Identification of a receptor-type protein tyrosine phosphatase expressed in postmitotic maturing neurons: its structure and expression in the central nervous system

Masatoshi TAGAWA*, Takuji SHIRASAWA†§, Yu-ichi YAHAGI†, Toshifumi TOMODA†, Hidehito KUROYANAGI†, Shinji FUJIMURA*, Shigeru SAKIYAMA‡ and Naoki MARUYAMA†

*Department of Biochemistry, School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba-260, Japan, †Department of Molecular Pathology, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo-173, Japan, and ‡Division of Biochemistry, Chiba Cancer Reseach Institute, 666-2, Nitona-cho, Chuo-ku, Chiba-260, Japan

We have isolated a rat cDNA encoding a receptor-type protein-tyrosine-phosphatase (RTP) expressed in brain and kidney (RPTP-BK) and characterized its expression in the developing central nervous system. RPTP-BK has seven fibronectin type III-like repeats in the extracellular region and a unique catalytic phosphatase domain in the cytoplasmic region. Bacterial expression of its phosphatase domain showed that the dephosphorylation of phosphotyrosine residues was mediated by the cytoplasmic catalytic domain. Sequence comparison revealed that RPTP-BK is homologous with GLEPP1, a rabbit PTP expressed in renal glomerular epithelia, and has the same phosphatase domain as murine PTPϕ expressed in macrophages. RPTP-BK has also significant homology with *Drosophila* DPTP10D in the phosphatase domain, whose expression is localized exclusively in growth cones of the embryonal brains. The gene for RPTP-BK is well conserved among other species, and the expression in the brain but not in the kidney is developmentally regulated during the neonatal stage. Hybridization *in situ* showed that RPTP-BK is highly expressed in the postmitotic maturing neurons of the olfactory bulb, developing neocortex, hippocampus and thalamus. Because the expression of RPTP-BK in the developing neocortex is correlated with the stage of axonogenesis in cortical neurons, RPTP-BK might be crucial in neural cell development of the mammalian central nervous system.

INTRODUCTION

Reversible phosphorylation of tyrosine residues in proteins is responsible for various vital processes such as signal transduction, cell growth and homeostasis in many organisms [1]. Much effort has been focused on the understanding of these events at molecular levels. Protein phosphorylation is biochemically mediated by the two opposing dynamic activities of protein kinases and phosphatases. Protein phosphatases are a diversified group of enzymes that are categorized into two major families according to their substrate specificities: serine/threonine phosphatases and protein tyrosine phosphatases (PTPs, protein tyrosine phosphate phosphohydrolase, EC 3.1.3.48); there is also another group designated as dual-specificity phosphatases that are structually related to PTPs but can dephosphorylate all the three phosphorylated residues.

Among protein phosphatases, PTP is the most numerous and ramified family, harbouring about 50 molecules currently identified. These PTPs are further divided into two main subgroups: the low-molecular-mass, cytoplasmic, PTPs containing a single catalytic domain, and the high-molecular-mass, membrane-spanning, receptor-type PTPs (RPTPs), many of which contain two tandem repeats of intracellular catalytic domains [2]. The catalytic domain consists of about 250 amino acid residues with several consensus sequences, allowing the reverse genetic approach to search for PTPs expressed in any tissues with reverse transcriptase–PCR (RT–PCR). The receptor-type PTP is characterized by its large extracellular motifs including immunoglobulin-like repeats and fibronectin type III (FN-III)-like repeats, which suggest ligand-binding activity. However, sequence similarities of the extracellular domain to neural cell adhesion molecules such as N-CAM, L1 and fasciclin II led us to speculate that homophilic or heterophilic cell-to-cell interactions rather than soluble ligands trigger the phosphatase activity as shown in PTPμ and PTPκ [3,4].

In the nervous system, the regulation of protein functions through tyrosine phophorylation catalysed by receptor tyrosine kinases is known to be crucial to the developmental process [5]. The function of these receptor tyrosine kinases has been extensively studied in *Drosophila* with several mutants [6]. However, the biological significance of PTP in the developing brain is not well understood. In a previous study we searched for PTPs expressed in rat embryonic brain by the RT–PCR method and detected the expression of several PTPs, including a novel PTP [7]. In the present study, we molecularly cloned this novel PTP from a rat embryonic brain cDNA library and characterized its structure and expression in the rat nervous system. This cDNA, designated as RPTP-BK because of its expression in the brain and kidney, encodes an RPTP and is homologous with rabbit GLEPP1 expressed in glomerular epithelia of kidney [8], and with murine PTPϕ expressed in haematopoietic cells [9]. RNA blot analysis and hybridization *in situ* revealed that the

Abbreviations used: FN-III, fibronectin type III; LAR, leucocyte-antigen-related; MBP, maltose-binding protein; PTP, protein tyrosine phosphatase; RPTP, receptor-type protein tyrosine phosphatase; RT–PCR, reverse transcriptase–PCR.

§ To whom correspondence should be addressed.

The nucleotide sequence reported will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number U28938.

expression in the brain is developmentally regulated in the postmitotic maturing neuronal cells.

MATERIALS AND METHODS

Isolation of rat RPTP-BK cDNA

A rat embryonic brain cDNA library [10] was screened by the PCR fragment of PTPD28 [7]. One of the positive clones harbouring the largest insert was subcloned, and the reaction for sequencing was done with PCR by using SequiTherm polymerase and fluorescein isothiocyanate-labelled primers in accordance with the manufacture's instructions (Epicentre Technologies, Madison, WI, U.S.A.). The nucleotide sequence in both strands was determined with a DSQ-1 autosequencer (Shimazu, Kyoto, Japan). Multiple alignments for PTP genes were adjusted by the Clustal method in Lasergene software packages (DNASTAR, London, U.K.).

