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Molecular cloning and expression of a protein-tyrosine phosphatase showing homology with transcription factors Fos and Jun

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A cDNA clone coding for a protein-tyrosine phosphatase (PTPase) was isolated from a rat spleen cDNA library. Nucleotide sequence of the clone showed an open reading frame coding for a polypeptide of 363 amino acids. Expression of this clone in *E. coli* in an expression vector showed PTPase activity. The non-catalytic region of this PTPase located at the carboxy terminus shows homology with the basic domains of transcription factors Fos and Jun. Northern blot analysis showed that a 1.7 kb transcript was present in many tissues and cells, the highest level being in macrophages. This PTPase is a rat homolog of human T-cell PTPase although it shows 3 large deletions in the carboxy terminal non-catalytic region.

Protein-tyrosine phosphatase; cDNA cloning; Gene expression; Transcription factor

1. INTRODUCTION

Protein phosphorylation at tyrosine residues has been implicated as an important regulatory component in cell growth, differentiation, malignant transformation by certain viruses and in signal transduction [1–4]. The level of phosphorylation of substrate proteins is determined by the relative activities of protein kinases and protein phosphatases. The study of protein phosphatases in general has received much less attention as compared to that of protein kinases. This was mainly due to the belief that protein phosphatases simply reverse the effects of protein kinases by dephosphorylating the substrates constitutively. Recent findings suggest that this may not be the case with many protein-serine phosphatases as well as with PTPases [5,6], certain cell cycle genes and a transcriptional regulatory gene code for protein-serine phosphatases [5]. The CD45 molecule which is a receptor-like transmembrane protein, has been found to have PTPase activity [7,8]. These findings suggest that phosphatases themselves might play central and specific roles in cellular physiology. The role of non-receptor-type PTPases, their mode of regulation and the substrates on which they act are also not known. PTPase activity is widely distributed in various tissues [9,10]. Rat spleen and brain are a rich source of this activity [9,10]. The genes for several receptor-type PTPases have been isolated [11–15]. The non-receptor-type PTPases which have been analysed for their cDNA include placental PTPase 1b [16,17], PTPase 1 from rat brain [18] and a PTPase from T cells [19]. An essential virulence determinant of Yersinia (a bacterium which is a causative agent of plague) has been shown to be a PTPase [20]. Here we report the isolation of a cDNA clone coding for a PTPase of 363 amino acids which shows homology in its non-catalytic domain with the basic domains of transcription factors Fos and Jun which are required for binding to DNA.

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Abbreviations: PTPase, Protein-tyrosine phosphatase; PTP-S, PTPase cloned from a rat spleen cDNA library

2. MATERIALS AND METHODS

2.1. *Construction and screening of cDNA library*

A cDNA library was constructed in λgt11 from poly (A)$^+$RNA using cDNA synthesis and cDNA cloning systems obtained from Amersham. Total RNA was isolated by the acid guanidinium thiocyanate method [21]. Poly (A)$^+$RNA was prepared by two cycles of binding to oligo (dT) cellulose [22]. The unamplified cDNA library was screened [22] using nick translated *Eco* RI–*Pst* I fragment of T-cell PTPase cDNA [20]. T-cell PTPase cDNA was kindly provided by Dr D.E. Cool (University of Washington, Seattle, USA). The hybridization for screening the cDNA library was carried out at 60°C with 5 × SSPE (1 × SSPE = 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA), 0.1% SDS, 5× Denhardt's (0.1% Ficoll, 0.1% polyvinyl pyrrolidone, 0.1% BSA), 0.1 mg/ml denatured salmon sperm DNA [22]. Filters were washed at 50°C in 1 × SSPE, 0.1% SDS.

2.2. *DNA sequencing*

Nucleotide sequence of the cDNA inserts was determined using the dideoxy chain-termination method of Sanger [23] as modified for double stranded DNA by Chen and Seeburg [24]. The cDNA inserts released from λgt11 clones were subcloned in plasmid pGEM3Z for sequencing. The complete nucleotide sequence of the full length cDNA clone was assembled by sequencing various restriction fragments and smaller clones.

