Subcellular localization of a protein-tyrosine phosphatase: evidence for association with chromatin

Vegesna RADHA, Sundaram NAMBIKIRAJAN and Ghanshyam SWARUP*

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

A non-transmembrane protein-tyrosine phosphatase, PTP-S, has been shown to bind to DNA *in vitro* through its C-terminal non-catalytic domain. The cellular PTP-S gene product was identified as 42–44 kDa polypeptides in the rat fibroblast cell lines Rat2 and F111, by immunoblotting using polyclonal and monoclonal antibodies. Indirect immunofluorescence-staining experiments showed that PTP-S was predominantly associated with the nuclear compartment in interphase cells, although some fluorescence was present in cytoplasm. This protein appeared to be uniformly distributed throughout the cell during mitosis. Subcellular fractionation followed by immunoblotting supports the

immunofluorescence data showing that PTP-S was present in the nucleus as well as cytoplasm. PTP-S could be released from the isolated nuclei by moderate salt concentration (400 mM NaCl) or mild treatment with DNAase I, suggesting that at least part of this protein present in the nucleus was associated with chromatin. PTP-S extracted from nuclei of Rat2 cells binds to DNA, as determined by DNA affinity chromatography. The nuclear location of this phosphatase, and its association with chromatin, provide support to our previous suggestion that the DNA-binding property of this phosphatase may be physiologically relevant.

INTRODUCTION

Phosphorylation and dephosphorylation of tyrosine residues is a regulatory change in proteins that is transient and under the control of two classes of enzymes, the protein-tyrosine kinases and the protein-tyrosine phosphatases (PTPases). This post-translational modification has been utilized by most eukaryotic cells in controlling various functions such as cell division and response to the external environment (Hunter, 1989; Fischer et al., 1991). PTPases not only dephosphorylate substrates of tyrosine kinases, but also dephosphorylate many protein kinases and thereby control their activities (Swarup and Radha, 1992). Deregulation of these controls is known to result in a variety of abnormalities, including malignant transformation of cells (Klarlund, 1985; Zheng et al., 1992). The transmembrane PTPases are localized at the cell membrane and possibly play a receptor-like role (Fischer et al., 1991; Saito and Streuli, 1991). The non-transmembrane forms of PTPases have a single catalytic domain; the non-catalytic regions possibly play a role in subcellular localization or in regulating enzyme activity (Cool et al., 1990; Fischer et al., 1991; Frangioni et al., 1992; Woodford-Thomas et al., 1992). The cellular targets of these enzymes and their roles in various physiological processes are yet to be identified (Brautigan, 1992; Pot and Dixon, 1992).

PTP-S, cloned from a rat spleen cDNA library, encodes a non-transmembrane PTPase that is expressed in various tissues (Swarup et al., 1991). Different forms of this PTPase, which arise due to alternative splicing, have been isolated by cDNA cloning (Cool et al., 1989; Champion-Arnaud et al., 1991; Swarup et al., 1991; Mosinger et al., 1992). Analysis of the cDNA-derived amino acid sequence revealed a C-terminal non-catalytic domain with putative nuclear location signal sequences which is similar to basic domain of transcription factors Fos and Jun (Swarup et al., 1991). Using various constructs of PTP-S in bacterial expression vectors, a DNA-binding domain has been defined within the C-terminal non-catalytic region of PTP-S (Radha et

al., 1993). The C-terminal 57 amino acids of PTP-S are necessary and sufficient for its DNA-binding property *in vitro*. We have now identified the native PTP-S gene product in rat fibroblasts and determined its subcellular location, using specific polyclonal and monoclonal antibodies. Indirect immunofluorescence staining showed that PTP-S was predominantly present in the nucleus in interphase cells. During mitosis, uniform staining was seen throughout the cell. The results of experiments designed to study its subnuclear location and possible association with chromatin, nuclear matrix and DNA are also presented.

