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cDNA Cloning and Characterization of a Novel Receptor-type Protein Tyrosine Phosphatase Expressed Predominantly in the Brain*

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Protein tyrosine phosphatase has the potential to control various cellular events by negatively regulating the extent of tyrosine phosphorylation. Here, we report the isolation of a murine receptor protein tyrosine phosphatase, PTPBR7, which is expressed almost exclusively in the brain. Though the cytoplasmic portion of PTPBR7 reveals high similarity to HePTP/LC-PTP and STEP, these are, unlike PTPBR7, non-receptor protein tyrosine phosphatases. Unlike most receptor protein tyrosine phosphatases, PTPBR7 has only one cytoplasmic phosphatase domain, and its extracellular domain reveals no obvious structural similarity to known molecules. Thus, PTPBR7 defines a new subfamily of receptor-type protein tyrosine phosphatases. The putative extracellular domain of PTPBR7 was expressed in COS-7 cells as a chimeric fusion protein with an immunoglobulin Fc portion (PTPBR7-Fc). PTPBR7-Fc was secreted in the culture supernatant, confirming the capability of the extracellular domain of PTPBR7 to translocate across the cytoplasmic membrane. The cytoplasmic portion of PTPBR7 was expressed as a fusion protein in bacteria and was demonstrated to have catalytic activity. The expression of PTPBR7 was detectable in brain and especially in cerebellum but undetectable in liver, lung, heart, kidney, thymus, bone marrow, and spleen. *In situ* hybridization analysis revealed the most prominent signal in Purkinje cells. The predominant expression of PTPBR7 in the brain suggests that PTPBR7 may have role(s) in neuronal cells.

Protein tyrosine phosphorylation plays a crucial regulatory role in various cellular events, including growth and differentiation. Compared with PTK[1] (protein tyrosine kinase), much less is known about PTP (protein tyrosine phosphatase), although both enzymes can regulate the tyrosine phosphorylation level.

There are many examples of the importance of PTK in the nervous systems. The trk family of PTKs is expressed almost exclusively in neurons and constitutes receptors of the nerve growth factor family of growth factors that regulate survival, neurite growth, and neurotransmitter production (reviewed in Ref. 1). The *Drosophila* homologue of *abl*, a non-receptor tyrosine kinase, is expressed in axons and can cause defects in the axon pathway formation if it is mutated in combination with the fasciclin I gene (2). It has been reported that the rat brain contains high levels of PTK activity, and one of the highest levels of activity was found in the cerebellum (3, 4).

Considering the importance of tyrosine phosphorylation, it is conceivable that PTPs are also involved in the biological processes of the nervous system. The family of PTPs has been growing rapidly, and there is a great diversity in the structure of PTPs (reviewed in Refs. 5–7). Except for the PTP domain(s), the rest of each PTP molecule is composed of diverse structural motifs that might be essential for a specific function of the PTP. Like PTKs, there are two forms of PTPs, namely, a receptor and a non-receptor form. Receptor PTPs are of special interest in the nervous system, where, as in the immune system, cell growth, survival, and differentiation are regulated by extracellular signals, and specific interaction or recognition of cells is required.

Receptor PTPs of various types are expressed in the nervous system. Receptor PTPs can be classified into five types based on the structure of the extracellular domain (5–9). Types II and III receptor PTPs have an extracellular domain composed of FN-III repeats. Type II receptor PTPs have additional Ig-like domains and, thus, resemble N-CAM, a cell adhesion molecule. In fact, two type II receptor PTPs, mRPTPμ and R-PTP-κ, have been shown to mediate homophilic cell adhesion (10–12). DPTP99A, DPTP10D, and DLAR are nervous system-specific types II and III receptor PTPs in *Drosophila*. These PTPs are expressed in developing axons or pioneer neurons in the embryonic central nervous system, and their involvement in axon outgrowth and guidance has been proposed (13, 14). LAR (15, 16), PTPδ (17, 18), and PTP NE-3/PTP-σ/PTP-P1/CPTP1 (19–22) are type II receptor PTPs in mammals, and these are expressed in neuronal tissues as well as in other tissues. Type IV receptor PTPs have a short extracellular domain of unknown function. LRP/RPTPα (17, 23–26) is a type IV receptor PTP that is distributed in a wide variety of tissues, including brain. Type V receptor PTPs have a carbonic anhydrase-like domain in addition to an FN-III domain (8, 9, 27). Recently, it has been reported that an extracellular variant of a type V receptor PTP interacts with an extracellular matrix protein and N-CAM (28, 29). RPTPβ/PTPζ and RPTPγ are type V receptor PTPs, and these are expressed in the brain in a highly specific or a less specific manner, respectively. The physiological role of these receptor PTPs remains to be elucidated.

Here, we report the cDNA cloning and characterization of a novel murine PTP, which we called PTPBR7. PTPBR7 is pre-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D31898.

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[1] The abbreviations used are: PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; FN-III, fibronectin type III; Ig, immunoglobulin; CAM, cell-adhesion molecule; PCR, polymerase chain reaction; ss-cDNA, single-stranded cDNA; SLIC, single strand ligation to ss-cDNA; GST, glutathione *S*-transferase; *p*-NPP, *p*-nitrophenyl phosphate; kb, kilobases.

dominantly expressed in the nervous system, and the most prominent signal was detected in the Purkinje cell. Unlike most receptor PTPs, PTPBR7 has only one cytoplasmic PTP domain and reveals no obvious similarity to known PTPs in the extracellular domain. Therefore, PTPBR7 defines a new subfamily of receptor PTP, which is expressed almost exclusively in the nervous system.

EXPERIMENTAL PROCEDURES

Materials—Female C57BL/6 mice (4–7 weeks old) were obtained from Shizuoka Experimental Animal Laboratory (Hamamatsu, Japan). Enzymes were purchased from Life Technologies, Inc., New England Biolabs (Beverly, MA), Promega (Madison, WI), and Stratagene (La Jolla, CA). Oligonucleotides were synthesized on an Applied Biosystems 380A oligonucleotide synthesizer. Radionucleotides were purchased from DuPont-NEN or Amersham. MRL104.8a, a thymic stroma cell line, was established in our laboratory (30).

Amplification of cDNA Fragments Encoding PTP Domains—Total RNA was isolated from adult brain of C57BL/6 mice, and first-strand cDNA was synthesized using mouse Molony leukemia virus reverse transcriptase and random hexamer primers. For PCR, the following degenerative oligonucleotides were designed based on the reported alignment of PTPase domains (31): PTP-A, 5′-CCGCATATGGA(T/C)-TT(C/T)-TGG-(C/A)(G/T/A)N-ATG-(A/G)TN-(T/G)(T/G)N-(G/C)A-3′ (sense), and PTP-B, 5′-CCGCAAGCTTT-CG-CCC-(G/A)A(C/T)-NCC-NGC-NC(T/A)-(G/A)CA-(G/A)TG-3′ (antisense). The primers contained restriction sites for *Nde*I or *Hin*dIII (underlined) and codons for the amino acids DFW(R/Q/M/L)M(I/V/L)(W/V)(E/Q/D) or HCSAG(V/I)GR, respectively.

cDNA was amplified with 40 amplification cycles (94 °C for 1 min, 37 °C for 2 min, and 72 °C for 3 min). PCR fragments of expected size (about 300 base pairs) were purified on 6% native polyacrylamide gel, reamplified, and cloned into *Sma*I/*Hin*dIII sites of pBluescript SK− plasmid (Stratagene).

