A novel receptor-type protein tyrosine phosphatase with a single catalytic domain is specifically expressed in mouse brain

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Protein tyrosine phosphatases (PTPases) are important regulatory proteins that, together with protein tyrosine kinases, determine the phosphotyrosine levels in cell signalling proteins. By PCR amplification of mouse brain cDNA fragments encoding the catalytic domains of these enzymes, we identified three novel members of the PTPase gene family. Northern-blot analysis showed that two of these novel clones represent brain-specific

PTPases, whereas the third originates from a large-sized mRNA that is more ubiquitously expressed. A full-length cDNA encoding one of the brain-specific PTPases, PTP-SL, was isolated. Sequence analysis revealed a transmembrane PTPase containing a single catalytic phosphatase domain that has 45 % homology to a rat cytoplasmic brain-specific PTPase named STEP. This suggests a role for PTP-SL in cell–cell signalling processes in the brain.

INTRODUCTION

Protein phosphorylation, the transfer of phosphoryl groups from ATP on intracellular proteins, is recognized as a key event in the transduction of extracellular signals [1,2]. Subsequent removal of phosphoryl groups from target proteins by protein phosphatases has long been considered a housekeeping process without any regulatory role in cell signalling. However, for many different organisms, such as *Yersinia* [3], yeast [4], *Dictyostelium* [5,6], *Drosophila* [7] and mouse [8,9], recent experiments have now revealed important roles for protein tyrosine phosphatases (PTPases) in the regulation of development and cellular proliferation. As a consequence, it has now become clear that the interplay of kinases and phosphatases is crucial for actuating cellular responses to extracellular signals. Structural information on PTPases has accumulated rapidly over the last few years, and many have been found to be transmembrane receptor-type molecules [10–12]. These enzymes thus represent a novel repertoire of signalling proteins the precise role of which in cell physiology, growth control and pathogenesis still has to be determined.

To obtain the molecular tools for further study of the bioactivity of PTPases in mouse development, we have cloned several PTPase sequences from murine brain employing degenerate oligonucleotide primers and PCR techniques. A novel PTPase (PTP-SL) was found and its full-length cDNA was isolated and characterized.

MATERIALS AND METHODS

PCR and subcloning of PTPase domains

Degenerate oligonucleotide primers 1 and 2 were based on consensus sequences for highly conserved amino acid stretches within the catalytic domains in PTPases: primer 1, 5'-GA(C/T)TT(C/T)TGG(A/C)(A/G/T)(A/G)ATG(A/G)T(A/C/T)TGG(G/C)A-3'; primer 2, 5'-C(G/T)CCC(A/T)(A/G)-C(A/G/C/T)CC(A/T)GC(A/G/C/T)CT(A/G)CAGTG-3'. DNA of a mouse brain cDNA phage library (Stratagene) was used as a template for PCR. Primers 1 and 2 (final concentration 7 ng/μl) were added to a 100 μl reaction mixture containing

20 mM Tris/HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 0.01 % BSA, all four dNTPs (each at 250 μM), 2 units of *Taq* polymerase (Perkin–Elmer Cetus) and 10 ng of phage DNA. Thirty-five cycles were performed on a Perkin–Elmer thermal cycler; each cycle involved incubation at 94 °C for 0.5 min, at 37 °C for 0.5 min and at 72 °C for 1 min. The PCR products were treated with proteinase K as described previously [13]. After DNA end-repair using Klenow (large fragment) DNA polymerase, the fragments were 5'-phosphorylated with T4 polynucleotide kinase and ATP, and analysed on a 1.5 % low-gelling-temperature agarose gel. Fragments of 350–400 bp were excised and subcloned into the *Sma*I site of plasmid pBlueScript by standard protocols [14]. Sequences were determined using the double-stranded DNA dideoxy sequencing method [15].