MATERIALS AND METHODS

Cell culture and cell fractionation

Rat embryo fibroblast cell lines (Rat2 and F111) and human cell line HeLa were grown as monolayers in Dulbecco's modified Eagle medium supplemented with 10% fetal-calf serum and antibiotics at 37 °C in a humidified 5% CO₂ atmosphere. Cells were washed with PBS and harvested by scraping and centrifugation. They were either put directly in SDS-sample buffer to make whole cell lysates or subjected to detergent lysis for preparation of nuclear and post-nuclear fractions. Briefly, 1×10^7 cells were suspended in 1 ml of ice-cold hypotonic buffer [10 mM Hepes, pH 8.0, 5 mM KCl, 2 mM MgCl₂, 2 mM phenylmethanesulphonyl fluoride (PMSF), 2 µg/ml soyabean trypsin inhibitor and 2 µg/ml leupeptin] containing 0.5% (v/v) Nonidet P-40, left on ice for 10 min and centrifuged at 1000 *g* for 5 min to pellet nuclei. The post-nuclear fraction was collected and the nuclei were washed with the same buffer. The purity of nuclei was checked by looking for the presence of malate dehydrogenase and esterase (microsomal marker), which were found to be absent from the nuclear fraction and present in the post-nuclear fraction, as determined by immunoblotting. Nuclei were used for extraction studies or boiled in sample buffer for fractionation of proteins by SDS/PAGE.

Treatment of nuclei with salt and DNAase I

Salt extraction was performed by suspending 1×10^6 freshly made nuclei in hypotonic buffer containing the desired salt concentration. After 10 min on ice, the post-nuclear supernatants and residual nuclei were separated by centrifugation and boiled in SDS-sample buffer before loading on gels for immunoblotting.

Nuclei were subjected to DNAase I treatment by suspending them in hypotonic buffer containing the desired concentration of DNAase I (Sigma) and incubating on ice for 10 min as described by Klempnauer and Sippel (1986). EDTA was added to a concentration of 5 mM and the mixture was left for a further 10 min on ice. The supernatants and residual nuclei were separated by centrifugation and analysed by Western blotting, or treated with proteinase K (after adding SDS to 0.5%) at 37 °C for 30 min, electrophoresed on 1.2%-agarose gel and stained with ethidium bromide.

Production of anti-PTP-S antibodies

Antibodies against the non-catalytic domain of PTP-S were raised by using a fusion protein (GST-NC.1) of glutathione S-transferase (GST) with the C-terminal 122 amino acids of PTP-S by constructing an expression vector pGEX-NC-1 (Radha et al., 1993). This fusion-protein band of 41 kDa was electroeluted from polyacrylamide gels and used to inject several Balb/c mice with complete Freund's adjuvant. Two further injections were given with Freund's incomplete adjuvant at 15-day intervals, and blood was collected 4 days after the third injection. Antibody titres were monitored by immunodiffusion and immunoblotting. Antibodies against the full-length PTP-S protein were raised in the same way by using the protein expressed in *Escherichia coli* cells carrying the vector pET-PTP (Radha et al., 1993).

Production of anti-PTP-S monoclonal antibodies

Splenocytes were collected from a mouse showing high PTP-S antibody titre after boosting with the bacterially expressed full-length PTP-S protein intraperitoneally. These splenocytes were fused with Sp2/0 myeloma cells by using the poly(ethylene glycol) method. Hybrid cells were dispersed in Iscove's modified Dulbecco's medium (IMDM) containing hypoxanthine, aminopterin and thymidine (HAT) over a layer of mouse peritoneal-macrophage feeder cells (Fazekas de St. Groth and Scheidegger, 1980). Supernatants from HAT-resistant colonies were checked for antibody secretion by enzyme-linked immunofiltration assay and Western blotting, and the positive clones were propagated. Cells giving a good positive reaction were cloned by limiting dilution. The hybridoma cell line was maintained in IMDM supplemented with 10% fetal-calf serum and antibiotics.