Southern blot analysis

Genomic DNA (10 μg) from Wistar rat liver was digested by several restriction enzymes. Digested DNA species were subjected to agarose-gel electrophoresis and were transferred to a nylon membrane. The 3.9 kb *Xho*I/*Xba*I fragment from RPTP-BK labelled with [^{32}P]dCTP was used as a probe. The hybridization was performed in a solution containing 6 × SSPE [where SSPE is 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA], 2 × Denhardt's solution, 0.5 % SDS and, 100 μg/ml salmon sperm DNA at 65 °C for 12 h. The filter was washed in a solution of 0.2 × SSPE/0.1 % SDS at 65 °C. The washed filter was exposed to Fuji X-ray film RX.

For zooblot analysis, the filter containing *Eco*RI-digested genomic DNAs from various species was purchased from BIOS Laboratory (New Haven, CT, U.S.A.). The 2.5 kb *Eco*RI/*Eco*RV fragment from RPTP-BK was used as a probe. Hybridization was performed as described above in the presence of 10 % (w/v) dextran sulphate. The filter was washed in a solution of 1 × SSPE,/0.1 % SDS at 65 °C and exposed to X-ray film.

RNA preparation and RNA blot analysis

Total RNA was extracted from rat tissues and cultured cells by the acid guanidinium thiocyanate/phenol/chloroform method [11]. The isolation of renal glomeruli was performed as described [12]. Messenger RNA species were prepared from rat embryonal, neonatal and adult brains with an RNA preparation kit (Pharmacia, Uppsala, Sweden). Isolated RNA (10 μg) or mRNA species (1 μg) were subjected to electrophoresis in a 1.2 % agarose/10 % formaldehyde gel, and blotted on a nylon membrane. The 3.9 kb *Xho*I/*Xba*I fragment labelled with [^{32}P]dCTP was used as a probe. Hybridization was performed in a buffer containing 50 % (v/v) formamide, 5 × SSPE, 0.1 % SDS, 5 × Denhardt's solution and 100 μg/ml salmon sperm DNA at 42 °C for 16 h. The blots were washed at 50 °C in a solution of 0.2 × SSC/0.1 % SDS (where SSC is 0.15 M NaCl/0.015 M sodium citrate) and exposed to X-ray film.

Preparation of maltose-binding protein (MBP)–PTP fusion protein

A fragment (nt 2830–3720) corresponding to the catalytic domain of RPTP-BK was subcloned into pMAL-c2 vector (New England Biolabs, Beverly, MA, U.S.A.). MBP–PTP and MBP–β-galactosidase fusion protein (as a control) were prepared in accordance with the manufacturer's protocol (New England Biolabs). Briefly, JM104 cells harbouring the plasmids were inoculated into Luria–Bertani medium at 1:10 dilution and grown at 37 °C for 2 h, and then 0.3 mM isopropyl β-δ-thiogalactoside was added. After the cells had been further propagated for 2 h they were suspended in a solution of 33 mM Tris/HCl (pH 7.5)/2.5 mM EDTA/10 mM 2-mercaptoethanol/1 mg/ml lysozyme/1 % (w/v) Triton X-100/10 μg/ml aprotinin/10 μg/ml leupeptin/1 mM PMSF, incubated at room temperature for 10 min and then sonicated on ice. The lysate was obtained by centrifugation at 10000 ***g*** for 10 min. Protein concentration was determined by the method of Bradford [13]. The aliquots were subjected to SDS/PAGE to confirm that they migrated appropriately for their expected molecular masses.

Assay for phosphatase activity

Phosphatase activity was assayed by dephosphorylating the synthetic peptide, Raytide (Oncogene Science, Uniondale, NY, U.S.A.) as described [14]. The peptide was labelled with [γ-^{32}P]ATP by p60$^{c\text{-}src}$(Oncogene Science) at 37 °C for 12 h. Reaction mixtures with ^{32}P-Tyr-Raytide (2×10^5 c.p.m.) and bacterial lysates were incubated in 25 mM Hepes (pH 7.3)/5 mM EDTA/10 mM dithiothreitol at 37 °C. The reaction was terminated with acidic charcoal mixture [0.9 M HCl/90 mM sodium pyrophosphate/2 mM NaH_2PO_4/4 % (v/v) Norit A]. After centrifugation the amount of radioactivity in the supernatant was measured and the phosphatase activity (released radioactivity) was expressed as a percentage of the total radioactivity used.

