

Expression of a truncated protein-tyrosine phosphatase mRNA in human lung

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Protein-tyrosine phosphatases (PTPases) are becoming an important family of enzymes that might regulate key events in cell growth and transformation. While isolating a new member of this family via amplification of human lung cDNA by the polymerase chain reaction, we found a clone identical to but truncated at the 3'-end of the coding region of human PTPase β (HPTP β) mRNA. This difference in sequence is situated in the most conserved part of the catalytic domain of the enzyme. The expression level of the truncated form of HPTP β mRNA in human lung was lower than its normal form

Protein-tyrosine phosphatase; mRNA; Human lung; Polymerase chain reaction

1. INTRODUCTION

The discovery of a family of enzymes catalyzing the dephosphorylation of phosphotyrosyl residues is very recent. These protein-tyrosine phosphatases (PTPases) are probably regulators of protein tyrosine kinase activities (PTKases), the latter ones often being described as activators of cell proliferation. In view of the association PTKase-oncogene, PTPases could be defined as anti-oncogenes, although their role is probably more complex and general than this [1]. PTPase activities have been found in numerous mammalian tissues (for a review, see [2]), but our knowledge about their role is still very limited.

A cytoplasmic PTPase activity from human placenta has been purified and sequenced [3,4]. This 37 kDa protein revealed to be homologous to the cytoplasmic part of CD45, a leukocyte surface antigen [5]. Since then, screening of cDNA libraries with specific degenerate oligonucleotides or low-stringency screening with specific DNA probes have revealed a whole family of PTPases in several human tissues [6-10] and different species, including *Drosophila* [11-13].

All members of the PTPase family share at least one very conserved domain which contains the catalytic site

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Abbreviations: PTPase, protein-tyrosine phosphatase; PTKase, protein-tyrosine kinase; PCR, polymerase chain reaction; RT, reverse transcription; HPTP β , human PTPase β ; Δ 3'-HPTP β , HPTP β deleted in the 3'-end; bp, base pair

of the enzyme [14]. Until now no ligands are known to bind to the extracellular domains of the transmembrane PTPases, but the hypothesis for extracellular factors regulating PTPase activity seems plausible in analogy with receptor-PTKases. The extracellular part of the LAR protein (leukocyte common antigen related molecule, a transmembrane PTPase) contains several N-CAM (neural-cell adhesion molecule) and fibronectin type III-like domains which could play a role in cell to cell signalling [7].

Using degenerate primers based on conserved amino acid sequences in the catalytic domain, we isolated via PCR and library screening a PTPase sequencing from human lung cDNA identical to HPTP β but truncated near its catalytic site. We report here the natural occurrence of the normal and of this truncated form of HPTP β mRNA in normal lung tissue.

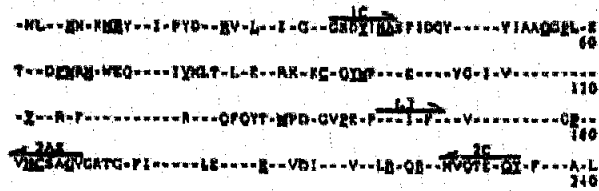
2. MATERIALS AND METHODS

2.1. Oligonucleotides and DNA purification

Oligonucleotides were synthesized on an Applied Biosystems 391 PCR-mate DNA synthesizer. Degenerate oligonucleotide primers to conserved sequences of the catalytic domain of the PTPase family were synthesized (Fig. 1a), followed by other primers that exactly match the determined sequence. One couple of oligonucleotides contained sequences complementary to M13 sequencing primers, which allowed rapid nucleotide determination of the fragment (Fig. 1b). A stock of λ gt11 DNA was prepared after amplification of a λ gt11 normal human lung cDNA library (Clontech Laboratories, USA), according to Maniatis et al. [15]. DNA was stored at -20°C in H_2O .

2.2. RNA purification

Postsurgical tissues were immediately N_2 -frozen and stored at -80°C . RNA was prepared from human lung and placenta by the guanidine thiocyanate/CsCl procedure of Chirgwin et al. [16].



Name	5' Sequence 3'	Fragment Length
1C	CG(N1)-UP17)GGIAGCTGAYTAYTCAATGC	sense 495 bp
2AS	CG(N1)-SSP15)ACICACGCACTGCANTOIAIC	antisense
L3	CACTCTCTGATCCAGTTCGTGAGA	sense 220 bp
2C	TATTCGTCTCTGTCGTGTGACCAT	antisense