Isolation and sequencing of mPTP13 cDNAs

The mPTP13 PCR fragment was isolated, labelled radioactively by random priming [16], and used to screen a mouse brain λ-ZAPIII cDNA phage library (Stratagene). Hybridization conditions were those of Church and Gilbert [17]: membranes were preincubated in hybridization buffer [7 % SDS, 0.5 M sodium phosphate buffer (pH 7.4), 1 mM EDTA] for 10–60 min at 65 °C. After probe denaturation and addition, membranes were hybridized overnight at 65 °C. Washing at high stringency [0.1 % SDS, 0.04 M sodium phosphate buffer (pH 7.4), 1 mM EDTA] was performed three times at 65 °C for 20 min. Autoradiography was on Kodak X-Omat S1 films at –70 °C for 1–2 days using Dupont Cronex intensifying screens. Positive phages were plaque-purified and inserts were rescued as pBlueScript SK plasmids according to the manufacturer's protocols. Nucleotide sequences were determined using the DNAase shotgun strategy [18] in combination with the double-stranded DNA dideoxy sequencing method [15]. DNA sequence gel readings were recorded, compared, edited and assembled using the IG-SUITE 5.35 package (Intelligenetics, Mountain View, CA, U.S.A.). Deduced protein sequences were analysed using the GCG package [19] and the SIGSEQ program [20] provided by the Dutch CAOS/CAMM Center.

Figure 1 Alignment of deduced amino acid sequences from PCR-derived mouse brain PTPase cDNA clones

The number above the alignment indicates the relative position within the region which starts immediately after the sequence DFWRM(I/V)W(E/D) found in most PTPase domains. The region ends just before the stretch that contains the essential cysteine residue [HCSAG(V/I)GR] in the catalytic domain. The number of gaps introduced was kept to a minimum and the gap around position 62 was introduced between amino acid residues the corresponding triplets of which are located in different exons of the *LCA* gene [25,26]; the other two gaps were arbitrarily chosen. The positions at which ten or more PTPase domains share identical residues are shown on a shaded background. mPTP1B is identical with part of PTP-1, the mouse homologue of PTP1B [27,28]. mLRP-1 and mLRP-2 were identical with the two LRP phosphatase domains [29,30]. mPTPδ-1 represents the first phosphatase domain of MPTPδ [31]. mPTPε-1 is identical with PTP-ty8 [27], the mouse homologue of the first phosphatase domain in human HPTPε [32]. mLAR-1 and mLAR-2 very closely resemble the first and second phosphatase domain respectively in human [33] and rat [34] LAR. Likewise, mBPTP-1 is the mouse homologue of human BPTP-1 [35] and rat rPTP-2 [36]. PTPNE-3 [37], PTPσ [38], CPTP1 [39] and PTP-P1 [40]. mPTPβ is the homologue of HPTPβ [32], and mPTPε-2 strongly resembles the second phosphatase domain in human HPTPε [32]. Six of these cDNA fragments have been published [41]. Finally, three clones (mPTP13, mPTP14 and mPTP38) are different from the phosphatase domains currently published.

RNA isolation and Northern-blot analysis

Total RNA was isolated from various sources by the LiCl/urea method [21] and stored as alcohol precipitates. Selection of polyadenylated [poly(A)⁺]RNA on oligo(dT)-cellulose was as described previously [14]. Poly(A)⁺ RNA (2 µg) was resolved on a 2.2 M formaldehyde/1% (w/v) agarose gel, transferred to Hybond-N⁺ membranes (Amersham) [14] and fixed by u.v. irradiation (Stratalinker type; Stratagene). PCR-derived PTPase inserts, labelled by random priming [16], were used as probes. Hybridization conditions were as described above. Autoradiography was on Kodak X-Omat S1 films for 1–4 days at –70 °C using Dupont Cronex intensifying screens.

Assay of phosphatase activity

A 1.9 kb *Sma*I–*Xho*I fragment (starting at nt. 866) of clone mPTP13-6 was treated with Klenow large-fragment DNA poly-

merase to create a blunt-ended fragment encoding the last 294 amino acids of PTP-SL. This fragment was cloned into the *Sma*I site of pGEX-2T [22] to create pGEX-SL. *Escherichia coli* DH5α (Gibco-BRL) transformed with pGEX-SL or the empty vector pGEX-2T were grown in 10 ml of Luria broth containing 100 µg/ml ampicillin until *A*₅₅₀ reached 0.7, and were induced with 0.1 mM isopropyl thiogalactoside for 4 h at 37 °C. Bacteria were transferred to Eppendorf tubes, pelleted, washed once with 200 µl of cold buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA), and resuspended in 250 µl of buffer (150 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄, pH 7.3) containing 1% Triton X-100 and 0.4 mM phenylmethanesulphonyl fluoride. After the addition of 25 µl of lysozyme solution (10 mg/ml), the mixture was incubated for 15 min at 37 °C. After three freeze-thaw cycles, 2 µl of DNAase I (1 µg/µl in 1 M MgCl₂) was added to the lysate and the mixture incubated for 15 min at 37 °C. After centrifugation (10 min at 10000 g) the supernatant was transferred to a new tube and protein concentration deter-

