Cloning of mouse prostaglandin transporter PGT cDNA: species-specific substrate affinities

MICHAEL L. PUCCI,1 YI BAO,1 BRENDA CHAN,1 SHIGEKAZU ITOH,1 RUN LU,1 NEAL G. COPELAND,2 DEBRA J. GILBERT,2 NANCY A. JENKINS,2 AND VICTOR L. SCHUSTER1

Departments of Medicine, Physiology, and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461; 2Mammalian Genetics Laboratory, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

Pucci, Michael L., Yi Bao, Brenda Chan, Shigekazu Itoh, Run Lu, Neal G. Copeland, Debra J. Gilbert, Nancy A. Jenkins, and Victor L. Schuster Cloning of mouse prostaglandin transporter PGT cDNA: species-specific substrate affinities. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R734–R741, 1999.—We recently identified and/or cloned the PG transporter PGT in the rat (rPGT) (Kanai, N., R., Lu, J., A. Satriono, Y. Bao, A. W. Wolkoff, and V. L. Schuster, Science 268: 866–869, 1995) and the human (hPGT) (Lu, R., and V. L. Schuster, J. Clin. Invest. 98: 1142–1149, 1996). Here we have cloned and expressed the mouse PGT (mPGT) cDNA. The tissue distribution of mPGT mRNA expression is significantly more restricted than that of rPGT and hPGT mRNA. Although the deduced amino acid sequence of mPGT is similar to the rat (91% identity) and human (82% identity) homologues, it has three regions of dissimilarity: amino acids 128–163 and 283–298, and valine 610 and isoleucine 611 (predicted to lie within putative transmembrane span 12). Affinities of hPGT, rPGT, and mPGT for several PG substrates differed, with hPGT having the highest [low Michaelis constant (Km)] and mPGT the lowest affinity. A chimeric protein, linking the N-terminal domain of mPGT with the C-terminal domain of hPGT, had affinity for PGE2 indistinguishable from that of hPGT, indicating that the C-terminal domain dictates Km. We mutagenized mouse valine 610 and isoleucine 611 to their corresponding human residues (methionine and glycine, respectively); however, these changes did not convert the inhibition constant of mPGT to that of hPGT. The mouse gene was localized to chromosome 9 in a region syntenic with the region of human chromosome 3 containing the hPGT gene. These studies highlight the species-dependence of tissue expression and function of PGT and lay the groundwork for the use of the mouse as a model system for the study of PGT function.

METHODS

Mouse cDNA library screening. A gel-purified fragment of rPGT cDNA (1.7 kb, from a HindIII digest of the full-length cDNA) was labeled with 32P-labeled deoxycytidine triphosphate ([α-32P]dCTP) by the random primer method (31) and subsequently used to probe a mouse brain cDNA library in the phagemid vector pBluescript (IZAP; Stratagene, Lajolla, CA). After plating and duplicate replica transfer to nylon membranes (Nytran Plus; Schleicher & Schuell, Keene, NH), the membranes were hybridized for 12–16 h at 42°C with the 32P-labeled probe in hybridization solution of the following composition: 5 X standard sodium citrate (SSC), 0.1% Na-laurylsarcosine, 0.02% SDS, and 2% blocking reagent (Genius System; Boehringer Mannheim Biochemicals, Indianapolis, IN). Membranes were subsequently washed as follows: twice with 2 X SSC at room temperature; once with 1 X SSC, 0.1% SDS at 42°C; and twice with 0.5 X SSC, 0.1% SDS at 42°C. Radiological film (X-Omat AR; Eastman Kodak, Rochester, NY) was exposed for 48–72 h at –70°C. Of approximately 2 X 106 plaques screened, a single positive plaque on duplicate membranes was picked and isolated by secondary screening. This clone was subjected to phagemid excision (Stratagene). By DNA sequencing and computer analysis, this 1.1-kb clone was found to have high homology (90%) to rPGT and was presumed to encode a portion of the mPGT cDNA.

This partial mPGT cDNA was then used as a probe to screen a mouse lung cDNA library (Clontech, Palo Alto, CA) in the phage vector λgt10. Screening procedures were similar to those described above with the following exceptions: 1) after hybridization and washing as above, the membranes

PROSTAGLANDINS (PGs) regulate or modulate diverse physiological and pathophysiological processes, including platelet aggregation, vascular tone, gastric cytoprotection, uterine contraction, and inflammatory responses (5, 13, 24, 28, 30, 33, 35). These arachidonic acid metabolites are charged organic anions at physiological pH (34) and do not readily transverse biological membranes by diffusion (4, 6, 8). Studies have demonstrated carrier-mediated PG transport in a variety of tissues, including the kidney, lung, liver, choroid plexus, uterus, and eye (reviewed in Ref. 32).