Hybridization *in situ*

Rat neonatal brain and kidney were fixed in 4 % (w/v) paraformaldehyde in PBS at 4 °C overnight, dehydrated with ethanol and embedded in paraffin. Serial sections (5 μm) were cut and mounted on poly(L-lysine)-coated slides. After the removal of wax, sections were fixed in 4 % (w/v) paraformaldehyde, treated with 0.25 % (v/v) acetic anhydride in 0.1 M triethanolamine and dehydrated again. As anti-sense probe pGEM7Z plasmid (Promega, Madison, WI) harbouring an 808 bp *Pvu*II fragment (nt 2012–2819) of RPTP-BK cDNA was linearized and transcribed by SP6 polymerase with ^{35}S-UTP (more than 37 Tbq/mmol; Amersham, Little Chalfont, Bucks, U.K.). Sense probe was synthesized by T7 polymerase after linearization. Hybridizations with 10^7 c.p.m./ml radiolabelled probe were performed at 50 °C for 16 h in 50 % (v/v) deionized formamide/10 mM Tris/HCl (pH 7.6)/1 mM EDTA/600 mM NaCl/0.25 % SDS/1 × Denhardt's solution/10 % dextran sulphate/10 mM dithiothreitol/200 μg/ml *Escherichia coli* tRNA. Samples were then treated with 12.5 μg/ml RNase A at 37 °C for 30 min. Washing was performed in a solution of 0.1 × SSC at 50 °C and slides were dehydrated with ethanol. Autoradiography was performed with Kodak NTB-3 emulsion diluted 1:1 with 2 % (v/v) glycerol in distilled water. Slides were developed with Kodak D-19 and fixed with Fujifix. Sections were counterstained with haematoxylin/eosin or Toluidine Blue.

RESULTS

Molecular cloning of RPTP-BK cDNA

We detected a novel PTP cDNA among the amplified genes for PTP from rat embryonic brain with the RT–PCR technique using degenerate primers corresponding to consensus motifs of the phosphatase domain [7]. To characterize the primary structure of this PTP molecule, we isolated a full-length cDNA clone from a rat embryonic brain cDNA library and designated it as

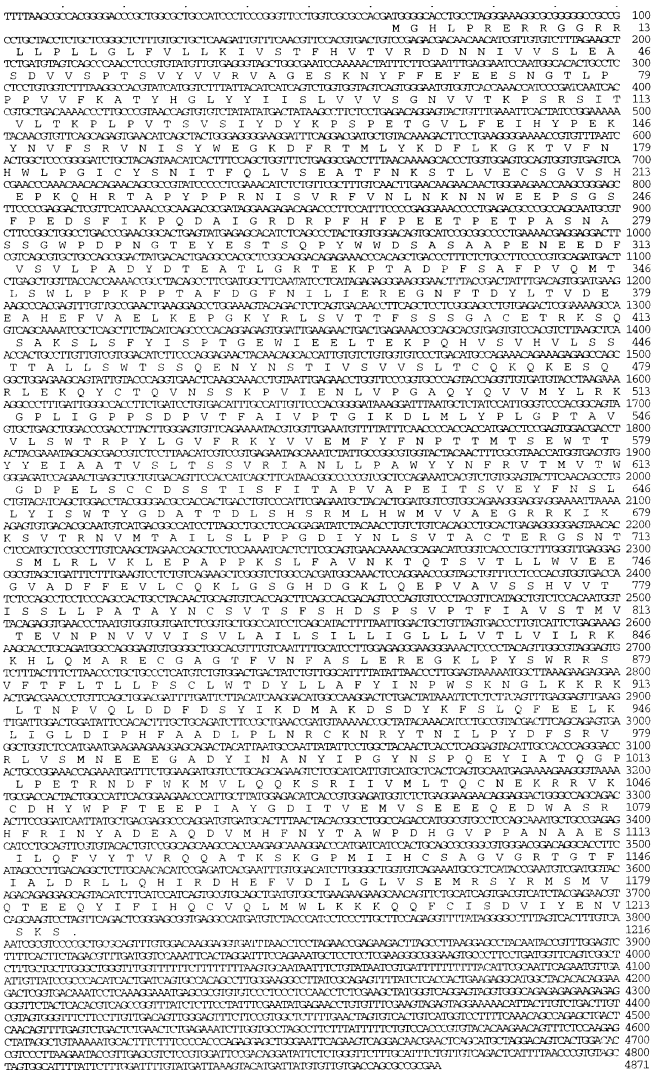

Figure 1 Nucleotide and deduced amino acid sequences of rat RPTP-BK

The amino acid sequence is shown below the nucleotide sequence. Nucleotide numbering begins with the first nucleotide of the cDNA clone and the amino acid sequence begins with the leader sequence.

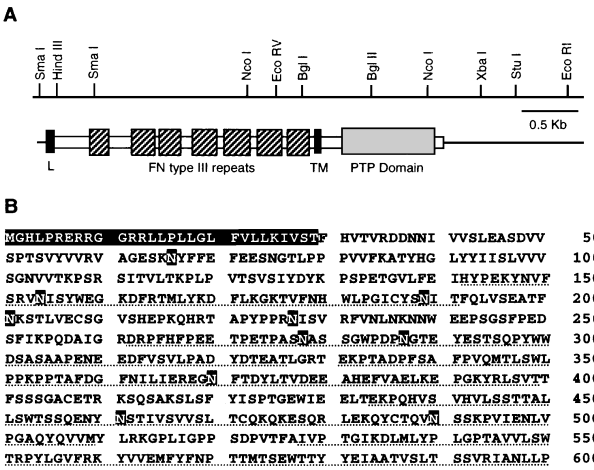

Figure 2 Primary structure of RPTP-BK

(**A**) Restriction enzyme map of RPTP-BK and scheme showing domain structures of RPTP-BK. The leader sequence (L) and the transmembrane region (TM) are indicated by filled boxes. FN-III-like repeats and the cytoplasmic phosphatase domain are shown by hatched boxes and a grey box respectively. (**B**) Amino acid sequence of RPTP-BK. The leader sequence and the transmembranous region are indicated by filled boxes. Asparagine residues for possible N-glycosylation sites are also indicated by filled boxes. The phosphatase domain is underlined with a solid line; FN-III-like repeats are underlined with dotted lines.