Indirect Immunofluorescence

Cells grown on cover-slips were washed with PBS and fixed in 3.7% formaldehyde for 10 min at room temperature. Permeabilization was done for 6 min at room temperature in PBS containing 0.5% Triton X 100 and 0.05% Tween 20. After washing in PBS, non-specific protein adsorption was blocked by incubation for 30 min in 2%-BSA/PBS. The cover-slips were then incubated with either control sera or anti-PTP-S antibodies overnight at 4 °C. Polyclonal antibodies were used at 1:100 dilution and hybridoma supernatant was used undiluted. After washing in PBS several times, fluorescein isothiocyanate-conjugated anti-mouse IgG (Sigma) was added to the cover-slips for 1 h incubation at room temperature. After final washes in PBS, cells were mounted in 90% glycerol in PBS. For the competition

experiments, 5 µg of electroeluted PTP-S protein was incubated with the diluted polyclonal antibody or hybridoma supernatant at room temperature for 30 min, and the clarified solution was used as primary antibody.

Immunoblotting

Proteins fractionated by SDS/PAGE were transferred electrophoretically to Immobilon-P membranes. The membranes were blocked with 1% horse serum in TBST/gelatin (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20 and 0.2% gelatin) for 1 h. The blots were incubated with either polyclonal antibody diluted 1:1000 or hybridoma supernatant diluted 1:10 for 2 h. After washing for 2 h, the blots were incubated with alkaline-phosphatase-conjugated anti-mouse IgG for 1 h. After washing, immunoreactive bands were revealed by using Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

For competition experiments, 10 µg of purified PTP-S protein or GST-NC.1 fusion protein of 41 kDa was incubated at room temperature for 30 min with 5 µl of antiserum in a volume of 500 µl and then used as primary antibody after dilution to 5 ml in TBST.

Northern blotting

RNA isolation and Northern blotting were carried out essentially as described previously (Swarup et al., 1991). RNA (10 µg) was fractionated under denaturing conditions in formaldehyde gels, transferred to Hybond N⁺ membranes (Amersham) and hybridized with the PTP-S cDNA probe at 65 °C for 16 h. Blots were washed with 2× and 0.5× SSPE (SSPE = 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA) containing 0.1% SDS and exposed for autoradiography.

Immunoprecipitation

Nuclei and post-nuclear supernatant from 5×10^6 Rat2 cells were used to immunoprecipitate and assay the PTP-S enzyme. Isolated nuclei were lysed in 0.5 ml of 2× IP buffer (1× IP buffer contains 150 mM NaCl, 1% Triton, 0.5% deoxycholate, 2 mM EDTA, 2 mM PMSF, 2 µg/ml soyabean trypsin inhibitor and 2 µg/ml leupeptin) on ice and centrifuged at 15000 g for 20 min. The nuclear extract and post-nuclear supernatant were made up to 1× IP buffer for immunoprecipitation. Incubation with antibody (30 µl of hybridoma supernatant or control medium) was carried out for 1 h at 4 °C by gentle mixing. Preswollen Protein A-Sepharose beads were added and further mixed for 30 min. The beads were centrifuged and washed repeatedly with IP buffer and assayed for PTPase activity as described previously (Swarup and Subrahmanyam, 1989) with phosphorylated poly(Glu⁴, Tyr¹) as substrate. Competition experiments were performed by pre-incubating the antibody with the electroeluted full-length PTP-S protein made in bacteria for 1 h at room temperature.