We recently identified a novel PG transporter (PGT) (20). PGT transports PGs of the D, E, and F series at a high rate, thromboxane at an intermediate rate, and prostacyclin analogs at a low rate (20, 25). PGT may play roles in the efflux of PG from cells and/or the clearance of PGs by the lung (32).

To examine the mouse as a potential model for studying PGT function, we used a probe from rat PGT (rPGT) to screen mouse cDNA libraries, doned a full-length mouse PGT (mPGT) cDNA from a mouse lung library, characterized the mouse cDNA structurally and functionally, and mapped the mPGT gene. We report here clear differences in the functional properties and pattern of tissue expression of the mPGT as opposed to the rat and human (hPGT) isofoms.
were additionally washed twice with 0.1 × SSC, 0.1% SDS at 42°C; 2) film was exposed for 48 h. Secondary screening under the same hybridization and washing conditions yielded 15 positive clones from an initial screening of 10⁶ plaques. After restriction analysis, the largest three of these clones (clone 3–1, 1.9 kb; clone 5–2, 2.1 kb; and clone 7–2, 2.9 kb) were excised from the xgt10 vector by restriction digest with EcoRI gel purified, and subcloned into the plasmid vector pSPORT-1 (GIBCO-BRL, Grand Island, NY).

DNA sequencing and computer analysis. All of both strands of the mouse brain CDNA clone, along with portions of clones 3–1 and 5–2 and all of both strands of clone 7–2 from the mouse lung CDNA library, were sequenced by means of the deoxynucleotide chain-termination method by primer walking (31) (Sequenase version 2.0 DNA sequencing kit; U.S. Biochemical, Cleveland, OH). Alignment of sequencing runs and comparisons of nucleotide and amino acid homologies between mPGT, hPGT, and rPGT were performed with MacVector (Eastman Kodak) and GeneWorks (Intelligenetics, Campbell, CA) software programs.

Preparation of chimeric CDNA. Using two conserved restriction enzyme sites (BstEI at bp 836 of hPGT and 866 of mPGT; EcoN1 at bp 1423 of hPGT and 1450 of mPGT), chimeric CDNAS linking the 5' fragment of mPGT or hPGT with the 3' fragment of the corresponding CDNA were prepared as follows. Restriction digestion of the CDNAS with BstEI at 65°C or EcoN1 at 37°C for 24 h was followed by agarose gel purification, dephosphorylation of the 5' fragments, overnight ligation of corresponding fragments, and subsequent transformation of resulting subclones in DH5α competent bacteria. Ligation of fragments in the correct orientation was confirmed by sequencing as described, across the restriction site from both directions and across the 5' and 3' ends of the CDNA clones. In transient expression assays as described in Transient expression of PGT in HeLa cells and transport assay, both the EcoN1 site chimera and the 5' human/3' mouse BstEI chimera were found to produce no measurable tracer PG uptake (data not shown). The 5' mPGT/3' hPGT chimera (m/hPGT) did produce tracer PG uptake in transient expression assays as described in RESULTS.

Site-directed mutagenesis of mPGT. Oligonucleotide mutagenesis primers were designed to introduce the separate changes in the mPGT amino acid sequence: Val610Met, Iso611Gly, and the double-mutant comprising both point mutations. Mutagenesis was accomplished by means of an oligonucleotide-directed mutagenesis system (Gene-Editor Site-Directed Mutagenesis System; Promega, Madison, WI). Briefly, selection and mutagenesis oligonucleotide were annealed to wild-type template, followed by synthesis of a mutant strand. Selection was based on competition of the ampicillin resistance gene to enhance resistance to β-lactam antibiotics. The single-strand mutant template was used to transform repair-deficient competent bacteria (BMH71–18 mutS) under ampicillin resistance gene to enhance the ampicillin resistance gene to enhance transformation efficiency. The resulting inhibition dose-response curves were analyzed by curve-fitting and calculation of a Ki value for each experiment (18). For each proteinase tested, K values were calculated from three to five separate transfections for mPGT, rPGT, and hPGT CDNA.

