Proliferating and Migrating Mesangial Cells Responding to Injury Express a Novel Receptor Protein-tyrosine Phosphatase in Experimental Mesangial Proliferative Glomerulonephritis*

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Matthew B. Wright‡‡, Christian Hugo®, Ronald Seifert‡, Christine M. Disteche‡, and Daniel F. Bowen-Pope‡**

From the ‡Department of Pathology and the ¶Division of Nephrology, University of Washington, Seattle, Washington 98105-7470

The mesangial cell provides structural support to the kidney glomerulus. A polymerase chain reaction-based cDNA display approach identified a novel protein-tyrosine phosphatase, rPTP-GMC1, whose transcript expression is transiently and dramatically up-regulated during the period of mesangial cell migration and proliferation that follows mesangial cell injury in the anti-Thy 1 model of mesangial proliferative glomerulonephritis in the rat. In situ hybridization analysis confirmed that rPTP-GMC1 mRNA is up-regulated specifically by mesangial cells responding to the injury and is not detectable in other cells in the kidney or in many normal tissues. In cell culture, rPTP-GMC1 is expressed by mesangial cells but not by glomerular endothelial or epithelial cells (podocytes). The longest transcript (7.5 kilobases) encodes a receptor-like protein-tyrosine phosphatase consisting of a single catalytic domain, a transmembrane segment, and 18 fibronectin type III-like repeats in the extracellular segment. A splice variant predicts a truncated molecule missing the catalytic domain. rPTP-GMC1 maps to human chromosome 12q15 and to the distal end of mouse chromosome 10. The predicted structure of rPTP-GMC1 and its pattern of expression in vivo and in culture suggest that it plays a role in regulating the adhesion and migration of mesangial cells in response to injury.

Glomerular disease is initiated by a variety of factors, including immunologic, infectious, and toxic agents, as well as nondynamic processes. A central pathological feature of many types of acute and progressive glomerular disease is injury of mesangial cells, which respond by proliferating as well as by secreting growth factor and extracellular matrix proteins. This contributes to resolution of glomerular damage but may also lead to fibrosis, which occurs in many chronic disease processes. The glomerular mesangial cell is a mesenchymally derived cell that shares properties with fibroblasts and smooth muscle cells and provides structural support to the glomerular tuft.

Experimental mesangial proliferative glomerulonephritis can be induced in rats by the injection of anti-Thy 1 antibody, which binds to the Thy 1 antigen expressed on the rat mesangial cell (2). Binding of the Thy 1 antibody results in a complement (C5b-9)-dependent killing of mesangial cells (mesangiolysis), which is followed by a transient phase of mesangial cell proliferation (days 2–7) and matrix expansion (days 7–42) resembling mesangial proliferative glomerulonephritis in man (2, 3). Recently, repopulation of the mesangium in the anti-Thy 1 model has also been shown to involve migration of surviving mesangial cells (days 2–5) from the hilus to the periphery of the glomerulus (5).

It has been well established that basic fibroblast growth factor (6), platelet-derived growth factor (7, 8), and transforming growth factor β (9, 10) play major roles in the mesangial cell proliferative response and extracellular matrix expansion in experimental glomerulonephritis. Despite the evident role of protein tyrosine phosphorylation in processes activated by growth factor receptors with protein-tyrosine kinase activity, little information regarding the expression and biological roles of protein-tyrosine phosphatases (PTPase)1 in glomerular disease has been reported. The potential importance of PTPases in the glomerulus has been underscored by the recent identification of GLEPP1, a type III receptor-like PTPase (rPTPase), which is localized to the specialized foot processes of the podocyte (11, 12). GLEPP1 has been proposed to play a role in the regulation of podocyte foot process structure and function. In support of this hypothesis, GLEPP1 protein levels are reduced in several types of human glomerular disease and in several animal models of glomerulonephritis (13).