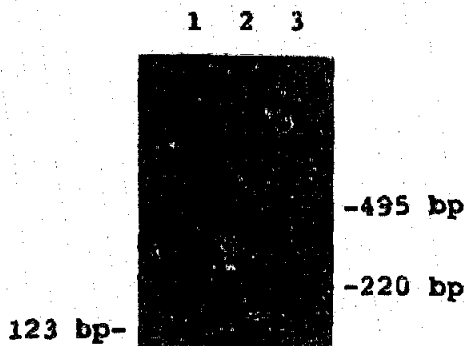


Fig. 1. (a) Consensus sequence of the PTPase core domain. The single-letter code is used. The 100% conserved amino acid residues are in bold type and underlined. Oligonucleotides synthesised are indicated above the amino acid sequence. This consensus sequence is adapted from Streuli et al. [11], the best one available at the time of oligonucleotide synthesis. (b) Sequence of oligonucleotides used for isolation of 495-bp and 220-bp fragments of HPTPβ. UP, universal primer; SSP, single stranded primer; Y, C, T 50% each, R, A, G 50% each; I, inosine. (c) 1.2% agarose gel stained with ethidium bromide. Lane 1: 123-bp DNA ladder, lane 2: the PCR-amplified 495-bp fragment from human lung cDNA library with primers 1C and 2A, lane 3: as in lane 2 but using primers L3 and 2C (220 bp).

Messenger RNA (mRNA) was isolated using and mRNA purification kit (Pharmacia France). All RNA stocks were stored at -80°C in the presence of 1 mM dithiothreitol and 40 U of RNasin.

2.3. PCR amplification

Primers (100 pmol) were used for PCR amplification of DNA isolated from a λgt11 cDNA library, performed in a Perkin-Elmer Cetus DNA thermal cycler. The 30-cycle program consisted of 1 min 94°C denaturation, 1 min 58°C annealing and 1 min 72°C extension steps. Amplified fragments were separated and purified by 1.2% agarose gel electrophoresis, excised and electro-eluted. Amplified DNA fragments were visualised in the gel by ethidium bromide staining.

2.4. DNA sequencing

Single-stranded DNA templates for sequencing were obtained by asymmetric PCR on purified fragments. Dideoxynucleotide chain-termination sequencing of single-stranded templates or fragments cloned in M13-mp18/19 vectors was performed using a T7 sequencing kit (Pharmacia) or the Gene-ATAQ kit (Pharmacia) for problematic templates [17]. Nucleotide sequences were analysed using PC-Gen software (Intelligenetics, Switzerland) coupled to the EMBL-SwissProt databank.

2.5. Library screening and hybridization

Screening of the cDNA library (50000 clones) was carried out using the ³²P-labeled 495-base-pair (bp) specific fragment (see Fig. 1c). Labelling was performed by the Random Priming method [18] (Amersham, France). High stringency hybridizations were performed overnight at 42°C in 50% formamide containing hybridization buffer [19]. Stringent washing was performed in 0.1 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at 60°C for 30 min. Autoradiography was done for 72 h at -80°C using intensifying screens. One positive clone was amplified by asymmetric-PCR and sequenced with λgt11-specific primers. For confirmation, the same clone was also inserted into phage M13 mp18/19 and sequenced.

2.6. Reverse Transcription-PCR (RT-PCR)

For reverse transcription, 3 μg of total RNA or 1 μg of mRNA were heated at 95°C in H₂O, 100 pmol of specific 3'-antisense oligonucleotide, 800 μM dNTP and reverse transcription buffer was added together with 200 U of MuMV reverse transcriptase (BRL, France) in a final volume of 20 μl. The reaction was incubated for 15 min at room temperature followed by 1 h at 42°C. The cDNA thus produced was directly submitted to PCR amplification by adding 100 pmol of a specific 5'-sense primer, Taq-polymerase buffer and 2.5 U of Taq polymerase (Beckman, France) in a final volume of 100 μl.