DNA-Sepharose chromatography

Rat-2 nuclei were suspended in 100 µl of extraction buffer (25 mM Tris, pH 7.4, 10 mM EDTA, 0.025% 2-mercaptoethanol, 1% Nonidet P-40, 400 mM NaCl, 2 mM PMSF, 2 µg/ml soyabean trypsin inhibitor and 2 µg/ml leupeptin) on ice, and the supernatant obtained after centrifuging at 15000 g for 10 min was diluted to 1 ml with the binding buffer (25 mM Tris, pH 7.4, 1 mM EDTA, 1% Nonidet P40, 40 mM NaCl and 0.025% 2-mercaptoethanol) without salt. This extract was loaded on to a DNA-Sepharose column equilibrated with binding buffer at 4 °C (Radha et al., 1993). DNA-Sepharose was prepared by coupling calf thymus DNA with CNBr-activated Sepharose 4B as de-

scribed previously (Radha et al., 1993). After washing, bound proteins were eluted with 1 ml portions of binding buffer containing 0.1 M, 0.2 M, 0.4 M, 0.6 M and 1 M NaCl. The flow-through, wash and eluted fractions were precipitated with trichloroacetic acid and the pellet was washed with acetone and fractionated by SDS/PAGE for immunoblotting.

RESULTS

Identification of cellular PTP-S gene product

In order to identify and study the cellular PTP-S gene product, specific polyclonal and monoclonal antibodies were prepared.

Figure 1 Identification of the PTP-S gene product in fibroblasts and characterization of antibodies

(a) Lysates of Rat2 cells (75 µg of protein) were fractionated by SDS/PAGE, transferred to Immobilon P membranes and probed with polyclonal antibodies against either the full-length protein (lane 1) or the non-catalytic domain (lane 3). Lanes 2 and 4 show blots probed with antibodies preincubated with the respective antigens. Whole-cell lysates of Rat2 and F111 fibroblasts were analysed by Western blotting using monoclonal antibody G11 (lanes 5 and 7). Arrow indicates position of the PTP-S polypeptides. Lane 6 is empty. The acrylamide concentration in the gel was 8% (lanes 1 and 2) or 10% (lanes 3–7). (b) Spent medium from anti-PTP-S hybridoma clone G11 was checked for reactivity against bacterially expressed proteins by Western blotting. A 1:100 dilution of the protein, seen in Coomassie Brilliant Blue-stained lanes (c), was used for the immunoblot, and hybridoma medium diluted 1:100 was used as primary antibody. Bacterial cultures were induced for 3 h, lysed in sample buffer and analysed by SDS/PAGE or Western blotting. The vectors pGEX, pGEX-NC.1 and pGEX-NC.2 direct the synthesis of control GST protein (27.5 kDa) and fusion proteins of 41 and 34 kDa respectively. Truncated form of PTP-S (ΔPTP-S, 45 kDa), full-length PTP-S (47 kDa) and PTP-1 (50 kDa) are enzymically active when expressed in *E. coli*. Lysate of cells carrying control vector (pET) is also shown.

Antibodies against the full-length PTP-S were prepared by immunizing with bacterially expressed PTP-S gene product. Antibodies against the non-catalytic domain of PTP-S were prepared by immunizing mice with a fusion protein of GST with the C-terminal 122 amino acids of PTP-S, as described in the Materials and methods section. Both the antibodies identified a major polypeptide of about 42 kDa in rat fibroblast cell lines, Rat2 and F111, by immunoblotting (Figure 1a). An additional band of about 44 kDa was also observed. These two bands could be due to alternatively spliced forms of PTP-S or to some post-translational modification such as phosphorylation. When antibodies were preincubated with the corresponding antigen, these polypeptides were not seen in the immunoblot (Figure 1a), confirming that these polypeptides are in fact the PTP-S gene product. The two bands of 42 and 44 kDa do not separate in some experiments (Figure 1a, lane 1). The relative amounts of the two bands are variable, but in most experiments the 42 kDa band is predominant.

