Two Splice Variants of a Tyrosine Phosphatase Differ in Substrate Specificity, DNA Binding, and Subcellular Location*

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Four different forms of a non-receptor type protein-tyrosine phosphatase are generated by alternative splicing; two of these forms (PTP-S2 and PTP-S4) are major forms, which are expressed in rat as well as human cells. Here we report that PTP-S2 binds to nonspecific DNA in vitro and localizes in the nucleus upon transfection in HeLa cells. PTP-S4 does not bind to nonspecific DNA and shows perinuclear and cytoplasmic localization. Removal of the C-terminal 34 amino acids of PTP-S4 gives rise to a truncated protein, which binds to nonspecific DNA and localizes to the nucleus. PTP-S4, but not PTP-S2, interacts strongly with the isolated nuclear matrix. The two forms of this tyrosine phosphatase show different substrate specificity in vitro, a feature novel to splice variants of tyrosine phosphatases. Mitogenic stimulation induces mRNAs for PTP-S2 as well as for PTP-S4 in the G1 phase during liver regeneration. These results suggest that alternative splicing gives rise to two protein-tyrosine phosphatases with distinct substrate specificities and subcellular locations. The 34 amino acids at the C terminus of PTP-S4 play a critical role in determining substrate specificity, subcellular location, and interaction with nuclear matrix and DNA.

The cloning and characterization of several genes encoding protein-tyrosine phosphatases (PTPs) has drawn attention to their significance in regulating various cellular processes by modulating the level of phosphorytrosine in the cell (along with the tyrosine kinases). In vivo, it is important that the substrate specificities and activities of these enzymes are stringently regulated. While the transmembrane receptor type tyrosine kinases are restricted in their spatial distribution to the cell surface, the intracellular enzymes appear to be more versatile in their association with various cellular organelles and macromolecules (1–6). Although the physiological substrates and in vivo functions for most of the intracellular PTPs are not known, the sequences in the non-catalytic domain have been suggestive of putative functions, as these domains have the ability to target the molecule to specific intracellular locations (2, 6–14).

Human T-cell phosphatase was the first non-transmembrane PTP to be cloned from a T-cell cDNA library (15). A variant of the T-cell phosphatase, named PTP-S (protein tyrosine phosphatase of spleen) was isolated by us from rat spleen cDNA library, which differs from the former in its C-terminal non-catalytic domain (16). Mouse and human variants of TC PTP have also been isolated (17, 18). We have recently shown that four different forms of PTP-S (named PTP-S1, PTP-S2, PTP-S3, and PTP-S4), which arise due to alternative splicing (19), are expressed in rat cells. PTP-S1 is the same as PTP-S, described previously. PTP-S4 is very similar to the human T-cell phosphatase or p48TC (15, 19). PTP-S2 is a polypeptide of 382 amino acids and is very similar to human p45TC or mouse homolog MPTP (17, 18). PTP-S2 and PTP-S4 are the major forms which are present in human cells also. PTP-S1 and PTP-S3 are minor forms and are expressed in rat and mouse cells but not in human cells, due to loss of an internal splice site by mutation (19). The last 6 amino acids of PTP-S2 are replaced by 34 amino acids (mostly hydrophobic) in PTP-S4. Previously it has been shown that PTP-S1 binds to nonspecific DNA through the C-terminal 57 amino acids (10), which are present in PTP-S2 as well.

Very little is known about the cellular function of any isoform of PTP-S. Mitogenic stimulation of a wide variety of cells such as T-lymphocytes, HeLa cells, fibroblasts, and liver cells results in an increase in mRNA levels during G1 phase, raising the possibility of a role in cell proliferation (20–22). In these studies individual isoforms were not analyzed; therefore, it is not known whether one or more isoforms are induced by mitogens. Major protein products of this PTP, recognized by a monoclonal antibody, are present in the cell nucleus largely in association with chromatin in rat fibroblasts (3). The mouse homolog MPTP is localized in the nucleus in transfected cells (22). Two splice variants of human T-cell PTP p45TC and p48TC have different subcellular locations in transfected cells; their enzymatic and other molecular properties have not been examined (23).

