIII-digested fragments of the THP-EGFP transgene in lines 1 and 6 precisely matched the predicted 4.0-kb (corresponding to head-to-tail orientation of two transgene copies inserted in tandem) and 5.5-kb (tail-to-tail orientation) sizes, respectively. The size of the transgene fragment in line 11 (12.0 kb) did not match predicted sizes, which suggests that the transgene was inserted as a single copy into the mouse genome.

To assess the pattern of transgene expression, we used Northern blotting to examine a variety of tissues from transgenic mice for the expression of the EGFP gene. EGFP mRNA was detected exclusively in the

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**Fig. 3. Alignment of the proximal 5′-flanking sequences of THP genes from different species.** Sequences (589 bp) from mouse, rat (GenBank accession no. S75965; Ref. 56), cow (GenBank accession no. S75961; Ref. 56), and human (GenBank accession no. S75968; Ref. 28) were aligned using PileUp software (SegWeb 1.2). Consensus sequences for known transcription factors are boxed. Nucleotides identical in all four species are marked by asterisks. Note the highly conserved nature of the 5′-upstream sequences of THP in different species.
kidney and not in any other tissues of the transgenic mice (Fig. 5). In addition, kidneys of the nontransgenic controls were completely negative for EGFP. The expression of EGFP was also assessed on a protein level by fluorescence microscopy. Cross sections of kidneys from all three transgenic lines but not from nontransgenic control mice exhibited strong fluorescence in the tubular cells (Fig. 6, A and B). The fluorescence was concentrated along the deep cortex and cortex-medulla junction, which is consistent with the localization of TALH and early distal tubules (2, 3, 40). Within the tubular cells, the fluorescence was uniformly cytoplasmic with the nuclei being entirely negative (Fig. 6). This was consistent with the fact that EGFP existed as a cytoplasmic protein when expressed in animal cells (29). Importantly, green fluorescence was not detected in any other tissues examined, including urinary bladder, liver, brain, small intestine, colon, stomach, prostate, testes, spleen, lungs, thyroid gland, heart, and thymus. To further establish the nephron-segment-specific expression of the 5′-flanking sequence, we stained the kidney sections of transgenic mice with an anti-THP antibody followed by a secondary antibody conjugated with rhodamine. The anti-THP staining colocalized precisely with the green fluorescence and thus established transgene expression only in those cells that endogenously express the THP protein; specifically, the TALH and early distal tubules (Fig. 7). The expression of EGFP in the functionally important TALH did not appear to have any untoward effect on renal function, because all transgenic animals thrived and bred normally.

**DISCUSSION**

*Identification of a kidney- and nephron-segment-specific promoter.* Via gene cloning and transgenic mouse analysis, the present study demonstrates that a 3.0-kb, 5′-flanking sequence of the mouse THP gene is capable of driving a heterologous reporter gene to express in a kidney-specific manner. Because such kidney specificity occurred consistently in all three transgenic lines, it is highly unlikely that the tissue-specific gene expression was due to chromosomal insert-site-dependent events of the THP-EGFP transgene; rather, it reflects the genuine promoter activity of the 5′-flanking sequence. These in vivo expression data therefore strongly indicate that the 3.0-kb upstream sequence of

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**Fig. 4.** Generation of transgenic mice harboring the THP-enhanced green fluorescent protein (EGFP) chimeric gene. A: transgene construct. A 3.0-kb PCR product containing a sequence from −3.0 kb of the 5′-flanking region to +9 bp of the first exon was inserted 5′-upstream of an EGFP reporter gene, the latter of which was supplemented with an SV40 poly A tail. Restriction enzymes include HindIII (H); ApeI (A); KpnI (K); NorI (N); and AfIII (Af). B: Southern blotting identification of transgenic mice. Genomic DNA purified from mouse-tail biopsies was digested with HindIII, resolved by agarose gel, transferred onto nylon membrane, and hybridized with a 32P-labeled probe located at the 5′-flanking region of the THP gene (“probe” in A). Note the detection of a predicted 1.8-kb endogenous DNA fragment (arrow) in all mice including the nontransgenic control (lane 1) and transgene fragments of 4.0 kb in lane 1 (lane 2; head-to-tail orientation), 5.5 kb in lane 6 (lane 3; tail-to-tail orientation), and 12.0 kb in lane 11 (lane 4; most likely a single transgene copy with unpredictable size). *Band (10 kb) present in all mice (most likely an incompletely digested endogenous THP gene fragment).