A monoclonal antibody against the PTP-S protein was prepared which also recognized the 42 kDa and 44 kDa polypeptides in Rat2 and F111 cells by immunoblotting (Figure 1a, lanes 5 and 7). This monoclonal antibody G11 recognizes an epitope located in the C-terminal 57 amino acids of the PTP-S protein. This was determined by using two fusion proteins of GST with the C-terminal 57 or 122 amino acids of PTP-S and a truncated form of PTP-S lacking the C-terminal 57 amino acids, all expressed in bacteria (Figures 1b and 1c). The fusion proteins with GST of 34 and 41 kDa were recognized by the G11 antibody, but not the GST itself or the truncated form of PTP-S (Figures 1b and 1c). This antibody does not recognize PTP-1, a non-transmembrane PTPase (Guan et al., 1990), which shows 65% identity with PTP-S (Figures 1b and 1c). Construction of vectors expressing the GST fusion proteins, PTP-1 and truncated form of PTP-S, and induction of cultures for protein expression have been described previously (Radha et al., 1993).

Expression of the PTP-S gene in fibroblasts has not been reported previously. Therefore, the presence of PTP-S mRNA was examined in various fibroblastic and other cell lines by Northern blotting using a PTP-S cDNA probe. PTP-S transcripts were observed in all the cell lines examined, such as Rat2, F111, HeLa, NIH3T3 and MDCK (results not shown). In HeLa cells, in addition to the 1.7 kb transcript, a second transcript of larger size was also observed.

Subcellular localization by indirect immunofluorescence

Indirect immunofluorescence staining of Rat2 and F111 cells grown on cover slips was done to find out the subcellular location of PTP-S. Figure 2 shows the results of immunofluorescence staining with polyclonal antisera against the full-length protein and the non-catalytic domain. Both antibodies showed a strong reaction in the nuclei of the cells. Examination of cells by immunostaining with antisera blocked by incubation with an excess of PTP-S protein did not result in any fluorescence in the nucleus or cytoplasm (Figure 2d). Figure 2 also shows the same cells viewed by phase-contrast microscopy. Staining appears to be distributed throughout the nuclear material. A very faint punctate pattern of staining was visible in the cytoplasm. A similar pattern of staining was visible with monoclonal antibody G11, although the staining was weaker (Figure 2). Mitotic cells showed staining throughout the cell (Figures 2c and 2j).

Cell-fractionation studies

The subcellular location of PTP-S was also determined by fractionating the Rat2 cells into nuclear and post-nuclear frac-

Figure 2 Subcellular localization of PTP-S by indirect immunofluorescence in Rat2 and F111 cells using polyclonal and monoclonal antibodies

Rat2 and F111 cells grown on coverslips were treated as described in the Materials and methods section and observed for the presence of fluorescence. Rat2 cells were treated in the absence of primary antibody (a), with polyclonal antibody against the non-catalytic domain of PTP-S (b), with polyclonal antibody against full-length protein (c) and with antibody against full-length protein competed with purified PTP-S protein (d). The middle panels (e–h) show phase-contrast micrographs of cells in the same fields as the upper panels. F111 cells fixed on coverslips were incubated with monoclonal antibody G11 (i) or polyclonal antibody (j) against non-catalytic domain of PTP-S. Magnification was $\times 1000$ (i) and $\times 400$ (all other panels). Arrowheads indicate cells in mitosis.

tions. Purity of nuclei was checked by looking for malate dehydrogenase and esterase; these enzymes were absent from the nuclear fraction. These fractions were then analysed by Western blotting using monoclonal antibody G11. It was observed that PTP-S was present in both these fractions (Figure 3), which is in agreement with the immunofluorescence data. The same result was obtained with F111 cells (results not shown).

The cellular PTP-S gene product was enzymically active. This was determined by immunoprecipitating the PTP-S protein and assaying the PTPase activity of the immunoprecipitate by using ^{32}P -labelled poly(Glu⁴,Tyr¹) as substrate, as described in the Materials and methods section. As shown in Table 1, both nuclear and cytoplasmic forms of PTP-S were active. Very little activity was seen in the immunoprecipitates when antibody

competed with the full-length PTP-S protein was used (Table 1). When antibody G11 was added to the bacterially expressed PTP-S, there was no change in enzyme activity, suggesting that this antibody does not inhibit enzymic activity of PTP-S under the conditions of our assay. About 70 % of PTP-S was precipitated by this antibody, as determined by immunoblotting.