To identify PTPases as potential mediators of the mesangial cell response in glomerular disease, we have evaluated PTPases expressed in the rat anti-Thy 1 model. This report describes the identification, molecular cloning, and characterization of rPTP-GMC1, a new receptor PTPase expressed by mesangial mesangial cells. Our results demonstrate that rPTP-GMC1 is highly restricted to the mesangial cell and that expression is acutely up-regulated in actively proliferating and migrating mesangial cells in the anti-Thy 1 model. rPTP-GMC1 is similar in structure to GLEPP1 and may sense or regulate cell-cell or cell-matrix interactions to mediate glomerular repair.

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‡ Current address: Dept. of Cardiovascular Research, F. Hoffmann-La Roche, 4070 Basel, Switzerland.
¶ Current address: Universität Erlangen-Nürnberg, Erlangen 91054, Germany.
** To whom correspondence should be addressed: Dept. of Pathology, University of Washington, Box 357470, Seattle, WA 98195-7470. Tel.: 206-685-2148; Fax: 206-543-3644; E-mail: bp@u.washington.edu.
**EXPERIMENTAL PROCEDURES**

*In Vivo Disease Model*—Intravenous injection of goat anti-rat thymocyte plasma (0.4 ml/100 g body weight) was given to 180–220-g male Wistar rats (Simonsen Laboratories, Gilroy, CA) to initiate experimental mesangial proliferative nephritis (anti-Thy 1 disease). Rats were sacrificed at days 2, 3, 5, 7, and 14. Renal tissue was fixed in 4% paraformaldehyde or methyl Carnoy’s solution and embedded in paraffin for in situ hybridization analysis and immunohistochemistry. For RNA isolation, renal tissue from 4–6 animals per time point was pooled and glomeruli were enriched by differential sieving through screens of decreasing mesh size. Glomerular preparations with less than 10% contamination with tubules, as assessed by light microscopy, were used for RNA preparation.

**Glomerular Cell Culture**—Rat mesangial cell and glomerular epithelial cell lines used in these experiments were maintained as described previously (14, 15). Glomerular endothelial cells were provided by Dr. Stephen Adler (New York Medical College, Westchester Medical Center, Valhalla, NY) and have been characterized previously(16).

**RNA Analysis**—Total RNA from glomeruli or cultured cells was isolated with Trizol (Life Technologies, Inc.) and poly(A)+ mRNA was purified on oligo(dT) cellulose columns (Stratagene). For Northern blot analysis, RNA was electrophoresed in MOPS/formaldehyde gels and transferred to nylon membranes that were hybridized with radiolabeled probes using Quikhyb (Stratagene) according to the suggested protocol.

**PCR Restriction Enzyme cDNA Fingerprint Display of PTPase Expression**—The essential methodology for PCR restriction enzyme cDNA fingerprint display of PTPase expression has been described elsewhere (17, 18). Three pairs of degenerate primers to conserved regions of PTPase catalytic domains were designed for the Thy 1 series to survey more PTPases than would be expected to be amplified with a single primer pair. The three sense primers were: F1, CTCTGGATCCACIGAA(G/A)TG(T/C)G(T/A)C(A/G)TA(T/C)TGGC, which corresponds to the amino acid sequence (S/T)DYINA; F2, CTCTGGATCCAA(G/A)TG(T/C)GT(G/A/T/C/T)TA(C/T)AT(ACT)AA(CT)GC, which corresponds to the amino acid sequence (S/T)DYINA; F3, CTCTGGATCCACIGAA(G/A)TG(T/C)G(T/A)C(A/G)TA(T/C)TGGC, corresponding to KCVKYWP. A single antisense primer to the sequence HCSAG-GGATCCAA(A/G)TG(T/C)GC(G/A/T)CA(A/G)TA(T/C)TGGC, corresponding to KCVKYWP; and F3, CTCTGGATCCACIGAA(G/A)TG(T/C)G(T/A)C(A/G)TA(T/C)TGGC, corresponding to KCVKYWP.