Northern blot analysis. A multiple mouse tissue Northern blot (Clontech) was probed with the mouse brain CDNA clone (1.1-kb fragment identical to the middle portion of the open reading frame of clone 7–2; see Fig. 2A) and separately with a β-actin CDNA probe (Clontech), each labeled by the random primer method with [α-32P]dCTP. The blot was hybridized overnight at 42°C in hybridization solution of the following composition: 5 × SSC, 50% formamide, 0.1% N-laurylsarcosine, 0.02% SDS, 0.01 M EDTA, and 2% blocking solution (Genius Systems; Boehringer Mannheim Biochemicals). The blot was then washed twice with each of the following wash solutions: 1 × SSC, 0.1% SDS, 0.01 M EDTA; 0.5 × SSC, 0.1% SDS, 0.01 M EDTA; and 0.1 × SSC, 0.1% SDS, 0.01 M EDTA. The membrane was exposed to film for 96 or 24 h for the blot probed with hPGT probe or mouse β-actin probe, respectively.

Interspecific mouse backcross mapping. Interspecific backcross progeny were generated by mating (C57BL/6 × Mus spretus)F1 females and C57BL/6 males as described (12). A total of 205 N₂ mice were used to map the Pgt locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (19). All blots were prepared with Hybond-N+ nylon membrane (Amer sham, Arlington Heights, IL). The probe, a 550-kb BamHI/EcoRI fragment of mouse CDNA, was labeled with [α-32P]dCTP with use of a nick translation labeling kit (Boehringer Mannheim Biochemicals). Washing was done to a final stringency of 0.5 × SSC, 0.1% SDS, 65°C. A fragment of 3.5 kb was detected in
Sphl-digested C57BL/6j DNA, and a fragment of 5.4 kb was detected in Sphl-digested M. spreitus DNA. The presence or absence of the 5.4-kb Sphl M. spreitus-specific fragment was followed in backcross mice.

A description of the probes and restriction fragment length polymorphisms (RFLPs) for the loci linked to Pgt, including Htr1b, Trf, and Gna12 has been reported previously (15, 21). Recombination distances were calculated with Map Manager, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Statistical analysis. Results are expressed as means ± SE. Data were analyzed by ANOVA. If differences were noted, Newman-Keuls modified t-test was used to make specific comparisons. The null hypothesis was rejected when the P value was <0.05.

RESULTS

Our initial attempts to isolate the mouse homologue of PGT from a mouse brain library yielded a single positive clone that encoded a 1.1-kb cDNA fragment with high sequence similarity (90%) to rPGT. Although repeated attempts to isolate other clones from brain were unsuccessful, this mouse brain cDNA clone was useful as a probe to examine the tissue expression of mPGT by Northern blot analysis of poly A+ RNA from various adult mouse tissues (Fig. 1). A 4.4-kb band that hybridizes strongly with the probe was observed in lung and liver, and, to a lesser extent, kidney and skeletal muscle. No discernible bands were present in brain, spleen, testis, or heart. Perhaps the apparent low level of expression of PGT mRNA in the mouse brain explains our difficulty in isolating other clones from the mouse brain library.

We used the PGT cDNA clone from mouse brain as a probe to screen a cDNA library generated from mouse lung, a tissue in which poly A+ RNA expression of PGT is very high (see Fig. 1). We isolated 15 clones and focused in more detail on three of these. Figure 2 illustrates the sequencing scheme and the alignment of the mouse brain cDNA clone with the three mouse lung clones. Partial sequencing of clones 3–1 and 5–2 demonstrated that they are overlapping, incomplete fragments of mPGT and that they are both represented within clone 7–2. Also illustrated is the alignment of the mouse clones with rPGT and hPGT. The rPGT and mPGT cDNAs are very similar in length as well as in nucleotide sequence homology (Fig. 2A). The mouse cDNA contains an ATG codon near the 5’ end that is in good context for translation initiation (22). Alignment of the mPGT and hPGT cDNAs also reveals a high level of homology.

