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The Src and Signal Transducers and Activators of Transcription Pathways As Specific Targets for Low Molecular Weight Phosphotyrosine-protein Phosphatase in Platelet-derived Growth Factor Signaling*

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Paola Chiarugi‡, Paolo Cirri‡, Fabio Marra§, Giovanni Raugei‡‖, Tania Fiaschi‡, Guido Camici‡, Giampaolo Manao‡, Roberto Giulio Romanelli§, and Giampietro Ramponi‡¶

From the ‡Dipartimento di Scienze Biochimiche, viale Morgagni 50, 50134 Firenze, Italy and §Istituto di Medicina Interna, Università di Firenze, viale Morgagni 85, 50134, Firenze, Italy

The low molecular weight phosphotyrosine-protein phosphatase (LMW-PTP) is a cytosolic phosphotyrosine-protein phosphatase specifically interacting with the activated platelet-derived growth factor (PDGF) receptor through its active site. Overexpression of the LMW-PTP results in modulation of PDGF-dependent mitogenesis. In this study we investigated the effects of this tyrosine phosphatase on the signaling pathways relevant for PDGF-dependent DNA synthesis. NIH 3T3 cells were stably transfected with active or dominant negative LMW-PTP. The effects of LMW-PTP were essentially restricted to the G_1 phase of the cell cycle. Upon stimulation with PDGF, cells transfected with the dominant negative LMW-PTP showed an increased activation of Src, whereas the active LMW-PTP induced a reduced activation of this proto-oncogene. We observe that c-Src binding to PDGF receptor upon stimulation is prevented by overexpression of LMW-PTP. These effects were associated with parallel changes in *myc* expression. Moreover, wild-type and dominant negative LMW-PTP differentially regulated STAT1 and STAT3 activation and tyrosine phosphorylation, whereas they did not modify extracellular signal-regulated kinase activity. However, these modifications were associated with changes in *fos* expression despite the lack of any effect on extracellular signal-regulated kinase activation. Other independent pathways involved in PDGF-induced mitogenesis, such as phosphatidylinositol 3-kinase and phospholipase C-γ1, were not affected by LMW-PTP. These data indicate that this phosphatase selectively interferes with the Src and the STATs pathways in PDGF downstream signaling. The resulting changes in *myc* and *fos* proto-oncogene expression are likely to mediate the modifications observed in the G_1 phase of the cell cycle.

Protein-tyrosine phosphorylation plays a key role in the regulation of many cellular processes in eukaryotes such as cellular metabolism, proliferation, differentiation, and oncogenic transformation (1). Accumulating evidence indicates that the contribution of phosphotyrosine-protein phosphatases (PTPs)[1] to the control of phosphorylation state is as relevant as that of phosphotyrosine-protein kinases. PTPs activity is carefully regulated and appears to be, in most cases, highly specific. The PTPs consist of a family of over 40 enzymes often classified into three groups: 1) receptor-like PTPs; 2) intracellular PTPs; and 3) dual specificity PTPs (2). All PTPs share the signature active site motif C*XXXXX*R, in which the catalytic cysteine residue is involved in formation of a phosphoenzyme reaction intermediate (3). The low molecular weight phosphotyrosine-protein phosphatase (LMW-PTP) is a cytosolic enzyme without extensive sequence homology with other PTPs family members, but it contains the C*XXXXX*R motif and shares the same catalytic mechanism of classical PTPs (4, 5). Furthermore, the LMW-PTP crystal structure (6) revealed a tridimensionally folded phosphate binding loop that is structurally identical to that contained in the human placenta PTP1B and *Yersinia* PTP (7, 8).

We have previously demonstrated that the mutation of the cysteine residue in the signature motif to serine (C12S), causes the complete loss of catalytic activity (9). Nevertheless, this dominant negative mutant (dnLMW-PTP) is still able to bind to specific substrates (10). Overexpression of the active phosphatase (wtLMW-PTP) causes a reduction of cell proliferation (11), whereas dnLMW-PTP induces a remarkable increase in DNA synthesis, indicating that this latter molecule behaves as a dominant negative *in vivo* (12). In addition we have demonstrated that this phenotype is associated with a specific and direct interaction between the active site of dnLMW-PTP and the activated platelet-derived growth factor receptor (PDGF-R) (13). These data indicate that the PDGF-R is a specific substrate of the LMW-PTP and that this phosphatase may be involved in the control of one or more signaling pathways triggered by PDGF-R activation.

Binding of PDGF induces receptor dimerization and autophosphorylation. Tyrosine phosphorylation sites in the PDGF-R function as high affinity binding sites for several molecules involved in downstream signal transduction (14). These proteins bind the phosphorylated receptor through their Src homology domain 2 or phosphotyrosine binding domains,

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‖ To whom correspondence should be addressed. Tel.: 39-55-413765; Fax: 39-55-4222725; E-mail: raugei@cesit1.unifi.it.

[1] The abbreviations used are: PTP, phosphotyrosine-protein phosphatase; LMW-PTP, low molecular weight PTP; dnLMW-PTP, dominant negative LMW-PTP; ERK, extracellular signal-regulated kinase; MAP-2, microtubule-associated protein 2; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; PI3K, phosphatidylinositol 3-kinase; PLCγ1, phospholipase C γ1; SIE, *sis*-inducible element; SIF, *sis*-inducible factor; SRE, serum response element; STAT, signal transducer and activator of transcription; wtLMW-PTP, wild-type LMW-PTP.

and in many cases are themselves substrates of the receptor kinase activity. These proteins include enzymes such as phospholipase C-γ1 (PLCγ1), phosphatidylinositol 3-kinase (PI3K), Src, or molecular adapters such as Shc, Grb2, and Nck, leading to different routes that mediate the biological effects of PDGF. One of the main routes leading to cell proliferation is the so-called Ras/extracellular signal-regulated kinase (ERK) pathway. Upon stimulation with growth factors, Ras activation can be achieved through a variety of signal transducers like Shc, Grb2, and Sos (15). Alternatively, Syp phosphatase may function as an adapter molecule for Grb2 and therefore activate Ras (16). Activated Ras triggers a kinase cascade with sequential activation of Raf-1, MAP/ERK, and ERK. Upon activation, ERK translocates to the nucleus where it induces *fos* transcriptional activation (17, 18). An alternative route leading to *fos* transcription independent of Ras/ERK, is the activation of the signal transducers and activators of transcription (STAT) pathway, leading to formation of three transcription factors that bind to the *fos* promoter. These transcription factors, known as *sis*-inducible factor (SIF) A, B, and C, are STAT-3 homodimers, STAT1/3 heterodimers, and STAT1 homodimers, respectively. In addition, another Ras-independent pathway relevant for mitogenesis is the activation of the cytosolic tyrosine kinase Src. Recently, Barone and Courtneidge (19) have demonstrated that the kinases of the Src family regulate *myc* activation, and that *myc* is necessary for PDGF-dependent DNA synthesis in NIH 3T3 cells. In fact, *myc* but not *fos* rescue the PDGF-signaling block caused by dominant negative Src. Both the Ras/ERK and the Src pathways are necessary for DNA synthesis in response to PDGF, epidermal growth factor, and colony-stimulating factor-1 (19). In addition, the Src and STAT pathways seems to be linked (20, 21), although it remains to be established if this connection is directly mediated by Src kinase. Finally, other pathways relevant for PDGF mitogenic signaling are PLCγ1 and PI3K. PLCγ1 catalyzes the breakdown of phosphatidylinositol bisphosphate, triggering a cytosolic calcium increase and protein kinase C activation. PI3K is a protein and lipid kinase that activates the product of the proto-oncogene *akt*, protein kinase Cζ, and other targets, which have been only partially elucidated.

In this study, we examine the role of the LMW-PTP in signaling pathways originated by PDGF-R activation. We report that LMW-PTP is involved in the regulation of Myc expression through Src activation, and of Fos through an ERK independent pathway mediated by the STAT proteins.

EXPERIMENTAL PROCEDURES

Materials—Unless specified all reagents were obtained from Sigma. NIH 3T3 cells were purchased from ATCC; human recombinant platelet-derived growth factor BB (PDGF-BB) was from Peprotech; RC20 anti-phosphotyrosine antibodies were from Affiniti; enhanced chemiluminescence kit was from Amersham; c-*fos* murine cDNA was from ATCC; human c-*myc* cDNA was a generous gift from G. I. Evans; and Src kinase assay kit, anti-Src antibodies, and PDGF receptor antibodies were from Upstate Biotechnology, Inc. Agarose-conjugated phosphotyrosine antibodies were purchased from Oncogene Science. Anti-ERK1, anti-phosphotyrosine, anti-PDGF receptor, and anti-PLCγ1 antibodies were from Santa Cruz Biotechnology, and anti-STAT1 and anti-STAT3 antibodies were from Transduction Laboratories.