For each experimental sample, cDNA was prepared from 1 μg of total glomerular RNA. The samples compared by the fingerprint display method were: total glomerular RNA from normal controls and 2, 5, 7, and 14 days after anti-Thy 1 injection. Primer R1 was radiolabeled with 32P-labeled PTPase PCR products obtained in the first PCR (19), equalized recovered cRNA synthesis efficiency, we gel-purified the PTPase products obtained in the first PCR (19), equalized recovered material between samples, and reamplified equal amounts for 25 cycles of 30 s, 94 °C; 30 s, 50 °C; 45 s, 72 °C in a 40-μl reaction with 1 μl of each of the reamplified PTPase fragments were digested to completion with restriction enzymes (HindIII, Rsal, TaqI, Sau3A1), and fragment patterns were resolved by gel electrophoresis and autoradiography.

5'- and 3'-RACE Cloning of rPTP-GMC1—Rat poly(A)+ glomerular RNA from day 5 of the anti-Thy 1 model was used as the template for combined 5' and 3' RACE cloning of rPTP-GMC1. 5'-RACE amplification was performed using the Clontech Marathon cDNA amplification kit as described in the product literature except that 25 cycles each were performed for the nested and primary PCRs. The 5'-RACE clones we obtained were not full-length, so further rounds of 5' RACE were performed using the most recently obtained rPTP-GMC1 sequence to derive new specific primers for PCR amplification. In all cases, primers were designed to maintain at least 100 bp of overlap between subsequent sets of 5'-RACE clones to verify that new clones were indeed derived from rPTP-GMC1.

PCR products were cloned using a TA vector (Invitrogen) and sequenced by the fluorescence sequencing facility in the Department of Biochemistry at the University of Washington. Sequence analysis was done with the Geneworks program (IntelliGenetics), and homology searches were performed using the BLAST program at the NCBI.

**In Situ Hybridization Analysis**—Antisense riboprobes were generated by *in vitro* transcription of linearized plasmid DNA with SP6 RNA polymerase in a 10-μl reaction at a concentration of 20 μM [32P]UTP (NEN Life Science Products, 2000Ci/ml). The probes were purified with G-50 NICK columns (Pharmacia) and then hydrolyzed to an average length of 150 bases.

Formaldehyde-fixed, paraffin-embedded tissue sections (6 μm sections) were deparaffinized and treated with 20 μg/ml proteinase K at 37 °C for 7.5 min. Hybridization with 5 × 105 cpm of probe/ml hybridization solution was done overnight at 65 °C. Slides were treated with RNase A after washing to remove unbound probe, dehydrated with EtOH, air-dried, and dipped in Kodak NTB2 emulsion. Slides were developed after 3 weeks in Kodak D19 and counterstained with hematoxylin and eosin.

**Combined Immunohistochemistry and In Situ Hybridization**—To identify the cells expressing rPTP-GMC1 mRNA, tissue was stained for a marker for activated mesangial cells (α-smooth muscle actin) and combined with *in situ* hybridization to detect rPTP-GMC1 transcripts. Immunoperoxidase staining for α-smooth muscle actin was performed

**FIG. 1. PCR restriction enzyme cDNA fingerprint display of PTPase expression in experimental glomerulonephritis.** Autoradiograms of denaturing polyacrylamide gels showing HindIII restriction fragment patterns of 32P-labeled PTPase PCR products during Thy 1 disease. The PTPase degenerate primers in each experiment are indicated above the panels. Abbreviations for the experimental samples are: N, normal glomeruli; 2, 5, 7, 14, days of Thy 1 disease. The PCR yields uniformly sized product representing many PTPases. Complete digestion of the 32P-labeled PCR product with a restriction enzyme yields a single radioactive fragment for each PTPase within the mixture due to incorporation of labeled downstream PCR primer. Most PTPases do not contain recognition sites for any single enzyme and remain undigested (*), while several produce unique fragments. Arrows indicate an approximately 85-bp HindIII fragment in all three of the restriction fragment displays. This band represents a PTPase (subsequently identified as rPTP-GMC1) that is expressed transiently in a temporal pattern similar to the mesangial proliferative phase of Thy 1 disease.