Figure 2B compares the deduced amino acid sequences of hPGT, rPGT, and mPGT beginning at comparable ATG translation initiation codons. The predicted proteins are very similar: rPGT and hPGT are 82% identical, rPGT and mPGT are 91% identical, and hPGT and mPGT are 82% identical. All three proteins are predicted to have 12 membrane-spanning domains based on the Kyte-Doolittle hydrophathy algorithm with a 13-residue window (23). Also conserved between the mouse, rat, and human isoforms are three extracytoplasmic asparagine consensus sites for glycosylation (mouse Asn 134, 477, and 490) and three charged residues within putative membrane spans (mouse glutamate 78, arginine 560, and lysine 613). Of note are two regions of striking dissimilarity, between amino acids 128 and 163, predicted to be on the extracellular face of the protein (32) and between amino acids 283 and 298, predicted to be on the intracellular face of the protein.

Figure 3 demonstrates that, when expressed in HeLa cells, mPGT, rPGT, and hPGT catalyze the rapid, time-dependent uptake of tracer PGE2. Although there are differences among the three species in the extent of uptake, especially at 2 and 5 min, it is unclear whether this relates to differences in the rate of uptake or to subtle differences in transfection efficiency. For all three homologues, cell-associated tracer PGE2 reaches equilibrium by 10 min.

To compare the binding characteristics of the three species homologues, competition studies with various PG analogs were performed. The observed differences in uptake, whether a result of variable maximal uptake velocity (Vmax) or to variable transfection efficiency, would not be expected to affect binding affinities because these are independent of Vmax. Figure 4 illustrates an example in which the effect of increasing concentrations of unlabeled PGE2 on tracer PGE2 uptake was determined. Although unlabeled PGE2 competed with high affinity against hPGT, rPGT, and mPGT, there were substantial quantitative differences among the three homologues.

As shown in Table 1, we explored this further using a variety of PG analogs known to have high affinities for rPGT (18, 20). There was a clear rank order to the Ki for the three species homologues. For PGE2 and PGF2α, all three transporters differed significantly from each other, with hPGT having the highest and mPGT the lowest Ki values. For PGD2, only the differences between mPGT vs. hPGT and mPGT vs. rPGT reached statistical significance. In general, inhibition constants varied 2.5- to 3-fold between hPGT and mPGT. In contrast, for the thromboxane agonist U46619, which inhibits PGT...
but is not transported by it (18), $K_i$ values showed no statistically significant interspecies variation. Previous work in our laboratory has established that amino acid residues important for the binding and transport of substrate lie within both the N- and C-terminal regions of the PGT protein (7–9). To begin examining the structural determinants responsible for these interspecies functional differences, we used a conserved BstEII restriction enzyme site near the 5' end of both hPGT and mPGT to produce a mouse/human chimeric cDNA. Figure 5A is a model of the putative protein structure of this m/hPGT chimera in the plasma membrane as deduced from the cDNA sequence and hydropathy profiles of mPGT and hPGT cDNAs. Based on sequence and open-reading frames of these cDNAs, the protein produced by the m/hPGT chimeric cDNA would be comprised of a 248-amino acid N-terminal domain from mPGT and a 395-amino acid C-terminal domain from hPGT. Figure 5B illustrates a competition study examining the effect of increasing concentrations of unlabeled PGE2 on tracer PGE2 uptake by HeLa cells expressing mPGT, mouse/human chimera (here called m/hPGT), or hPGT. Although the earlier described differences between mPGT and hPGT are apparent, the inhibitory profile of the mouse/human chimera is indistinguishable from that of hPGT. The inhibitory constant for mPGT differed significantly from those of m/hPGT and hPGT (see Fig. 6 legend).

mPGT amino acids 610 and 611 (valine and isoleucine) differ from rPGT (methionine and valine) and hPGT (methionine and glycine) in terms of side-chain...
Fig. 3. Tracer PGE$_2$ uptake as a function of time by mPGT (Δ), antisense mPGT (rev mPGT, ○), rPGT (□), and hPGT (○) expressed in HeLa cells. Data points represent means ± SE of 4 experiments done in duplicate.

The present study describes the cloning, expression, and characterization of mPGT cDNA. Although the deduced amino acid sequence of mPGT exhibits significant structural similarity to hPGT and rPGT, the mouse transporter has affinities for several prostanoids that are substantially and reproducibly lower than those of the rat and human homologues. The structural basis for these functional differences can be localized to a region beyond amino acid 248 but cannot be attributed to species-specific structural variations at mouse residues valine 610 and isoleucine 611. In addition, the mRNA tissue distribution of the mouse transporter is significantly more restricted than that of the rat and human isoforms. We have, to date, no evidence that alternative isoforms of PGT in mouse tissues could account for the differences in distribution and function. The mouse gene is located on chromosome 9 in a region syntenic with human chromosome 3q21.