Cell Culture and Transfections—NIH 3T3 cells were routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in 5% CO_2 humidified atmosphere. 10 μg of pSVT7PTPC12S (12) or pSVT7PTP (22) and 0.5 μg of pSV2neo, conferring neomycin resistance, were cotransfected in NIH 3T3 cells using the calcium phosphate method. Stable transfected clonal cell lines were isolated by selection with G418 (400 μg/ml). Control cell lines were obtained by transfecting 2 μg of pSV2neo alone. The clonal lines were screened for expression of the transfected genes by *a*) Northern blot analysis and *b*) enzyme-linked immunosorbent assay using polyclonal anti-LMW-PTP rabbit antibodies, which do not crossreact with murine endogenous LMW-PTP.

Immunoprecipitations and Western Blot Analysis—1×10^6 cells were seeded in 10-cm plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells were serum-starved for 24 h before receiving PDGF-BB. Cells were then lysed for 20 min on ice in 500 μl of modified RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 2 mM EGTA, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Lysates were clarified by centrifugation and immunoprecipitated for 4 h at 4 °C with 5 μg of the specific antibodies. Immune complexes were collected on protein A-Sepharose (Pharmacia Biotech Inc.), separated by SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose (Sartorius). Immunoblots were incubated in 3% bovine serum albumin, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.1% Tween-20, for 1 h at room temperature and then probed first with specific antibodies and then with secondary antibodies conjugated with horseradish peroxidase, washed and developed with the enhanced chemiluminescence kit (Amersham).

Cell Cycle Analysis—Growth data were obtained plating 2×10^5 cells for 48 h. Cell number was estimated by counting in a Burker chamber. Cell doubling times (*cdt*) were calculated as: $cdt = 48/(b - a)$, where a is $\log_2$ of the cell number at time of plating and b is $\log_2$ of cell number at 48 h of standard exponential growing conditions.

Cytofluorimetric analysis was performed according to Liu (23). Briefly, 2×10^5 cells were seeded into 6-cm dishes the day before analysis to obtain exponentially growing cultures. Cells were rinsed twice with cold phosphate-buffered saline and harvested after trypsinization in 1 ml of 50 mg/liter of propidium iodide. Analysis was performed in a Becton Dickinson FACScan using the Lysis II and Cell Fit cell analysis software according to the manufacturer's procedure.

mRNA Analysis—1×10^7 cells were serum-starved for 24 h and than stimulated with 30 ng/ml of PDGF. Total RNA was purified according to a method previously described (24). 10 μg of total RNA were separated onto a denaturing 15% formaldehyde-agarose gel and blotted onto nitrocellulose filters using standard methods. Hybridization was performed overnight in 4× standard saline citrate, 0.1% SDS, 5× Denhart's, 1 mM EDTA, 20 mM sodium phosphate, pH 7.2, at 65 °C using a random priming labeled cDNA with specific activity of about 10^9 cpm/μg. Washings were performed twice in 0.5× standard saline citrate, 0.1% SDS at 55 °C; the filter was then autoradiographed and the signals quantitated with a densitometer. Normalization was done on the basis of a human actin cDNA probe hybridization.

ERK Activity Assay—ERK activity was assayed by phosphorylation of microtubule-associated protein 2 (MAP-2) as described by Mihasaka (25). Briefly, 3×10^5 cells were seeded in 6-cm dishes and serum-starved for 24 h. Cells stimulated with 5–50 ng/ml PDGF were harvested by scraping in 0.2 ml of lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EGTA, 1 mM orthovanadate, 0.1 mM molybdate, 1% Triton X-100, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin). 10 μl of cell lysate (1.5 mg/ml of total protein) were incubated with 2 μg of MAP-2 for 10 min at 30 °C in a final volume of 25 μ l, containing 50 mM Tris-HCl, pH 7.4, 2 mM EGTA, 10 mM $MgCl_2$, 40 μM [γ-^{32}P]ATP (1 μCi, 3000 Ci/mmol). The reaction was stopped by the addition of 4× Laemmli SDS-polyacrylamide gel electrophoresis sample buffer. Phosphorylated MAP-2 was resolved by SDS-polyacrylamide gel electrophoresis (7.5% acrylamide gel). Coomassie Blue-stained bands containing MAP-2 protein were excised from the gel, and incorporated radioactivity was measured by scintillation counting. ERK activity was also measured by an *in vitro* kinase assay after immunoprecipitation. 70 μg of total proteins in modified RIPA lysis buffer were immunoprecipitated with anti-ERK1 polyclonal antibodies and with the use of protein A-Sepharose. After five washings with 1 ml of modified RIPA buffer, the immunobeads were incubated in 25 μl of a buffer containing 15 mM Tris-HCl, pH 7.4, 15 mM $MgCl_2$, 0.5 mM EGTA, 40 mM ATP, 1 μCi [γ-^{32}P]ATP (3000 Ci/mmol), and 10 μg of a peptide derived from myelin basic protein (Santa Cruz Biotechnology). After 5 min of incubation at 30 °C the reaction was stopped by adding 20 μl of 40% trichloroacetic acid. 25 μl of the reaction mixture were spotted onto 2 × 2-cm phosphocellulose disks, washed four times in 0.75% phosphoric acid and once in acetone. Radioactivity was evaluated by scintillation counting. The amount of immunoprecipitated ERK1 from each sample was evaluated by anti-ERK1 immunoblot. Densitometric analysis of the immunoblot was used for normalization of the immunokinase assay.

Cell Motility Assay—Migration of NIH 3T3 cells expressing wtLMW-PTP or dnLMW-PTP was assayed with the Boyden chamber system for chemotaxis (Nucleopore Corp., Pleasanton, CA) equipped with 8-μm pore polyvinylpyrrolidone-free polycarbonate filters (13-mm diameter) (26). Polycarbonate filters were precoated with human type I collagen

(20 μg/ml in phosphate-buffered saline, pH 7.4) for 30 min at 37 °C and placed between the chemoattractant (lower chamber) and the upper chamber. The lower chamber was filled with medium supplemented with different concentrations of PDGF-BB. Cells cultured in serum-free Dulbecco's modified Eagle's medium were suspended by trypsinization, and 3×10^4 cells in 200 μl was added to the top wells and incubated at 37 °C in 5% CO_2 for 6 h. After incubation the cells that had attached to the upper side but had not migrated through the filter were mechanically removed using cotton swabs. The filters were fixed in 96% methanol and stained with Harris hematoxylin solution. Chemotaxis was quantitated by counting the cells that had migrated to the lower surface of the polycarbonate filters. For each filter the number of cells in six randomly chosen fields was determined, and the counts were averaged (mean ± S.D.).

Phosphatidylinositol 3-Kinase Activity—PI3K assay was performed as described elsewhere (27, 28). Briefly, serum-starved cells were incubated with 30 ng/ml of PDGF for 10 min and then lysed in RIPA buffer. Equal amounts of protein were immunoprecipitated using an agarose-conjugated anti-phosphotyrosine antibody. After washing, the immunobeads were resuspended in 50 μl of 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.5 mM EGTA. 0.5 μl of 20 mg/ml phosphatidylinositol was added, mixed, and incubated at 25 °C for 10 min. 1 μl of 1 M $MgCl_2$ and 10 μCi of $[\gamma\text{-}^{32}\text{P}]$ATP (3000 Ci/mmol) were then added simultaneously and incubated at 25 °C for an additional 10 min. The reaction was stopped by the addition of 150 μl of chloroform, methanol, 37% HCl, 10:20:0.2. The samples were extracted with chloroform and dried. Radioactive lipids were separated by thin-layer chromatography using chloroform, methanol, 30% ammonium hydroxide, water 46:41:5:8. After drying, the plates were autoradiographed. Identity of the 3-OH phosphorylated lipids after separation by thin-layer chromatography has been previously confirmed using high-pressure liquid chromatography (28). The radioactive spots corresponding to phosphatidylinositol phosphate were scraped and counted in a liquid scintillation counter.