Isolation of cDNA Clones—A murine brain cDNA library (primed with oligo(dT), kindly provided by Dr. K. Kato) was screened using probes radiolabeled by random priming (32). Probes (2×10^5 cpm/ml) were hybridized to the phage DNA immobilized on nylon filters (Hybond-N+, Amersham) at 65 °C in 5 × SSPE (0.9 M NaCl, 50 mM NaH_2PO_4, pH 7.7, 5 mM EDTA), 5 × Denhardt's reagent, 0.5% SDS, and 20 μg/ml denatured salmon sperm DNA. The initial probe was an ~350-base pair insert from the PCR clone with which we isolated PBR7–7 (Fig. 1*A*). Then, PBR7–17, and -26 were isolated using PBR7–7 as a probe. Using the 5′-end fragment of PBR7–26 (nucleotides 272–632) as a probe, PBR7–47 was isolated. Finally, using the 5′-end fragment of PBR7–47 (nucleotides 44–618) as a probe, we screened another brain cDNA library (primed with oligo(dT) plus random hexamer, kindly provided by Dr. H. Yamamoto), but we could not obtain a clone containing more 5′ sequence than PBR7–47.

To obtain the 5′-end cDNA fragment of PTPBR7, the cDNA of murine whole brain was amplified, using SLIC (single strand ligation to single-stranded cDNA (ss-cDNA))-PCR as described (33). The strategy is summarized in Fig. 1*B*. Briefly, a specific primer (PBR7-B3, 5′-TACAGAAC-CGAGCACCTGCTTCCTCT-3′) was used to synthesize ss-cDNA. Then, an anchor-oligonucleotide (SLIC-1, 5′-ACTTAACCAGGCTGAACTT-GCTACCCTGGAAGAAATACTCAT-3′) was ligated to the 3′ end of ss-cDNA using T4 RNA ligase. ss-cDNA was amplified by PCR (40 cycles; 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min) using a second specific primer (PBR7-B1, 5′-AGAGGAAGCAGGTGCTCGGT-TCTGTA-3′) and an anchor-primer (5′-GAGAATTCAGCAAGTTCAGC-CTGGTTAAGT-3′). Amplified cDNA was cloned into a plasmid vector. Clones were screened by colony hybridization using the 5′ end of PBR7–47 (nucleotides 44–618) as a probe, and about 50 out of 2000 colonies showed positive signals.

DNA Sequencing and Analysis—Sequencing was carried out by the dideoxy chain-termination method, using a Taq dye primer cycle sequencing kit and ABI 373A DNA sequencer (Applied Biosystems). The final sequence was confirmed from both strands. Sequence analysis or comparison was done using NCBI E-mail server, FASTA, and BLAST E-mail server (34, 35) on GenomeNet and molecular analysis systems in DNA data bank of Japan.

RNA Analysis—Total RNA was prepared from various cell lines and murine organs by the acidic guanidium isothiocyanate-phenol-chloroform extraction method (36). An aliquot of each RNA sample was electrophoresed on a 1% agarose gel, and intactness of the RNA was confirmed by visualizing 18 and 28 S rRNA bands.

To analyze the expression of PTPBR7 in various tissues and cell lines, RNase protection assay was performed as described (37) with minor modifications. Briefly, RNA probes were synthesized using [α-^{32}P]UTP and purified by 6% polyacrylamide gel electrophoresis. Then, the probe ($1–2 \times 10^5$ cpm) was hybridized to total RNA samples (10 μg) in 15 μl of hybridization buffer. After hybridization for 12–20 h at 50 °C, samples were digested with RNase A and T1 at 37 °C for 60 min and separated on a 6% polyacrylamide/7 M urea gel.

For Northern hybridization analysis, 4 μg of poly(A)$^+$ RNA was separated on a 1% agarose gel and transferred to a nylon filter (Hybond-N, Amersham). RNA on the filter was stained by methylene blue to confirm even transfer. The filter was then hybridized to probes at 45 °C for 18 h in 5 × SSPE, 50% formamide, 5 × Denhardt's reagent, 0.5% SDS, 20 μg/ml of denatured salmon sperm DNA, and 5×10^6 cpm/ml of probes radiolabeled by random priming method.

In Situ Hybridization Analysis—Mice were killed under anesthesia and perfused with 20 ml of 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4) (fixation solution) from left ventricle to right atrium. Organs excised were further fixed in fixation solution for 3 h at 4 °C, soaked in 30% sucrose in 0.1 M sodium phosphate overnight at 4 °C, and cut on a cryostat into 10-μm thick sections. Sections were then hybridized with antisense RNA probes labeled with [α-^{35}S]UTP as described (38). Hybridization was done in 100 μl of 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM EDTA, 10% dextran sulfate, 1 × Denhardt's solution, 0.5 mg/ml yeast tRNA, 10 mM DTT, and 5×10^5 cpm of RNA probe for 16 h at 55 °C. The sections were then washed once in 5 × SSC, 10 mM DTT at 50 °C for 20 min, once in 50% formamide, 2 × SSC, 100 mM DTT (high stringency wash solution) at 65 °C for 30 min, and four times in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 M NaCl (RNase buffer) at 37 °C, for 10 min. After digestion with 10 μg/ml of RNase A in RNase buffer at 37 °C for 10 min, sections were washed once in high stringency wash solution at 65 °C for 30 min, once in 2 × SSC at room temperature for 10 min, and once in 0.1 × SSC at room temperature for 10 min. After dehydration, signals were visualized by autoradiography using Ilford K-5 emulsion.

Expression of Chimeric Protein of PTPBR7—A chimeric gene (see Fig. 4*A*), encoding a fusion protein (PTPBR7-Fc) of the extracellular portion of PTPBR7 and the Fc portion of human IgG_1, was constructed by replacing the *Cla*I/*Pst*I fragment of the mFas-Fc plasmid (39) with a partial cDNA fragment of the extracellular portion of PTPBR7 flanked by *Cla*I and *Nsi*I sites. Note that the DNA end after *Pst*I digestion is compatible with that after *Nsi*I digestion. *Cla*I and *Nsi*I sites were created at the ends of the cDNA fragment by PCR using primers PBR7-A1 (5′-TATCGATTGCACACACTATGAGGAGAGCG-3′) and PBR7-B4 (5′-GAATGCATCTTTGCTCCAGATCTTGTCTGCT-3′). *Cla*I and *Nsi*I sites in the primers are underlined. The plasmid was transfected into COS-7 cells in a 60-mm tissue culture dish by DEAE-dextran methods (32). After overnight culture in 4 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, culture medium was replaced by 4 ml of serum-free Dulbecco's modified Eagles medium and incubated for an additional 5 days. The culture supernatant was then removed, and cells were lysed in phosphate-buffered saline containing 1% Triton X-100. Fusion protein was purified from culture supernatant and cell lysate separately, using Protein A-Sepharose beads (10 μl, pellet volume).