Sub-nuclear localization

Within the nucleus, a protein may be associated with the chromatin, the inner nuclear membrane or the structure referred to as the nuclear matrix. Since PTP-S protein binds to DNA *in vitro*, and shows similarity to basic DNA-binding domains of transcription factors, its possible interaction with chromatin was

Figure 3 Subcellular fractionation of Rat2 cells

Nuclear and post-nuclear fractions of Rat2 cells were prepared by the detergent lysis method as described in the Materials and methods section, and analysed by SDS/PAGE (b) for Western blotting (a) performed using monoclonal antibody. Lanes 1, whole-cell lysate; 2, nuclear fraction; 3, post-nuclear fraction. The gel (b) was stained with Coomassie Brilliant Blue.

Table 1 PTPase activity of PTP-S in the immunoprecipitates

Nuclear extract and post-nuclear supernatant of Rat2 cells were prepared and subjected to immunoprecipitation with monoclonal antibody G11 or control hybridoma supernatant as described in the Materials and methods section. PTPase activity is expressed as pmol of [32 P]P_i released per assay per 10⁶ nuclei (or equivalent extracts), and are means $\pm$ S.E.M. ($n = 4$). For competition experiments, the antibody was preincubated with the bacterially expressed PTP-S, which was electroeluted from polyacrylamide gels and was enzymically inactive.

Fraction	Competing ligand	PTPase activity in the immunoprecipitate (pmol/assay)
Nuclear	—	2.58 $\pm$ 0.50
	+	0.53 $\pm$ 0.12
Post-nuclear	—	4.33 $\pm$ 0.20
	+	0.56 $\pm$ 0.10

examined. The nuclei were isolated from Rat2 or F111 cells, treated with NaCl or digested with DNAase I at 0 °C for 10 min and centrifuged as described in the Materials and methods section; the resulting supernatant and pellets were analysed by Western blotting for the presence of PTP-S. Treatment of isolated nuclei with moderate salt concentration released most of the PTP-S protein from the nuclei (Figure 4). Digestion of nuclei

Figure 4 Release of PTP-S protein from isolated nuclei by salt

Nuclei purified by detergent lysis method from F111 cells were treated for 10 min on ice with hypotonic buffer containing no NaCl or 100, 200, 300, 400 or 600 mM NaCl. Pellets (N) and supernatants (Sup) were separated by centrifugation and analysed by Western blotting using anti-PTP-S monoclonal antibody. The arrow indicates the position of PTP-S protein.

Figure 5 Release of PTP-S from nuclei on digestion with DNAase I

Isolated nuclei were incubated in buffer containing either no DNAase I or 10, 30 or 100 μ g/ml of DNAase I (final concn.) for 10 min on ice. EDTA (final concn. 5 mM) was added, and the mixture was further incubated for 10 min on ice. Pellets (N) and supernatants (Sup) were analysed by Western blotting using monoclonal antibody to detect the release of PTP-S from the nuclei (a). The pellets and supernatants, after proteinase K treatment, were electrophoresed on 1.2% agarose gel to detect the extent of DNA digestion and release from the nuclei (b). The agarose gel was stained with ethidium bromide. The arrow indicates the position of PTP-S protein.

with DNAase I under mild conditions (at 0 °C) released 30–50 % of the PTP-S polypeptides (Figure 5) in the supernatant fluid. A part of these samples was analysed for the presence of DNA (Figure 5b), and it was found that the nucleolytic cleavage of DNA was not complete. Incubation of nuclei with 100 μ g/ml RNAase did not solubilize any PTP-S, indicating that RNA was not required for retaining PTP-S in the nuclei (results not shown).