Gel Mobility Shift Assay—This assay has been carried out as described previously (29). Briefly, double-stranded synthetic oligonucleotides corresponding to the high affinity m67 oligonucleotide 5′-CAGTTCCCGTCAATC (30) were synthesized using a 329 DNA/RNA synthesizer (Applied Biosystems). The single-stranded oligonucleotides were annealed and labeled using T4 polynucleotide kinase and $[\gamma\text{-}^{32}\text{P}]$ATP (3000 Ci/mmol). Labeled DNA was separated from the unincorporated radioactivity using an ion-exchange column (Schleicher & Schuell). 10 μg of total cell extracts were incubated in binding buffer (35 mM HEPES, pH 7.8, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, and 250 mM spermidine) containing 10 μg/ml of poly(dI·dC) (Pharmacia) and 50,000–100,000 cpm of radiolabeled DNA for 30 min at 25 °C.

The samples were then separated on a native 5% polyacrylamide gel (50 mM Tris, 380 mM glycine, 10% glycerol). After electrophoresis, the gel was dried and autoradiographed. For "supershift" experiments, the cell extracts were incubated with a monoclonal anti-STAT1 or a polyclonal anti-STAT3 antibody for 30 min on ice before the binding reaction.

Src Kinase Assay—1.5×10^6 cells were seeded in 10-cm dishes, serum-starved for 24 h and then stimulated with 50 ng/ml of PDGF for 5 min at 37 °C. Cell were lysed in modified RIPA buffer (50 mM Tris-HCl, pH 7.5, 2 mM EGTA, 1 mM orthovanadate, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Lysates were clarified by centrifugation and assayed for protein content. 5 μg of anti-Src antibodies were added to 500 μg of total proteins. The reaction mixtures were incubated overnight at 4 °C. Immune complexes were collected using 50 μl of protein A-Sepharose resin that had been preincubated with 5 μg of rabbit anti-mouse IgG. The beads were incubated in the reaction mixture for 1 h at 4 °C. The beads were washed three times with 500 μl of modified RIPA buffer and once with 1 ml of cold phosphate-buffered saline. The beads were then incubated for 15 min at 30 °C in 40 μl kinase buffer (25 mM Tris-HCl, pH 7.2, 30 mM $MgCl_2$, 6 mM $MnCl_2$, 0.5 mM EGTA, 0.1 mM orthovanadate, 0.5 mM dithiothreitol, 125 μM $[\gamma\text{-}^{32}\text{P}]$ATP, corresponding to 1 μCi) containing 10 μg of a Src specific peptide. The reaction was stopped by adding 20 μl of 40% trichloroacetic acid. After centrifugation 25 μl of supernatant were spotted onto 2 × 2-cm phosphocellulose paper, that was subsequently washed in a 0.75% phosphoric acid solution and then in pure acetone. Radioactivity was quantitated in a scintillation counter. The amount of immunoprecipitated c-Src from each sample was evaluated by anti-c-Src immunoblot. Densitometric analysis of the immunoblot was used for normalization of the immunokinase assay.

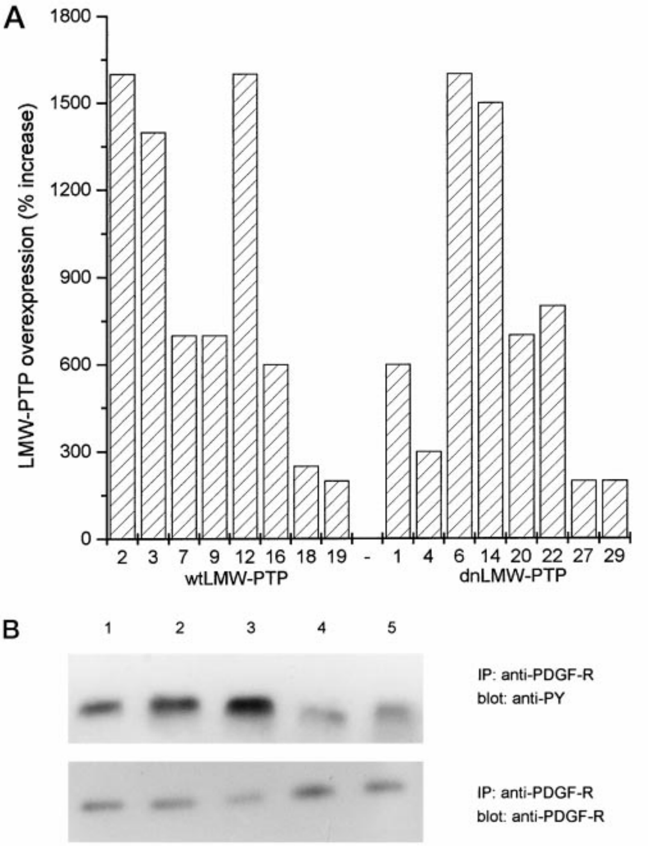

FIG. 1. **LMW-PTP expression in NIH 3T3 affect PDGF-R tyrosine phosphorylation.** *A*, NIH 3T3 cells were transfected with expression plasmids coding for dnLMW-PTP and wtLMW-PTP. Independent G418-resistant selected clones were grown to near-confluence and lysed in phospho-saline buffer by sonication. LMW-PTP expression was evaluated by an enzyme-linked immunosorbent assay test with polyclonal anti-LMW-PTP antibodies. LMW-PTP basal expression in neomycin cells was taken as 100%. Clone numbers are reported in abscissa. *B*, NIH 3T3 cells were serum-starved for 24 h and then incubated with 30 ng/ml of PDGF for 5 min. Cell lysates were used for anti-PDGF-R immunoprecipitation with polyclonal antibodies. Anti-phosphotyrosine and anti-PDGF-R immunoblots were performed as reported under "Experimental Procedures." The same blot reprobed with anti-PDGF-R antibodies is presented below. *Lane 1*, neomycin cells; *lane 2*, dnLMW-PTP cl.6; *lane 3*, dnLMW-PTP cl.14; *lane 4*, wtLMW-PTP cl.2; *lane 5*, wtLMW-PTP cl.12. This a representative of three independently performed experiments. *IP*, immunoprecipitation.

RESULTS

LMW-PTP Expression—NIH 3T3 cell line was chosen to assess the role of LMW-PTP in PDGF signaling. Expression plasmids containing the coding sequence for either wtLMW-PTP and dnLMW-PTP, in which the critical active site cysteine residue had been mutated to serine (C12S), were transfected into the NIH 3T3 cell line. Clonal selection of neomycin-resistant cells resulted in the establishment of cell lines overexpressing active LMW-PTP or the dominant negative form of LMW-PTP, respectively. To verify the overexpression of LMW-PTP and to choose clones with comparable LMW-PTP expression for subsequent studies, we used polyclonal anti-LMW-PTP antibodies in an enzyme-linked immunosorbent assay analysis of whole cell lysates from each of the derivative cell lines in comparison to neomycin cells. Enzyme-linked immunosorbent assay quantification data of LMW-PTP overexpression are presented in Fig. 1*A*. For further analysis, we chose four NIH 3T3-transfected clones with comparable LMW-PTP expression levels (wtLMW-PTP cl.2 and cl.12 and dnLMW-PTP cl.6 and cl.14, respectively). We previously demonstrated that overexpression of wtLMW-PTP and dnLMW-PTP in NIH 3T3 cells

TABLE I
Effects of the overexpression of dnLMW-PTP or wtLMW-PTP in NIH 3T3 cells

Percentages of cells in different cell cycle phases in exponentially growing cultures in complete medium were calculated by flow cytometry as described under "Experimental Procedures." The deduced duration of single phases is indicated. Data are mean ± S.E. of twelve independent determinations.

Transfection	Neomycin	dnLMW-PTP	wtLMW-PTP
Cell doubling time	20.7 ± 0.4	17.6 ± 0.4	29.9 ± 0.5
G_1			
%	56.3 ± 1.8	48.7 ± 4.0	68.0 ± 1.4
hours	11.7 ± 0.4	8.5 ± 0.3	20.4 ± 0.4
S			
%	32.6 ± 1.8	38.7 ± 5.0	13.9 ± 1.2
hours	6.7 ± 0.3	6.8 ± 0.8	4.1 ± 0.3
G_2/M			
%	11.2 ± 0.9	12.8 ± 1.2	18.0 ± 0.9
hours	2.3 ± 0.14	2.25 ± 0.1	5.4 ± 0.3

resulted in an association between the phosphatase and the PDGF receptor, leading, in the case of wtLMW-PTP, to receptor dephosphorylation (12, 13). We analyzed the PDGF receptor tyrosine phosphorylation of both cl.2 and cl.12 overexpressing wtLMW-PTP and cl.6 and cl.14 overexpressing dnLMW-PTP, respectively. PDGF receptor was immunoprecipitated from cells stimulated with 30 ng/ml PDGF-BB for 5′ and then its tyrosine phosphorylation was analyzed by anti-phosphotyrosine immunoblotting. Fig. 1*B* presents the results obtained. Scanning densitometry of these data revealed that both dnLMW-PTP-overexpressing clones showed an increased tyrosine phosphorylation of PDGF receptor (*lanes 2* and *3*) with respect to control (*lane 1*). In contrast, clones overexpressing the active phosphatase (*lanes 4* and *5*) showed the opposite phenotype, displaying a decreased level of receptor phosphorylation. As already published, overexpression of the dnLMW-PTP in NIH 3T3 cells causes an increased mitogenic response to PDGF, whereas overexpression of the wtLMW-PTP has an opposite effect (11, 12). Moreover, we observed that cell morphology, viability, and protein content were not affected by LMW-PTP overexpression. In any case, no difference among clones overexpressing either wtLMW-PTP or dnLMW-PTP was ever observed. For these reasons, clones 2 and 14 were randomly chosen for further analysis.