PGs are locally acting fatty acids that are synthesized on demand and released from the cell across the plasma membrane. They then bind to cell-surface receptors and activate a broad variety of cellular events (32). PGs also cross the plasma membrane on uptake before

<table>
<thead>
<tr>
<th>cDNAs</th>
<th>PGE$_2$</th>
<th>PGF$_{2\alpha}$</th>
<th>PGD$_2$</th>
<th>U46619</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPGT</td>
<td>110±9</td>
<td>104±10</td>
<td>98±17</td>
<td>90±28</td>
</tr>
<tr>
<td>rPGT</td>
<td>43±4*</td>
<td>45±5*</td>
<td>45±7*</td>
<td>60±16</td>
</tr>
<tr>
<td>hPGT</td>
<td>25±2†</td>
<td>23±2†</td>
<td>38±9*</td>
<td>39±7</td>
</tr>
</tbody>
</table>

**Table 1. Inhibition constants of various PGs for mouse, rat, and human PGT**

Uptake of tracer PGE$_2$ for 2 min was determined in presence and absence of unlabeled PGs as described in METHODS. Inhibition constants ($K_i$) were calculated based on exponential curve-fitting and represent means ± SE of 4–7 separate experiments performed in duplicate. *$P < 0.05$ vs. $K_i$ for mouse PG transporter (mPGT); †$P < 0.05$ vs. $K_i$ for rat PG transporter (rPGT). hPGT, human PGT.
intracellular oxidation. There is good evidence that a carrier(s) mediates this process (32). PGT is a broadly expressed, 12-membrane-spanning protein that catalyzes the rapid, specific, and high-affinity uptake of PGE2, PGF$\text{a}_2$, PGD$\text{a}_2$, 8-iso-PGF$\text{a}_2$, and thromboxane B$\text{a}_2$ (20, 25). Data from our laboratory from a monoclonal antibody to rPGT are consistent with a role of PGT in the release of newly synthesized PGs (i.e., immunocytochemical localization to cell types that release large amounts of PGs and thromboxanes) (3). In addition, the substrate specificity and inhibitor profile for rPGT match remarkably well with in situ studies on the metabolic clearance of PGs by the isolated perfused rat lung. Because rPGT expression is especially high in the rat lung, it is likely that PGT mediates the membrane step in PG clearance by the pulmonary circulation of the rat.

Given these hypotheses about the function of PGT, it is of interest that PGT mRNA expression in the mouse (primarily lung and liver) is much more limited than that in the rat or human (20, 25). This restricted expression of mPGT suggests that, in the mouse, PGT may play a predominant role only in PG metabolism. In the human and rat, the available evidence suggests that PG metabolic clearance is similar in the two species and occurs by selective uptake across the plasma membrane followed by nonselective intracellular oxidation (reviewed in Ref. 32). We are aware of no data on PG metabolic clearance in the mouse. Further studies will be required to address this issue experimentally.

There has been relatively little attention given to interspecies variation in the function of membrane transport proteins. Pajor and Sun (29) reported that the rabbit and human sodium-dependent dicarboxylate cotransporters differed about four- to eightfold in their affinities for citrate, and about twofold in their affinities for sodium, but not in their affinities for succinate and glutarate. No structural basis was experimentally shown for these interspecies differences. Similarly, Hirayama et al. (16) reported significant differences in both the kinetics and substrate specificities of the human, rabbit, and rat Na$^+$-glucose cotransporter SGLT-1. For example, the substrate concentration at half-maximal current for hexoses varied from 0.2 to >40 mM, depending on the species and the sugar, and the affinity constant for the inhibitor phlorizin varied over two orders of magnitude. Analysis of amino acid differences suggested that residues 548–644 might be responsible for the interspecies differences in affinities, but no experimental testing of this hypothesis was reported (16).

In the present study, we found that the inhibitory constants of PGT for the transported substrates PGE2, PGF$\text{a}_2$, and PGD$\text{a}_2$ were reproducibly 2.6- to 4-fold higher in the mouse compared with the human and rat (Table 1). Inspection of the mouse protein sequence compared with hPGT and rPGT sequences reveals three major regions of difference: 1) between amino acids 128 and 163, predicted to be on the extracellular face of the protein (32); 2) between amino acids 283 and 298, predicted to be intracellular; and 3) the residues valine 610 and isoleucine 611 (in the human these are methionine and glycine, respectively, and in the rat methionine and valine, respectively).

