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# cDNA isolated from a human T-cell library encodes a member of the protein-tyrosine-phosphatase family

**(tyrosine phosphorylation/dephosphorylation/phosphatase/T-cell cDNA)**

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**ABSTRACT** **A human peripheral T-cell cDNA library was screened with two labeled synthetic oligonucleotides encoding regions of a human placenta protein-tyrosine-phosphatase (protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48). One positive clone was isolated and the nucleotide sequence was determined. It contained 1305 base pairs of open reading frame followed by a TAA stop codon and 978 base pairs of 3′ untranslated end, although a poly(A)$^+$ tail was not found. An initiator methionine residue was predicted at position 61, which would result in a protein of 415 amino acid residues ($M_r$, 48,400). This was supported by the synthesis of a $M_r$ 48,000 protein in an *in vitro* reticulocyte lysate translation system using RNA transcribed from the cloned cDNA and T7 RNA polymerase. The deduced amino acid sequence was compared to other known proteins revealing 65% identity to the low $M_r$ PTPase 1B isolated from placenta. In view of the high degree of similarity, the T-cell cDNA likely encodes a newly discovered protein-tyrosine-phosphatase, thus expanding this family of genes.**

Protein-tyrosine-phosphatases (PTPases; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) are a family of proteins described in the accompanying paper (1) that specifically remove phosphatate from tyrosyl residues in proteins. Two forms of the enzyme have been purified to homogeneity: a low $M_r$ enzyme ($M_r$, 37,000) from human placenta (PTPase 1B) and a high $M_r$ transmembrane receptor-linked glycoprotein ($M_r$, 180,000–220,000) designated the leukocyte common antigen, CD45, found in hematopoietic cells. The complete amino acid sequence of the low $M_r$ PTPase 1B is presented in the preceding article (1); sequences of human (2), rat (3), and mouse (4) CD45 were deduced from cDNAs isolated from lymphocyte libraries. CD45 contains in its cytoplasmic segment two ≈300-residue domains, I and II, which bear 37% and 32% sequence identity to PTPase 1B, respectively (5).

The precise role of protein tyrosine phosphorylation in mediating signal transduction, cell cycle, and transformation remains to be established. An essential component of these investigations will be to discern the effects of PTPases. For instance, injection of PTPase 1B into oocytes delayed maturation induced by insulin and blocked insulin-stimulated S6 peptide phosphorylation (ref. 6 and unpublished data). Likewise, CD45 was shown to effect T- and B-cell activation when conjugated to cell-surface antigens including CD2, CD3, CD4, and CD19 (7).

To further our understanding of the structure, regulation, and tissue distribution of PTPases, a cDNA was isolated from a human T-cell cDNA library. Synthetic oligonucleotides encoding conserved amino acid segments among the PTPase domains were used as probes under low-stringency hybridization conditions. This manuscript reports the identification of a single cDNA clone that encodes a low $M_r$ protein§ displaying 65% identity to the placenta PTPase 1B.¶

## MATERIALS

All restriction and modifying enzymes and *in vitro* transcription and translation systems were purchased from Stratagene. The human peripheral T-cell cDNA library (8) was provided by R. Perlmutter (University of Washington). Oligonucleotides were synthesized with an Applied Biosystems 380A DNA Synthesizer (Howard Hughes Medical Institute DNA Synthesis Facility, University of Washington). Radionucleotides were from NEN. Sequenase was provided by United States Biochemical.

**Screening of a cDNA Library with Synthetic Oligonucleotides.** A set of complementary overlapping oligonucleotides were synthesized for each of the protein segments Lys-120–Asn-139 and Gly-209–Phe-225 from the human placenta PTPase 1B (1). Prediction of the DNA sequence was based on the optimal codon choice for human amino acid sequence data (9). Oligonucleotides in set 1 (5′-AAGTGTGCACAGTACTGGCCGCAGAAGGAA-3′ and 5′-GTTGGTATCCTCAAAGATCATCTCCTTCTCTTCCTTCTGC-3′) or in set 2 (5′-GGTCCTGTGGTGGTGCACTGCAGTGCTGGT-3′ and 5′-AAGGTTCCAGTGCGCCAATACCAGCACTG-3′) were annealed and labeled using [$\alpha$-$^{32}$P]dATP and [$\alpha$-$^{32}$P]dCTP and the large fragment of DNA polymerase I (Klenow fragment), producing radioactive double-stranded DNA with a specific activity of $4 \times 10^7$ cpm/pmol bp.