RPTP-BK (receptor-type PTP expressed in brain and kidney; see Figure 8).

The nucleotide sequence of 4871 bp RPTP-BK cDNA was determined and an open reading frame predicted the polypeptide of 1215 amino acid residues with a molecular mass of 137939 Da and an isoelectric point of 6.08 (Figure 1). The sequence around a putative initiator methionine codon is consistent with the Kozak consensus sequence [15]; however, we failed to detect polyadenylation signals in the 3′ untranslated region. The nucleotide and deduced amino acid sequences were compared with the sequences in the GenBank (Release 91.0) and SWISS-PROT (Release 32.0) databases respectively. The survey suggested that RPTP-BK is a rat homologue of GLEPP1, a rabbit or a human PTP expressed predominantly in glomerular epithelia of kidney [8,16]. The homology between RPTP-BK and rabbit GLEPP1 or human GLEPP1 was 81.4% and 82.5% respectively at the nucleotide level, and 89.0% and 90.7% respectively at the amino acid level. The sequence alignment also showed that RPTP-BK has an additional 28 amino acid residues (877–904) in the juxtamembrane cytoplasmic region compared with GLEPP1.

Characterization of primary structure of RPTP-BK

The deduced amino acid sequence of RPTP-BK showed a putative signal peptide sequence (residues 1–29), a single hydrophobic amino acid stretch compatible with a transmembrane portion (residues 819–843) and a unique catalytic domain (residues 917–1192) (Figure 2). Thus RPTP-BK is classified as type III RPTP [17], including HPTPβ [18] and DPTP10D [19,20], which is expressed specifically in a subset of developing axons in the *Drosophila* central nervous system. In the extracellular domain there are seven repeats of FN-III-like domain (Figures 2 and 3), whereas HPTPβ and DPTP10D have 16 and 12 FN-III-like repeats respectively.

We compared the primary structure of the catalytic domain of RPTP-BK with those of other RPTPs. As shown in Figure 4, the phosphatase domain of RPTP-BK is highly homologous with GLEPP1 (95% amino acid similarity) and to DPTP10D (48%). It also possesses about 35% homology with each domain of HPTPβ, leucocyte-antigen-related (LAR) [21], PTPδ [22], PTPμ [23] and CD45 [24]. Crystallographic studies on PTP1B showed that the C-terminal region of this domain forms the binding pocket for phosphotyrosine [25,26]. The residues at the base of the binding pockets were well conserved among RPTPs, from Trp^{1007} to Pro^{1013} and from His^{1135} to Ile^{1147} (Figure 4), although RPTP-BK showed Ala-Leu^{1012} instead of the conserved Gly-Val^{1012}.

Figure 3 Alignment of FN-III-like repeats in the extracellular domain of RPTP-BK

Seven FN-III-like repeats in RPTP-BK are aligned in accordance with the consensus sequence [30]. Amino acid residues matched with the consensus are indicated by filled boxes. Gaps have been introduced to maximize the alignment.

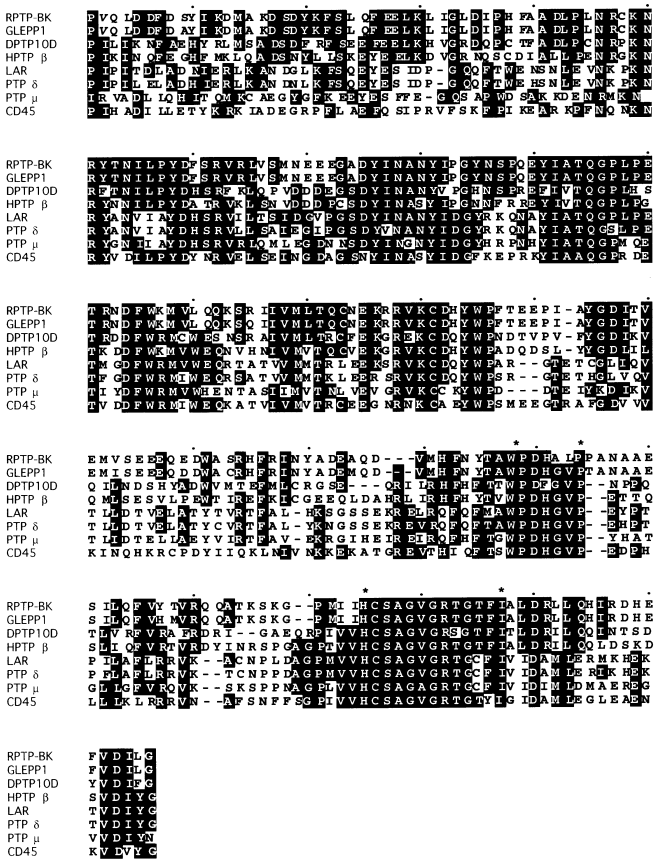

Figure 4 Alignment of phosphatase domains of RPTP-BK and other PTPs

Primary sequences of phosphatase domains from RPTP-BK, GLEPP1, DPTP10D, HPTPβ, LAR, PTPδ, PTPμ and CD45 were aligned by the Clustal method. Amino acid residues that showed at least four identical residues among PTP members are indicated by filled boxes. Gaps have been introduced to maximize the alignment. Asterisks denote the segments corresponding to the binding pocket for phosphotyrosine [25,26].