![Image](https://example.com/image.png)
with a murine IgG, monoclonal antibody (21) (Sigma) essentially as described previously (22, 23). The primary antibody was incubated overnight at 4 °C, followed sequentially by biotinylated rabbit anti-mouse IgG (Zymed Laboratories Inc.), avidin D peroxidase, and finally mouse IgG (Zymed Laboratories Inc.), avidin D peroxidase, and finally 

**Mapping by Interspecific Mouse Backcross Analysis—**The rPTP-GMC1 gene was mapped using the Mouse Backcross DNA Panel Mapping Resource of the Jackson Laboratory (Bar Harbor, ME) (24). To genotype the mice, we looked for useful rPTP-GMC1 polymorphisms between the parental strains (Mus spretus and C57BL/6J). PCR primers based on rat rPTP-GMC1 cDNA were used to amplify M. spretus and C57BL/6J genomic DNA across a putative rPTP-GMC1 splice junction within the signature motif containing the essential catalytic cysteine of PTPases. We found an approximately 570-bp intron at this position, which exhibited multiple sequence polymorphisms between M. spretus and C57BL/6J (Fig. 8A). A HaeIII site was detected in the C57BL/6J intron that was not present in the M. spretus intron. All of the backcross panel mice were genotyped by HaeIII restriction digestion of rPTP-GMC1 PCR products, which yielded a 0.64-kb product from M. spretus DNA and a 0.31-kb doublet from C57BL/6J DNA (Fig. 8B). A total of 188 mice were used to map the rPTP-GMC1 locus (BSB and BSS backcross panels, 94 mice in each panel). The PCR primers used for genotyping are indicated in Fig. 8A. Cycling protocol was: denaturation for 3 min at 94 °C and then 40 cycles of 30 s, 94 °C; 30 s, 60 °C; 3 min, 72 °C with a final elongation step of 5 min, 72 °C.

Recombination distances with respect to previously mapped loci were calculated at the Jackson Laboratory using the program Map Manager (25).

**Mapping by Fluorescence in Situ Hybridization (FISH) to Human Chromosome 12—**A 2.8-kb fragment of human rPTP-GMC1 was obtained by RT-PCR of poly(A+) RNA from human kidney (CLONTECH) and subcloned into the PCR2.1 vector (Invitrogen). The whole plasmid was labeled with biotin-11-dATP by nick translation (Life Technologies, Inc.). The probe was hybridized to metaphase chromosomes from lymphocytes of a human male that were prepared by hypotonic lysis in 0.075 M KCl followed by fixation in methanol:acetic acid (3:1, v/v). The hybridization was performed essentially as described by Edelhoff et al. (26), and hybridization signals were detected using immunological reagents from Vector Laboratories (Burlingham, CA). The chromosomes were banded by Hoechst 33258-actinomycin D staining and counterstained with propidium iodide. The chromosomes and hybridization signals were visualized by fluorescence microscopy using a dual band pass filter (Omega, Brattleboro, VT).

# RESULTS

**PCR Restriction Enzyme cDNA Display Reveals a Novel PT-Pase Expressed in Experimental Glomerulonephritis—**We used the restriction enzyme cDNA fingerprint display method to identify differentially expressed PTPases in the rat anti-Thy 1 model of mesangial proliferative glomerulonephritis. PCR amplification of PTPases with degenerate primers yielded uniformly sized products representing the conserved catalytic domains of many expressed PTPases (data not shown). We assessed expression of individual PTPases by digesting the radiolabeled PCR products with restriction enzymes to produce fragment patterns (cDNA fingerprint). Each fragment in the cDNA fingerprint represents a specific expressed PTPase, and the intensity of that fragment reflects the representation of the amplified sequence within the total PCR product (Fig. 1). As equivalent amounts (cpm) of the total PCR product are analyzed for each experimental sample, variation in fragment intensity between samples suggests differential expression of the corresponding PTPase. This method does not provide information about absolute transcript abundance. However, the cDNA fingerprints obtained in this manner provide a good estimate of the relative expression of specific transcripts over an experimental time course.

We found several restriction fragments that varied in intensity during the response to anti-Thy 1 injury, suggesting differential expression of PTPases (Fig. 1). The most striking was an 85-bp HinfI fragment, which was induced in a manner paralleling the mesangial cell proliferative and migratory phase (days 2–7) of the Thy 1 model (5). We observed similar temporal expression profiles for this PTPase in three separate cDNA fingerprinting experiments, each performed with different PCR primers in independent reactions (Fig. 1). We also observed similar levels of expression in duplicate RNA preparations from normal and day 5 anti-Thy 1 glomeruli.