Here we have analyzed the DNA binding properties of the two major splice variants, PTP-S2 and PTP-S4, determined their subcellular location, and also studied their enzymatic and other molecular properties. Our results show that although there is only a small difference in the primary structure of these two proteins, their substrate specificity, subcellular location, and other molecular properties are quite different.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors—PTP-S2 and PTP-S4 cDNAs, described previously, were cloned by amplifying mRNA using reverse transcription and PCR (19). These were cloned in the BamHI site of
plasmid vector pET-3a (24) for expression of the protein in Escherichia coli. The C-terminal end of the C-terminal end of PTP-S4 was created by amplifying the desired region (amino acids 1–376) by PCR using sense primer RR11 (5’-CGGGATCCATGCTCGGATCCAGGCGG-3’) and anti-sense primer SK6 (5’-CGGGATCCATGCTCGGATCCAGGCGG-3’) with BamHI sites. The resulting cDNA was digested with BamHI, cloned in pUC18, sequenced, and then cloned in pET-3a. This construct named ΔPTP-S4 gives rise to a protein lacking the C-terminal 34 amino acids of PTP-S4 (Fig. 1D). These cDNAs were also cloned in pCB6 vector in the BglII site for expressing in eukaryotic cells. This vector drives the expression using cytomegalovirus promoter (25). Two deletion mutants of PTP-S2 lacking the C-terminal 40 or 68 amino acids (Δ40 PTP-S2 and Δ68 PTP-S2) were made by amplifying the required region by PCR using appropriate primers.

Expression of E. coli and Southwestern Blotting—BL-21 (DE3) was transformed by the pET expression plasmids, and proteins were expressed by induction with isopropyl-1-thio-β-D-galactopyranoside (10). Cell extracts were prepared, and DNA binding experiments by Southwestern blotting were carried out as described (10). Bacterial cell lysates expressing PTP-S2, PTP-S4, ΔPTP-S4, or uninduced controls were fractionated by SDS-PAGE, proteins transferred to Immobilon P membrane, and incubated with 32P-labeled DNA, after renaturation blocking overnight. Single-stranded probe was made by heating the double-stranded labeled probe at 100 °C for 10 min and then cooling on ice immediately.

Nuclear Matrix Preparation and Binding of PTP-S2, PTP-S4, and ΔPTP-S4—Nuclear matrix was prepared as described previously (26, 27). The nuclear matrix preparation was suspended in buffer A (10 mM HEPES, pH 8.0, 5 mM KCl, 3 mM MgCl2, 1 mM phenylmethylsulfonl fluoride, 2 μg/ml soybean trypsin inhibitor, 2 μg/ml leupeptin and aprotinin) containing 150 mM NaCl, and incubated on ice for 30 min with various amounts of either PTP-S2, PTP-S4, or ΔPTP-S4 (or control buffer). These samples were then centrifuged for 5 min at 10,000 rpm. The pellet was washed three times with buffer A containing 400 mM NaCl, suspended in SDS sample buffer, and fractionated on SDS-PAGE, followed by immunoblotting with PTP-S monoclonal antibody.

Transfection of Cells, Immunofluorescence, and Subcellular Fractionation—HeLa or COS-1 cells grown as monolayer on coverslips or Petri dishes to about 70% confluence were used for transient transfection using the Lipofectin reagent (Life Technologies, Inc.) according to manufacturer’s instructions. For better expression, 16 h before fixation the cells for immunofluorescence, sodium butyrate was added to the medium to a final concentration of 5 mM. The cells were then processed for immunofluorescence as described earlier (3), using anti-PTP-S monoclonal antibody. For obtaining stable cell lines expressing PTP-S2, HeLa cells were transfected with the plasmid pCB6-PTP-S2 using Lipofectin reagent. After 24 h of transfection, the cells were replated into medium containing 500 μg/ml Geneticin (Life Technologies, Inc.). After 2 weeks, neomycin-resistant clones were isolated and checked for PTP-S2 protein expression by immunofluorescence and Western blotting. Subcellular fractionation of stably transfected clone D3 expressing PTP-S2 or COS-1 cells transiently transfected with PTP-S4 was carried out essentially as described (3, 26).