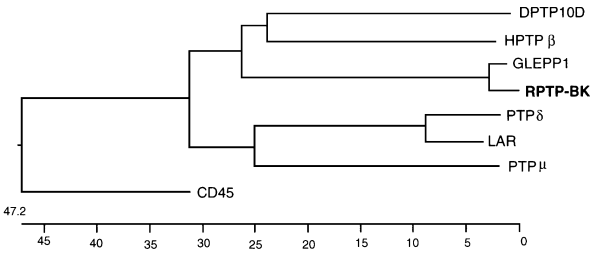

Figure 5 Phylogenetic tree of PTPs

Genetic distance was calculated on the aligned sequences of phosphatase domains examined in Figure 4. The scale beneath the tree shows the relative distance between sequences.

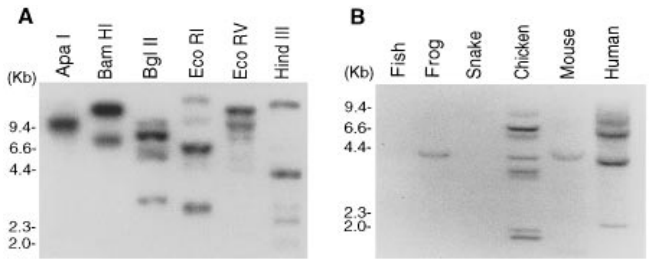

Figure 6 Southern blot analysis of RPTP-BK gene

(**A**) Rat genomic DNA (10 μg) digested by *Apa*I, *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV and *Hin*dIII were separated by agarose-gel electrophoresis, transferred to a nylon membrane and hybridized with an RPTP-BK cDNA probe. (**B**) Genomic DNA species (10 μg) from fish, frog, snake, chicken, mouse and human were digested by *Eco*RI. The blot was hybridized with an RPTP-BK cDNA probe.

To analyse the molecular evolution of RPTP-BK-related phosphatases, we calculated the genetic distances on the aligned sequences of the catalytic domain and made a phylogenetic tree (Figure 5). The tree suggests that RPTP-BK has evolved from the same ancestor gene as have DPTP10D and HPTPβ, although the extracytoplasmic domains among these molecules are less similar than the cytoplasmic domains (results not shown). The structural divergence in the extracellular domain thus implies that a putative ancestral PTP gene underwent complex evolutionary processes.

The deduced amino acid sequence suggests that RPTP-BK has 14 possible N-linked glycosylation sites in its extracellular domain and two sites in its cytoplasmic domain (Figure 2B). We also found two putative tyrosine kinase phosphorylation sites at Tyr^{67} and Tyr^{567}, and a cAMP-dependent protein kinase phosphorylation site at Thr^{915}. There are also 13 and 19 potential sites for phosphorylation by protein kinase C and casein kinase II respectively (results not shown).

RPTP-BK is well conserved in various animals

We performed a Southern blot analysis to examine the genomic organization of RPTP-BK in rat and detected a single *Apa*I fragment (10 kb) and two *Bam*HI fragments (15 and 7 kb) (Figure 6A). This implies that RPTP-BK gene is a single copy and there is at least one intron sequence within the RPTP-BK genomic locus. Because we observed multiple bands in DNA digested with other restriction enzymes, we needed a more detailed map to determine the whole genomic organization. In zooblot analysis, a rat RPTP-BK cDNA probe detected a unique

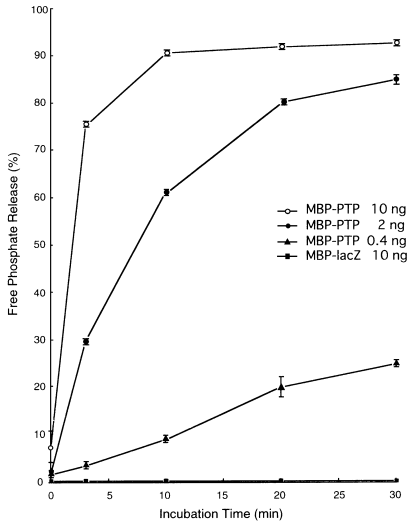

Figure 7 Phosphatase activity of RPTP-BK

A recombinant phosphatase domain from RPTP-BK was produced in *E. coli* as a fusion protein with MBP. Bacterial lysates (10, 2 and 0.4 ng) were assayed for phosphatase activity against ^{32}P-labelled Raytide peptide as a substrate. Bacterial lysate containing MBP–lacZ (10 ng) was used as a control. (The line for MBP–lacZ runs along the *x*-axis.) The activity (free phosphate released) is expressed as a percentage of the total radioactivity used.

band in *Eco*RI-digested genomic DNA from mouse and frog, whereas multiple bands were observed in DNA from human and chicken (Figure 6B). No homologous DNA from fish and snake was hybridized under mild washing conditions. The results obtained here suggest that the RPTP-BK gene is well conserved among mammalian species and in some species of birds and reptiles.