λgt10 recombinant cDNA phage (500,000) prepared from human peripheral T-cell poly(A)$^+$ mRNA (8) was plated at a density of 50,000 phage per plate. Duplicate nitrocellulose filter lifts were taken from each plate and hybridized with the oligonucleotide probes ($2.5 \times 10^6$ cpm per filter) in 20% formamide/5× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate)/2.5× Denhardt's solution (10)/1 mM sodium pyrophosphate/50 mM sodium phosphate buffer, pH 6.8, at 37°C for 18 hr. The filters were washed in 2× SSC/0.2% SDS at 42°C and subjected to autoradiography for 3 days with an intensifier screen at −70°C.

**DNA Sequence Analysis.** An *Eco*RI fragment isolated from a recombinant phage was ligated into the Bluescript plasmid vector (Stratagene) using T4 DNA ligase under reaction conditions suggested by the supplier. The DNA in the ligation mixture was transfected into an *Escherichia coli* host (XL-

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Abbreviation: PTPase, protein-tyrosine-phosphatase.
‡To whom reprint requests should be addressed.
§The protein encoded by this cDNA is designated here as the T-cell PTPase; however, the protein itself has not been isolated and directly demonstrated to possess intrinsic PTPase activity.
¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25393).

Blue, Stratagene) and colonies containing the correct DNA inserts were selected and amplified. Closed circular plasmid DNA was purified by CsCl density centrifugation. The DNA (1 μg) was denatured and hybridized to synthetic deoxyribonucleotide primers (15-mers). The DNA sequence was determined by the chain-termination method of Sanger *et al.* (11) using the Sequenase reaction mixtures recommended by the supplier.

***In Vitro* Translation of T-Cell PTPase mRNA.** The Bluescript plasmid containing the T-cell cDNA was made linear by *Hin*dIII restriction endonuclease digestion. mRNA was synthesized *in vitro* from 1 μg of plasmid DNA using the T7 polymerase promoter and Stratagene's assay condition. The DNA was degraded with DNase I and the RNA was purified. The mRNA (1 μg) was added to 20 μl of a rabbit reticulocyte translation system in the presence of [$^{35}$S]methionine and protein synthesis was allowed to proceed for 30 min. The control reaction mixture contained mRNA produced from linearized vector. The products were analyzed on a 10% SDS/polyacrylamide gel (12) and subjected to autoradiography for 18 hr.

**Northern Blot Analysis.** Total RNA was extracted (13) from monkey brain, spleen, and thymus; human RNA was from placenta and T cells. Poly(A)$^+$ mRNA was purified by oligo(dT) column chromatography as described (10). Poly(A)$^+$ mRNA (10 μg) from brain, spleen, thymus, and placenta and 20 μg of the total T-cell mRNA were subjected to Northern blot analysis using $^{32}$P-labeled cDNA insert from the T-cell clone as a probe. The hybridization conditions were the same as those described for the screening of the library except that