For the construction of a plasmid encoding GST-PTPBR7, the pGEX-3X vector (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) was digested with *Bam*HI, filled with Klenow fragment, and digested with *Eco*RI. The cDNA insert of PBR7–47, subcloned in pBluescript SK plasmid, was digested with *Sma*I and *Not*I. Then, the cDNA fragment encompassing nucleotides 1440–2844 of PTPBR7 was separated by agarose gel electrophoresis. This cDNA fragment was ligated to the vector treated as described above with a *Not*I/*Eco*RI fragment as a filler. *Escherichia coli* (XL1-Blue) transformed with the plasmid was cultured at 29 °C in 200 ml of LB medium, and expression of the chimeric gene was induced by adding 0.1 mM of isopropyl-1-thio-β-D-galactopyranoside when absorbance at 600 nm of the culture reached 0.40. After a 3-h incubation, cells were centrifuged, and the pellet was resuspended in 4 ml of 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride. The bacteria was then lysed by sonication and centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was mixed with 200 μl (pellet volume) of glutathione-Sepharose beads suspended in phosphate-buffered saline (1:1, v/v) and rocked for 1 h at 4 °C. The beads were washed five times with 1 ml of 50 mM Hepes pH 7.6, 150 mM NaCl, 0.1% Triton X-100 and once with the phosphatase assay buffer described below. Finally, the beads were suspended in the phosphatase assay buffer. An aliquot was mixed with an equal volume of 2 × SDS-PAGE sample buffer containing β-mercaptoethanol, boiled for 3 min, electrophoresed on a 10% polyacrylamide

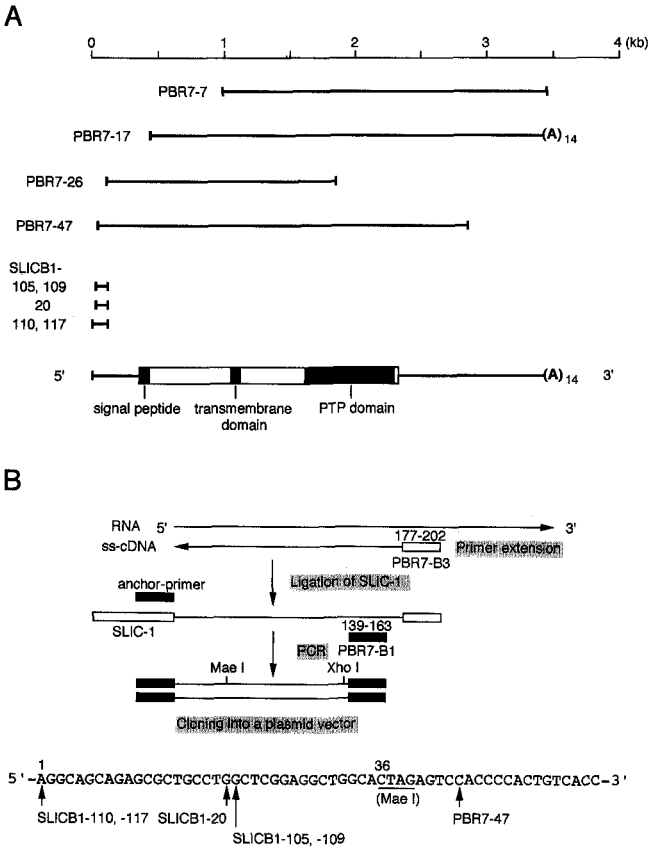

FIG. 1. **Overlapping cDNA clones of PTPBR7.** *A*, four overlapping clones isolated from a murine brain cDNA library, five independent clones isolated by SLIC-PCR, and the schematic structure of PTPBR7 are shown. *B*, a schematic drawing of the strategy of SLIC-PCR is shown in the *upper* part (see also "Materials and methods" and text). PBR7-B1 and PBR7-B3 are specific antisense primers and cover nucleotides 139–163 and 177–202, respectively. In the *lower* part, *arrows* below the 5'-end sequence of PTPBR7 indicate the starting position of the five independent SLIC-PCR clones. The *number above* the sequence indicates the nucleotide position. An *Mae*I site is *underlined*.

gel, and stained with Coomassie Brilliant Blue. The amount of GST-PTPBR7 protein captured on the beads was estimated by comparing the stained band with that of a known amount of purified glutathione *S*-transferase protein.

Phosphatase Assay—The catalytic activity of GST-PTPBR7 was assayed using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate (40, 41), with minor modifications. In the preliminary experiment, the catalytic activity was measured using either pH 7.4 assay buffer (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, and 1 mM EDTA) or pH 5.0 assay buffer (0.1 M sodium citrate, pH 5.0, 1 mM DTT, and 1 mM EDTA). We found that the activity detected with pH 7.4 assay buffer was approximately 300-fold higher than that detected with pH 5.0 assay buffer. Therefore, we used pH 7.4 assay buffer in the following experiments. The beads capturing 0.8–12.8 μg of GST-PTPBR7 protein were suspended in 50 μl of pH 7.4 assay buffer and the reaction was initiated by adding 200 μl of pH 7.4 assay buffer containing 15 mM *p*-NPP with or without 3 mM Na_3VO_4. After a 10-min incubation at 37 °C, the reaction was stopped by adding 50 μl of 2.5 N NaOH. The amount of *p*-nitrophenol released was determined from absorbance at 405 nm and using a molar extinction coefficient of 18,000 M^{-1}, cm^{-1}. The nonenzymatic hydrolysis of the substrate was corrected by measuring the optical density without the recombinant protein (beads alone).

RESULTS

cDNA Cloning of a Protein Tyrosine Phosphatase, PTPBR7—We amplified the cDNAs encoding a part of the PTP domain by PCR. Degenerative primers that corresponded to conserved amino acid sequences in the PTP domains were used. The amplified PCR fragments were cloned into a plasmid vector and identified by DNA sequencing. One PCR clone that we amplified from murine brain defined a novel murine PTP. Here, we report the structure and characterization of this PTP, which we called PTPBR7.

Four overlapping cDNA clones (PBR7–7, -17, -26, and -47) were isolated from a murine brain cDNA library (Fig. 1*A*). The length of the consensus sequence generated from these clones agreed with the size of the longest transcript detected by Northern hybridization analysis (see Fig. 6*B*). However, there was no in-frame stop codon preceding the first ATG in the longest open reading frame, and it was possible that the coding region was extending further in the 5′ direction. Therefore, we screened more than one million clones from a brain cDNA library (primed with oligo(dT)) and an additional one million clones from another brain cDNA library (primed with oligo(dT) plus random hexamer), but we could not isolate a clone containing more upstream sequence.