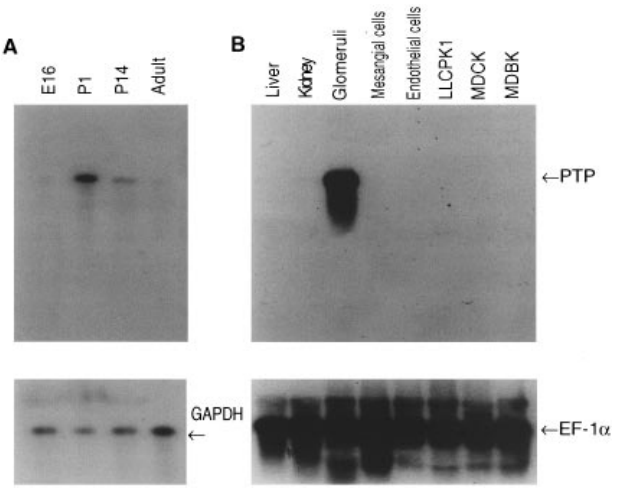

Figure 8 Expression of RPTP-BK in developing brain and renal glomeruli

(**A**) mRNA (1 μg) from rat embryonal (E16), juvenile (P1 and P14) and adult brains was separated by formaldehyde gel electrophoresis, blotted on a nylon membrane and hybridized with a RPTP-BK cDNA probe. The same blot was rehybridized with a probe of glyceraldehyde-3-phosphate dehydrogenase cDNA as a control. (**B**) A blot carrying 10 μg of total RNA from rat liver, kidney, isolated glomeruli, cultured mesangial cells, cultured glomerular endothelial cells, LLCPK1, MDCK and MDBK was hybridized with an RPTP-BK cDNA probe. The same blot was rehybridized with a probe of rat EF-1α cDNA as a control.

Phosphatase activity of recombinant RPTP-BK

We prepared recombinant protein in which the catalytic domain (Asp^{924} to Ser^{1216}) from RPTP-BK was fused to MBP, to examine the phosphatase activity of RPTP-BK. As shown in Figure 7, MBP–PTP, harbouring the catalytic domain of RPTP-BK, showed phosphatase activity for ^{32}P-Tyr-Raytide in a dose-dependent manner, whereas the control, MBP–lacZ, failed to show phosphatase activity. In the assay, 10 ng of bacterial lysate containing the recombinant catalytic domain released 90% of the incorporated phosphate from the labelled Raytide peptide in 10 min and the reaction reached a plateau. In contrast, 0.4 ng of bacterial lysate showed a time-dependent increase in released phosphate up to 30 min. These results indicate that RPTP-BK has an intrinsic enzyme activity of tyrosine phosphatase in its cytoplasmic region, like other receptor-type PTPs.

RPTP-BK is expressed in developing brain and renal glomeruli

Northern blot analysis showed that the hybridized band migrated at 5.6 kb, corresponding to the cDNA obtained, and revealed the restricted expression of RPTP-BK in brain and kidney (Figure 8). Transcription in brain is dynamic during the neonatal stage. We detected hardly any expression in embryos at day 16 *post coitum*, but significant expression in 1-day-old pups. The transcript decreased 14 days after birth, and was further down-regulated in adult brains but still detectable in a trace amount (Figure 8A). We also investigated expression in renal tissues and various renal tubular cell lines. RPTP-BK was specifically expressed in isolated glomeruli, but not in mesangial cells, glomerular endothelial cells or tubular cell lines such as LLCPK1, MDCK and MDBK (Figure 8B).

To analyse the spatial and temporal regulation of RPTP-BK we investigated the expression of RPTP-BK in rat neonatal brain and kidney by hybridization *in situ*. In the brains of 3-day-old pups, we observed the strongest expression in the olfactory bulb and cerebral cortex and definitive signals *in situ*, although less strong, in the thalamus, hippocampus, midbrain regions such as the superior colliculus, inferior colliculus and interpeduncular nucleus, and the brainstem (Figure 9A). In the olfactory bulb the signals were specifically localized to the granular cell layer and the mitral cell layer but were hardly detected in precursor neurons of the subventricular zone (Figure 9B). This suggests that RPTP-BK mRNA is specifically transcribed in postmitotic maturing neurons. In addition to the expression in the olfactory bulb, predominant expression of RPTP-BK was also localized in the olfactory tubercle (Figure 9A) and the piriform cortex (results not shown), suggesting that RPTP-BK is involved in the olfactory–rhinencephalon system. In the kidney, RPTP-BK expression was detected exclusively in the renal glomeruli of newborns (Figures 9C and 9D) and of adult rats (results not shown). The expression in the kidney is not developmentally regulated but is constitutive irrespective of the developmental stage.

DISCUSSION

In the present study we isolated a full-length RPTP-BK cDNA that encodes a type III RPTP with seven FN-III-like repeats in the extracellular region, a single transmembrane domain and one catalytic phosphatase domain in the cytoplasmic region. Thomas et al. [8] proposed eight repeats of the FN-III domain in GLEPP1, a rabbit homologue of RPTP-BK, but the first repeat in the most N-terminal portion seems to be aberrant and does not conform to the consensus. Genomic Southern blot analysis suggests that the gene for RPTP-BK is a single-copy gene in rodents' genomes