Extraction and Purification of Enzymes—E. coli cells carrying the appropriate expression plasmid were induced in the mid log phase with 1 mM isoprpyl-1-thio-β-D-galactopyranoside for 4 h at 37 °C. Cells from 45 ml of induced culture were suspended in 15 ml of extraction buffer (50 mM Tris-Cl, pH 8.0, 10 mM EDTA, 0.1% Triton X-100, 200 mM NaCl) and incubated for 30 min at 25 °C. The supernatant obtained after centrifugation at 15000 rpm for 20 min at 4 °C was chromatographed on a Sephadex G-75 column (1.6 × 65 cm) equilibrated with column buffer (25 mM Tris, pH 7.1, 1 mM EDTA, 0.1% Triton X-100, 0.025% 2-mercaptoethanol, 200 mM NaCl, 10% glycerol). PTP-S4 was chromatographed on a Sephacryl S-200 column under the same conditions. Column fractions were assayed for enzyme activity using pNPP as substrate. Peak fractions were stored in aliquots at −70 °C. The columns were calibrated with Pharmacia molecular weight markers. The position of markers was determined by analyzing the column fractions by SDS-PAGE. The marker proteins used were catalase (232,000), aldolase (158,000), bovine serum albumin (67,000), ovalbumin (45,000), and chymotrypsigen (25,000).

PTP assay and Substrates—PTP assay was carried out as described (28) using poly(Glu4,Tyr1) or MBP as substrate. MBP (Sigma) was phosphorylated using a tyrosine kinase from rat spleen in the same manner as described for the phosphorylation of poly(Glu4,Tyr1) previously (28). The PTP assay buffer contained 25 mM Tris, pH 7.1, 20 mM NaCl, 0.2 mM EDTA, 0.005% 2-mercaptoethanol, 0.02% Triton X-100, 2% glycerol, and indicated amounts of substrate. After incubating for 5 min at 30 °C, the reaction was stopped by adding trichloroacetic acid. Assay with pNPP was also carried out under the same conditions as described (28).

Quantitation of PTP-S mRNAs during Liver Regeneration—Partial hepatectomy, RNA isolation from liver biopsies, and quantitation of PTP-S transcripts by RNA-PCR was performed essentially as described earlier, using the primers RR6, RR7, and RR8 for 28 cycles (19, 21). The PTP-S mRNAs were coinmplied with the mRNA for GAPDH as control for 23 cycles (which is within the logarithmic scale of amplification for GAPDH mRNA; data not shown). For this purpose, the primers RR15 (5’-GACCCCTTCCATGGACCTCAAGTC-3’) and RR17 (5’-ACCTGGCA-CAGCCTTGCGAG-3’) were used equally. This last 6 amino acids of PTP-S2 (exon G) are replaced by 34 amino acids (exon F2) in PTP-S4. These two proteins were enzymatically active and could dephosphorylate 32P-labeled poly(Glu4,Tyr1) and pNPP. DNA binding properties of PTP-S2 and PTP-S4 were examined by Southwestern blotting using 32P-labeled non-specific DNA (pUC18). PTP-S2 could bind to single-stranded as well as single-stranded DNA (Fig. 1, B and C, lane 2). Surprisingly, PTP-S4 did not show any interaction with
DNA (Fig. 1, B and C, lane 3) even after longer exposure (data not shown). A comparison of the amino acid sequences of PTP-S2 and PTP-S4 in the C-terminal region (Fig. 1D) suggests that either the last 6 amino acids of PTP-S2 are critical for DNA binding or the last 34 amino acids of PTP-S4 are inhibiting interaction with DNA. Therefore, we made a vector expressing a truncated form of PTP-S4 lacking the C-terminal 34 amino acids (Fig. 1A, lane 4). This protein showed PTP activity and could interact with double-stranded as well as single-stranded DNA (Fig. 1, B and C, lane 4). This suggests that C-terminal 34 amino acids inhibit interaction of PTP-S4 with nonspecific DNA. The C-terminal 6 amino acids of PTP-S2 do not appear to be critical for interaction with DNA, although they contribute to some extent to this interaction (Fig. 1, B and C, lanes 2 and 4). Human T-cell phosphatase (p48 TC) expressed in E. coli did not show any interaction with DNA, but a deletion mutant lacking the C-terminal 34 amino acids showed interaction with DNA as determined by Southwestern blotting (data not shown).