Finally, to obtain the 5′-end cDNA fragment of PTPBR7, we employed SLIC-PCR (33) (Fig. 1*B*) using the whole brain RNA. The 5′-end fragment of the cDNA was specifically synthesized by reverse transcription with a specific primer (PBR7-B3), which was complementary to the sequence near the 5′ end of PBR7–47. Because the amount of the cDNA generated was very small, the cDNA was amplified by PCR, using an anchor primer and the second specific primer (PBR7-B1). After cloning into a plasmid vector, clones containing the 5′-end sequence of PBR7–47 were determined by colony hybridization. We randomly picked up 39 positive clones and mapped the *Xho*I and *Mae*I sites in each insert. The *Xho*I site was present at nucleotide 122, and a preliminary sequencing experiment of one of the PCR clones showed that there was an *Mae*I site (CTAG) at nucleotide 36. Twenty-two of 39 clones had expected *Mae*I and *Xho*I sites, showing that these clones contained the 5′ sequence at least up to nucleotide 36. The size of the insert was then compared, and five clones that had inserts longer than or equal to others were analyzed by sequencing. The result is summarized in Fig. 1*B*. We found two independent clones (SLICB1–110 and -117) starting from nucleotide 1. The other three clones started at nucleotide 20 or 21.

Sequence Analysis of PTPBR7—The nucleotide sequence and estimated amino acid sequence of PTPBR7 are shown in Fig. 2. A putative polyadenylation signal (AATAAA) is found at nucleotides 3411–3416. The longest open reading frame of PTPBR7 encodes a protein of 656 amino acids. Although there is no in-frame stop codon preceding the first ATG, the nucleotide sequence flanking the ATG codon matches the consensus sequence of the translation start site described by Kozak (42). The N-terminal amino acid sequence resembles a signal peptide (43), and there is a stretch of hydrophobic amino acid at amino acids 226–248. Thus, PTPBR7 has the typical structural features of a class I integral membrane protein with a putative PTP domain in the cytoplasmic portion (Fig. 2). A data base search showed that PTPBR7 has high similarity to previously reported PTPs, HePTP/LC-PTP (44, 45), and STEP (46). However, the similarity is restricted to the PTP domain and to a short stretch (approximately 90 amino acids) preceding the PTP domain (Fig. 3, *A* and *B*). The percent homology of PTPBR7 (amino acids 328–656) compared with HePTP/LC-PTP (amino acids 13–339) or STEP (amino acids 39–369) is 55 or 59%, respectively. Moreover, unlike PTPBR7, HePTP/LC-PTP and STEP are non-receptor PTPs (see "Discussion"). Thus, it is clear that PTPBR7 is not the murine homologue of human HePTP/LC-PTP or STEP.

In addition, we found a sequence of 104 amino acids in the EMBL data (Hendriks, W., Brugman, C., Zeeuwen, J., Schepens, J., and Wieringa, B., accession No. S40280), which has 100% identity to a part of the PTPBR7 sequence (amino acids