2.3. *Northern blotting*

Poly (A)$^+$RNA (5 μg) or total RNA (10 μg) was fractionated under denaturing conditions in formaldehyde gels and transferred to nitrocellulose [22]. Hybridization was carried out with 5 × SSPE, 5 × Denhardts, 0.1% SDS, 100 μg/ml salmon sperm DNA, 30% formamide at 50°C for 16 h. Blots were washed with 0.5 × SSPE, 0.1% SDS at 50°C and kept for autoradiography at −70°C with intensifying screens.

2.4. *Expression of PTPase in E. coli*

The plasmid PTP-S was digested with *Bam* H1 and filled in by using Klenow fragment of DNA polymerase I followed by religation. Transformation of competent *E. coli* cells (DH5α) gave a plasmid without *Bam* H1 site and extra 4 nucleotides. This was confirmed by sequence analysis. This plasmid was digested with *Hind* III and the *Hind* III fragment was electroeluted and ligated with the *Hind* III digested expression vector pKK233-2 (Pharmacia). The plasmid containing the insert in correct orientation (pKK-PTP) showed PTPase activity. Orientation of the insert was checked by restriction digestion. Assay for PTPase activity was carried out using ^{32}P-labelled poly (Glu, Tyr) as substrate as described previously [10].

3. RESULTS AND DISCUSSION

A rat spleen cDNA library (λgt11) was screened with the *Eco* RI–*Pst* I fragment (1,38 kb) of human T-cell PTPase cDNA. This fragment contains the entire coding region for PTPase. The screening of over 200 000 recombinants produced 3 positive clones, one of which (PTP-S) contained a full length cDNA insert of 1.5 kb (Fig. 1). The other two clones were smaller in size and corresponded to nucleotides 254–884 (clone 2) and 750–1287 (clone 3) of the largest clone. The nucleotide sequence showed an open reading frame coding for a polypeptide of 363 amino acids. The first ATG codon may serve as the translation initiation codon at position 26 although the sequence surrounding it does not match perfectly with the proposed translation start consensus sequence A/GNNATGG [25]. The open reading frame is terminated by a stop codon TAA at nucleotide 1115. A polyadenylation signal AATAAA is present at nucleotide 1368.

The PTP-S shows a high level of homology with human T-cell PTPase in the catalytic domain [19]. Out of the first 275 amino acids which include the catalytic domain, 261 are identical (95% homology) in the two proteins. This high level of homology suggests that PTP-S may be a rat homolog of T-cell PTPase. A comparison of the carboxy-terminal sequence of PTP-S in the non- catalytic domain with human T-cell PTPase (Fig. 2) shows that there are 3 large deletions after amino acid 288 (19 amino acids), 308 (5 amino acids) and 363 (28 amino acids). These carboxy-terminal differences are perhaps not due to cloning artefacts since one other independent clone also had the same sequence (clone 3, nucleotides 750–1287). The C-terminal differences between PTP-S and human T-cell PTPase may be due to alternative splicing.

In the carboxy-terminal region outside the catalytic domain, there is a region rich in basic amino acids. This basic region is organised as cluster–spacer–cluster in the same pattern as found in many sequence specific DNA-binding proteins which function as transcription factors [27]. An alignment of the carboxy-terminal 56 amino acids of the PTP-S with segments of the Fos [28] and Jun [29] proteins containing the basic domain is shown in Fig. 3. Out of 37 residues of Fos protein, 18 are identical with PTP-S and 5 other residues are similar in character. Jun protein shows 15 residues (out of 30) which are identical with PTP-S and 6 other residues are similar. The level of homology was also assessed by comparing the alignment scores obtained by using the program ALIGN as described by Dayhoff et al. [26]. These scores were similar for each of the 3 pairs; PTP-S with Fos (3.34), PTP-S with Jun (4.18) and Fos with Jun (3.49). Thus the level of homology between PTP-S and Fos (or Jun) is the same as between Fos and Jun in the basic regions. The gene coding for PTP-1 (or placental PTPase 1b) does not possess any such basic domains in the non-catalytic region of the molecule as shown in Fig. 2. However this basic region is conserved in human T-cell PTPase (Fig. 2.).