mined as described by Bradford [23]. Lysate supernatants containing glutathione S-transferase (GST) or GST-SL fusion protein were used to determine tyrosine phosphatase activity with the colorimetric substrate *p*-nitrophenyl phosphate. Reactions were performed with lysate amounts and substrate concentrations that showed a linear reaction velocity with respect to time. Each 200 μ l of reaction mixture contained 50 mM imidazole, pH 7.0, 5 mM *p*-nitrophenyl phosphate, 10 mM dithiothreitol, 10 mM EDTA and 0.5 μ g of protein. Potential modifiers of phosphatase activity were added to some reaction mixtures. Reaction mixtures were incubated for 30 min at 37 °C and the reaction was terminated by adding 800 μ l of 0.2 M NaOH. Absorbance was measured at 410 nm. Two phosphotyrosine-containing peptides were also used in a non-radioactive phosphatase assay carried out as described by the manufacturer (Boehringer-Mannheim).

In vitro translation

The mPTP13-6 cDNA clone was linearized with *Xho*I and used for *in vitro* transcription with T3 RNA polymerase (Pharmacia) according to the manufacturer's protocols. RNA (200 ng) was heated for 10 min at 67 °C and added to a rabbit reticulocyte lysate (Promega) for *in vitro* translation in the presence or absence of canine pancreatic microsomal membranes (Promega) as described by the manufacturer. The [³⁵S]methionine-labelled products were analysed by SDS/PAGE on a 10% polyacrylamide gel [24] and visualized, after treatment with dimethyl sulphoxide/2,5-diphenyloxazole and drying of the gel, by autoradiography on Kodak X-Omat S1 film for 16 h.

RESULTS

Cloning of three novel PTPase cDNA fragments

To isolate murine cDNA clones encoding PTPases, we designed degenerate oligonucleotide primers based on sequences conserved in the core region of the catalytic phosphatase domain of most known PTPases. Using mouse brain cDNA as template, 29 clones with PTPase domain-like inserts, grouped into 13 different sequences, were identified (Figure 1). Computer-assisted comparison with published PTPase domain sequences was performed to reveal the possible identity of the clones. Five sequences (mPTP1B, mLRP-1 and -2, mPTP δ -1 and mPTP ϵ -1) contained regions that were identical with reported murine PTPases [27–31]. Five sequences (mLAR-1, mLAR-2, mBPTP-1, mPTP β and mPTP ϵ -2) probably represent mouse homologues of human or rat PTPases [32–39] for which no mouse clones have been identified until now. Finally and most interestingly, three clones with only weak homology to currently published phosphatase domains were found: mPTP13, mPTP14 and mPTP38. Still, the deduced protein sequences for these three clones clearly display the consensus features of tyrosine phosphatase domains (Figure 1). We conclude therefore that these sequences represent new PTPase family members.

To investigate the nature and expression patterns of the transcripts from which these three novel PTPase cDNA fragments originate, PCR fragments were used as probes on Northern blots containing RNA samples of various murine tissues. The mPTP13 probe hybridized to mRNA species of 4.1 and 3.2 kb in adult brain. A very weak signal of the 4.1 kb mRNA was detectable in 13.5-day embryos (Figure 2a). Also mPTP38 is exclusively expressed in brain, and originates from a single 4 kb mRNA species (Figure 2c). No hybridizing signal could be obtained with RNA isolated from 13.5-day embryos. Southern blots from different digests of murine genomic DNA yield distinct hybrid-

Figure 2 Northern-blot analysis of mPTP13, mPTP14 and mPTP38 mRNA

Poly(A)⁺ RNA samples from adult mouse brain (lane 1), skeletal muscle (lane 2), heart (lane 3), spleen (lane 4), pancreas (lane 5), liver (lane 6), lung (lane 7) and kidney (lane 8), as well as from a Balb/c mouse keratinocyte cell line (lane 9) and 13-day mouse embryos (lane 10) were loaded. The cDNA fragments mPTP13 (a), mPTP14 (b) and mPTP38 (c) were used as probes (Figure 1). (d) Ethidium bromide-staining pattern of the RNA gel. The 28S and 18S ribosomal bands, corresponding to 4.6 and 1.9 kb transcript lengths respectively, are indicated.

ization patterns for both probes (results not shown), indicating that the two PCR fragments indeed represent independent members of the PTPase family.