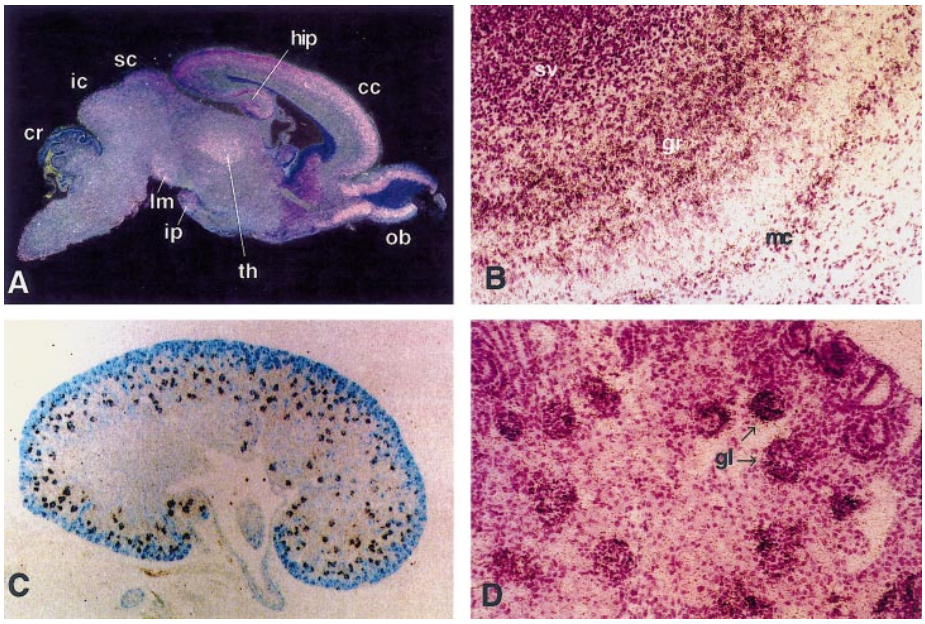

Figure 9 Localization of RPTP-BK mRNA in neonatal brain and kidney

Hybridization *in situ* for RPTP-BK gene in a sagittal section of 3-day-old rat brain (**A**), coronal section of olfactory bulb (**B**), kidney (**C**) and renal cortex (**D**). (**A**) Dark-field photomicrograph showing the localization of RPTP-BK mRNA in the olfactory bulb (ob), developing cerebral cortex (cc), hippocampus (hip), thalamus (th), superior colliculus (sc), inferior colliculus (ic), interpeduncular nucleus (ip), lateral mammillary nucleus (lm) and brainstem, but almost no signal in the cerebellum (cr). (**B**) Bright-field photomicrograph showing the RPTP-BK mRNA in the mitral cell layer (mc) and granular cell layer (gr) but not the subventricular zone (sv) of the olfactory bulb. RPTP-BK mRNA was specifically localized in glomeruli in the low-power field (**C**) and glomerular epithelium (gl) in the high-power field (**D**). Magnifications: (**A**) ×8, (**B**) ×100, (**C**) ×10, (**D**) ×165.

and is well conserved among various species of animal. RNA blot and *in situ* hybridization analyses showed that RPTP-BK is expressed in the developing brain as well as the renal glomerular epithelia, but not in lung, skeletal muscle or intestine (results not shown).

Pixley et al. [9] recently reported a murine PTP, PTPϕ, identified from a macrophage cell line, whose intracytoplasmic region including phosphatase domain is homologous with that of RPTP-BK. They described three isoforms of PTPϕ, two membrane-spanning forms and a cytoplasmic form, the three molecules sharing the same phosphatase domain. The difference of 28 amino acid residues in the juxtamembrane region between the two forms of membrane-spanning PTPϕ (expected molecular masses 47 and 43 kDa) exactly corresponds to the additional amino acid stretch of RPTP-BK, which is not found in GLEPP1. Thus the 47 kDa PTPϕ corresponds to RPTP-BK, and the 43 kDa PTPϕ to GLEPP1, as regards the intracytoplasmic portion. Because three isoforms of PTPϕ, including a cytoplasmic form (33 kDa), are considered to be generated by alternative splicing with different usage of an initiation codon [9], RPTP-BK and GLEPP1 might be generated by a differential splicing mechanism. In addition, the proximal 5′ untranslated sequence of PTPϕ (nt 112–380) is well aligned with the extracellular domain of RPTP-BK (nt 2227–2495), whereas the distal 5′ untranslated region of PTPϕ (nt 1–111) showed a diverged sequence compared with the corresponding sequence of extracellular domain of RPTP-BK (nt 2116–2226). The point of the divergent site could be a splice site, because the corresponding RPTP-BK site is likely to represent an acceptor consensus sequence.

Expression of RPTP-BK was limited to brain and kidney in our study, and small bands corresponding to PTPϕ forms were not detected (Figure 8). The expressed size of the GLEPP1 transcript is not reported [8] but Pixley et al. showed that the expression of PTPϕ in macrophages is limited to 2.8 kb, whereas they detected only a 5.5 kb band in brain and kidney [9]. Thus the tissue specificity of the expression seems to be correlated with different-sized transcriptions. Further study is necessary to clarify the transcriptional control. Elson and Leder recently reported that two isoforms of PTPϵ, the membrane-spanning isoform and the cytoplasmic isoform, were generated by an alternative use of 5′ exons [27]. Thus the underlying molecular mechanism of generating isoforms might not be unprecedented in PTP genes.

In the present study we examined the temporal and spatial expression of RPTP-BK in the developing nervous system and demonstrated that the transcript was localized in neurons of the olfactory bulb, cerebral cortex and other regions at the neonatal stage. The expression in the cerebral cortex of neonatal brain was down-regulated thereafter, but it remained unchanged during and after the maturation of the olfactory bulb and allocortex. The constitutive expression was also observed in the kidney irrespective of developmental stage (results not shown).