To identify the differentially expressed PTPase, we subcloned the PCR product obtained with the F1/R1 primers from day 5 anti-Thy 1 glomerular RNA (Fig. 1). PCR analysis of the cloned plasmid DNA of 80 independent subclones was performed with the F1/R1 primers, followed by HinfI digestion to identify clones that produced the 85-bp HinfI fragment. The resulting HinfI fragment patterns suggested that at least eight different PTPase sequences were represented by the 80 clones and revealed that seven clones represented the regulated PTPase (data not shown). We selected a single representative
clone from each of the eight groups for DNA sequence analysis. The seven clones, which produced HinF1 digestion fragments other than the 85-bp fragment, represented the known PT-Pases: PTPα, PTPβ, PTP1B, GLEPP1, SHP1, SHP2, and PTP-PEST. A remaining clone, which represented the differentially expressed PTPase in the anti-Thy 1 model, was a novel PTPase sequence. We have designated this PTPase rPTP-GMC1 for receptor-like protein-tyrosine phosphatase expressed by glo-

**FIG. 3.** In situ hybridization analysis of rPTP-GMC1 transcript expression in experimental glomerulonephritis. The rPTP-GMC1 antisense riboprobe was produced from the 1448-bp rPTP-GMC1 fragment in clone 23-4 (Fig. 6). The left panels are darkfield micrographs of renal cortex containing several glomeruli. Right panels are higher magnification micrographs of individual glomeruli, which are oriented with the juxtaglomerular apparatus in the lower left corner in order to demonstrate positional hybridization patterns. Results are representative of at least six experimental ani-

**Multiple rPTP-GMC1 Transcripts Are Expressed in Experimental Glomerulonephritis**—The PCR fingerprint displays suggested that highest expression of rPTP-GMC1 occurred at day 5 after initiation of experimental glomerulonephritis. We isolated poly(A⁺) mRNA from rat glomeruli at day 5 of Thy 1 disease and performed Northern blot analysis with two independent cDNA probes (Fig. 2). Both probes revealed four hybridizing transcripts of approximately 1.8, 3.3, 5.0, and 7.5 kb in the injured glomeruli (Fig. 2A). No rPTP-GMC1 transcripts were detected in a Northern blot containing poly(A⁺) RNA from normal kidney or from heart, liver, spleen, brain, thymus, lung, skeletal muscle, intestine, or testis (Fig. 2B).

**In Situ Hybridization Demonstrates That rPTP-GMC1 mRNA Is Transiently Expressed by Mesangial Cells in Experimental Glomerulonephritis**—In situ hybridization of tissue sections was performed to determine which glomerular cells were expressing rPTP-GMC1 transcripts during the course of anti-Thy 1 disease. Fig. 3 shows that rPTP-GMC1 mRNA dramatically increased during the time course of mesangial cell activation. Cells expressing rPTP-GMC1 appeared at day 3 in the glomerulus near the juxtaglomerular (JG) apparatus (Fig. 3). This positional hybridization pattern is consistent with the location of proliferating and migrating mesangial cells at this time in anti-Thy 1 disease (5). By day 5, hybridizing cells were evident throughout the glomerulus. Expression abruptly declined by day 7. Although rPTP-GMC1 expression appeared slightly less per cell at day 5 compared with day 3, total expres-
activated mesangial cells identified four major rPTP-GMC1 transcripts expressed by activated mesangial cells co-distributes with rPTP-GMC1 expression in a typical mesangial pattern throughout the glomerulus.

To demonstrate that activated mesangial cells are the major source of rPTP-GMC1 mRNA expression, immunohistochemistry for α-smooth muscle actin, was combined with rPTP-GMC1 mRNA in situ hybridization. Fig. 4 shows a glomerulus at day 5 of Thy 1 disease. Significant immunoreactivity for α-smooth muscle actin was observed in a characteristic mesangial pattern. The silver grains, indicating the presence of rPTP-GMC1 transcripts, were localized to the activated mesangial regions, indicating that activated mesangial cells are the primary source of rPTP-GMC1 expression in the glomerulus.