In contrast, mPTP14 is more broadly expressed. Weak signals corresponding to an mRNA size of around 8 kb are found in RNA isolated from 13.5-day embryos and brain, muscle, heart and kidney of adult mice. A very strong signal was obtained using murine keratinocyte RNA (Figure 2b). Sequence comparison showed the highest homology for mPTP14 with the PTPases MEG [42] and PTPH1 [43] (29% and 31% identity respectively). MEG and PTPH1 contain one catalytic phosphatase domain and an N-terminal stretch with significant similarity to band 4.1, ezrin and talin. This class of proteins is thought to mediate interactions between integral plasma-membrane proteins and the cytoskeleton [44]. However, the length of the mPTP14 transcript (8 kb) is significantly larger than those reported for MEG and PTPH1 (4–4.4 kb) and probably accommodates a much larger protein product. Isolation and elucidation of the full-length PTP14 cDNA sequence is needed to reveal the

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1 GCTAGCTCCA GGTGACTAAA CGAGGCTTCA GCTCGCAGGC TTCTGATTA GCCCAGCTAG CAATGGTAA GAGTAACAA GAAGAACA GATGGAAGTC GG
103 ATGCATCGCAACTCGGTCAGTTTCCACA CCGACACTGCAATGGATATAACCAAGCTG AACATAACCTGCTTCGGATCTTCGCCCAA GGAGTAGCTGCAGCCCTGGGACTCTTACCT
1 M H R N T R S V S T P T L 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
223 CAGCAAGTGACATTAAACGGCTCATTGAA AAGAAGAACCGGTTGAGTTGTTGTGTCT CCCGAAACCGGAAACAGGAGAAACGCAG GCCCTGCAGGCTGAGGAAGTGCTCGTTC
41 Q Q V H I N R L I E K K N 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
343 CTCAATGTGGATGGTTTGCATCAGAGTTTA CCACAGTTTGAATTACAGACTGCGCCCT GAGAAAAATGTTTACAAGGCGAGCAGAA GCAGACAAGATCTGGAGCAAGAAGGATT
81 L N V D G L H Q S L P Q F G I T D C A P E K N V L Q G Q H E A D K I W S K E G F
463 TACGCTGCTGTCATCTTCTCAGCATCTT ATCATCATAGTAACCTGTTTGATGATTAT TACAGGTAAAAGAAAGGCTTCAGCTTTC TTAAGACAAGATAAAGAGAAAAACAGGAG
121 Y A V V I F L S I F I I I 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
583 ATCCACCTATCCCATTCACGCGCAGCAA GCACAATCGGAGGCCAAGACGACCCACAGC ATGGTCCAGCCGATCAGGCGCCAAAGGTG CTGAACGTGGTTGTGGACCTCAAGGCCAA
161 I H L S P I A R Q Q A Q S 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
703 TGCACCTCTGAGATTGGAACAGCACCTCC ACCTCTGCTGCTCCCTTCTCCCTCAGAAATG AAGCCATAGGACTCCAGGAGCGACGAGGT TCCATGTATCTCTTACGTGGACATGAGT
201 C T P E I R N S T S T S V 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
823 AGCCTGGGCGAGTGGAAACCTTTGTGGCC GTCTCAACCCCCGGGAGAAGTAGCCATG GAATACCTGCAGTCAGCCAGCGAGTTCTC ACACGGTCACAGCTGAGGAGCGTCTGGCA
241 S L G S V E P F V A V S T 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
943 AGTTCACCTACTTCAAAGTGAATTCATG GAAATACCAATGAATTTGTGGATCCAAA GAAATGTATATCCACGTACGGAACATAA AATCGTTATAAGACCAATTTGCCAAATCC
281 S S H L L Q S E F M E I P 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
1063 CTCAGCAGAGTGTGCTTAAGACCAAAAAAT ATAACCGATTCCCTGAGTACTTACATAAT GCTAACTATATCGGGGCTACAGTGGTAAG GAGAAAGCCTTCAATGCCACCCAGGCCCC
321 L S R V C L R P K N I T D 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