In order to confirm that this cDNA clone codes for enzymatically active PTPase we have expressed it in *E. coli*. The cDNA was cloned after appropriate modification (to align the reading frame) in *E. coli* expression vector pKK 233-2 which uses a hybrid promotor *trc*. The strategy for construction of pKK-PTP is described in section 2. The *E. coli* cell extracts containing control plasmid do not show any detectable PTPase activity whereas cells expressing pKK-PTP showed significant PTPase activity (Fig. 4). Complete dephosphorylation of the substrate could be obtained if time of incubation or the amount of extract was increased (not shown).

Expression of the transcripts for PTP-S was studied by Northern blot analysis of mRNA or total RNA isolated from various rat tissues. A major transcript of about 1.7 kb was observed in spleen, brain and thymus (Fig. 5A) and in some other tissues. The size of this transcript is in agreement with the size of the cDNA insert. When an identical RNA blot was analysed by a full length T-cell PTPase probe, a 1.7-kb transcript was observed; the result was same as that obtained with PTP-S probe (figure not shown). These results suggest that PTP-S is the rat homolog of human T-cell PTPase. The high level of homology between the two supports this conclusion.

The expression of PTP-S transcript was compared in splenic lymphocytes with that in peritoneal macrophages. Surprisingly macrophages showed a much higher level of PTP-S transcripts than lymphocytes (Fig. 5C). The results of expression studies suggest that this PTPase does not show tissue- or cell-type specificity although levels of transcription show variability.

In order to see the effect of an inhibitor of protein synthesis on the expression of PTP-S transcripts, rats were injected with cycloheximide (50 mg/kg body weight) and RNA was isolated from spleen and thymus