Reasoning that the structural determinants of the functional diversity between mPGT, rPGT, and hPGT might lie within one of these areas of variability, we generated chimeric cDNAs linking the N-terminal do-
mains of mPGT or hPGT with the corresponding fragment of the other. Of four possible chimeric proteins, only the mouse/human chimera at the BstEII site produced a protein that mediated PG uptake. However, it is clear from studies with this chimera that the residues determining binding affinity lie within the carboxy-terminal portion of the protein. Because this chimeric protein links mPGT and hPGT between proline 248 and glycine 249, the region of sequence variability between amino acids 128 and 163 is likely not important in determining the interspecies differences in binding affinity. It is unclear why the chimeric cDNAs at the EcoN1 site and the human/mouse chimera at the BstEII site did not produce functional transporters.

Val 610 and Iso 611 lie in putative transmembrane span 12, very near K613, a residue that is highly conserved as cationic (arg or lys) in this gene family (see alignment in Ref. 32). In the case of rPGT, we have shown that K613 is essential for function (7). Also, we have identified several cysteine residues in adjacent transmembrane span 10 whose susceptibility to thiol-reactive agents is substrate protectable (9). Taken together, these data suggest that spans 10 and 12 may be important in substrate binding by PGT. Therefore, we hypothesized that the interspecies differences in PGT substrate affinity seen here might be a result of the substitutions at positions 610 and 611. Unfortunately, mutagenesis of these two mouse amino acids to correspond to those of the human transporter failed to change the substrate affinity. Further mutagenesis around this site and the other variable site (amino acids 283–298) will be required to understand the structural basis of the interspecies differences in substrate affinities reported here.

In contrast to the native PGs PGE2, PGF2α, and PGD2, the bicycloendoperoxide U46619 failed to discriminate among the three transporters by affinity measurements (Table 1). We have previously shown that, although unlabeled U46619 strongly inhibits transport of tracer PGE2 by rPGT, tracer U46619 is not transported (18). One interpretation of these data is that PGT has several different substrate binding sites such that, although U46619 binds tightly to one or more sites, these sites do not result in substrate translocation across the membrane. Such a multiple binding site model is emerging in the case of substrate binding to prostanooid receptors (1, 2, 10, 11, 17, 27). If this is the case with PGT, then the data of Table 1 suggest that binding site(s) for U46619 will be found among protein sequences that are conserved across the three species. Further experiments will be required to test this hypothesis.

The mPGT gene maps to the distal region of mouse chromosome 9, which shares regions of homology with human chromosomes 3 and 6 (summarized in Fig. 6). In particular, Trf has been mapped to 3q21. The close linkage between Trf and Pgt in mouse is consistent with our recent mapping of the hPGT gene to human chromosome 3q21 (26).

We have compared our interspecific map of chromosome 9 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database of human linkage information maintained by William H. Welch Medical Library of Johns Hopkins University (Baltimore, MD)).

Discrete points for human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME; http://www.informatics.jax.org/). The mutations in the region of the Pgt gene include tk (tail-kinks), fd (fur deficient), E11 (epilepsy 1), tip (tippy), sr (spinner), and pcy (polycystic kidney disease, recessive). Based on our present knowledge of PG physiology and of PGT expression in the kidney (Y. Bao and V.L. Schuster, unpublished observations), it is unlikely that any of these result from a defect of PGT. However, neither can we exclude the possibility at this time.
In summary, we have cloned and functionally expressed the mouse homologue of PGT. The mouse transporter has a distinctly lower affinity for several prostanoid substrates and has a more narrow tissue mRNA expression pattern compared with the rat and human counterparts. The mouse cDNA will facilitate generating targeted mutations of the PGT gene.

We thank Deborah B. Householder for excellent technical assistance. A portion of the work was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-49688) and the American Heart Association (New York City Affiliate) and, in part, by the National Cancer Institute, Department of Health and Human Services, under contract with ABL.

Address for reprint requests and other correspondence: V. L. Schuster, Renal Division, Ullman 615, 1300 Morris Park Ave., Bronx, NY 10461 (E-mail: schuster@aecom.yu.edu).

Received 23 July 1998; accepted in final form 27 April 1999.