1183 ATGATCAACACTGTGAATGACTTTCGGCAG ATGGTGTGGCAAGACAGCTCCCGTATT GTGATGATCAGGAACTCAAGAGAAAAAT GAGAAATGTGCTCTACTGCGCAGAAAG
361 M I N T V N D F W Q M V W 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
1303 ACAGCGATTACGCGCAGGTTGAGGTTCTG GTACCGGTTGTGACCAATGTGATAACTAC ACCATCCCAACCTCTCTTAAGCAAGGA ATTCACACCAACATGTGAAGCACTACTG
401 R G I Y G K V E V L V T G 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
1423 TACACTTCATGGCCGATCATAAGACTCCA GACAGTGCCAGCCCTTCTGCACTCATG TTGGATGTGGAAGAGACAGACTGBCCTCT GAAGCCGAGGCGCTGTGGTTGTCTCACTGC
441 Y T S W P D H K T P D S A 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
1543 AGTGCGGGGATTGGGAGAAGTGGGTGTTTC ATCGCTACATCCATTGGCTGTCAACAATTG AAAGAAGAAGGAGTTGTAGCGCACTAAGT ATTGTCTGCCAGCTTCGTGTAGACAGGGGT
481 S A G I G R T G C F I A T 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
1663 GGTATGGTCCAACAGCGAGCAGTATGAA TTTGTGCACCATGCTCTGTGCTGTTTCAG AGCAGACTTTCACCAGAACTGTGAGTGA CTCCGAAG ATTTACCAGA
521 G M V Q T S E Q Y E F V H H A L C L F E S R L S P E T V E *
1771 GTGCAATCT CTCACCGGT GATTATCAAA ATTACCTACC AAGAGCTCCA AGAGGGGCTC CTTGCAATGG ACGAGGAGGC TCTAAAGCCA GCCTAAGCCA CTGATTGTGG
1881 AAGATCTGGC AACATGAAAG ATTGCCAGCC TTTGTGTATA GGAATGCGTT CGTAGGCATC CCCCAGTTA TTCTGAAGGT CTTGTGCTGA TGGAGGCATG ACAGTCTCT
1991 CGCAGCCTGA GGAGGGTTTT GTTCTGTCTG TGTGCTCT CCGCCGACA GAATCGATGT GTGTGATAT CAGAGACTGG AGGAGCTGCT GACATTATCC TGTGTTTGA
2101 TGCTTTAATG TTCATAAAGC CTGCTCTGTG ACTGGACTGT CAGGTGTCCA ACTGTCCCTG TTGTAAGTC TATTAATAT CTCAGTTACC AGAATCTTGC TGCTCAAGTT
2211 TCGAGTGATC GACAATGGAT TTTTAACCAA GACATTTTGT TTTTGGAAA AAAAAAAAAA ATCAATGCA GTGACTATTT TATATGGACA TGTGCTTGA TTATATCTAT
2321 TAAGTGTGCA TTATAGTATC TCCCTATGGG ATCAATTACA GAGCACAATG ACTGTCCGTT GGATATATAT GTATATGAT ATGTATATTA TATGTATATG TACACATATA
2431 TATTCTATTA TTAGGCATAC AAGTGGCTCC TGTTCACAAA TACTAATAC TATATTCTA CATTTGACT TGTTTACTT TAAAGGGGA TAAACAAAT TATAGCAACT
2541 ATAAAGTATC AATAATTTTA AATATCATC TCTTTTTA TAAGAAGGA TTTTAAATA TGGTGTCTAC CTGGTATCTC TCTCCCAATA AGAAGCAGGT GCCTTTGGGG
2651 AATGCTCAA CCTGTTTCAT CCCCAAAAGT TTTCCGAGG AACTGTCTAT GCACGACCTC ACATTTCTAA AAGACCTCT TCTCAAGAG ACAAATGAAG GCAAAATAAT
2761 CACACTCAGC AAACAGGAGA TGTTCTGCTC AGATATGAAT TTTCAATGCA GCAAAGCTGA CTTTCTTTCG TACTCCCAAT AAATGACTCT TAACAGTA

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Figure 3 Complete nucleotide sequence of mPTP13-6 and deduced amino acid sequence of PTP-SL

The asterisk indicates a stop codon. The four putative translation initiation codons discussed in the text and the preceding in-frame stop codons are underlined. The signal sequence (Sig), transmembrane domain (TM) and poly(A)⁺ signal (pA) are indicated by open boxes. The arrow marks the putative cleavage site within the signal sequence. The sequence present in mPTP13 is shown on a shaded background.

nature of this novel PTPase. While this manuscript was in preparation, Maekawa and co-workers [45] published a human sequence with close homology to mPTP14. They predict an open reading frame which contains a band-4.1-like domain and, in addition, GLGF repeats.