The inability to detect rPTP-GMC1 transcripts in other normal tissues by a poly(A⁺) RNA tissue Northern blot does not preclude the possibility of expression by a restricted subset of cells within a specific tissue. However, in situ hybridization analysis of multiple sections of rat brain, liver, spleen, skeletal muscle, lung, heart, prostate, aorta, esophagus, stomach, and small intestine, in two separate experiments, did not detect rPTP-GMC1 expression by any cell type other than proliferating mesangial cells (data not shown).

rPTP-GMC1 Expression in Cultured Glomerular Cells—We isolated total RNA from cultured rat glomerular epithelial cells, endothelial cells, and mesangial cells (immortalized and low passage) and performed RT-PCR analysis for rPTP-GMC1 expression. The expected 716-bp product was obtained from cultured mesangial cells but not from cultured endothelial or glomerular epithelial cells (Fig. 5). This result further supports the mesangial cell specificity of rPTP-GMC1 expression and suggests that cultured mesangial cells more closely resemble the activated rather than quiescent state observed in vivo. By contrast, PTP-1B, a ubiquitously expressed PTpase that we evaluated as a positive control, was expressed by all of the cultured mesangial cell types (Fig. 5).

rPTP-GMC1 Cloning and Structure—Northern blot analysis identified four major rPTP-GMC1 transcripts expressed by activated mesangial cells in vivo (Fig. 2). We cloned full-length rPTP-GMC1 encoded by the longest transcript (estimated by Northern analysis to be approximately 7.5 kb) by 5' and 3'-RACE PCR of rat glomerular mRNA from day 5 of the anti-Thy 1 model (Fig. 6). Our clones predict that rPTP-GMC1 is a large receptor PTpase of 2302 amino acids that consists of an N-terminal hydrophobic signal peptide, followed by 18 fibronectin type III-like adhesion domains, a transmembrane segment, and a single cytosolic PTpase domain (Fig. 7). The ectodomain has 39 potential N-linked glycosylation sites, and the intracellular region has 9 potential sites for protein kinase C phosphorylation and 6 putative sites for phosphorylation by casein kinase II. Thus, it is a new member of the type III class of PTpases, which contains rPTPβ, DPTP10D, GLEPP1, Dep1, and Sap1 (11, 27–31). As expected, a search for similar sequences in public data bases revealed significant relatedness to other PTpases. In addition, the search also revealed a high level of homology with several partial human cDNA clones. The longest of these sequences (GenBank accession no. AF063249) predicts a polypeptide that is about 94% identical with the 800 C-terminal amino acids of rPTP-GMC1. However, no full-length clones have yet been reported for rPTP-GMC1 in any species.

Fig. 6B is an alignment of the fibronectin type III-like segments of rPTP-GMC1 with a representative domain of fibronectin. Each repeat has a characteristic hydrophobic core flanked by highly conserved tryptophan and tyrosine residues (32). The FN-III-like repeats in rPTP-GMC1 are 90–110 amino acids in length, as is characteristic of this structural domain, with the exception of repeats 2 and 18. The second predicted FN-III-like domain has about 50 additional amino acid residues inserted within the hydrophobic core. The 18th and final FN-III domain has an additional 60 amino acid residues just prior to the hydrophobic core. This is also the case for the most membrane proximal FN-III domain of Drosophila DPTP10D, which is also unusually long (29).

Fibronectin type III domains are characteristic of cell adhesion molecule ectodomains and are found by themselves or in tandem with immunoglobulin (Ig)-like domains (33). FN-III
domains are found in many diverse proteins but only in several cases have specific functions been assigned (34). The most well studied is domain 10 of fibronectin, which contains the sequence RGD, which binds integrins (32). Thus, FN-III domains are most likely involved in cell adhesion, but so far no ligands have been identified for this element in receptor PTPases so their specific functions remain obscure.