Yang et al. [20] identified a *Drosophila* PTP, DPTP10D, and showed that it was selectively expressed in a subset of developing axons and pioneer neurons in the embryonic central nervous system. Because its temporal expression is correlated with the development of major axon tracts, Yang et al. speculated that it plays definitive role(s) in axon outgrowth and/or neuronal guidance. In our study the expression of RPTP-BK in the developing central nervous system coincided with the stage of neuronal axonogenesis [28]. It is interesting to note that RPTP-BK is dominantly expressed in the regions where neuronal axonogenesis takes place, but not in precursor neurons that failed to show axonogenesis, such as those in the subventricular

zone. Because RPTP-BK is highly similar to DPTP10D, RPTP-BK might play pivotal role(s) in axon outgrowth and neuronal guidance in the mammalian central nervous system.

Recently, Desai et al. [29] characterized *Drosophila* mutants lacking PTPs DPTP69D and DPTP99A, and showed that they were required for motor axon guidance. They argued that these RPTPs might be involved in the defasciculation of bundled axons for proper pathfinding in the peripheral nervous system. It prompts us to speculate that the central nervous system has similar mechanisms for pathfinding in axonogenesis. Considering the biological significance of RPTP-BK, mutant mice lacking RPTP-BK would shed light on these issues in future.

We thank Dr. T. Karasawa, Dr. H. Takagi, Dr. T. Watanabe, Dr. T. Takahashi, Dr. H. Mori, Dr. T. Kondo and Dr. E. Moriizumi for their helpful discussions and technical assistance.

REFERENCES

1 Mourey, R. J. and Dixon, J. E. (1994) Curr. Opin. Genet. Dev. **4**, 31–39
2 Tonks, N. K. (1993) Semin. Cell Biol. **4**, 373–377
3 Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Etten, I. and Moolenaar, W. H. (1993) J. Biol. Chem. **268**, 16101–16104
4 Sap, J., Jiang, Y.-P., Friedlander, D., Grumet, M. and Schlessinger, J. (1994) Mol. Cell. Biol. **14**, 1–9
5 Chao, M. V. (1992) Neuron **9**, 583–593
6 Shilo, B. Z. (1992) FASEB J. **6**, 29125–2922
7 Tagawa, M., Shirasawa, T., Fujimura, S. and Sakiyama, S. (1994) Biochem. Mol. Biol. Int. **33**, 1221–1227
8 Thomas, P. E., Wharram, B. L., Goyal, M., Wiggins, J. E., Holzman, L. B. and Wiggins, R. C. (1994) J. Biol. Chem. **269**, 19953–19962
9 Pixley, F. J., Lee, P. S., Dominguez, M. G., Einstein, D. B. and Stanley, E. R. (1995) J. Biol. Chem. **270**, 27339–27347
10 Shirasawa, T., Akashi, T., Sakamoto, K., Takahashi, H., Maruyama, N. and Hirokawa, K. (1993) Dev. Dyn. **198**, 1–13
11 Chomczynski, P. and Sacchi, N. (1995) Anal. Biochem. **162**, 156–159
12 Takahashi, T., Shirasawa, T., Miyake, K., Yahagi, Y., Maruyama, N., Kasahara, N., Kawamura, T., Matsumura, O., Mitarai, T. and Sakai, O. (1995) Biochem. Biophys. Res. Commun. **209**, 218–226
13 Bradford, M. N. (1976) Anal. Biochem. **72**, 248–254
14 Streuli, M., Krueger, N. X., Thai, T., Tang, M. and Saito, H. (1990) EMBO J. **9**, 2399–2407
15 Kozak, M. (1986) Cell **44**, 283–292
16 Wiggins, R. C., Wiggins, J. E., Goyal, M., Wharram, B. L. and Thomas, P. E. (1995) Genomics **27**, 174–181
17 Fischer, E. H., Charbonneau, H. and Tonks, N. K. (1991) Science **253**, 401–406
18 Krueger, N. X., Struli, M. and Saito, H. (1990) EMBO J. **9**, 3241–3252
19 Tian, S. S., Tsoulfas, P. and Zinn, K. (1991) Cell **67**, 675–680
20 Yang, X. H., Seow, K. T., Bahri, S. M., Oon, S. H. and Chia, W. (1991) Cell **67**, 661–673
21 Longo, F. M., Martignetti, J. A., Le Beau, J. M., Zhang, J. S., Barnes, J. P. and Brosius, J. (1993) J. Biol. Chem. **268**, 26503–26511
22 Yan, H., Grossman, A., Wang, H., D'Eustachio, P., Mossie, K., Musacchio, J. M., Silvennoinen, O. and Schlessinger, J. (1993) J. Biol. Chem. **268**, 24880–24886
23 Gebbink, M. F. B. G., van Etten, I., Hateboer, G., Suijkerbuijk, R., Beijersbergen, R. L., van Kessel, A. G. and Moolenaar, W. H. (1991) FEBS Lett. **290**, 123–130
24 Thomas, M. L., Barclay, A. N., Gagnon, J. and Williams, A. F. (1985) Cell **41**, 83–93
25 Jia, Z., Barford, D., Flint, A. J. and Tonks, N. K. (1995) Science **268**, 1754–1758
26 Barford, D., Jia, Z. and Tonks, N. K. (1995) Nature Struct. Biol. **2**, 1043–1053
27 Elson, A. and Leder, P. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 12235–12239
28 Agmon, A., Yang, L. T., O'Dowd, D. K. and Jones, E. G. (1993) J. Neurosci. **13**, 5365–5382
29 Desai, C. J., Joseph, G., Gindhard, J., Goldstein, L. S. B. and Zinn, K. (1996) Cell **84**, 599–609
30 Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K. and Baralle, F. E. (1985) EMBO J. **4**, 1755–1759

Received 13 June 1996/4 September 1996; accepted 20 September 1996