**Subcellular Location of PTP-S2 and PTP-S4 in Transfected Cells**—Subcellular location of these proteins was examined in transfected cells by immunofluorescence microscopy, using a monoclonal antibody. This monoclonal antibody, which has been characterized previously (3), recognized rat and mouse PTP-S proteins but not the human protein from HeLa cells, as determined by immunofluorescence staining and immunoblotting (data not shown). This antibody could interact with PTP-S2 as well as PTP-S4. Transfected HeLa cells showed that PTP-S2 was present mainly in the nucleus (Fig. 2B), whereas PTP-S4 showed prominent perinuclear and cytoplasmic staining (Fig. 2D). In addition, the cells transfected with PTP-S4 plasmid showed a distinct reticular pattern of staining of the area surrounding the nucleus extending throughout the cytoplasm. Except for the nuclear membrane, no prominent staining was visible within the nucleus (Fig. 2D). This pattern of staining suggests that PTP-S4 is located in the endoplasmic reticulum and nuclear membrane. In order to determine the role of the C-terminal hydrophobic domain in subcellular localization, HeLa cells were transfected with a vector carrying ΔPTP-S4 cDNA. This protein localized to the nucleus (Fig. 2F) like the PTP-S2 isoform, showing that the C-terminal 34 amino acids prevent PTP-S4 from entering the nucleus or are essential for its cytoplasmic localization. Within the nucleus, PTP-S2 as well as ΔPTP-S4 showed more prominent staining of nucleoli (Fig. 2, B and F).

**Subcellular Localization by Biochemical Fractionation**—To further investigate and confirm the subcellular distribution of PTP-S2 and PTP-S4 the cells were subjected to biochemical fractionation and the distribution of protein was determined by immunoblotting. For this purpose a stable cell line (D3) of HeLa cells expressing rat PTP-S2 was obtained. No stable transfectant of HeLa cells expressing PTP-S4 could be obtained, in spite of repeated efforts. Therefore for PTP-S4, transiently transfected COS-1 cells were used for biochemical fractionation, where the transfection efficiency was higher (20–30% as compared to 1–3% of HeLa cells) with a much higher level of protein expression. Cells were fractionated into a nuclear and post-nuclear fraction by the detergent lysis method. PTP-S2 was present in both the nuclear and post-nuclear fractions, whereas PTP-S4 was enriched in the post-nuclear fraction (Fig. 3, B and D). The nuclei were further fractionated into chromatin fraction, a high salt-extractable fraction, and the residual insoluble pellet called the nuclear matrix. It was observed that PTP-S2 was predominantly associated with the chromatin fraction, and a very small amount was present in the nuclear matrix (Fig. 3, B and C). In contrast, little PTP-S4 was present in the nuclear fraction, most of which was associated with the nuclear matrix (Fig. 3, E and F). In the immunofluorescence staining experiment, very little extranuclear staining could be seen in PTP-S2 transfected cells, while this protein was present in the post nuclear supernatant after cellular fractionation. This may be due to leakage of the protein from the nuclei when the cells are lysed in the presence of detergent, which is also likely to remove the outer nuclear membrane.
PTP-S Splice Variants Differ in DNA Binding, Enzyme Activity