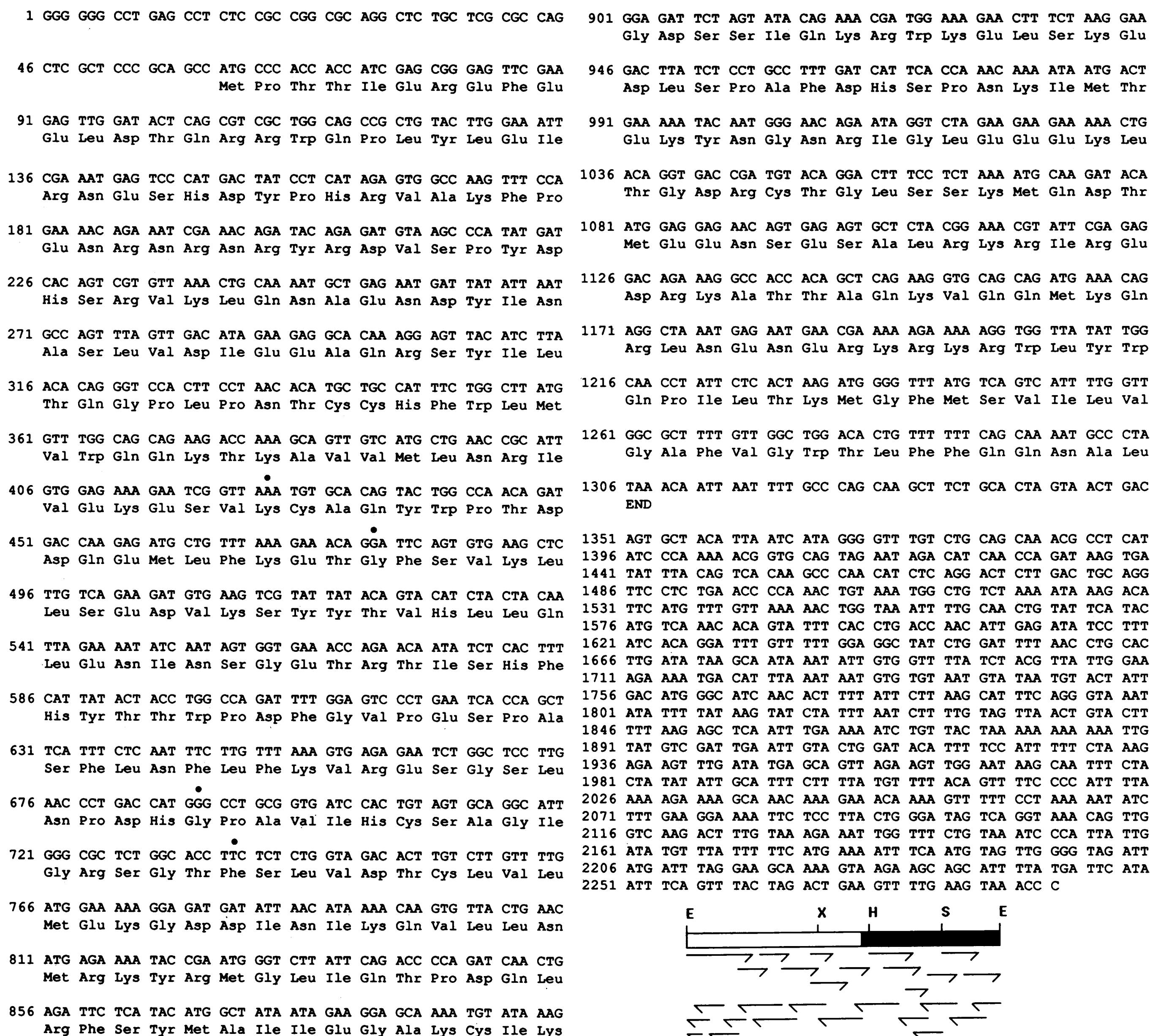


FIG. 1. Sequencing strategy, nucleotide, and deduced amino acid sequence of human T-cell cDNA T-cell PTPase. The predicted amino acid sequence of the open reading frame is shown below the nucleotide sequence. The oligonucleotide sequences used for screening the library are indicated by dots [e.g., between nucleotides 425 and 479 (probe 1), and 689 and 737 (probe 2)]. The TAA stop codon is located at nucleotide 1306 followed by a 3′ untranslated end containing two possible polyadenylylation sites AATAAA at 1521 and 1677. The schematic diagram below the nucleotide sequence depicts the sequencing strategy used. Open bar, open reading frame; solid bar, 3′ untranslated end. Arrows indicate the length of sequence obtained from different sequencing oligonucleotide primers. E, *Eco*RI; H, *Hin*dIII; S, *Sst* I; X, *Xba* I. The scale at the bottom represents 200 nucleotides (in kbp).

the blot was washed in 0.1× SSC/0.2% SDS at 50°C. The gel was exposed to film for 3 days with an intensifier screen at −70°C.