LMW-PTP Acts Specifically in G_1 Phase—To investigate the role of LMW-PTP in the downstream signaling events originating from PDGF-R, we analyzed the possible variations in cell cycle phases distribution in response to LMW-PTP overexpression. The cellular doubling time during exponential growth in complete medium was 20.7 ± 0.4 h (mean ± S.D.) for neomycin cells (expressing neomycin resistance alone), 17.6 ± 0.4 and 29.9 ± 0.7 h for NIH 3T3 cells overexpressing dnLMW-PTP or wtLMW-PTP, respectively. These data are in agreement with our previous results clearly showing an opposite phenotypic effect of dnLMW-PTP with respect to wtLMW-PTP. The effects on cell cycle distribution was analyzed by flow cytometry, and results are presented in Table I. dnLMW-PTP-overexpressing cells showed a shorter G_1 phase in comparison to neomycin cells, whereas the duration of the other phases remained almost identical. In contrast, cells transfected with wtLMW-PTP showed a marked increase in the duration of G_1 phase with respect to neomycin cells but also presented modifications in S and G_2/M phases, suggesting the existence of possible additional effects of wtLMW-PTP overexpression.

To confirm the influence of LMW-PTP on G_1 phase, cell synchronization was performed by aphidicolin treatment in dnLMW-PTP-overexpressing cells and neomycin cells. Results of this experiment confirmed a shortening of G_1 phase of about 3 h in response to dominant negative overexpression (data not shown).

LMW-PTP Modulates Src Activity and Myc Expression—The cytosolic tyrosine kinase Src is recruited and activated by phosphorylated PDGF-R. To investigate the possible interaction of LMW-PTP with this pathway, we evaluated the effects of phosphatase overexpression in NIH 3T3 cells on Src activity in response to PDGF stimulation. Overexpression of the dnLMW-PTP (Fig. 2*A*) greatly increased Src activity upon stimulation with PDGF in comparison to neomycin cells (about 60% increase), whereas overexpression of wtLMW-PTP resulted in a dramatic decrease of Src kinase activity (less than 30% with respect to neomycin cells). No significant change was observed in the Src activity of serum-starved unstimulated cells.

Growth factor-mediated activation of the Src pathway has recently been shown to be responsible for the activation of *myc* proto-oncogene (19). Therefore, we evaluated whether the observed changes induced by LMW-PTP on Src activity were paralleled by comparable changes in Myc expression. A Northern blot analysis using a human c-*myc* cDNA probe is shown in Fig. 2*B*. Using total RNA from neomycin cells (*lanes 1–3*), the expected increase in the specific transcript level (about 4.5-fold) was observed 1 and 3 h after stimulation with PDGF. Overexpression of the dnLMW-PTP (*lanes 7–9*) resulted in an increased response to PDGF both 1 and 3 h after stimulation. Conversely, overexpression of the wtLMW-PTP nearly abolished *myc* mRNA expression (*lanes 4–6*). These data indicate that LMW-PTP controls Myc expression through the regulation of Src activity.

Furthermore we have explored the possibility that LMW-PTP, while interacting directly with PDGF receptor, could prevent the recruitment of Src kinase by the receptor upon activation. For this purpose, PDGF receptor was immunoprecipitated from cell lysates after PDGF stimulation. Anti-Src immunoblot revealed that LMW-PTP really precludes Src association with the receptor (Fig. 3). In cells overexpressing dnLMW-PTP (*lanes 1* and *2*) we observed a dramatic increase in Src recruitment with respect to neomycin cells (*lanes 3* and *4*), whereas in cells overexpressing wtLMW-PTP the association of Src with the receptor was almost completely prevented. Taken together these data indicate that LMW-PTP regulation of Src pathway is at the very beginning in the signal transduction route leading to Myc activation. It is likely that LMW-PTP interferes with the binding of Src to PDGF receptor.

LMW-PTP Affects PDGF-stimulated Chemotaxis—Recent evidences indicates that the Src tyrosine kinase directly phosphorylates the PDGF-R in Tyr-934 (31). This leads to a negative modulation of the signal transduction pathway leading to motility response and shifts the response to increased mitogenicity. Since LMW-PTP appears to modulate the Src kinase activity, dnLMW-PTP or wtLMW-PTP-overexpressing cells were analyzed for their ability to migrate against a gradient of PDGF-BB, employing the leading front assay in a modified Boyden chamber. Measurements were done also in comparison with NIH 3T3 overexpressing c-Src. Cells at a density of 1.5 × 10^5/ml were resuspended after trypsinization and centrifugation in Dulbecco's modified Eagle's medium and seeded in the upper part of the modified Boyden chamber, while medium containing PDGF-BB was added below the 150-μm thick micropore filter. Compared with neomycin cells (Fig. 4), dnLMW-PTP-overexpressing cells displayed decreased PDGF-stimulated chemotaxis, whereas the wtLMW-PTP showed a clear extension in chemotactic response. As expected, c-Src-overexpressing cells showed a clear decrease in cell mobility as previously reported by Hansen (31). These data suggest that LMW-PTP influences the PDGF receptor-regulated chemotaxis. LMW-PTP probably modulates a signal transduction