Analysis of mPTP13 cDNA

Northern-blot analysis had suggested the possibility of developmentally regulated alternative splicing for the mPTP13 cor-

responding gene. To obtain full-length cDNA clones, a mouse brain cDNA library was screened with the PCR-derived mPTP13 fragment as a probe. Eleven overlapping clones were obtained and the longest insert (2.9 kbp) present in clone PTP13-6, was sequenced completely. All other clones were shown by end-in sequencing to represent shorter versions of the same transcript species. The nucleotide sequence and deduced amino acid sequence are shown in Figure 3. An open reading frame is found that spans 549 residues and is preceded by several in-frame stop codons. Therefore we conclude that mPTP13-6 represents a full-

Figure 4 Analysis of PTP-SL *in vitro* translation products

PTP-SL mRNA, produced by *in vitro* transcription of mPTP13-6 using T3 RNA polymerase, was translated in a rabbit reticulocyte lysate in the presence of [35 S]methionine. Protein products were analysed by SDS/PAGE and visualized by autoradiography. Lane 1, PTP-SL; lane 2, luciferase as a control (61 kDa). Molecular-mass markers are indicated on the left.

length cDNA clone of the smaller (3.2 kb) transcript found in adult mouse brain. None of the other isolated mPTP13 clones seemed to represent cDNA copies of the larger transcript.

Comparison of the open reading frame in PTP13-6 with all currently available mammalian PTPase sequences shows closest resemblance (45% amino acid identity over the segment displayed in Figure 1) to the brain-specific rat PTPase STEP [46]. Therefore we propose the name PTP-SL (STEP-like PTPase) for the protein encoded by mPTP13-6. STEP is expressed within dopaminergic neurons of the basal ganglia and related structures and appears in three cytoplasmic isoforms of molecular mass 46, 37 and 33 kDa [47]. From analogy with the STEP protein, the methionine at position 181 (nt 643–645) could be the first amino acid residue of PTP-SL. Three other potential translation start sites are present upstream of this AUG codon in mPTP13-6 (Figure 3). *In vitro* translation of mPTP13-6-derived RNA revealed a protein with an apparent molecular mass of 60 kDa (Figure 4), demonstrating that it is either the first (nt 103–105) or, more likely, the second (nt 145–147) AUG codon that is used as the translation start codon (resulting in 62 and 60 kDa proteins respectively).

A single conserved tyrosine phosphatase catalytic domain, which contains the highly conserved sequence VHCXAGXXR, is found at the C-terminus of PTP-SL. To demonstrate that PTP-SL indeed has tyrosine phosphatase activity, a fragment encoding the last 294 residues was cloned into the bacterial expression vector pGEX-2T [22]. The predicted 60 kDa recombinant GST fusion protein was detected on SDS/PAGE when transformed bacteria were grown in the presence of isopropyl thiogalactoside (results not shown). The fusion protein was predominantly present in inclusion bodies, but still contributed to about 1% of the Triton X-100-soluble protein fraction. With *p*-nitrophenyl phosphate as substrate, the GST–SL fusion protein, but not GST alone, displayed significant phosphatase activity which appeared to be sensitive to known PTPase inhibitors such as vanadate and phenylarsine oxide [48]. PTP-SL activity was not inhibited by Zn^{2+} under the conditions used or by the serine/threonine phosphatase inhibitor okadaic acid (Table 1). Furthermore two different phosphotyrosine-containing peptides could be dephosphorylated by the GST–SL fusion protein as determined using a non-radioactive assay (results not shown), clearly demonstrating the phosphotyrosine phosphatase activity of PTP-SL.

Table 1 Phosphotyrosine phosphatase activity of GST–SL fusion protein in the presence or absence of various potential inhibitors

All assays were performed in triplicate. An activity of 100% corresponds to an A_{410} of 0.301 after a 30 min incubation, as outlined in the Materials and methods section.