**Fig. 5.** Expression of EGFP reporter gene in transgenic mouse tissues. Total RNA was isolated from the kidneys of two nontransgenic littermates (lanes 1 and 3) and the kidney of a transgenic mouse of line 1 (lane 2) as well as from other tissues of a male and a female transgenic mouse including forestomach (lane 4), stomach (lane 5), spleen (lane 6), small intestine (lane 7), large intestine (lane 8), forebrain (lane 9), hindbrain (lane 10), lung (lane 11), thymus (lane 12), liver (lane 13), urinary bladder (lane 14), heart (lane 15), uterus (lane 16), seminal vesicle (lane 17), testis (lane 18), thyroid gland (lane 19), and skin (lane 20). A sample of total RNA (15 μg) from each tissue was subjected to Northern blotting using an EGFP probe. Note the specific detection of EGFP mRNA in the kidney but not in any other tissues of the transgenic mice. EGFP was also absent in the kidneys of the nontransgenic controls.

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the mouse THP gene contains all required cis elements that govern the kidney-specific gene transcription. Moreover, the transgene was expressed exclusively in the renal tubular cells that endogenously express the THP protein, namely, the TALH and early distal tubules. This latter finding indicates that cis elements that control the nephron segment-specific expression must also reside within the 3.0-kb, 5’-flanking se-

Fig. 6. Detection of EGFP in transgenic mice. Frozen sections of paraformaldehyde-fixed mouse tissues were directly examined with fluorescence microscopy after a brief nuclear staining with propidium iodine (red). Renal tubules in deep cortex and cortex-medulla junction of the two transgenic F1 mice [line 1 (A); and line 6 (B)] but not those of a nontransgenic control mouse (C), were strongly positive for green fluorescence. Fluorescence was uniformly cytoplasmic with the nuclei being completely negative. Other tissues from the transgenic mice including urinary bladder (D), liver (E), brain (F), and stomach (G) were all negative for green fluorescence. All panels were of the same magnification (×400).

Fig. 7. Segment-specific expression of THP-EGFP transgene. Kidney sections of the transgenic mice were stained with an anti-THP antibody followed by a rhodamine-conjugated secondary antibody. Digital images from the same field were taken using a green (A) or red (B) filter, and the two images were overlaid (C). Transgene expression (represented by green fluorescence) colocalized precisely with the endogenous THP expression (shown by red fluorescence). EGFP, the transgene product, showed uniform cytoplasmic staining, whereas the endogenous THP showed preferentially apical membrane staining (arrowheads). All panels were of the same magnification (×600).
quence. The existence of several stretches of repetitive sequences, which can potentially serve as “insulators” for position-independent gene transcription (44, 50), further supports the idea that the most critical cis elements of the THP gene are located within the 3-kb upstream region.

Although the specific cis elements responsible for the above-mentioned kidney- and nephron-segment-restricted expression are yet to be identified, the proximal promoter of the mouse THP gene does contain several canonical binding sites for known transcription factors. These include the TATA and CAATT boxes, both of which are frequently present in promoters of tissue-specific genes and are usually absent in ubiquitously expressed genes. Also of interest is the presence of the AP-2 binding sites, which are in close proximity to the TATA box. It is worth noting that the TATA and CAATT boxes and the first (distal) AP-2 site are also species conserved (see Fig. 3), which suggests a possible role in conferring tissue specificity. There is mounting experimental evidence which indicates that these cis elements are indeed indispensable for keratinocyte-specific gene expression (22). Given that cells of the TALH are keratinocytes in nature and thus most likely express the keratinocyte-specific transcription factors, these proximal cis elements may well be involved in renal tubule-specific transcription. Finally, the THP gene promoter contains, in the more distal portion (−952 bp), an AML-1a binding element that could potentially interact with Runt-domain-containing transcription factors. Initially identified in the hematopoietic cells, the Runt-domain proteins are capable of regulating a variety of tissue-specific genes by acting as an organizer to bring together other transcription factors (49).