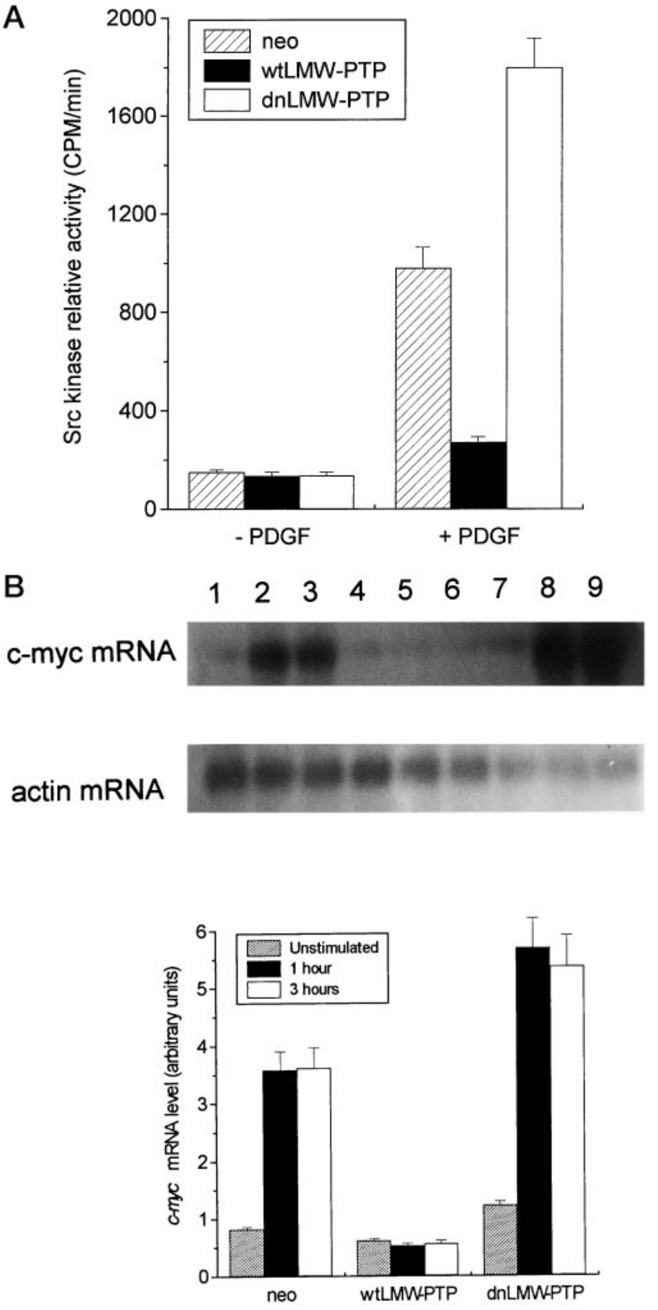

FIG. 2. **LMW-PTP modulates Src activity and Myc expression.** *A*, NIH 3T3 cells transfected with dnLMW-PTP or wtLMW-PTP or neomycin resistance alone (*neo*) were serum-starved for 24 h and then incubated with 30 ng/ml of PDGF for 5 min. Cell lysates were immunoprecipitated with anti-Src antibodies, and Src activity was assayed as described under "Experimental Procedures." Data have been normalized by anti-Src immunoblot of the immunoprecipitates. Data are the mean ± S.D. of three independent experiments. *B*, serum-starved cells were incubated for the indicated time with 30 ng/ml of PDGF. 10 μg of total RNA were analyzed by Northern blot using a human c-*myc* cDNA probe. Neomycin cells unstimulated (*lane 1*), stimulated for 1 h (*lane 2*), and for 3 h (*lane 3*); wtLMW-PTP-transfected cells unstimulated (*lane 4*), stimulated for 1 h (*lane 5*), and for 3 h (*lane 6*); dnLMW-PTP-transfected cells unstimulated (*lane 7*), stimulated for 1 h (*lane 8*), and for 3 h (*lane 9*). The blot was then stripped and reprobed with a human actin cDNA probe. Quantitation of three independent experiments by laser densitometry and normalization with actin is presented below. S.D. is indicated.

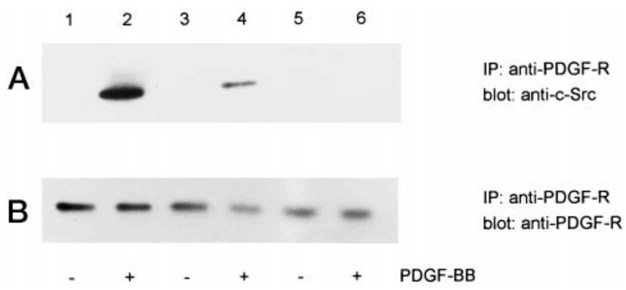

FIG. 3. **LMW-PTP prevents c-Src recruitment by the PDGF-R.** NIH 3T3 cells overexpressing dnLMW-PTP (*lanes 1* and *2*), neomycin cells (*lanes 3* and *4*), and wtLMW-PTP (*lanes 5* and *6*) were serum-starved for 24 h and then incubated with 30 ng/ml of PDGF for 2 min where indicated. Cell lysates were immunoprecipitated with polyclonal anti-PDGF-R antibodies and subjected to anti-Src immunoblot as described under "Experimental Procedures" (*A*). The same blot was reprobed with anti-PDGF-R antibodies for normalization (*B*). *IP*, immunoprecipitation. The results presented are based on three independent experiments (mean ± S.D.).

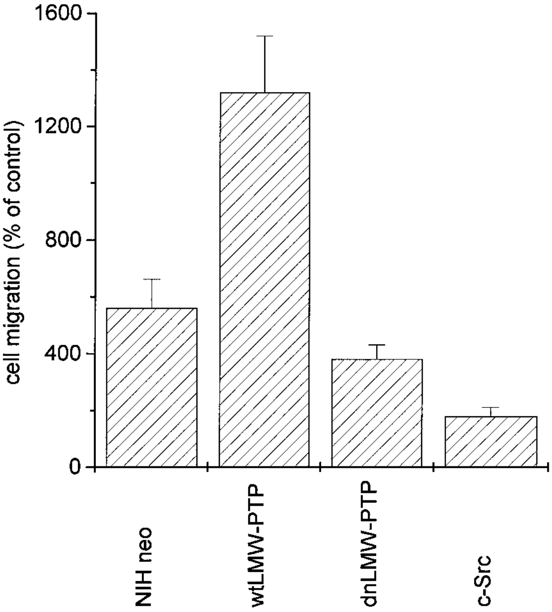

FIG. 4. **LMW-PTP affects the PDGF-stimulated chemotactic response.** The migration of NIH 3T3 cells overexpressing wtLMW-PTP or dnLMW-PTP or c-Src tyrosine kinase was measured in response to 10 ng/ml of PDGF-BB by the leading front assay using a modified Boyden chamber. Migration of cells under conditions where no PDGF-BB was added to the medium below the micropore filter was used as a measure of random migration and set as 100%. The results presented are based on three independent experiments (mean ± S.D.).

pathway leading to increased motility response and to a decreased mitogenic response.

LMW-PTP Is Not Involved in the Regulation of the Ras/ERK Pathway—The kinase cascade originating by activation of Ras results in ERK activation, which migrates to the nucleus and phosphorylates different transcription factors. The Ras/ERK pathway has been shown to be necessary for growth factor-dependent DNA synthesis. Therefore, we evaluated whether the observed effects of LMW-PTP on cell proliferation could be mediated by actions on this signaling pathway. Three different methods were used to address this issue. First, we assayed ERK activity by measuring the ability of total cell lysates to phosphorylate MAP-2, a specific ERK substrate. As shown in Fig. 5*A*, PDGF rapidly stimulated the enzyme activity in all cell lines, reaching a maximum within 5 min and slowly declining thereafter. No significant differences were observed in MAP-2 phosphorylation comparing neomycin cells with dnLMW-PTP- or wtLMW-PTP-overexpressing cells.

The possible influence of LMW-PTP on the PDGF dose-dependent ERK stimulation in cells overexpressing either

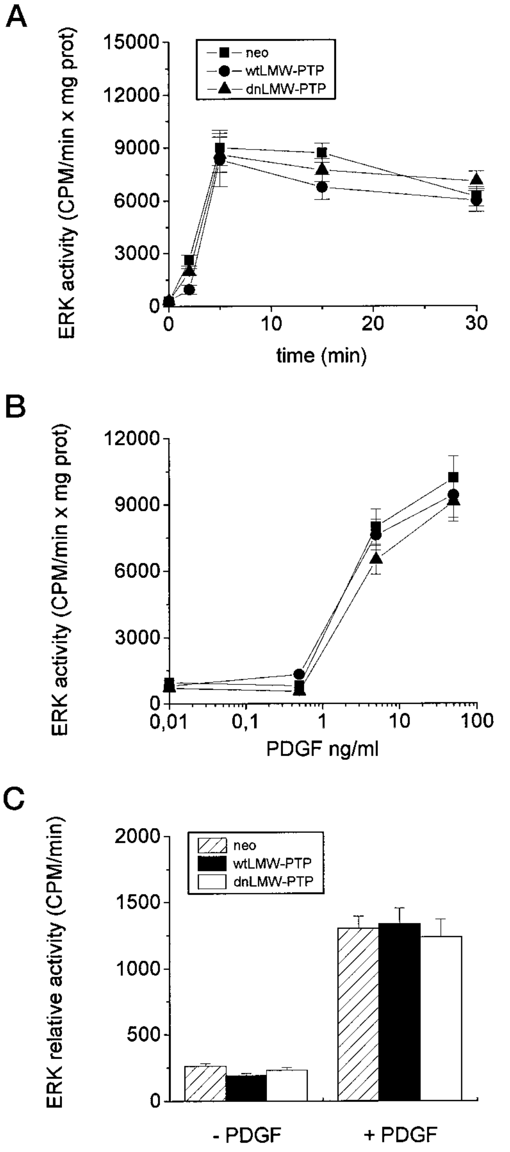

FIG. 5. **The LMW-PTP does not interfere with the Ras/ERK pathway.** *Panel A*, NIH 3T3 cells transfected with dnLMW-PTP or wtLMW-PTP or neomycin resistance alone (*neo*) were serum-starved for 24 h and then incubated with 30 ng/ml of PDGF for the indicated time. Cells were lysed and 15 μg of total proteins were used to measure the ability to phosphorylate the specific ERK substrate MAP-2. *Panel B*, the experiment was carried out as described for *panel A*, but the cells were incubated for 5 min with the indicated concentrations of PDGF. *Panel C*, transfected cells were incubated with 30 ng/ml of PDGF for 5 min and then lysed; 70 μg of total proteins were immunoprecipitated with polyclonal anti-ERK1 antibodies. The immunobeads were washed and used for an immune complex assay as indicated under "Experimental Procedures." Data have been normalized by anti-ERK1 immunoblot of the immunoprecipitates. Data are the mean ± S.D. of three independent experiments.

dnLMW-PTP or wtLMW-PTP was also evaluated (Fig. 5*B*). Maximal PDGF stimulation of ERK activity was always achieved with about 5 ng/ml of the growth factor. It is also evident that the potency of PDGF on ERK activation was not influenced by overexpression of LMW-PTP either in its dominant negative or active form.