Interaction of PTP-S4 and PTP-S2 with Isolated Nuclear Matrix in Vitro—We examined whether bacterially expressed purified PTP-S2 or PTP-S4 could interact with the nuclear matrix isolated from rat fibroblasts. Accordingly, nuclear matrix preparation was incubated with various amounts of PTP-S2 or PTP-S4 (or control buffer) at 0°C for 30 min on ice. After washing bound PTP-S proteins were analyzed by immunoblotting. Lane 1, control buffer; lanes 2, 4, and 6, with 30, 15, and 7.5 ng of PTP-S2; lanes 3, 5, and 7, with 30, 15, and 7.5 ng of PTP-S4. Panel C shows a comparison of nuclear matrix-binding property of PTP-S4 and ΔPTP-S4. Lane 1, control buffer; lanes 2 and 4, with 30 and 7.5 ng of PTP-S4; lanes 3 and 5, with 30 and 7.5 ng of ΔPTP-S4.

Enzymatic Properties of PTP-S2 and PTP-S4—In order to examine the enzymatic and molecular properties of these two PTPs, bacterially expressed PTP-S2 and PTP-S4 were chromatographed on a Sephadex G-75 column. PTP-S2 eluted as a monomer at the expected elution volume (Fig. 5A). However, PTP-S4 eluted in the void volume, suggesting that it was forming a high molecular weight complex (Fig. 5B). The apparent molecular weight of PTP-S4 by gel filtration on Sephacryl S-200 column was estimated to be about 200,000, suggesting that it forms a tetramer. Truncated PTP-S4 lacking the C-terminal 34 amino acids eluted at the expected position as a monomer (Fig. 5D). These observations suggest that the C-terminal 34 amino acid region is required for oligomerization of PTP-S4. All the three G-75 purified PTPs: PTP-S2, PTP-S4, and ΔPTP-S4 (Fig. 5D), were recognized by the monoclonal antibody (Fig. 5E) against PTP-S. Both PTP-S2 and PTP-S4 were inhibited by a micromolar concentration of orthovanadate, a known inhibitor of most of the PTPs (10, 28, 30). The substrate specificity of PTP-S2 and PTP-S4 was determined in vitro by using poly(Glu₄,Tyr₁) and MBP as substrate. PTP-S2 preferentially dephosphorylated poly(Glu₄,Tyr₁), whereas PTP-S4 preferred MBP as substrate (Fig. 6). The difference in the substrate specificity is also seen from the Vₘₙₙ and Kₘ of these two isoforms for the two substrates, as shown in Table I. With MBP as substrate, the enzymatic properties of
In order to determine the relative levels of these two mRNAs, reverse transcription coupled with PCR was used. In each PCR reaction, the levels of GAPDH mRNA was also determined by including appropriate primers. Since GAPDH mRNA levels are relatively constant in many tissues, this was used as a reference for quantitation. Amplification of PTP-S2 as well as PTP-S4 was linear up to 28 cycles (data not shown).

The highest level of PTP-S2 mRNA was found in the rat spleen (Table II). PTP-S4 level was generally 4–10 times lower as compared to PTP-S2. Comparison of the levels in different tissues and cells involves the assumption that the GAPDH expression is the same in all these cells. However, this assumption is not required for the quantitation of relative levels of the isoforms in a given tissue or cell. PTP-S1 and PTP-S3 mRNAs could be detected in rat tissues and cell lines, but their levels were less than 5% of PTP-S2 levels (data not shown).