Fig. 2a

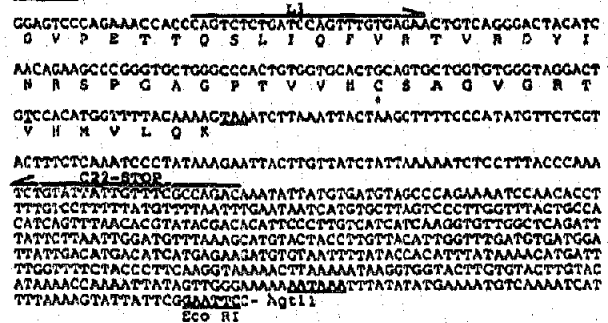


Fig. 2b

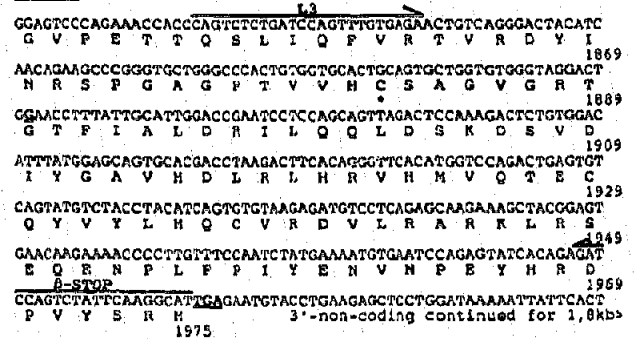


Fig. 2. (a) Nucleotide and deduced amino acid sequence of Δ3'-HPTPβ (clone C22:3'-end). Deduced amino acid sequences are shown under the nucleotide sequences using the single-letter code. The conserved amino acid residues of the catalytic site are in bold type and the indispensable cysteine residue marked with an asterisk. The first divergent nucleotide with HPTPβ, STOP codon and polyadenylation signal are in bold type and underlined. Positions of primers are above the nucleotide sequence. (b) As in (a) but for HPTPβ. Amino acid positions of the deduced protein, according to Krueger et al. [20], are shown to the right below each row.

3. RESULTS

Using a couple of degenerate primers (1C and 2A2) and purified cDNA of λ gt11 human lung library, a PCR-fragment of about 495 bp was produced. By using primers L3 and 2C, a second 220-bp fragment was found to contain the 3'-extension of the 495-bp fragment (Fig. 1c). Sequence analysis of these fragments revealed an open reading frame which contained the very conserved amino acids of the PTPase family together with new amino acid residues. The recent publication of Krueger et al. [20] showed that these fragments were identical to the cDNA sequence of the catalytic domain of HPTP β (Fig. 2b).

By subsequent screening of the λ gt11 human lung cDNA library with the 495-bp probe, we isolated a 1.2-kb clone (C22) that contained a good part of the 3' extremity of the HPTP β mRNA sequence. This cDNA clone contained an open reading frame of 627 nucleotides coding for 209 amino acids. The first 606 nucleotides and their corresponding 202 amino acids (up to residue Thr¹⁸⁹⁰ as in Krueger et al. [20]) were in complete agreement with the published deduced amino acid sequence of HPTP β . Interestingly, the nucleotide sequence of this clone showed an in-phase STOP codon (TAA) in the most conserved region of the PTPase family, near the putative catalytic site of the enzyme (Fig. 2a). Our deduced protein sequence lacked the 86 C-terminal residues of HPTP β (Gly¹⁸⁹¹-His¹⁹⁷⁵). The STOP codon was followed by a typical A/T-rich 3' non-coding sequence of 533 bp, whereafter the *Eco* RI cloning site of the λ gt11 vector was found. A putative polyadenylation signal sequence was located 40 nucleotides upstream of this *Eco* RI cloning site. The 3' non-coding sequence did not belong to any known mRNA (EMBL 21 databank, release 3.0, February 1990), neither to the PTPase family of mRNAs described until now.

Verification of the expression of the truncated type HPTP β mRNA, for which we propose the name Δ 3'-HPTP β , was performed by RT-PCR. This technique nowadays seems more powerful than classical Northern blot analysis to detect the presence of rare mRNAs, provided that PCR-amplified fragments are subsequently verified by nucleotide sequencing. We used antisense primers C22-STOP and β -STOP (see Fig. 2), respectively to the Δ 3'-HPTP β and HPTP β mRNA sequences, for reverse transcription followed by PCR with the L3 sense primer (Fig. 3a). Total RNA from human placenta and from human lung tissue was submitted to RT-PCR with the β -STOP oligonucleotide specific for the HPTP β C-terminal extremity and with a specific sense primer (L3). Fig. 3b shows the theoretically expected 368-bp fragment for HPTP β mRNA in both tissues, demonstrating for the first time the expression of this mRNA in human lung. We have always found a lower expression level in the latter

Fig. 3a

Name	5' Sequence 3'	Fragment length
C22-STOP	CTCTCCGAAACCATAATACAG	antisense 3' 245 bp
L3	CAGTCTCTGATCCAGTTTCTGAGA	sense 3' 368 bp
β -STOP	CGCAAGCTTATCCCTTCAATACACTGGATC Hind III	antisense

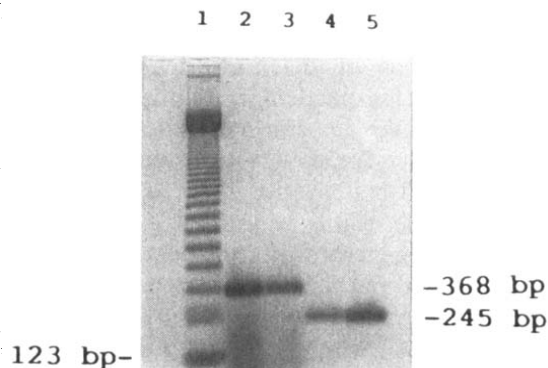


Fig. 3. (a) Sequences of oligonucleotides used for the isolation of 245-bp (Δ 3'-HPTP β specific) and 368-bp (HPTP β specific) fragments. The *Hind* III site in β -STOP is for subsequent cloning facilitation. (b) 1.2% agarose gel stained with ethidium bromide. Lane 1: 123-bp DNA ladder, lane 2: RT-PCR on 3 μ g human lung RNA with β -STOP and L3 primers, lane 3: RT-PCR on 3 μ g human lung RNA with β -STOP and L3 primers, lane 4: RT-PCR on 1 μ g human lung mRNA with C22-STOP and L3 primers, lane 5: PCR on clone C22 DNA with C22-STOP and L3 primers.