DNAase-digestion and salt-extraction experiments suggested that a significant part or most of the PTP-S protein present in the nucleus was associated with chromatin; less release upon DNAase digestion was perhaps due to incomplete digestion of DNA under the conditions of our experiments. To find out whether a small part of PTP-S could be associated with the

Figure 6 Sequential extraction of PTP-S from nuclei with DNAase I and NaCl

Rat2 nuclei (2×10^6) were incubated in hypotonic buffer containing 100 $\mu\text{g}/\text{ml}$ DNAase I for 10 min at 0 °C. The reaction was stopped by addition of EDTA (final concn. 5 mM), and centrifuged after 10 min on ice. The residual nuclei were then sequentially extracted with 400 mM and 2 M salt in hypotonic buffer. The supernatant collected after each incubation and the residual nuclei were analysed by Western blotting. Lanes: 1, untreated nuclei; 2, supernatant after DNAase I digestion; 3, supernatant after 0.4 M NaCl treatment; 4, supernatant after 2 M NaCl treatment; 5, residual nuclear pellet after sequential treatment with DNAase, 0.4 M NaCl and 2 M NaCl. (a) and (b) show Western blot and Coomassie Blue staining respectively of the same samples.

Figure 7 DNA-binding activity of PTP-S

Nuclear extract from Rat2 cells was chromatographed on a DNA–Sepharose column as described in the Materials and methods section. Fractions from this column, flow-through (FT) and wash were precipitated with trichloroacetic acid and analysed by Western blotting using monoclonal antibody G11.

nuclear matrix, the nuclei were digested with DNAase I, followed by extraction with 0.4 M and 2 M NaCl sequentially, and the resulting pellet and supernatants were analysed by Western blotting. As shown in Figure 6, a small fraction of PTP-S could not be extracted with these treatments, and may be associated with the nuclear matrix. Treatment of nuclei with 0.1% SDS resulted in release of all the PTP-S into the supernatant (results not shown). Nuclear matrix prepared by the method of Belgrader et al. (1991) also showed the presence of PTP-S protein, as determined by immunoblotting (results not shown).

Binding of PTP-S to DNA

The PTP-S gene product expressed in bacteria has been shown to bind to DNA by various methods, such as DNA affinity chromatography and Southwestern blotting (Radha et al., 1993). In order to determine whether cellular PTP-S protein can bind to DNA, extracts were prepared from nuclei isolated from Rat2 cells and assayed for DNA-binding activity by using DNA–Sepharose chromatography after adjusting the NaCl concen-

tration to 40 mM. Most of the PTP-S protein present in the extract was retained by DNA–Sepharose and could be eluted, after washing, with increasing NaCl concentration (Figure 7).

DISCUSSION

In order to identify the PTP-S gene product and to study its subcellular location, we prepared antibodies against the non-catalytic domain of this protein, as well as against the full-length protein. A monoclonal antibody which recognizes an epitope in the C-terminal 57 amino acids of PTP-S protein was also prepared. All these antibodies identified polypeptides of 42 and 44 kDa in Rat2 and F111 fibroblasts. These polypeptides are products of the PTP-S gene; this is supported by the observation that preincubation of monoclonal or polyclonal antibodies with the PTP-S protein made in bacteria abolishes the 42 and 44 kDa bands in the immunoblots. These two polypeptides may be coded by two different mRNAs generated by differential splicing, or a post-translational process such as phosphorylation may generate these two bands, due to differential modification. Differentially spliced forms of this PTPase are known from cDNA-cloning experiments which differ in their C-terminal domain (Cool et al., 1989; Champion-Arnaud et al., 1991; Swarup et al., 1991; Mosinger et al., 1992).