Mitogenic Stimulation Induces PTP-S2 and PTP-S4 Isoforms—Mitogenic stimulation of various cells leads to increase in mRNA levels of PTP-S (20–22). However, since full-length cDNA probes were used for Northern blot or dot blot analysis, in these studies it is not known whether one form or both the major forms are induced by mitogens. Using quantitative PCR methods, we observed that both PTP-S2 and PTP-S4 are induced during rat liver regeneration after partial hepatectomy (Fig. 7). Relative quantitation of these isoforms showed that PTP-S4 was induced somewhat more than PTP-S2 isoform. As a control, expression of GAPDH mRNA was also analyzed during liver regeneration, which showed a small increase (about 3–4-fold) over control liver. Increase in level of GAPDH mRNA during liver regeneration has been reported earlier (31). When PTP-S2 and PTP-S4 mRNA levels were normalized to GAPDH mRNA it was found that both PTP-S2 and PTP-S4 still showed 5–6-fold increase in their levels after 6 h of partial hepatectomy.

**DISCUSSION**

To our knowledge, PTP-S is the first PTP that has been shown to generate, by alternative splicing, two forms with inherent differences in their substrate specificity. These forms differ in their enzymatic activity toward phosphorylated protein substrates but not with pNPP as substrate, suggesting thereby that the amino acid residues in the non-catalytic domain are important in determining the interaction with polypeptide substrates even in vitro. This conclusion is also supported by the observation that deletion of C-terminal amino acids of PTP-S2 results in much higher activity toward poly(Glu⁴,Tyr¹) as substrate, but the activity toward MBP does not increase. Previously it has been reported that alternative

**TABLE II**

| Tissue/cell line | Species/type | Relative level of PTP-S isoforms |
|------------------|--------------|----------------------------------|
|                  |              | PTP-S2⁺ | PTP-S4⁺ |
| Spleen           | Rat          | 100     | 27     |
| Thymus           | Rat          | 86      | 20     |
| Testis           | Rat          | 44      | 10     |
| Brain            | Rat          | 47      | 12     |
| Liver            | Rat          | 18      | 3      |
| F111             | Rat fibroblast| 42    | 10     |
| Rat2             | Rat fibroblast| 46      | 11     |
| HeLa             | Human epithelial| 20      | 8      |
| CCRF             | Human lymphoma| 16      | 5      |
| A375             | Human melanoma| 71      | 13     |
| SW720            | Human colon cancer| 47 | 5 |
| M14              | Human melanoma| 30      | 5      |
| MDA-MB-453       | Human breast cancer| 17 | 3      |
| SNB19            | Human central nervous system| 12      | 1     |
| A549             | Human lung cancer| 12      | 1     |

⁺ Relative mRNA levels are expressed as percentage of PTP-S2 in spleen.

these two enzymes are easily distinguishable. PTP-S4 shows strong preference for the basic protein, MBP (higher $V_{max}$ lower $K_m$, Table I). Removal of the C-terminal 34 amino acids of PTP-S4 results in a phosphatase that shows a 4-fold decrease in $V_{max}$ and 27-fold increase in $K_m$ for MBP as substrate (Table I). With pNPP as substrate, there was very little difference in the enzymatic properties of these forms of PTP-S (Table I).

In order to analyze the role of C-terminal sequences of PTP-S2, two deletion mutants were made in E. coli, which lacked 40 or 68 amino acids. Both of these mutants showed increased activity toward poly(Glu⁴,Tyr¹) as substrate but their activity toward MBP was not increased (Table I).

**Expression of PTP-S2 and PTP-S4 in Different Tissues and Cell Lines**—In order to determine the relative levels of these polypeptide substrates even **in vitro**. This conclusion is also supported by the observation that deletion of C-terminal amino acids of PTP-S2 results in much higher activity toward poly(Glu⁴,Tyr¹) as substrate, but the activity toward MBP does not increase. Previously it has been reported that alternative