**Southern Blot Analysis.** Human genomic DNA was cleaved with the restriction endonucleases *Bam*HI, *Eco*RI, and *Hin*dIII. The blot was hybridized to the labeled insert of the cDNA and washed as described for the Northern blot analysis and subjected to autoradiography for 3 days with an intensifier screen at −70°C. It was then reprobed with the labeled cDNA using the same hybridization conditions as above, but washed under less stringent conditions, such as 2× SSC/0.2% SDS and 45°C.

## RESULTS

**Isolation and DNA Sequence Analysis of a Human T-Cell cDNA Encoding an Isoform of Placenta PTPase 1B.** Two synthetic $^{32}$P-labeled oligonucleotides representing different segments of the low $M_r$ placenta PTPase 1B were used as probes to screen duplicate plaque lifts containing 500,000 recombinant phage from a λgt10 cDNA library prepared from human peripheral T-cell mRNA (8). Although many recombinant phage hybridized to each probe, only one overlapping positive clone bound to both oligonucleotides. Restriction enzyme analysis of the purified recombinant cDNA revealed a single *Eco*RI cDNA insert 2.3 kilobase pairs (kbp) long (Fig. 1). The entire nucleotide sequence of the *Eco*RI fragment was obtained in duplicate on both strands. The sequencing strategy is shown in Fig. 1; short regions of DNA sequence represented by the overlapping arrows were obtained with different primers in the extension reactions. Sequence analysis (Fig. 1) shows that the T-cell PTPase cDNA contains an open reading frame of 1305 nucleotides. A consensus sequence [CC($^{A}_{G}$)CCAUG(G)] for eukaryotic initiation sites described by Kozak (14) is found at nucleotides 56–64 encoding a putative initiator methionine. The open reading frame terminates with a TAA stop codon followed by 978 bp of 3′ untranslated end. However, neither a polyadenylylation site nor a 3′ poly(A)$^+$ tail was observed. There are two possible AATAAA polyadenylylation signals (15) at sites 213 and 369 bp past the stop codon (nucleotides 1521–1526 and 1677–1682, respectively).

**Identification of an Initiator Methionine Residue.** mRNA was synthesized *in vitro* from the T7 polymerase promoter in the Bluescript vector in which the T-cell cDNA was subcloned. Using the rabbit reticulocyte lysate translation system, a protein product with an estimated $M_r$ of 48,000 was produced (Fig. 2). Since its apparent $M_r$ agreed closely with that predicted from the cDNA sequence, it is probable that the putative initiator methionine at nucleotide 61 is being recognized as a translation initiator codon in the *in vitro* system. The translation reaction was carried out in the presence of [$^{35}$S]methionine and the band of labeled protein was excised and counted. The amount of protein synthesized, estimated at 2.5 pg, was not sufficient to detect PTPase activity under our assay conditions (16).

**The T-Cell cDNA Sequence Is Present in Other Tissues.** Northern blot analysis of mRNA extracted from monkey brain, spleen, and thymus and human T cell and placenta reveals multiple bands of hybridization (Fig. 3). The most abundant transcript of ≈2.3 kb was found in all the above tissues, although the level of expression in brain was quite low. Comparison of the thymus poly(A)$^+$ mRNA with the T-cell total mRNA shows at least a 20-fold enrichment of the transcript. The predominant message, whose precise length cannot be determined in the agarose gel, seems likely to represent the T-cell PTPase cDNA since the expected length of this transcript is at least 2.5 kb including a 200-base poly(A)$^+$ tail (17).