Furthermore, we also measured ERK activity in immunoprecipitates obtained with the use of anti-ERK antibodies. An immune complex kinase assay using a peptide derived from myelin basic protein as a substrate was performed (Fig. 5*C*). No significant difference is present in the different samples.

ERK is activated by phosphorylation on tyrosine and threonine residues by the dual-specificity MAPK/ERK kinase (32). We evaluated the electrophoretic mobility of ERK as an index of its phosphorylation state since phosphorylated ERK shows a reduced mobility. Also in this case, the shift induced by treatment with PDGF was identical, irrespective of LMW-PTP overexpression (data not shown). Taken together, these data indicate that the phenotypic effect of the LMW-PTP is not mediated by its interference on the Ras/ERK pathway.

LMW-PTP Modulates Fos Expression via STAT1 and STAT3 Activation—Several soluble mediators activate the so called STATs pathway with differing specificity with respect to the activated kinases and the STAT protein complexes formed (33). PDGF and epidermal growth factor induce activation of STAT1 and STAT3 through tyrosine phosphorylation, which binds to the promoter of *fos* as homo- and heterodimers known as SIF-A, SIF-B, and SIF-C (34). We analyzed STAT1 and STAT3 tyrosine phosphorylation after PDGF stimulation for 15 min. (Fig. 6). In dnLMW-PTP-overexpressing cells both STAT1 and STAT3 showed an increased tyrosine phosphorylation with respect to mock transfected cells, whereas in wtLMW-PTP-overexpressing cells a decrease of STAT1 and STAT3 tyrosine phosphorylation was observed. In addition, we analyzed STAT1 and STAT3 activation in a gel mobility shift assay using a high affinity mutated oligonucleotide from the *fos* promoter (m67) according to Wagner (30). Formation of the three DNA binding complexes in response to PDGF stimulation (Fig. 7*A*) was markedly increased in cells expressing the dnLMW-PTP (*lane 6*), whereas it was dramatically reduced in those transfected with wtLMW-PTP (*lane 5*). As expected, no complex was formed in the absence of PDGF stimulation (*lanes 1* to *3*). Competition with an excess of unlabeled m67 oligonucleotide resulted in the disappearance of the three complexes (*lanes 10–12* and *16*), whereas addition of an unrelated oligonucleotide (specific for NFκB, *lane 17*) did not affect the DNA binding. The identity of the proteins involved in DNA binding was confirmed by preincubating the cell lysates with anti-STAT1 (*lanes 7–9*) or STAT3 antibodies (*lane 14*). Anti-STAT1 antibodies resulted in a further reduction ("supershift") of the electrophoretic mobility of SIF-B and SIF-C, the two complexes which contain STAT1. Anti-STAT3 antibodies caused the disappearance of the two slower migrating complexes corresponding to SIF-A and SIF-B, which contain STAT3. As a control, non-immune mouse IgG did not affect DNA binding of any of the SIF complexes.

Signals generated by PDGF at the cell membrane and transduced through the Ras/ERK or the STATs pathways converge at the level of the *fos* promoter, leading to the transcriptional activation of this oncogene. ERK and STATs pathways act on different regulatory elements of the *fos* promoter. Our results indicate that whereas LMW-PTP does not modify the Ras/ERK activation cascade, it modulates the STATs pathway and that this effect should be responsible for Fos induction. We evaluated the *fos* mRNA level in response to PDGF stimulation by Northern blot analysis (Fig. 7*B*). As expected, an increase of *fos* mRNA was observed in serum-starved neomycin cells as early as 5 min after the addition of the growth factor (*lanes 1–3*). This effect was clearly more pronounced in cells transfected with the dnLMW-PTP (*lanes 7–9*). On the other hand, in wtLMW-PTP-overexpressing cells this effect was observed as having a marked reduction of *fos* transcript accumulation (*lanes 4–6*) 15 and 30 min after the mitogenic stimulation with respect to neomycin cells. According to these data, it is very likely that modulatory activity of LMW-PTP on PDGF-regulated STAT activation is sufficient to induce changes in Fos expression.

PI3K or PLCγ1 Are not Targets of the LMW-PTP—PI3K and PLCγ1 have been shown to contribute in the transduction of the

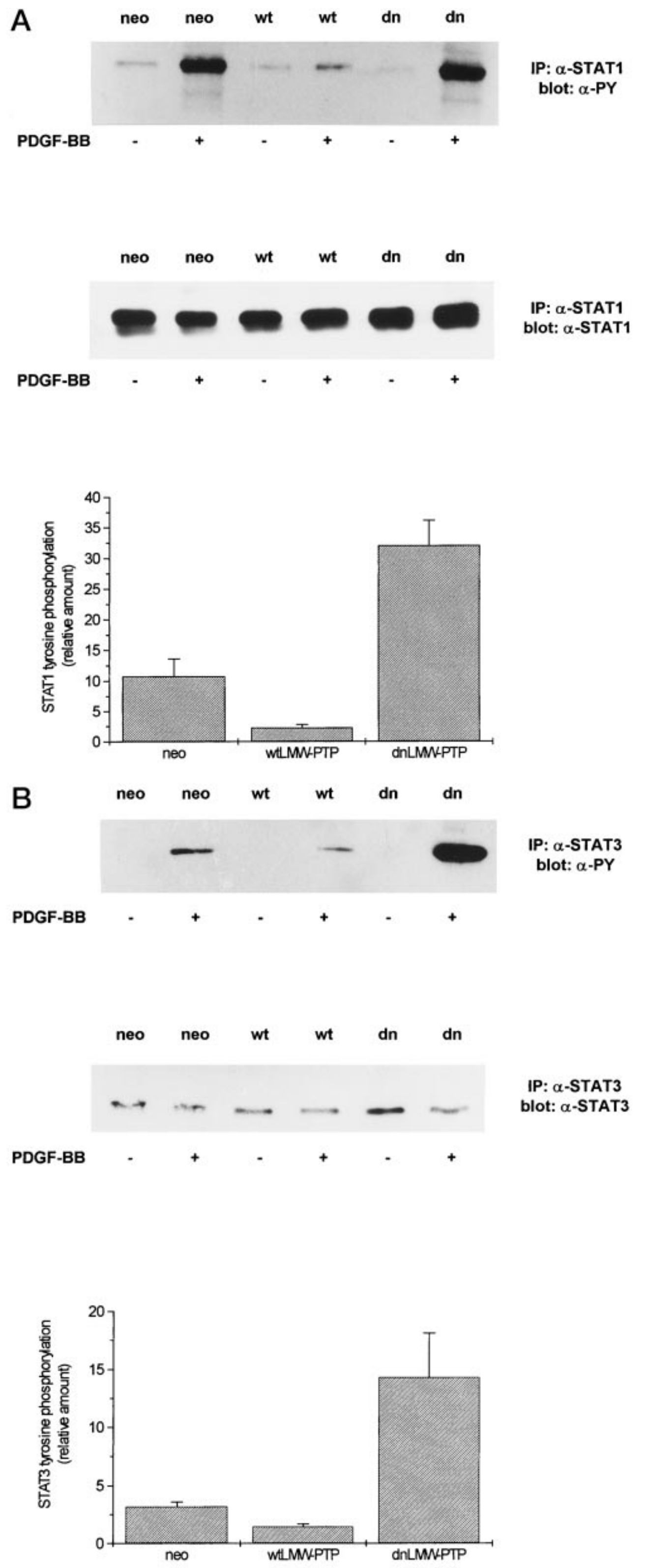

FIG. 6. **LMW-PTP affects the tyrosine phosphorylation level of both STAT1 and STAT3.** NIH 3T3 cells overexpressing dnLMW-PTP, wtLMW-PTP, or neomycin cells were serum-starved for 24 h and then incubated with 100 ng/ml of PDGF for 15 min where indicated. 250 μg of cell lysates were immunoprecipitated with polyclonal anti-STAT1 (*panel A*) or STAT3 (*panel B*) antibodies and subjected to anti-phosphotyrosine immunoblot as described under "Experimental Procedures." The same blots were reprobed with anti-STAT1 (*panel A*) or anti-STAT3 (*panel B*) antibodies for normalization. Normalized data obtained from densitometric analysis is shown. *IP*, immunoprecipitation; *PY*, phosphotyrosine. Data are the mean ± S.D. of three independent experiments.

mitogenic signal originating from PDGF-R activation (14). To investigate if LMW-PTP overexpression caused perturbations in these pathways, we analyzed PI3K and PLCγ1 activity. PDGF induced a dramatic up-regulation of PI3K activity (Fig. 8*A*), but this variation was not significantly influenced by the overexpression of either dnLMW-PTP or wtLMW-PTP (*lanes 5* and *6*) in comparison to neomycin cells (*lane 4*). Furthermore, we evaluated the amount of receptor-recruited PLCγ1. Immunoprecipitates obtained with anti-PDGF receptor antibodies were analyzed on Western blot using anti-PLCγ1 antibodies (Fig. 8*B*). No marked difference was present in the three samples.