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1       AGGCAGCAGAGCGCTGCCTGGCTCGGAGGCTGGCACTAGAGTCCACCCCACTGTCACCACCGGCTGCGGGCTTCCAGAAGGCCAAACTCTCACCTCTTCTGAGCGTCTTGCCCCC   115
116 ATCAGCCTCGAGTCCCCATCTCCCGCAGCATCCTACTCTCGTATCTCCCCATCCCTTGCGGAGAGGAAGCAGGTGCTCGGTTCTGTAGGCACGCCACAGACTGCTCTGTGTACCCCAGCG   235
236 GAGGACCCTGTCATCGGATGCCCGGGATCAAAGGACTCTTGGCAGTGGTTCTCTCAAATAGCACTTAGCCTAGAGTGGGGTTGCGGCGGAGTTTCTCCTCTGACCCTCAATGCACACACT   355
356 ATGAGGAGAGCGGTCGGCTTCCCTGCGCTGTGCCTGCTTCTTAATCTTCATGCTGCAGGTTGTTTTTCCAGAAACAATGATCACTTTTTGGCTATTCGTCAAAAGAAGAGTTGGAAGCCG   475
  1  M  R  R  A  V  G  F  P  A  L  C  L  L  L  N  L  H  A  A  G  C  F  S  R  N  N  D  H  F  L  A  I  R  Q  K  K  S  W  K  P    40
476 GTGTTCATTTATGACCATTCACAGGATATCAAGAAGAGCCTGGACATCGCACAAGAGGCATACAAACATAACTACCACTCCCCTTCCGAAGTTCAAATAAGCAAACATCACCAGATTATT   595
 41  V  F  I  Y  D  H  S  Q  D  I  K  K  S  L  D  I  A  Q  E  A  Y  K  H  N  Y  H  S  P  S  E  V  Q  I  S  K  H  H  Q  I  I    80
596 AATTCAGCATTTCCTAGACCTGCATATGACCCGTCTCTTAATCTGTTGGCTGAGTCTGATCAAGATCTTGAAATAGAAAATCTCCCAATTCCAGCAGCAAATGTGATTGTGGTGACACTG   715
 81  N  S  A  F  P  R  P  A  Y  D  P  S  L  N  L  L  A  E  S  D  Q  D  L  E  I  E  N  L  P  I  P  A  A  N  V  I  V  V  T  L   120
716 CAAATGGATATAACCAAGCTGAACATAACCCTGCTTCGGATCTTCCGCCAAGGAGTAGCTGCAGCCCTGGGACTCTTACCTCAGCAAGTGCACATTAACCGGCTCATTGAAAAGAAGAAC   835
121  Q  M  D  I  T  K  L  N  I  T  L  L  R  I  F  R  Q  G  V  A  A  A  L  G  L  L  P  Q  Q  V  H  I  N  R  L  I  E  K  K  N   160
836 CAGGTTGAGTTGTTTGTGTCTCCCGGAAACCGGAAACCAGGAGAAACGCAGGCCCTGCAGGCTGAGGAAGTGCTGCGTTCCCTCAATGTGGATGGTTTGCATCAGAGTTTACCACAGTTT   955
161  Q  V  E  L  F  V  S  P  G  N  R  K  P  G  E  T  Q  A  L  Q  A  E  E  V  L  R  S  L  N  V  D  G  L  H  Q  S  L  P  Q  F   200
956 GGAATTACAGACGTCGCCCCTGAGAAAAATGTTTTACAAGGGCAGCACGAAGCAGACAAGATCTGGAGCAAAGAAGGATTTTACGCTGTCGTCATCTTCCTCAGCATCTTTATCATCATA  1075
201  G  I  T  D  V  A  P  E  K  N  V  L  Q  G  Q  H  E  A  D  K  I  W  S  K  E  G  F  Y  A  V  V  I  F  L  S  I  F  I  I  I   240
1076 GTAACCTGTTTGATGATTATTTACAGGTTAAAAGAAAGCTTCAGCTTTCCTTAAGACAAGATAAAGAGAAAAACCAGGAGATCCACCTATCACCCATTGCACGGCAGCAAGCACAATCG  1195
241  V  T  C  L  M  I  I  Y  R  L  K  E  R  L  Q  L  S  L  R  Q  D  K  E  K  N  Q  E  I  H  L  S  P  I  A  R  Q  Q  A  Q  S   280
1196 GAGGCCAAGACGACCCACAGCATGGTCCAGCCCGATCAGGCGCCAAAGGTGCTGAACGTGGTTGTGGACCCTCAAGGCCAATGCACTCCTGAGATTCGAAACAGCACCTCCACCTCTGTC  1315
281  E  A  K  T  T  H  S  M  V  Q  P  D  Q  A  P  K  V  L  N  V  V  V  D  P  Q  G  Q  C  T  P  E  I  R  N  S  T  S  T  S  V   320
1316 TGCCCTTCTCCCTTCAGAATGAAGCCCATAGGACTCCAGGAGCGACGAGGTTCCAATGTATCTCTTACGCTGGACATGAGTAGCCTGGGCAGTGTGGAACCCTTTGTGGCCGTCTCAACC  1435
321  C  P  S  P  F  R  M  K  P  I  G  L  Q  E  R  R  G  S  N  V  S  L  T  L  D  M  S  S  L  G  S  V  E  P  F  V  A  V  S  T   360
1436 CCCCGGGAGAAGGTAGCCATGGAATACCTGCAGTCAGCCAGCCGAGTTCTCACACGGTCACAGCTGAGGGACGTCGTGGCAAGTTCCCACCTACTTCAAAGTGAATTCATGGAAATACCA  1555
361  P  R  E  K  V  A  M  E  Y  L  Q  S  A  S  R  V  L  T  R  S  Q  L  R  D  V  V  A  S  S  H  L  L  Q  S  E  F  M  E  I  P   400
1556 ATGAATTTTGTGGATCCCAAAGAAATTGATATTCCACGTCACGGAACTAAAAATCGTTATAAGACCATTTTGCCAAATCCCCTCAGCAGAGTGTGCTTAAGACCAAAAAATATAACCGAT  1675
401  M  N  F  V  D  P  K  E  I  D  I  P  R  H  G  T  K  N  R  Y  K  T  I  L  P  N  P  L  S  R  V  C  L  R  P  K  N  I  T  D   440
1676 TCCTTGAGTACTTACATAAATGCTAACTATATTCGGGGCTACAGTGGTAAGGAGAAAGCCTTCATTGCCACCCAGGGCCCCATGATCAACACTGTGAATGACTTCTGGCAGATGGTGTGG  1795
441  S  L  S  T  Y  I  N  A  N  Y  I  R  G  Y  S  G  K  E  K  A  F  I  A  T  Q  G  P  M  I  N  T  V  N  D  F  W  Q  M  V  W   480
1796 CAAGAAGACAGTCCCGTGATTGTGATGATCACGAAACTCAAAGAGAAAAATGAGAAATGTGTGCTCTACTGGCCAGAAAAGAGAGGGATTTACGGCAAGGTTGAGGTTCTGGTCACCGGT  1915
481  Q  E  D  S  P  V  I  V  M  I  T  K  L  K  E  K  N  E  K  C  V  L  Y  W  P  E  K  R  G  I  Y  G  K  V  E  V  L  V  T  G   520
1916 GTGACCGAATGTGATAACTACACCATCCGCAACCTCGTCTTAAAGCAAGGAAGTCACACCCAACATGTGAAGCACTACTGGTACACTTCATGGCCGGATCATAAGACTCCAGACAGTGCC  2035
521  V  T  E  C  D  N  Y  T  I  R  N  L  V  L  K  Q  G  S  H  T  Q  H  V  K  H  Y  W  Y  T  S  W  P  D  H  K  T  P  D  S  A   560
2036 CAGCCCCTTCTGCAGCTCATGTTGGATGTGGAAGAAGACAGACTGGCCTCTGAAGGCCGAGGGCCTGTGGTTGTCCACTGCAGTGCGGGGATTGGGAGAACTGGGTGTTTCATCGCTACA  2155
561  Q  P  L  L  Q  L  M  L  D  V  E  E  D  R  L  A  S  E  G  R  G  P  V  V  V  H  C  S  A  G  I  G  R  T  G  C  F  I  A  T   600
2156 TCCATTGGCTGTCAACAATTGAAAGAAGAAGGAGTTGTAGACGCACTAAGTATTGTCTGCCAGCTTCGTGTAGACAGGGGTGGTATGGTCCAAACCAGCGAGCAGTATGAATTTGTGCAC  2275
601  S  I  G  C  Q  Q  L  K  E  E  G  V  V  D  A  L  S  I  V  C  Q  L  R  V  D  R  G  G  M  V  Q  T  S  E  Q  Y  E  F  V  H   640
2276 CATGCTCTGTGCCTGTTCGAGAGCAGACTTTCACCAGAAACTGTCGAGTGACTCCGAAGATTTACCAGAGTGTCAATCTCTCACCGGGTGATTCATCAAATTACCTACCAAGAGCTCCAA  2395
641  H  A  L  C  L  F  E  S  R  L  S  P  E  T  V  E  *                                                                      656
2396 GAGGGGCTCCCTGCAATGGACGAGGAGGCTCTAAAGCCAGCCTAAGGCACTGATTGTGGAAGATCTGGCAACATGAAAGATTGCCAGCCTTTGTGTATAGGACTGCGTTCGTAGGCATCC  2515
2516 CCCCAGTTATTCTGAAGGTCCTGTGCTGATGGAGGCATGACAGTCCTCTCGCAGCCTGAGGAGGGTTTTGTTCTGTCTGTGTCGCCTCTCCGCCGCACAGAATCGATGTGTGTGATATTC  2635
2636 AGAGACTGGAGGAGCTGCTGACATTATCCTGTGTTTAGATGCTTTAATGTTCATAAAGCCTGTCTTGTGACTGGACTGTCAGCTGTCCAACTGTCCCTGTTGTAAGTGCTATTAACTATC  2755
2756 TCAGTTACCAGAATCTTGCTGCTCAAGTTTCGAGTGATCGACAATGGATTTTTAACCAAGACATTTTTGTTTTTGGAAAAAAAAAAAAAAAATCAAATGCAGTGACTATTTTATATGGACA  2875
2876 TGTGTCTTGATTATATCTATTAAGTGTCATTTATAGTATCTCCTATGGATCAATTACAGAGCACAATGACTGTCGTTGGATATATATGTATATGTATATTATATGTATATGTA  2995
2996 CACATATATATTCTATTATTAGGCATACAAGTGGCTCCTGTTCCACAATACTAACTACTATATTTCTACATTGTGACTTGTTTTACTTTAAAAGGGGATAAACAAATTTATAGCAACTAT  3115
3116 AAAGTATCAATAATTTTAAATACTCATCTCTCTTTTACTAAGAAGGGATTTTTAAATATGGTGTCTACCTGGTATCTCTCTCCCAATAAGAAGCAGGTGCCTTTGGGGAATGCTCCAACC  3235
3236 TGTTTCATCCCCAAAAGTTTTTCCGAGGACACTGTCATGCACGACCTCACATTTCTAAAAGACCTCCTTCCTCAAGAGACAAATGAAGGCAAAATAATCACACTCAGCAAACAGGAGATG  3355
3356 TTCTGCTCAGATATGAATTTTCAATGCAGCAAAGCTGACTTTCTTTCGTACTCCCAATAAATGACTCTTAACAGTAAAAAAAAAAAAAA                              3444
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FIG. 2. **Nucleotide and predicted amino acid sequence of PTPBR7.** The deduced amino acid sequence of PTPBR7 is shown *below* the nucleotide sequence. The putative signal peptide sequence is *underlined*, the transmembrane domain is *double underlined*, and the approximate location of the putative PTP domain is *shaded*. The cleavage site of the signal peptide was estimated using a scoring method as described (43). Equally high scores (8.68 and 8.13) were observed when the C at position 21 and the S at position 23 were assigned the −1 position of the cleavage site. The C at position 21 showed a much lower score in the c-region than in the h-region (1.66 *versus* 7.02), whereas the S at position 23 showed a score in the c-region comparable with that in the h-region (4.86 *versus* 3.27). Thus, we estimated the −1 position of the cleavage site to be the S at position 23.