Our clones also predict a truncated rPTP-GMC1 protein. Two clones predicted a protein with a consensus PTPase catalytic domain (Fig. 6). Six additional clones contain a 109-nucleotide deletion just prior to the PTPase domain, which predicted a frameshift resulting in a truncated protein with an alternative C terminus (Fig. 7). Interestingly, the position at which the two predicted isoforms diverge is immediately prior to a helix-loop-helix motif that is conserved in transmembrane but not cytosolic PTPases. This helix-loop-helix motif has been postulated to play a role in regulating PTPase enzymatic activity in a model of ligand-induced dimerization of rPTPases (35).

The shorter rPTP-GMC1 transcripts are more abundantly expressed in the Thy 1 model than the 7.5-kb transcript. However, we did not identify additional alternatively spliced forms among our RACE clones which would account for these smaller transcripts.

Chromosomal Location of rPTP-GMC1 in Mouse and Human—rPTP-GMC1 was genetically mapped in mice by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × M. spretus)F1 × C57BL/6J] (BSB panel) mice and the reciprocal backcross [(C57BL/6JEi × SPRET/Ei)F1 × SPRET/Ei] (BSS panel) mice (24). The BSS panel has been typed for over 3,000 loci that are distributed over all of the autosomes and the X chromosome, and the BSB panel has been typed for over 750 loci. C57BL/6J × M. spretus genomic DNA was amplified by PCR with rPTP-GMC1-specific oligonucleotide primers which were designed to span a potential splice junction that is conserved in other PTPases (36). As shown in Fig. 8A, a 571-bp intron is present in M. spretus (GenBank AF073999) but not in C57BL/6J (GenBank AF073998). The HaeIII polymorphism was used to follow the

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**Fig. 6. Cloning and deduced structure of rPTP-GMC1.** A, the upper part of the diagram depicts the rPTP-GMC1 cDNA clones used to derive the 7396-bp sequence (GenBank accession number AF063249) corresponding to the longest transcript found in the Thy 1 model. Clone PCR1 is from the original restriction enzyme display obtained by degenerate PCR. The remaining clones were obtained by 3'-RACE PCR (clone numbers beginning with 3'), 5'-RACE PCR (clone numbers beginning with 5'), or by PCR with rPTP-GMC1-specific primers (23-4, 23-5) from day 5 anti-Thy 1 glomerular mRNA. The lower part of the upper panel depicts the deduced protein structures of the two rPTP-GMC1 isoforms encoded by our cDNA clones. The ectodomain consists of a hydrophilic signal peptide followed by 18 tandem fibronectin type-III-like repeats, a 23-amino acid hydrophobic segment (putative transmembrane domain, TM), a juxtamembrane segment (JM) of 51 amino acids, and a consensus PTPase catalytic domain. A truncated isoform is predicted by a 109-nucleotide deletion in the cDNA, which predicts a frameshift and premature truncation just prior to the catalytic domain. B, amino acid alignment of the 18 FN-III-like repeats of rPTP-GMC1 with the seventh type-III domain of fibronectin. The beginning of each repeat is numbered to the left in parentheses. The consensus line shows only those residues present in at least 10 of the 19 aligned sequences.
The segregation of the rPTP-GMC1 alleles in the backcross mice (Fig. 8B). All 94 mice in each panel were analyzed for the indicated markers shown in Fig. 8C. The few missing typings for some loci were inferred unambiguously to be nonrecombinant based on data for surrounding loci. The ratios of the total number of mice exhibiting recombinant chromosomes to the total numbers of mice analyzed for each pair of loci and the most likely gene order and genetic distances are shown in Fig. 8C. The mapping results placed rPTP-GMC1 in the distal region of chromosome 10 linked to a large number of mapped loci, a representative selection of which is depicted in Fig. 8D. The approved mouse locus designation for rPTP-GMC1 is Ptprq.

rPTP-GMC1 was mapped in human metaphase chromosomes by FISH using a 2.8-kb cDNA probe. Metaphase chromosomes of 106 cells were examined; of these, 80 (75.5%) exhibited hybridization signals on both chromatids of chromosome 12 at band q15 (Fig. 9). The distal half of mouse chromosome 10, to which rPTP-GMC1 maps, is homologous with human chromosome 12.