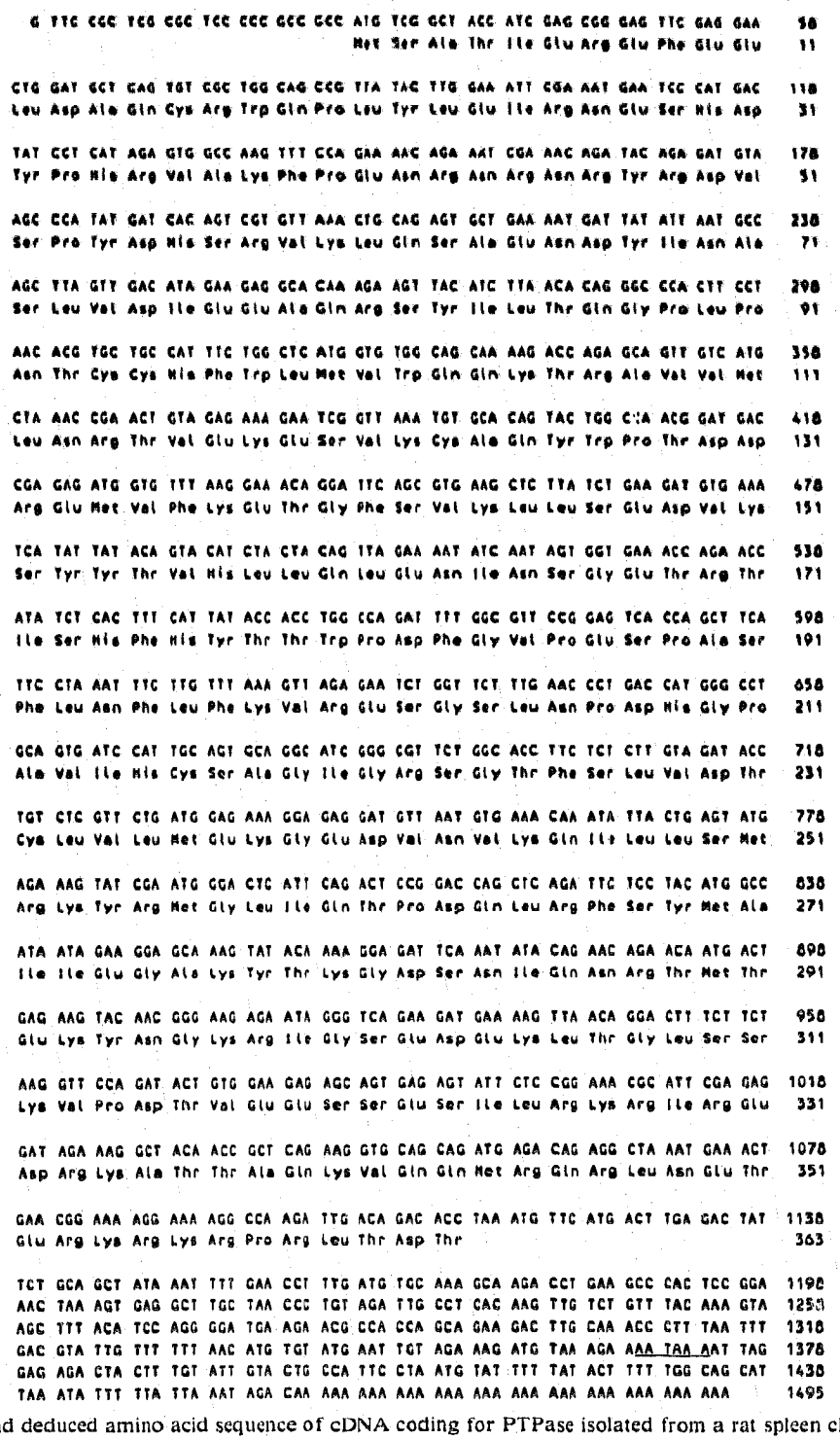

Fig. 1 Nucleotide and deduced amino acid sequence of cDNA coding for PTPase isolated from a rat spleen cDNA library (PTP-S).

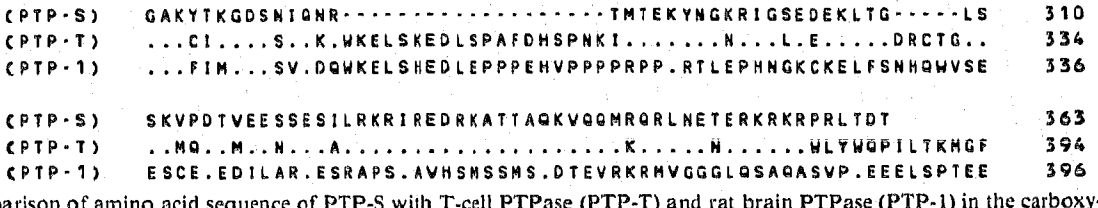

Fig. 2 A comparison of amino acid sequence of PTP-S with T-cell PTPase (PTP-T) and rat brain PTPase (PTP-1) in the carboxy-terminal non-catalytic region. Gaps introduced for alignment are indicated by dashes. Dots indicate identical residues.

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(PTP)  GLSSKVPDTVEESSESILRKRIREDRKATTAQKVQQMRQRLNETERKRKRPRLTDT

(Fos)  GRRGKVEQLSPEEEE---KRRIRRERNKMAAAKC-----------RNRRRE-LTDT

(PTP)  ESSESILRKRIREDRKATTAQKVQQMRQRLNETE-RKRKRPRL

(Jun)  ESQE-----RIKAERK--------RMRNRIAASKCRKRKLERI
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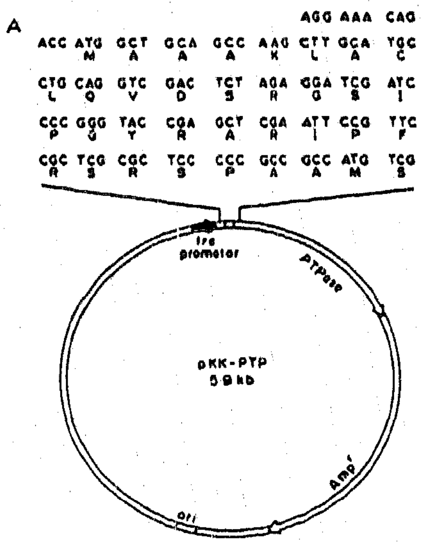
Fig. 3. Alignment of the carboxy-terminal 56 amino acids of PTP-S with the basic regions of Fos (124-164) and Jun (251-280) proteins. The alignment was done using the program ALIGN of Dayhoff et al. (26). Gaps are indicated by dashes. Two dots indicate identical residue. One dot indicates residues which are similar in character.