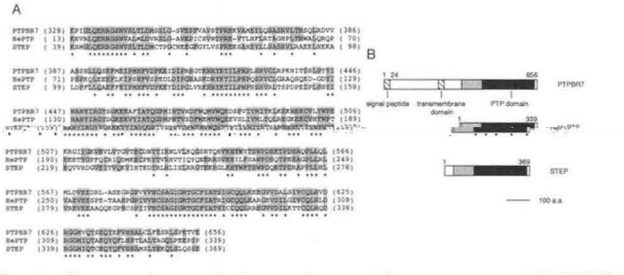

FIG. 3. **Comparison of the amino acid sequence of PTPBR7 with that of HePTP and STEP.** *A*, an alignment of PTPBR7 with two cytoplasmic PTPs, HePTP and STEP, is shown. The amino acid residues conserved in at least two of the three PTPs are *shaded*. The *stars* beneath the amino acid sequence indicate an amino acid residue identical in all three proteins. The *numbers* in *parentheses* indicate the amino acid position in the deduced protein sequences. *B*, schematic representations of PTPBR7, HePTP, and STEP. The *black box* indicates the PTP domain. The *shaded box* indicates the region of approximately 90 amino acid long, which shows high similarity. The *hatched box* shows the signal peptide or transmembrane domain as indicated. The *numbers* indicate the amino acid positions.

482–585). This sequence most likely represents a partial PTP domain sequence of PTPBR7.

PTPBR7 has only one PTP domain, whereas most receptor PTPs have two. So far, only three receptor PTPs, HPTPβ, DPTP10D, and SAP-1 (13, 14, 17, 40), are known to have one cytosolic PTP domain. These three PTPs have a large extracellular domain composed of FN-III repeats, and these structural features define the type III receptor PTP. We could not find apparent amino acid sequence similarity in the extracellular domain of PTPBR7 with HPTPβ, DPTP10D, SAP-1, or any other molecules in the NCBI data base. In the PTP domain, the similarity of PTPBR7 to HPTPβ, DPTP10D, or SAP-1 was lower than that to HePTP/LC-PTP or STEP.

Expression of the Extracellular Domain or the PTP Domain of PTPBR7 as a Chimeric Protein—The N-terminal sequence of PTPBR7 resembles a signal peptide. If the sequence were really a signal peptide, the putative extracellular domain of PTPBR7 would have the capability to translocate across the plasma membrane. To test this capability, we constructed a chimeric fusion gene, in which the cDNA encoding the extracellular domain of PTPBR7 was fused to hinge, CH2, and CH3 regions of the human IgG_1 heavy chain gene (39, 47) (Fig. 4*A*). This gene encodes a protein, PTPBR7-Fc, in which the extracellular domain of PTPBR7 (224 amino acids) was tagged with the immunoglobulin Fc portion (232 amino acids). The chimeric gene was transiently expressed in COS-7 cells, and PTPBR7-Fc was purified from either the culture supernatant or cell lysate using Protein A-Sepharose beads. PTPBR7-Fc was recovered mainly from the culture supernatant rather than from cell lysate (Fig. 4*B*, *lanes 3* and *6*), demonstrating the translocating

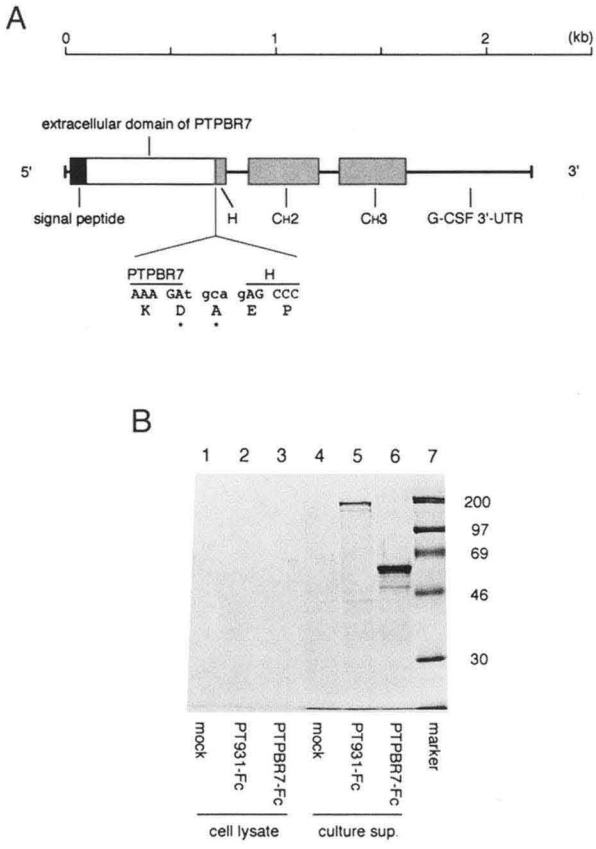

FIG. 4. **Expression and secretion of PTPBR7-Fc in COS-7 cells.** *A*, a schematic drawing of a chimeric gene encoding PTPBR7-Fc is shown. The nucleotide and amino acid sequences of the junction connecting the extracellular domain of PTPBR7 (nucleotides 346-1029) and the hinge region of human IgG_1 are shown *below*. Added nucleotides or amino acids to connect the sequences are indicated by *small letters* or an *asterisk*, respectively. *B*, the chimeric genes were transiently expressed in COS-7 cells. The fusion protein was prepared from cell lysate (*lanes 1–3*) or culture supernatant (*lanes 4–6*) and analyzed by SDS-PAGE under reducing conditions. *Lanes 1* and *4*, mock transfection (negative control); *lanes 2* and *5*, transfection with a construct encoding a mRPTP-σ-Fc, in which the extracellular portion of the receptor PTP (amino acids 1–1256) (53) was fused to the Fc portion of IgG_1 (positive control, ~190 kDa); *lanes 3* and *6*, transfection with a construct encoding PTPBR7-Fc; *lane 7*, marker. The size (kDa) of the marker is shown on the *right*.