Protein	Modifier	Activity (% of GST–SL)
GST	–	< 1
GST–SL	–	100
GST–SL	0.5 mM vanadate	40
GST–SL	2.5 mM vanadate	5
GST–SL	0.1 mM phenylarsine oxide	42
GST–SL	0.1 mM ZnCl_2	96
GST–SL	1.0 mM ZnCl_2	98
GST–SL	0.1 μM okadaic acid	97
GST–SL	1.0 μM okadaic acid	102

Computer analysis of the deduced PTP-SL protein sequence revealed a signal sequence and a hydrophobic transmembrane region, indicating that PTP-SL is a type-I transmembrane protein (Figure 3). To test whether the predicted signal sequence cleavage site [20] is used, an *in vitro* translation experiment was performed in the presence of pancreatic microsomes. Although the control protein (β -lactamase) was correctly processed, no change in mobility could be observed for PTP-SL (results not shown). Also the use of a truncated version of PTP-SL, made by introducing a stop codon after residue 260, did not show altered mobility as a result of signal-peptide cleavage. We can therefore conclude that either the PTP-SL signal sequence is not normally cleaved or the specific endopeptidase involved is not present or active in the microsomal fraction used. Future experiments employing peptide-specific antibodies will be necessary to reveal the precise processing and membrane orientation of PTP-SL.

DISCUSSION

Using degenerate oligodeoxynucleotides and PCR, probes were obtained for three novel putative PTPases. For one of these, mPTP13, Northern-blot analysis suggested developmentally regulated alternative splicing of the corresponding brain-specific primary transcript. A full-length cDNA was subsequently isolated from a mouse brain cDNA library, representing the smaller, 3.2 kb, messenger and encoding PTP-SL. In embryonic tissue only the larger, 4.1 kb, mRNA species could be detected with the mPTP13 probe (Figure 2a). The length and terminal sequences of the inserts from the different cDNA clones indicated that all originate from the shorter transcript. Using several different cDNA fragments as probes on Northern blots of murine brain mRNA, we attempted to reveal internal sequence differences between the two transcripts. All probes, however, gave identical hybridization patterns (results not shown) making it very difficult to speculate on the origin of the two hybridizing mRNA species. They might result from the use of alternative promoters, differential splicing or polyadenylation, or the expression of recently duplicated genes. Southern-blot analysis of mouse genomic DNA digests reveals a rather simple hybridization pattern (results not shown) arguing against the latter possibility. Alternative splicing is a more likely explanation because this phenomenon has been previously demonstrated for many PTPase genes. Interestingly, in rat brain samples, both a 4.4 kb and a more prominent 3 kb mRNA species are recognized by STEP-specific probes and these have been suggested to be the result of alternative splicing of transcripts derived from a single gene [46]. Clearly, a more

detailed characterization of the PTP-SL gene structure or cloning of the larger mRNA species will be necessary to establish the precise relationships between the two transcripts.

From the mPTP13-6 cDNA sequence it can be deduced that PTP-SL is a transmembrane receptor-type PTPase containing one conserved phosphatase catalytic domain. This is a rather unusual conformation as single phosphatase domains are mainly restricted to cytoplasmic PTPases, receptor-type PTPases consistently having two tandem repeated domains. Thus far, only two exceptions have been reported: the receptor-type PTPases HPTP β [32] and PTP-PS [40]. PTP-PS lacks the second phosphatase domain that is present in PTP-P1 because of alternative splicing of a single precursor transcript. In the light of this, it will be interesting to investigate whether the 4.1 kb transcript encodes a PTP-SL isoform with two phosphatase domains. The extracellular portions of PTP-PS, PTP β and PTP-SL are completely different. PTP-PS contains four fibronectin type-III repeats and three immunoglobulin-like domains. HPTP β has eight fibronectin type-III repeats. In contrast, PTP-SL has a relatively short extracellular part (Figure 3) which displays no obvious similarities to other proteins.

Many receptor-type molecules, such as the cytokine receptors, have cytoplasmic portions that do not appear to contain domains with recognizable catalytic functions. These ligand-binding molecules are thought to mediate the actual signalling effect via association, direct or indirect, with proteins that do contain such catalytic signalling functions [49]. PTP-SL has a rather short extracellular domain, like the transmembrane PTPases LRP/HPTP α and HPTP ϵ [32], and perhaps lacks ligand-binding capability. It is tempting to speculate that PTP-SL may be a catalytic component of an as yet unknown complex in which other transmembrane proteins contribute this ligand-binding feature. Future experiments will be aimed at identification of proteins that associate with PTP-SL in order to unravel its role in the functioning and development of the brain.

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