In addition to these putative tissue-specific elements, the mouse THP gene promoter harbors several interspersed heat shock factor binding motifs. It is possible that some of these motifs may be involved in stress-related modulation of THP gene expression, given the fact that THP can undergo quantitative changes during oxidative stress such as acute renal failure (23, 24, 42). The functional significance of any of these known cis elements along with those unknown but highly species-conserved sequences (see Fig. 3) will require further experimental verification. By further dissection of the promoter region of the THP gene using deletion and mutation approaches, it should be possible to narrow down the minimal promoter elements that are necessary for THP gene expression. Such experiments may also unveil novel cis elements and transcription factors for kidney-specific and segment-restricted expression. The identification of the THP gene promoter that functions in vivo has set a stage for studying kidney-specific gene regulation.

Although it is well known that different segments of the nephronal tubules perform distinct functions in absorption and secretion, it was only relatively recently that segment-specific markers began to be identified and characterized. Representative examples include the renal-specific oxidoreductase specific for the proximal convoluted tubules (54), the ClC-K1 chloride channel for the thin ascending limb of Henle’s loop (48), and the aquaporin-2 water channel for the collecting duct (1, 31). Besides the THP gene, the only other known gene that is specifically expressed in the TALH is the NKCC2 gene (18). It encodes a (diuretic) bumetanide-sensitive Na+-K+-Cl− cotransporter that is located at the apical membrane of the TALH (18). This multiple membrane-spanning protein mediates coupled transport of Na+, K+, and Cl−. Igarashi and coworkers (18) recently isolated the mouse NKCC2 gene promoter and found that a 280-bp proximal DNA fragment was sufficient to confer specific reporter gene expression in a TALH-derived cell line. It is of interest that the NKCC2 gene promoter also contains the TATA and CAATT boxes and the AP-1 and AP-2 binding sites. It is presently unknown (but will certainly be interesting to examine) whether these shared binding sites will turn out to be important for TALH-specific transcription.

Potential applications of the mouse THP gene promoter. The availability of the mouse THP gene promoter should enhance our understanding of the molecular mechanisms underlying kidney-specific gene transcription and facilitate kidney- and nephron-segment-specific gene targeting. Biologically active molecules can be specifically introduced into the TALH using the transgenic mouse approach, and the effects on renal physiology and pathology can be investigated systematically in an in vivo setting. For example, genes that are not normally expressed in the TALH can be ectopically targeted to evaluate their ability to alter the TALH-associated functions. In addition, genes that are naturally expressed at the TALH such as the Na+-K+-Cl− cotransporter can be overexpressed to determine the impact on ion reabsorption. Conversely, mutated or truncated molecules that exert dominant negative effects can be expressed in the TALH to examine the pathophysiology as a result of their deficiency. The THP gene promoter can also be used to drive oncogenes to study the contribution of TALH cells in the tumorigenesis of renal cell carcinoma, for which the cellular origin remains elusive (12). Finally, genes of particular importance but having wide tissue distribution can be ablated in a TALH-specific fashion. This can be accomplished by generating a transgenic mouse expressing the Cre recombinase under the control of the THP gene promoter and then by breeding such a mouse with another transgenic mouse having a loxP-flanked target gene (21). Many of these transgenic approaches can be carried out in conjunction with an inducible gene expression strategy, so that the analysis can be done at postnatal stages (36, 38). Together, these studies will undoubtedly shed new light on renal-specific gene expression and renal functions.

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