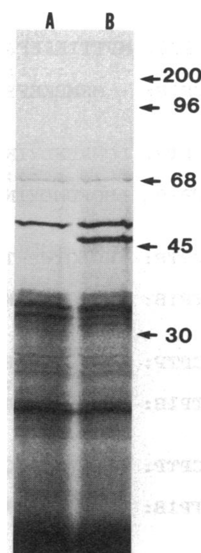


FIG. 2. SDS/PAGE analysis of the $^{35}$S-labeled protein following translation *in vitro* from T-cell PTPase mRNA. Capped mRNA (1 μg) synthesized from the T7 polymerase promoter in the Bluescript plasmid vector containing the T-cell cDNA (or the plasmid alone) was added to 25 μl of a rabbit reticulocyte *in vitro* translation system and incubated for 30 min at 30°C in the presence of [$^{35}$S]methionine. The extract (10 μl) was boiled with 20 μl of 2× sample buffer and a third of the sample was applied to a 10% SDS/polyacrylamide gel, according to Laemmli (12). The newly synthesized $^{35}$S-labeled proteins were detected after 16 hr of autoradiography. Lanes: 1, control containing mRNA synthesized from the Bluescript vector DNA; 2, from vector containing T-cell PTPase cDNA. Molecular weight markers (×$10^{-3}$) are indicated by arrows and numbers.

The Northern blot also reveals other species of poly(A)$^+$ mRNA that are of higher molecular weight than those described above. Poly(A)$^+$ mRNA from the various tissues all have a hybridizing transcript at the 28S marker not observed in the total T-cell RNA; this 4.7-kb species could encode a high $M_r$ transmembrane molecule related to CD45. Also, a very high molecular weight message (>7 kb) detected in all the poly(A)$^+$ purified samples but not in total RNA could be due to LAR, which has a transcript of 8 kb (18), or other as yet unreported PTPases.

**Relationship Between the T-Cell PTPase and the Low Molecular Weight Placenta Enzyme.** The preceding article (1)

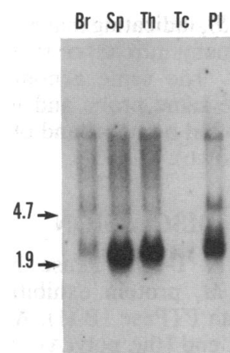


FIG. 3. Detection of the T-cell transcript in tissue. Ten micrograms of poly(A)$^+$ mRNA from monkey brain, spleen, or thymus and from human placenta mRNA and 20 μg of total human T-cell RNA were subjected to electrophoresis in a 1% formaldehyde-agarose gel and transferred to nitrocellulose paper. The RNA blot was hybridized with $^{32}$P-labeled cDNA insert from T-cell PTPase and subjected to autoradiography for 3 days. Br, brain; Sp, spleen; Th, thymus; Tc, T cell; Pl, placenta. The 28S and 18S ribosomal bands are indicated and correspond to 4.7- and 1.9-kb transcript lengths, respectively.

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                                                      ↓
TCPTP:  MPTTIEREFEELDTQRRWQPLYLEIRNESHDYPHRVAKFPENRNRNRYRDVSPYDHSRVK   60
          :.::: .:    :  .: .:: :  :.: :::: : :.::::::::::::.:::::.:
PTP1B:    MEMEKEFEQIDKSGSWAAIYQDIRHEASDFPCRVAKLPKNKNRNRYRDVSPFDHSRIK   58

TCPTP:  LQNAENDYINASLVDIEEAQRSYILTQGPLPNTCCHFWLMVWQQKTKAVVMLNRIVEKES  120
        : . .::::::::.  ::::::::::::::::::: ::: ::: ::...:::::::. :: :
PTP1B:  LHQEDNDYINASLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVVMLNRVMEKGS  118