**TABLE I**

| Substrate          | Enzyme | $K_m$ (μM) | $V_{max}$ (nmol min⁻¹ mg⁻¹) |
|--------------------|--------|------------|-----------------------------|
| 1. Myelin basic protein | PTP-S2  | 1.3        | 1447                        |
|                    | PTP-S4  | 0.11       | 11,168                      |
|                    | ΔPTP-S2 | 3.2        | 2894                        |
|                    | Δ40PTP-S2 | 1.2      | 803                         |
|                    | Δ68PTP-S2 | 1.2      | 1071                        |
| 2. Poly(Glu⁴,Tyr¹) | PTP-S2  | 0.13       | 10,721                      |
|                    | PTP-S4  | 0.12       | 2226                        |
|                    | ΔPTP-S4 | 0.16       | 12,902                      |
|                    | Δ40PTP-S2 | 0.08     | 24,027                      |
|                    | Δ68PTP-S2 | 0.12     | 49,469                      |
| 3. pNPP            | PTP-S2  | 1920       | 15,860                      |
|                    | PTP-S4  | 2800       | 14,600                      |
|                    | ΔPTP-S4 | 1530       | 17,360                      |

FIG. 6. Dephosphorylation of myelin basic protein and poly(Glu⁴,Tyr¹) by PTP-S2 and PTP-S4. PTP assays were carried out for indicated period of times using 72 pg of PTP-S2 or 48 pg of PTP-S4 per assay. The concentration of substrates used was 0.53 μM phosphorylated poly(Glu⁴,Tyr¹) and 0.41 μM phosphorylated MBP.
splicing generates two forms of a PTP in Drosophila (dPTP61F), which show different subcellular location (nucleus and mitochondria) but show similar phosphatase activity as judged by the use of pNPP as substrate (14). The enzyme activity of these forms was not compared using polypeptide substrates.

The most unexpected observation we have made is that PTP-S4 does not interact with nonspecific DNA activity of these forms was not compared using polypeptide substrates.

PTP-S4 localizes mainly in the cytoplasmic and nuclear membranes. Its staining pattern is very similar to that of human T-cell phosphatase (p48Tc), which is located in the endoplasmic reticulum and nuclear membrane (23, 32). Another related phosphatase, PTP-1B, has been shown to be located in the endoplasmic reticulum (2, 6). The staining pattern of PTP-S4 differs from that of PTP-1B in having prominent staining of nuclear membrane. Interaction of PTP-S4 with nuclear matrix is much stronger than that of PTP-S2 and requires the C-terminal 34-amino acid domain. The association of PTP-S4 with nuclear matrix is of particular interest, since this subnuclear structure is involved in several nuclear functions such as DNA replication, transcription, etc. (33). Recently tyrosine kinase activity has been found in the nuclear matrix, which shows cell cycle-dependent changes that are maximal during G1/S transition (27). One of the tyrosine kinases found tightly associated with nuclear matrix is Lyn, a member of the Src family (27).

We have shown previously that PTP-S mRNA levels increase upon mitogenic stimulation of T lymphocytes and that the increase is due to post-transcriptional stabilization of mRNA in the stimulated cells (20). PTP-S mRNA was superinduced in lymphocytes in the presence of cycloheximide (20). Both the major isoforms, PTP-S2 and PTP-S4, are induced upon mitogenic stimulation of liver cells (this study). The timing of induction of PTP-S2 and PTP-S4 suggests that these are delayed early genes. Higashitsuji et al. (34) have shown that during mouse liver regeneration, PTP-PEST and PTP-RL9 (a novel intracellular phosphatase) are induced to peak levels at 6 h after partial hepatectomy, while another enzyme, PTP-RL10, increases and peaks only after the first round of cell division, i.e. 48–72 h. PRL-1, MKP-1, Pac-1, and VH1 family members are examples of tyrosine and dual specificity phosphatases that behave as immediate early genes upon mitogenic stimulation (35–37). Upon growth factor stimulation of quiescent cells, one of the isoforms of PTP-1B is induced, reaching peak levels by late G1, but the generation of this isoform requires protein synthesis (38). Thus, different PTPs show distinct pattern of induction in response to mitogenic stimulation, but their role in cell proliferation remains to be elucidated.

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