According to these data, it is very likely that the effects of the LMW-PTP on mitogenesis are not mediated by PI3K and PLCγ1 activation.

DISCUSSION

Recent data from our laboratory have provided evidence for a role of LMW-PTP in the regulation of PDGF-dependent mitogenesis. In addition, we have reported that the PDGF receptor is a specific *in vivo* substrate of the LMW-PTP in NIH 3T3 cells and that this phosphatase physically associates with the activated receptor (12). However, no data were available regarding the molecular mechanisms by which the LMW-PTP can shut off the mitogenic signals initiated by PDGF. In this report, we first established that the overexpression of dnLMW-PTP induces a significant shortening of G_1 cell cycle phase whereas S and G_2/M remain almost unchanged, indicating that the LMW-PTP acts specifically in G_1 phase. This is in agreement with the hypothesis that LMW-PTP acts on the activated PDGF receptor (11, 12), which is a specific effector of G_1 progression. We have also observed that overexpression of the wtLMW-PTP markedly prolongs the doubling time although the changes are not uniquely restricted to the G_1 phase. It is possible that overexpression of the active enzyme can artifactually interact with other cellular districts and/or functions, whereas the dnLMW-PTP would cause fewer and more specific perturbations of the physiology of the cells. However, actions on phases of the cell cycle other than G_1 cannot be completely ruled out by the present data.

The main finding of this study is that in cells transfected with the LMW-PTP not all the pathways originating from the activated PDGF receptor are affected. Rather, this phosphatase appears to be highly specific in modulating the Src and the STATs pathways without interacting with other important routes such as Ras/ERK, PI3K, and PLCγ1. The effects on ERK activation are particularly striking since many extracellular and intracellular effectors may converge at this level. Along these lines, the lack of changes in ERK activity further confirms that neither PI3K nor PLCγ1 are involved in mediating the effects of the LMW-PTP. In fact, PI3K has been shown to signal through routes that are at least in part dependent on Ras (35), and activation of PLCγ1 results in the generation of signals that ultimately lead to ERK activation via Ras-dependent and -independent pathways (36, 37). Overexpression of the active and inactive forms of the LMW-PTP determine a reduced or increased activation of both Src and STATs pathways. Furthermore, LMW-PTP prevents the direct association of Src with the tyrosine-phosphorylated PDGF receptor. These modifications are associated with similar changes in the nuclear targets of these signaling pathways, namely *myc* and *fos*. Therefore, not only LMW-PTP modulate post-receptor signaling, but the resulting modifications are sufficient to affect activation of specific transcription factors.

Src kinase transduces PDGF signaling in a ERK-independent manner (19). Recent results indicate that Src is the starting point of a route that leads to Myc expression, whereas it is not necessary for Ras activation. This so called "Src pathway" is in

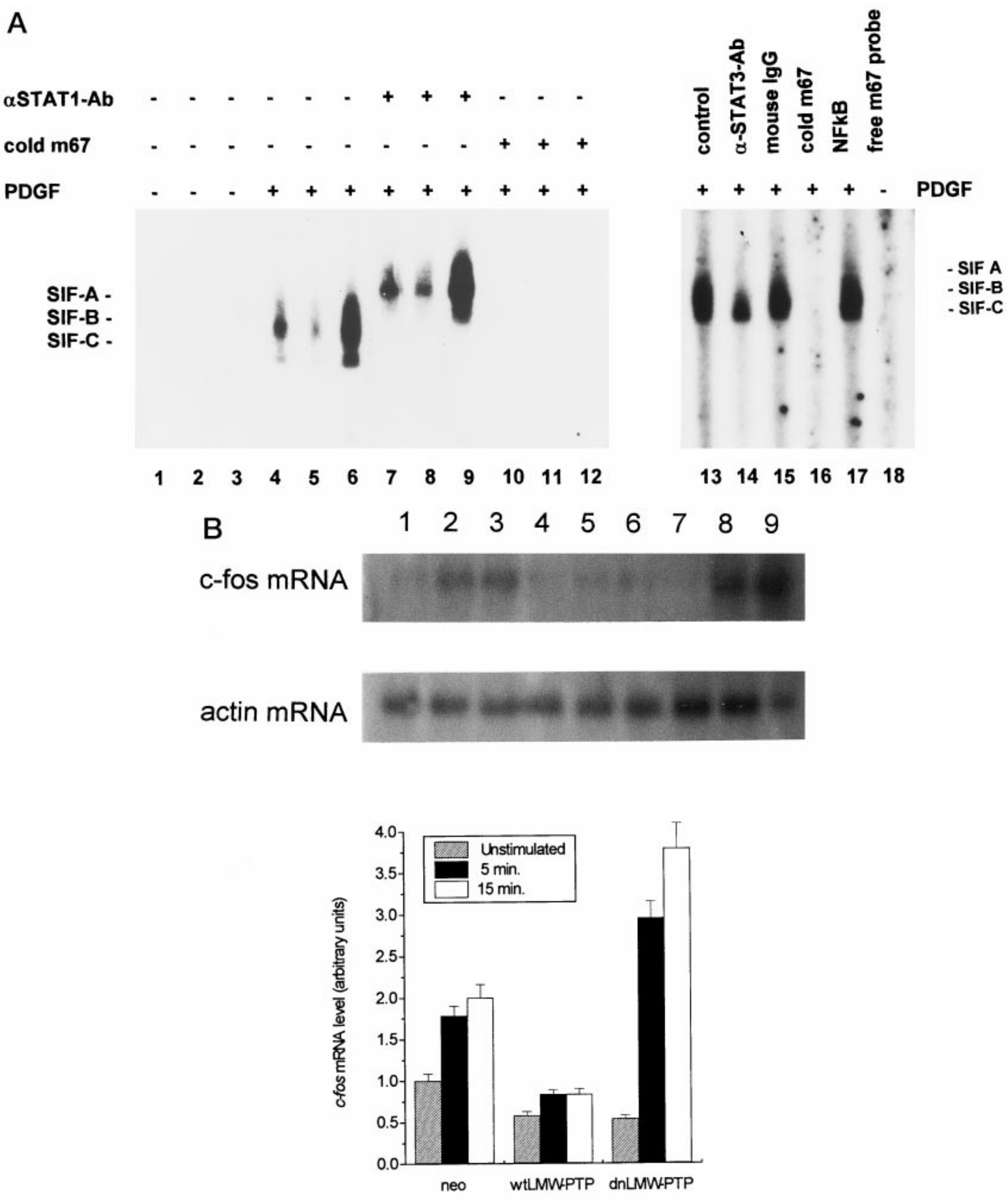

FIG. 7. **LMW-PTP modulates STAT activation through their tyrosine phosphorylation and c-*fos* expression.** *A*, NIH 3T3 cells transfected with dnLMW-PTP or wtLMW-PTP or neomycin resistance alone (*neo*) were serum-starved for 24 h and then incubated with 100 ng/ml of PDGF for 15 min where indicated. 10 μg of total proteins from cell lysate were used for a gel mobility shift assay using a radiolabeled oligonucleotide (m67) as described under "Experimental Procedures." In the indicated *lanes*, the lysates were preincubated with a 100-fold molar excess of unlabeled m67-SIE or an unrelated oligonucleotide (*NFκB*). In addition, we incubated the lysates with monoclonal anti-STAT1 or anti-STAT3 antibodies or with an unrelated anti-mouse IgG (*Ab*). *Lanes 1*, *4*, *7*, and *10*, lysates from neomycin NIH 3T3 cells; *lanes 2*, *5*, *8*, and *11*, lysates from cell overexpressing wtLMW-PTP; *lanes 3*, *6*, *9*, and *12–17*, lysates from cell overexpressing dnLMW-PTP; *lane 18*, free m67-SIE probe. The three complexes specifically interacting with the oligonucleotide are indicated on the *left*. *B*, serum-starved cells were incubated for the indicated time with 30 ng/ml of PDGF. 10 μg of total RNA were analyzed by Northern blot using a murine c-*fos* cDNA probe. Neomycin cells unstimulated (*lane 1*), stimulated for 5 min (*lane 2*), and for 15 min (*lane 3*); wtLMW-PTP-transfected cells unstimulated (*lane 4*), stimulated for 5 min (*lane 5*), and for 15 min (*lane 6*); dnLMW-PTP-transfected cells unstimulated (*lane 7*), stimulated for 5 min (*lane 8*), and for 15 min (*lane 9*). The blot was then stripped and reprobed with a human actin cDNA probe. Quantitation of three independent experiments by laser densitometry and normalization with actin is presented below. S.D. is indicated.