tissue. We only detected a very faint band of the expected size for Δ 3'-HPTP β mRNA (245 bp) in total RNA from human lung (not shown). By enrichment to purified mRNA from human lung tissue we were able to detect clearly the truncated Δ 3'-HPTP β mRNA (Fig. 3b). This result reflects the low level expression of Δ 3'-HPTP β mRNA in human lung. Nucleotide sequencing confirmed the presence of the (TAA)-STOP codon in this fragment. We also verified the presence of this sequence in the cDNA library by PCR amplification and nucleotide sequencing (Fig. 3b).

4. DISCUSSION

Although discovered much more recently, the PTPases are emerging as large a family as the PTKases. It is becoming clear that in the field of signal transduction PTPases will have to be considered as important as PTKases, with both types of enzymes taking part in the complex regulation of a proliferative signal. One example of such a regulatory mechanism is provided by a couple PTPase-PTKase in T-cells: the CD45 antigen activates the PTKase pp56^{lck} by dephosphorylation of its tyrosine-505 residue, activation of pp56^{lck} by CD45 appearing to play a crucial role in T-cell activation [21].

Until very recently it was believed that the PTPase family could be subdivided into two classes: transmembrane PTPases containing two repetitive homologous domains and cytoplasmic PTPases containing one such

domain. The recent finding of the HPTP β sequence, a transmembrane PTPase with only one homologous domain, makes this subdivision somewhat artificial [20].

The PTPase activity of HPTP β seems to be very high relative to other PTPases, as measured *in vitro* using phosphotyrosyl protein substrates [20]. The physiological substrates of this enzyme have not been found yet. Because HPTP β contains only one catalytic domain, mutations or deletions near the conserved catalytic site in this particular PTPase will probably dramatically change its enzymatic activity. This has already been shown for the LAR protein where PTPase activity was abolished by site-directed mutagenesis of the cystein residue in this region [14].

The use of degenerate oligonucleotide primers for PCR to conserved parts of the catalytic domain of the PTPase family revealed to be successful for isolating a new member of this family in human lung. Recently, by using the same approach, 11 different partial cDNA clones belonging to the PTPase family were isolated from human liver, including HPTP β [22].

The isolation of a variant HPTP β clone in a human lung cDNA library, truncated near the catalytic site of the enzyme, seems very important to us. We demonstrated the natural existence of this variant mRNA ($\Delta 3'$ -HPTP β) in a fresh RNA preparation of lung tissue by RT-PCR. The normal HPTP β mRNA is expressed at a higher level than the $\Delta 3'$ -HPTP β mRNA in lung tissue. Indeed, we noted a more intense band (ethidium bromide staining) for the 368-bp fragment (HPTP β) than for the 245-bp band ($\Delta 3'$ -HPTP β - hardly visible), both being amplified from the same amount of total RNA. In fact we needed 1 μ g of mRNA to clearly visualize the $\Delta 3'$ -HPTP β specific fragment.

As to the origin of $\Delta 3'$ -HPTP β mRNA, this might occur by alternative splicing in a way similar to leukocyte antigen related protein (LRP), where an alternate insertion was found to disrupt the first PTPase domain [12]. However it will be necessary to characterise this fragment on the genomic DNA level to know whether only one gene is expressed.

The physiological meaning of the difference in mRNA expression level between the two forms is still unclear, but if a difference in enzymatic activity between them also exists, the regulation of certain specific substrates intervening in cell growth might be disturbed. From these types of experiments it is hard to tell if both mRNAs are present at the same time in one cell, but 'in situ' hybridizations may answer this question.

One can hypothesize two ways about this truncated PTPase: either it has lost part or all of its enzymatic activity due to disruption of the catalytic site environment, or it has become constitutively active. It could be possible that part of the regulation of HPTP β activity resides in the C-terminal extremity through protein phosphorylation. We noted that a putative casein kinase II phosphorylation site is present on serine

residue 1949 of HPTP β [23]. We are actually analysing the PTPase activity of the $\Delta 3'$ -HPTP β catalytic domain in comparison with its HPTP β analog.

Another important research axis, which is part of our future studies, is to analyse the expression levels of the $\Delta 3'$ -HPTP β mRNA and protein in tumoral tissue compared to normal tissue.

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