TCPTP:  VKCAQYWP-TDDQEMLFKETGFSVKLLSEDVKSYYTVHLLQLENINSGETRTISHFHYTT  179
        .:::::::   .. ::.: .:    . :.:::.::::::  : :::. . ::: : ::::::
PTP1B:  LKCAQYWPQKEEKEMIFEDTNLKLTLISEDIKSYYTVRQLELENLTTQETREILHFHYTT  178

TCPTP:  WPDFGVPESPASFLNFLFKVRESGSLNPDHGPAVIHCSAGIGRSGTFSLVDTCLVLMEKG  239
        ::::::::::::::::::::::::::: :.::: :.::::::::::::: : ::::.:::.:
PTP1B:  WPDFGVPESPASFLNFLFKVRESGSLSPEHGPVVVHCSAGIGRSGTFCLADTCLLLMDKR  238
                                               ↓
TCPTP:  DD---INIKQVLLNMRKYRMGLIQTPDQLRFSYMAIIEGAKCIKGDSSIQKRWKELSKED  296
         :    . :: ::: :::.::::::: ::::::: :.::::: : ::::.:  ::::: ::
PTP1B:  KDPSSVDIKKVLLEMRKFRMGLIQTADQLRFSYLAVIEGAKFIMGDSSVQDQWKELSHED  298

TCPTP:  LSPAFDHSPNKIMTEKYNGNRIGLEEEKLTGDRCTGLSSKMQDTMEENSESALRKRIRED  356
        : :  .: :       :
PTP1B:  LEPPPEHIPPPPRPPKRILEPHN                                       321

TCPTP:  RKATTAQKVQQMKQRLNENERKRKRWLYWQPILTKMGFMSVILVGAFVGWTLFFQQNAL   415
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FIG. 4. Alignment of amino acid residues for T-cell PTPase and placenta PTPase 1B using the single-letter amino acid code. The arrows demarcate the 236-residue core segment found in all PTPases described in the preceding manuscript (1).

defines the minimum segments of sequence similarity among various members of the family of PTPase-related proteins. Fig. 4 illustrates that the structural relationship between the two low $M_r$ proteins extends beyond this core structure toward the N and C termini. The alignment score (1) between the two proteins is very high (46.7). Percentage sequence identities for the three regions of the molecule are as follows: N terminus, 43; core structure, 72; C terminus, 46. When sequence similarities (defined as conservative substitution) are included, these percentages increased to 56, 84, and 51, respectively. Fig. 4 also supports the assignment made for the initiating methionine at nucleotide 61 in T-cell PTPase, which corresponds closely to the N-terminal methionine in PTPase 1B. The next possible methionyl residue is located well within the conserved core structure.

**Southern Blot Analysis of the T-Cell PTPase Gene.** A Southern blot of human genomic DNA from two individuals was hybridized with $^{32}P$-labeled cDNA insert from T-cell PTPase. Autoradiography of the blot revealed several bands of hybridization (Fig. 5), indicating that either the gene is very large (>70 kbp with many introns) or that there are multiple genes in this family. The same genomic DNA blot was rehybridized with the same probe and washed under low-stringency conditions but no new band of hybridization was detected (data not shown).

## DISCUSSION

A human T-cell cDNA, T-cell PTPase, has been described that encodes a low $M_r$ protein exhibiting 65% sequence identity to the placenta PTPase 1B (1). Although the cDNA has an incomplete 3′ end [the poly(A)$^+$ tail is missing] the T-cell PTPase probably represents most of the transcript since the predominant band detected in Northern analysis is approximately the same length as the cDNA insert. *In vitro* translation of the mRNA synthesized from the cDNA produces a single protein ($M_r$, 48,000), implying that the proposed initiator methionine residue can be recognized by the rabbit reticulocyte translation system. This result suggests that *in vivo* expression of the cDNA using eukaryotic vectors and transfection into culture cells should be possible.