Subcellular localization by indirect immunofluorescence suggests that PTP-S protein is present predominantly in the nucleus and to some extent in the cytoplasm. Subcellular-fractionation experiments also suggest that it is present in the nuclear compartment as well as the cytoplasm. The presence of PTP-S in the nucleus as well as the cytoplasm raises the possibility of nuclear–cytoplasmic exchange; it could possibly transmit signals from cytoplasm to the nucleus. Changes in subcellular localization in response to external stimuli is one of the strategies used by the cell for the regulation of activity of certain proteins (Whiteside and Goodbourn, 1993).

Sub-nuclear localization

The PTP-S protein present in the nucleus appears to be largely associated with chromatin, since salt and DNAase I treatment could release it. Less release upon DNAase I digestion is perhaps due to incomplete digestion of DNA under the conditions of our experiments. A small fraction of PTP-S may be associated with the nuclear matrix. Since the PTP-S protein possesses DNA-binding ability and is released from nuclei upon digestion with DNAase, it is likely that its interaction with chromatin is through DNA. This is supported by the observation that the salt concentration required for its elution from DNA–Sepharose is the same as that required for its release from the nuclei.

Some nuclear proteins, which bind to DNA, are known to be phosphorylated on tyrosine. These include members of the nuclear receptor superfamily: the oestradiol receptor (Migliaccio et al., 1984), the glucocorticoid receptor (Rao and Fox, 1987) and the β 1 thyroid-hormone receptor (Lin et al., 1992). Cells transformed by Abelson murine leukaemia virus have been found to contain elevated levels of several phosphotyrosyl proteins which bind to DNA (Bell et al., 1987). Recently it has been shown that α -interferon, upon binding to its membrane receptor, activates a latent cytoplasmic factor which becomes phosphorylated on tyrosine; subsequently this factor translocates to the nucleus, where it activates transcription of specific genes (Schindler et al., 1992). Several growth factors and cytokines can also activate putative transcription factors by tyrosine phosphorylation (Lerner et al., 1993; Ruff-Jamison et al., 1993; Silvennoinen et al., 1993; Sadowski et al., 1993). Although tyrosine phosphorylation of some of these proteins occurs in the

cytoplasm, followed by their translocation to the nucleus, some tyrosine kinases, e.g. those encoded by *c-abl* and *FER*, are located in the nucleus (Van Etten et al., 1989; Dhut et al., 1991; Hao et al., 1991). Since phosphorylation of proteins is a reversible process, dephosphorylation of these and other phosphotyrosyl proteins present in the nucleus would require PTPases in the nucleus. Only a few examples of PTPases in the nucleus are known. A nuclear phosphatase from uterus had been partially purified, which dephosphorylates the oestradiol receptor (Auricchio et al., 1987). The mammalian homologue of yeast *cdc25* protein is a nuclear PTPase which plays a critical role in the entry of cells into mitosis (Miller et al., 1991). Recently a nuclear PTPase named PAC-1 has been reported, which is transiently expressed upon mitogenic stimulation of T-lymphocytes (Rohan et al., 1993). In *Drosophila*, alternative splicing gives rise to two forms of a PTPase, one of which locates in the nucleus (McLaughlin and Dixon, 1993). In addition to these, the presence of PTPase activity has been reported in purified brain and liver nuclei (Swarup et al., 1991). A PTPase in the nucleus will have access to potential substrate proteins such as those that regulate the cell-division cycle or transcription. Whether PTP-S acts on any of these proteins is not known.

In T-lymphocytes the level of PTP-S mRNA increases upon mitogenic stimulation by concanavalin A for 72 h (Rajendra Kumar et al., 1993). This was due to an increase in half-life of PTP-S mRNA, from 25 min in resting cells to about 5 h in stimulated lymphocytes. The short half-life of PTP-S mRNA, increased expression on mitogenic stimulation, association of PTP-S protein with chromatin and nuclear matrix, and interaction with DNA, are some of the properties which suggest an important regulatory role for this protein in mammalian cells. The role of PTP-S or its cellular substrates is not known at present. Subcellular-localization studies presented in this paper suggest that the predominant site of action of this protein is likely to be within the cell nucleus.

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