turn independent of Ras and appears to be required for PDGF-induced DNA synthesis. In fact, microinjection of a dominant negative Myc (Myc In 373) completely inhibits the mitogenic response to PDGF (19). Furthermore, microinjection of antibodies directed against all three Src kinase family proteins blocks both PDGF-induced Myc expression and entry of NIH 3T3 cells into S phase (38). Hence, these data strongly support the hypothesis that *myc* transcription is under the control of Src family kinases and is required for mitogenesis. Data presented herein indicate that the LMW-PTP is involved in the modulation of the Src pathway activated by the PDGF-R since Src recruitment by the phosphorylated PDGF-R is modified by LMW-PTP overexpression. Src kinase receptor association directly up-regulates its tyrosine kinase activity. It is likely that the modulation in the Src kinase activity observed in LMW-PTP-overexpressing cells is mediated by the LMW-PTP preven-

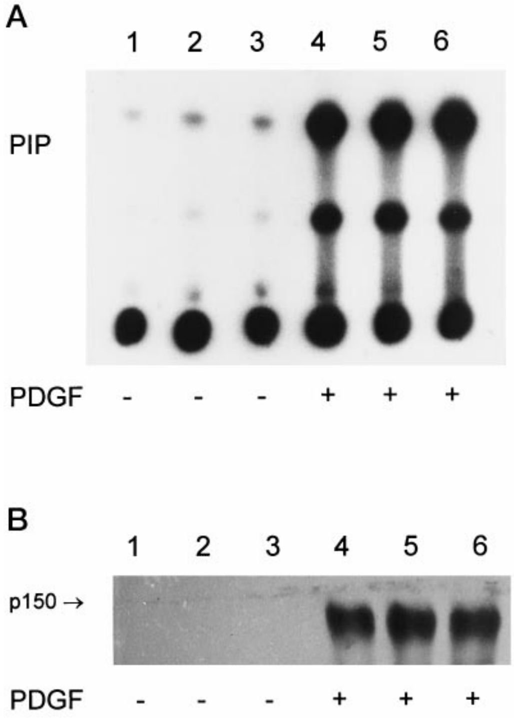

FIG. 8. **The LMW-PTP does not interact with PI3K or PLCγ1.** *A*, NIH 3T3 cells transfected with dnLMW-PTP or wtLMW-PTP or neomycin resistance alone (*neo*) were serum-starved for 24 h and then incubated with 30 ng/ml of PDGF for 10 min. 150 μg of total proteins from cell lysate were immunoprecipitated with anti-phosphotyrosine antibodies and used for PI3K assay as described under "Experimental Procedures." The spots corresponding to phosphatidylinositol 3-phosphate (*PIP*) from a representative experiment are shown. The intermediate spots are side products due to substrate contamination with phosphatidylinositol 2-phosphate, which is rapidly phosphorylated in position 3 by PI3K. *Lanes 1–3*, PI3K assay on immunoprecipitates from unstimulated neomycin, wtLMW-PTP-, and dnLMW-PTP-transfected cells; *lanes 4–6*, PI3K assay on immunoprecipitates from neomycin, wtLMW-PTP-, and dnLMW-PTP-transfected cells, respectively, stimulated with PDGF. Data from three separate experiments showed that no significant differences were present comparing the three cell lines stimulated by PDGF. *B*, transfected NIH 3T3 cells were serum-starved for 24 h and then incubated with 30 ng/ml PDGF for 10 min. 500 μg of total proteins from cell lysate were immunoprecipitated with anti-PDGF-β receptor antibodies, separated by SDS-polyacrylamide gel electrophoresis, and immunoblotted using polyclonal anti-PLCγ1 antibodies. *Lanes 1–3*, immunoprecipitates from unstimulated neomycin, wtLMW-PTP-, and dnLMW-PTP-transfected cells; *lanes 4–6*, immunoprecipitates from neomycin, wtLMW-PTP-, and dnLMW-PTP-transfected cells, respectively, stimulated with PDGF. Data reported are representative of three independent experiments.

tion of binding of Src to the receptor. Recent data reported by our group indicate that LMW-PTP is a target of v-Src *in vitro* (39). However, we failed to observe a physical interaction between c-Src and either dnLMW-PTP or wtLMW-PTP *in vivo*. A recent paper reports that the recruitment of the Src tyrosine kinase by PDGF-R leads to decreased PDGF-stimulated chemotaxis directly mediated by PDGF-R Tyr-934 phosphorylation by Src kinase (31). Our data on the modulation of the PDGF chemotactic response by LMW-PTP are in agreement with a specific action of LMW-PTP on the Src-mediated pathways leading to increased mitogenicity through Myc activation and to decreased PDGF-stimulated chemotaxis.

Fos activation appears to be strongly implicated in PDGF mitogenic signaling (17, 18). Activation of *fos* transcription by PDGF is mediated by the serum response element (SRE) and the *sis*-inducible element (SIE) of the promoter. The SRE interacts with the ternary complex factor, whose components Elk-1 and SAP-1 are direct targets of ERK (18). Conversely, SIE interacts with proteins of the STAT family, STAT1 and STAT3, which form three transcription factors, namely SIF-A, -B, or -C (30). We find that the LMW-PTP modulates Fos expression, which is greater in cells transfected with the dnLMW-PTP and reduced in those expressing wtLMW-PTP. These changes are associated with a modulation of both tyrosine phosphorylation and DNA binding activity of STAT1 and STAT3. On the other hand, ERK activity, measured by three independent methods, is unaffected by the overexpression of the LMW-PTP. The SRE and the SIE are both able to drive *fos* transcription in studies using deletion mutants of the promoter and a reporter gene (30). However, the relative contribution of the SIE to *fos* transcription is controversial (40). Data from the present study provide evidence that Fos expression can be modulated by the STATs pathway *in vivo* in the absence of any changes in the ERK/SRE pathway, even though the possible contribution of other factors cannot be completely ruled out. Therefore, the effects of the LMW-PTP suggest that both the SRE and the SIE are necessary to achieve optimal *fos* transcription.

The mechanisms involved in the reduced activation of STAT proteins by the LMW-PTP remain to be clarified. Three different Jak kinases, Jak-1, Jak-2, and Tyk-2 have been shown to interact with the PDGF receptor (36). However, none of these enzymes is necessary *per se* to obtain STATs phosphorylation. Recent data indicate that v-Src transformed cells show greater activation of STAT3 (20, 21), indicating a possible cross-talk between the Src and the STATs pathways. In addition, we have observed that in PDGF-stimulated NIH 3T3 cells c-Src overexpression leads to an up-regulation of both STAT1 and STAT3 (41).

In this study we present evidences showing that the activation of both STAT1 and STAT3 are clearly affected by the LMW-PTP, a finding that could be explained by the concomitant action of LMW-PTP on Src activity, thus suggesting that the STATs and Src pathways could be related. However, the direct LMW-PTP action on a Src-independent STATs pathway cannot be excluded.

In summary, the results of this study indicate that LMW-PTP decreases PDGF mitogenic signaling by selectively interacting with the Src and the STATs pathway, resulting in modulated expression of *myc* and *fos*, two proto-oncogenes crucial for G_1 progression. Whether the LMW-PTP affects PDGF receptor signal transduction pathways through the dephosphorylation of different receptor tyrosines remains to be established. On the other hand, this phosphatase may directly and specifically interact with only one phosphorylated tyrosine, leading to pleiotropic effects. The available data indicate that LMW-PTP may act as a physiological modulator of the effects of PDGF on cell growth.

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The Src and Signal Transducers and Activators of Transcription Pathways As Specific Targets for Low Molecular Weight Phosphotyrosine-protein Phosphatase in Platelet-derived Growth Factor Signaling

Paola Chiarugi, Paolo Cirri, Fabio Marra, Giovanni Raugei, Tania Fiaschi, Guido Camici,
Giampaolo Manao, Roberto Giulio Romanelli and Giampietro Ramponi

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