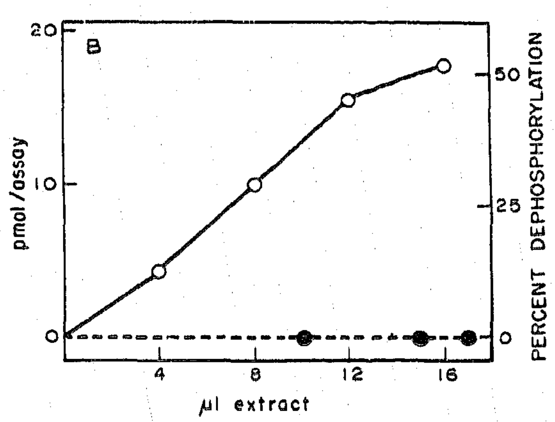


Fig. 4 Expression of PTP-S in *E. coli* regulated by the *trc* promotor. (A) The cDNA for PTP-S was cloned behind the hybrid *trc* promotor as described in section 2. This construct contains 33 additional amino acids (shown here) before the initiator methionine of PTP-S. The remaining 363 amino acids correspond to PTP-S. (B) PTPase activity of *E. coli* extracts expressing PTP-S. Extracts from bacteria containing pKK-PTP (solid line) and from the control (broken line) carrying an insert in the opposite orientation were used.

after 3 h and analysed for expression of PTP-S transcripts. Treatment with cycloheximide resulted in about a 10-fold increase in the level of 1.7 kb transcript (Fig. 5B). In addition, a new transcript of about 3 kb was observed in cycloheximide-treated samples. This transcript is perhaps coded by some other PTPase since it was not observed when a similar blot was probed with a probe lacking catalytic domain of PTP-S (nucleotides 943–1495). No significant effect of cycloheximide treatment was observed on the expression of *hck* or ribophorin transcripts (data not shown).

What is the function of clusters of basic amino acids in the non-catalytic region of PTP-S? Does the homology with transcription factors indicate any functional significance? One possibility is that the clusters of basic amino acids might serve as a signal for localization in a subcellular compartment such as the nucleus [30]. In this connection it may be relevant to point out that

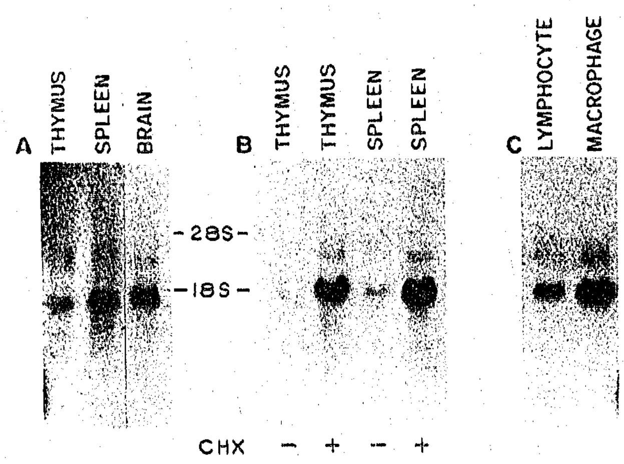


Fig. 5. Northern blot analysis of RNA from various tissues and cells using a full length PTP-S probe. (A) Northern blot of mRNA (5 μg) from rat tissues. (B) Northern blot of total RNA (10 μg) from cycloheximide (CHX) treated and control rat tissues. (C) Northern blot of total RNA (10 μg) from splenic lymphocytes and peritoneal macrophages. Splenic lymphocytes were prepared by lysis of erythrocytes with ammonium chloride. Peritoneal macrophages were incubated at 37°C in tissue culture Petri-dishes to remove non-adherent cells.

PTPase activity is present in purified rat liver and brain nuclei (unpublished observations from our laboratory). The strong conservation of clusters of basic amino acids in the non-catalytic region suggests that these residues have an important function.

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