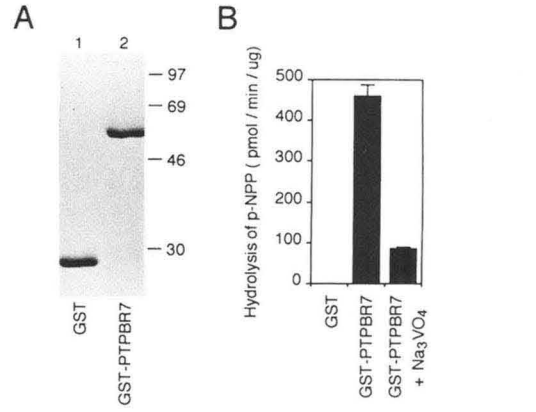

FIG. 5. **Catalytic activity of the GST-PTPBR7 fusion protein.** *A*, 2 μg of purified glutathione *S*-transferase or GST-PTPBR7 protein, which was used for the phosphatase assay, was electrophoresed on a 10% polyacrylamide gel and stained with Coomassie Blue. Molecular sizes (kDa) are shown on the *right*. *B*, catalytic activity of glutathione *S*-transferase or GST-PTPBR7 was measured at pH 7.4 as described under "Materials and Methods." In a reaction (*third column*), 3 mM Na_3VO_4 was included.

capability of PTPBR7-Fc across the plasma membrane. The apparent molecular mass of the major band was about 56 kDa on SDS-PAGE, whereas 48 kDa was expected for the core protein of mature PTPBR7-Fc. This minor difference could be explained by glycosylation, because there are possible glycosylation sites in both the extracellular domain of PTPBR7 (N at position 128) and the immunoglobulin Fc.

To test the catalytic activity of the putative PTP domain, we constructed a chimeric gene encoding a fusion protein, GST-PTPBR7, in which the putative PTP domain was fused C-terminally to glutathione *S*-transferase. The chimeric gene was expressed in *E. coli*, and the phosphatase activity was measured using *p*-NPP as a substrate (Fig. 5, *A* and *B*). The phosphatase activity was measured at pH 7.4, because, in the preliminary experiment, the activity detected with the pH 7.4 assay buffer was approximately 300-fold higher than that detected with the pH 5.0 assay buffer. The catalytic activity was inhibited by 3 mM Na_3VO_4, a tyrosine phosphatase inhibitor (Fig. 5*B*, *third column*).

Tissue Distribution of PTPBR7—Expression of PTPBR7 in various tissues and cell lines was studied by RNase protection assay. Radiolabeled antisense RNA, covering a part of the PTP domain, was used as a probe. Although PTPBR7 was expressed in both cerebellum and cerebrum, the expression level was higher in the cerebellum compared with the cerebrum (Fig. 6*A*). The expression of PTPBR7 was undetectable in thymus, spleen, bone marrow, kidney, liver, lung, and heart. As shown in Fig. 6*B*, the expression of PTPBR7 in cerebellum but not in liver was further confirmed by Northern hybridization analysis using a probe that did not contain the sequence of the PTP domain.

The expression of PTPBR7 was undetectable in all the cell lines tested, which included T-lineage (EL4 and 2B4), B-lineage (70Z/3 and NS-1), myeloid (WEHI3), mastcytoma (P815), thymic stroma (MRL104.8a), and fibroblast (BALB3T3) cell lines (Fig. 6*A*).

In Situ Hybridization Analysis of the Expression of PTPBR7—As described above, the PTPBR7 was expressed almost exclusively in the brain. To more precisely localize the expression in adult brain, we performed *in situ* hybridization analysis. Strong signals were detected in the Purkinje cells, and signals of weaker but above background level were observed in the granule cell layer in cerebellum (Fig. 7*A*). Outside the cerebellum, clear signals were detected in the habenula region (Fig. 7*B*), and weaker signals were detectable in the hippocampal formation (Fig. 7*C*). The higher level of expression of PTPBR7 in cerebellum agrees with the results of the RNase protection analysis described above. A probe for CD45, which is expressed exclusively upon cells of the hematopoietic system (reviewed in Ref. 48), was used as a control (Fig. 7, *D–F*).

DISCUSSION

We have cloned a cDNA of a novel PTP, PTPBR7, which is expressed exclusively in the brain. PTPBR7 possesses typical features of a type I membrane protein. There is a stretch of hydrophobic amino acid, a putative transmembrane domain. The N-terminal amino acid sequence deduced from the putative translation initiation site resembles a signal peptide. The capability of the extracellular domain of PTPBR7 to translocate across the cytoplasmic membrane was confirmed using a chimeric PTPBR7 tagged with immunoglobulin Fc (PTPBR7-Fc). Taken collectively, we conclude that PTPBR7 belongs to the receptor PTP family.

The extracellular domain of PTPBR7 shows no apparent structural similarity with known molecules, including reported receptor PTPs. Thus, PTPBR7 defines a new

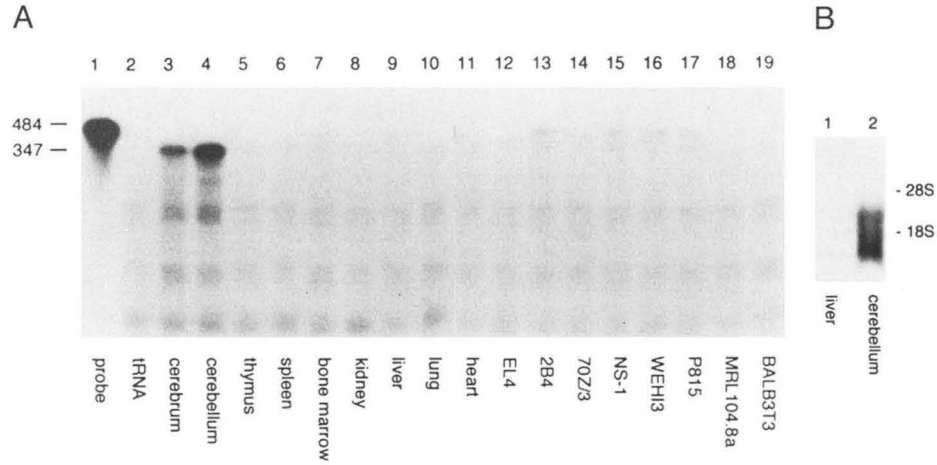

FIG. 6. **RNase protection analysis of the expression of PTPBR7 in various tissue and cell lines.** *A*, 10 μg of total RNA from indicated organs or cell lines was hybridized to the ^{32}P-labeled antisense RNA probe, coding for a part of the PTP domain (nucleotides 1755–2122). The size of the undigested probe is 426 nucleotides, and protection of PTPBR7 transcripts yields a 348-nucleotide fragment. For each RNA sample, an aliquot was electrophoresed on a 1% agarose gel, and intactness of the RNA was confirmed by visualizing 18 and 28 S rRNA bands (data not shown). 200 cpm of undigested probe (*lane 1*) and yeast tRNA (*lane 2*) were included as controls. The positions of the markers are indicated on the *left*, and the *numbers* indicate the size (in nucleotides). *B*, 4 μg of poly(A)$^+$ RNA immobilized on filters was probed with ^{32}P-labeled cDNA fragments covering nucleotides 122-1538 of PTPBR7. Positions of murine 28 S (4.7 kb) and 18 S (1.9 kb) rRNAs are indicated on the *right*.