The discovery in T cells of a PTPase 1B isoform expands the family of PTPase-related proteins of which we propose to consider the placenta enzyme as a prototype. It is anticipated that other cDNAs for members of this family of enzymes will be identified since multiple species displaying various enzymatic and physical properties were observed in kidney (19), placenta (16, 20, 21), spleen (22–24), and brain (25). Furthermore, Northern blot analysis reveals variation in abundance and size of mRNA transcripts in brain, placenta, spleen, and thymus. This family includes transmembrane proteins such as CD45 and LAR (18), a molecule homologous to CD45 but with a different extracellular domain related to the neural cell

FIG. 5. Southern blot analysis of the human T-cell PTPase gene. Restriction endonuclease-treated DNA (10 μg) from two individuals was transferred to nitrocellulose from a 0.6% agarose gel and probed with a $^{32}P$-labeled cDNA insert from T-cell PTPase. The blot was exposed to film for 3 days with an intensifier screen at −70°C. Lanes: 1 and 2, *Hin*dIII; 3 and 4, *Eco*RI; 5 and 6, *Bam*HI. Numbers indicate marker fragments generated from λ DNA cleaved by *Hin*dIII.

adhesion molecule N-CAM (26). The existence of multiple forms may suggest differences in substrate specificity and function.

The low $M_r$ placenta enzyme displays unusually high specific activity and affinity toward artificial substrates (16). By contrast, CD45 has only 1% of the activity of PTPase 1B under our standard assay conditions (24). This could be due to loss of activity during purification, differing substrate specificity, lack of a ligand binding to the external domain or, as in the case of the protein tyrosine kinases, to the fact that the receptor-linked forms seem to be intrinsically less active than their cytoplasmic counterparts (24). With 65% sequence identity between the two low $M_r$ proteins and up to 85% sequence similarity within the 236-residue core described in the preceding article (1) as shown in Fig. 4, it could be anticipated that the T-cell PTPase protein would exhibit similar enzymatic activity to the placenta enzyme. However, these two proteins display significant differences in their carboxyl termini. The placenta enzyme is smaller ($M_r$, 37,000 vs. 48,000) and ends with a segment in which 10 of 21 residues are prolyl. The T-cell protein has an extension ($M_r$, ≈ 11,000) with a highly charged segment (Lys-284 to Lys-390) followed by a region of 25 uncharged residues. It will be interesting to determine whether this C-terminal extension possesses some regulatory function such as exerting a negative influence on enzyme activity. Cleavage of such a structure in a posttranslational event or during purification might derepress the enzyme and contribute to the high specific activity of PTPase 1B. Alternatively, it would serve to localize the protein in specific intracellular elements or compartments.

The role of low $M_r$ PTPases in cell signaling, growth, and transformation is not known. They may be responsible for ensuring the transient nature of tyrosine phosphorylation events in response to certain external stimuli. Considering the discrepancy between the activity of the protein tyrosine kinases and phosphatases (20), it would be expected that the latter are under tight control or confined to specific compartments within the cell.

Many multigene families, such as those for the serine proteases (27, 28) or protein kinase (29), arose from duplications of an ancestral gene. These families can often be characterized by the degree of conservation of both the number and position of introns in the coding region (28, 30–32). The intron/exon gene organization has been described completely for CD45 (33) but only partially for LAR (18). Frequency and position of introns present in segments of the gene encoding the homologous cytoplasmic regions of CD45 and LAR are not totally conserved. For example, within CD45, there are seven introns in domain I and six in domain II, with evidence of intron sliding (34) in one of the conserved insertions. Furthermore, LAR has two fewer introns than CD45 in the PTPase-related cytoplasmic domains between exons III and VIII (18). Determination of the gene structure of T-cell PTPase may help to establish the evolutionary relationship between the low $M_r$ and the integral membrane proteins.

Cell signaling through certain hormone and growth factor receptors, or transformation by a number of oncogenic retroviruses involves the phosphorylation of proteins on tyrosyl residues. Overexpression of a PTPase should cause a perturbation of the system in favor of the dephosphorylated state and thus may help clarify the role of protein tyrosine phosphorylation in the control of cellular processes.

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