**FIG. 7. rPTP-GMC1 nucleotide and amino acid sequence.** The 7396-bp nucleotide sequence was derived from the clones described in Fig. 6. The hydrophilic signal peptide is single underlined. The putative transmembrane domain is boxed. Two isoforms differ by a 109-bp segment (shown in bold type) in the intracellular region and the alternative C terminus, for the isoform in which this segment is deleted, is italicized and shown below the amino acid sequence of the intact isoform. The predicted PTPase domain is double underlined. The positions of the 18 FN III-like repeats in the ectodomain are described in Fig. 6.

The approved mouse locus designation for rPTP-GMC1 is Ptprq.
man chromosome 12, q13-q24 (37). The approved human locus designation for rPTP-GMC1 is PTPRQ.

**DISCUSSION**

Many types of human renal disease, such as diabetic nephropathy, acute glomerulonephritis, IgA nephropathy, and acute lupus nephritis, share similarities with the anti-Thy 1 glomerulonephritis model. Loss of mesangial cells results in dissolution of the glomerular architecture, which stimulates a response to injury mediated by repopulating mesangial cells (3, 38). In the rat anti-Thy 1 nephritis model, the period of mesangiolysis is followed by intense mesangial cell proliferation, which
is mediated by polypeptide growth factors including platelet-derived growth factor and basic fibroblast growth factor (8, 39, 40). Resonding mesangial cells appear at the glomerular hilus and migrate to the glomerular periphery (5). During this period, they a contractile phenotype with de novo expression of α-smooth muscle actin and increased expression of surface adhesion and extracellular matrix proteins (2, 23, 41).

We have cloned and characterized a new type III receptor protein-tyrosine phosphatase in experimental glomerulonephritis. rPTP-GMC1 exhibits a striking pattern of expression which is tightly coupled to the kinetics of the migrating mesangial cell in the rat anti-Thy 1 model. Following the acute mesangiolysis, the first identifiable mesangial cells consistently originate from the glomerular hilus or extraglomerular mesangium (5). These actively proliferating cells dramatically up-regulate expression of rPTP-GMC1. From 36 h to 7 days after disease induction, the responding mesangial cells participate in a coordinated wave of proliferation and migration which distributes them throughout the glomerulus (5). By day 7, the proliferative/migratory phase is essentially complete and this is coincident with loss of rPTP-GMC1 expression.

A role for rPTP-GMC1 is supported by genetic studies, which have provided good evidence for receptor PTPases as important regulators of cell migration and axon guidance. For example, four Drosophila PTPases (DLAR, DPTP10D, DPTP96D, and DPTP99A) are expressed specifically in the nervous system in overlapping subsets of neurons and play important roles in growth cone guidance and target innervation (42). Drosophila DPTP96D null mutants express motor neuron growth cones that do not target properly and either overshoot their muscle targets or stop prematurely (43). Mutants in DLAR result in motor axons that fail to reach their targets due to improper directional decisions at key checkpoints (44).

Migrating cells interact with the extracellular matrix via focal adhesions (FA), which are contact points that link actin stress fibers to the membrane cytoskeleton and to transmembrane integrins (45). It is well established that protein tyrosine phosphorylation plays a key role in regulating these structures. For example, LAR, a broadly expressed receptor PTPase, localizes to FAs in migrating cells but seems to be excluded from developing FAs at extending lamellipodia (47). This is consistent with a role of this receptor PTPase in FA disassembly by serving to dephosphorylate components that were activated initially by phosphorylation.

Mesangial cells in culture migrate toward a variety of factors including platelet-derived growth factor, fibronectin, and thrombospondin, which may also be important stimulators of mesangial cell migration in the Thy 1 model (4, 48, 49). Our results suggest that rPTP-GMC1 may play a role in cell migration by either regulating the formation of cell-cell or cell-matrix adhesion sites or providing a pathfinding or context-recognition function that targets the mesangial cell to its correct association with other glomerular cells. The loss of rPTP-GMC1 expression, concurrent with the end of the proliferative/migratory phase in the Thy 1 model, suggests that it plays a dynamic, rather than constitutive, role in formation of glomerular structure.

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