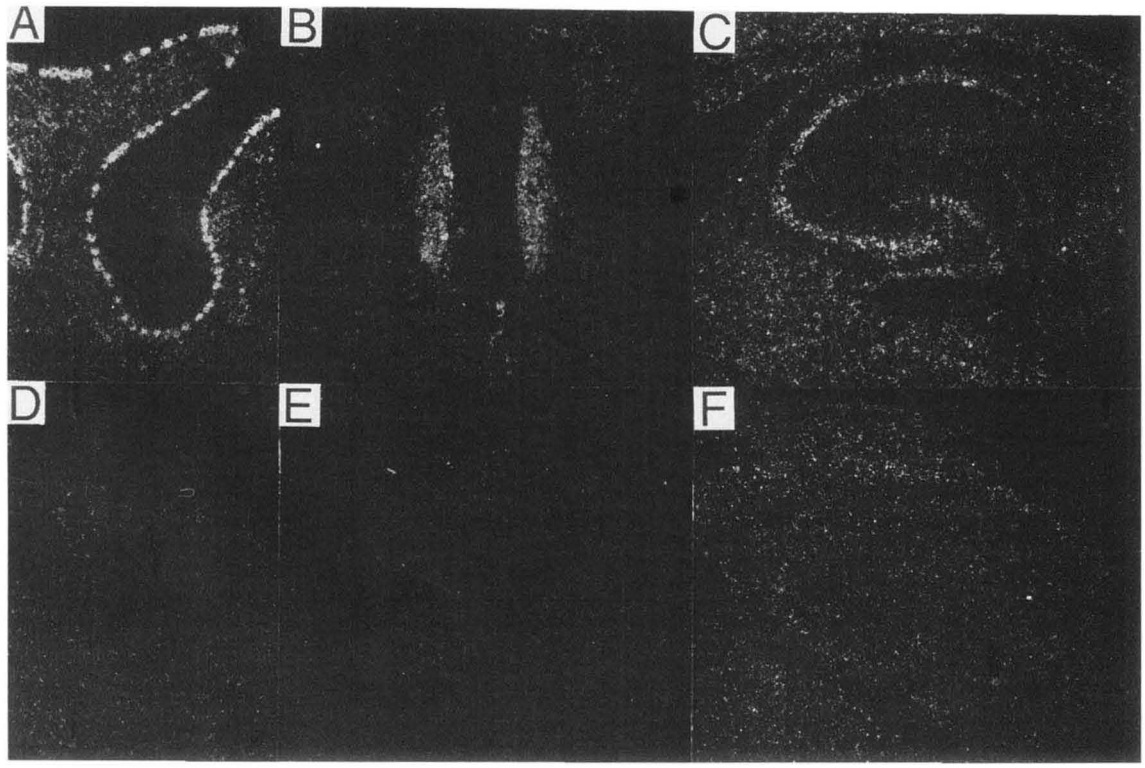

FIG. 7. **In situ hybridization analysis of the expression of PTPBR7.** Horizontal 10-μm sections of adult mouse brain were hybridized with a ^{35}S-labeled antisense RNA probe of PTPBR7 (*A–C*). A probe for CD45, which is specifically expressed on hematopoietic cells, was used as a negative control (*D–F*). *A* and *D*, cerebellum; *B* and *E*, regions of the habenula; *C* and *F*, regions of the hippocampal formation.

subfamily of receptor PTPs.

Data base searches revealed that the cytoplasmic portion of PTPBR7 has high similarity with previously reported PTPs, HePTP/LC-PTP (45) and STEP (46), in the PTP domain and in a short stretch (approximately 90 amino acids) preceding the PTP domain. Both HePTP and LC-PTP are specifically expressed in lymphoid cells and show an extremely high sequence similarity. The minor differences in the reported sequence of HePTP and LC-PTP could be explained as allelic variation of the same gene. STEP is expressed predominantly in striatum. Based on the differences in the sequence and in the tissue distribution pattern, it is clear that PTPBR7 is not the murine homologue of human HePTP/LC-PTP or rat STEP.

HePTP/LC-PTP and STEP are, unlike PTPBR7, non-receptor PTPs. This is surprising, because usually PTPs with high homology in the PTP domain have a similar overall structure. The reported sequences for HePTP and STEP are clearly not the partial cDNA sequences of receptor PTPs, because there are in-frame stop codons preceding the initiation codon. Interestingly, LC-PTP and STEP have two kinds of transcript, which are different in size, and the reported cDNA sequences are in good accordance with shorter transcript (2.9 kb for LC-PTP and 3 kb for STEP) in size. One attractive hypothesis is that the longer transcripts (4.0 kb for LC-PTP and 4.4 kb for

STEP), generated by alternative splicing or different usage of the transcription initiation sites, are encoding receptor forms. So far, there is no evidence for the presence of receptor forms of HePTP/LC-PTP and STEP.

Several PTPs, for example, DLAR, DPTP10D, and DPTP99A in *Drosophila* (13, 14, 49) and RPTPβ/PTPζ (9, 27) and STEP (46) in mammals, are expressed primarily in the nervous system. Among these PTPs, RPTPβ/PTPζ has been reported as the first cloned receptor PTP of which expression is restricted to the nervous system. PTPBR7 is a new example of a receptor PTP primarily expressed in the nervous system.

There is an apparent overlap in the expression of PTPBR7 and RPTPβ/PTPζ (9, 27). Both are expressed in Purkinje cell layer of cerebellum and the hippocampal formation. The differences between PTPBR7 and RPTPβ/PTPζ in the structure might result in different functions of these PTPs in a neuron. For example, the difference in the extracellular domains could imply a difference in (putative) ligand-specificity. In addition, PTPBR7 has one cytoplasmic PTP domain, whereas RPTPβ/PTPζ has two. The catalytic activity of the second PTP domain of receptor PTPs is usually low or absent, and its regulatory role or binding to tyrosine-phosphorylated proteins has been proposed. (6, 19, 50, 51). Thus, PTPBR7 lacking the second PTP domain might be involved in a signal transducing pathway different from that of RPTPβ/PTPζ.

The physiological role of PTPs in the nervous system remains to be elucidated. Because tyrosine phosphorylation by PTKs plays a critical role in the proliferation, survival, and differentiation of neuronal cells, PTPs might have regulatory function through dephosphorylation of the target protein. We found that PTPBR7 is expressed predominantly in the cerebellum. Interestingly, one of the highest levels of PTK activity in the brain has been found in the cerebellum (3). It was also reported that nerve growth factor, a ligand of a receptor-PTK, has effects on the survival or morphogenesis of Purkinje cells (52).

It is also possible that PTPBR7 is primarily active in immature nervous systems rather than in adult brain. During embryonic development, receptor/ligand systems seem to be involved in the specific guidance of migrating neurons and outgrowing axons, neuron survival, and proliferation. For example, three transmembrane PTPs, DLAR, DPTP10D, and DPTP99A are expressed on axons of developing nervous systems in *Drosophila*, and their involvement in axon outgrowth and guidance has been suggested (13, 14). Therefore, it would be important to investigate the expression of PTPBR7 more precisely in adult and developing nervous systems as well as to determine the distribution of PTPBR7 within a neuron, *i.e.* whether it is expressed in the cell body, axon, or both.

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