The Carboxyl-terminal Tyrosine Residue of Protein-tyrosine Phosphatase α Mediates Association with Focal Adhesion Plaques*

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The receptor protein-tyrosine phosphatase α (PTPα) is involved in the activation of c-Src kinase as well as in down-regulation of the insulin signal. To investigate the role of PTPα in activation of the Src kinase in more detail we tried to overexpress this phosphatase in NIH3T3 fibroblasts. Although PTPα has been overexpressed in rat embryonic fibroblasts and in embryonic carcinoma cells and should increase mitogenic responses we were not able to detect a measurable overexpression. In contrast, expression of partially (C442S) or completely inactive (C442S,C732S) PTPα or of phosphatase active PTPα containing mutation Y781F or Y798F was possible. The level of expression, however, was reduced to background after several passages of lines expressing PTPαC442S,C732S and PTPαY781F. When employed in a focus formation assay, only infection with virus encoding PTPαY798F induced Src-dependent formation of foci. In immunofluorescence studies, PTPαC442S and PTPαY781F but not PTPαY798F localized with proteins found in focal adhesion plaques. Treatment of PTPαC442S-overexpressing cells with vanadate abolished this colocalization and led to proteolytic processing of the phosphatase. We conclude that tyrosine 798 in PTPα is important for localization at focal adhesion plaques. Inhibition of phosphatases by vanadate treatment releases PTPα from focal adhesions.

Tyrosine phosphorylation regulates many cellular signaling pathways including those involved in mitogenesis, differentiation, and metabolic processes. Transmembrane and intracellular tyrosine kinases are activated by extracellular signals and generate phosphorylated tyrosines, either by auto- or substrate phosphorylation. These serve as docking sites or change the catalytic activity of the phosphorylated protein, respectively. The family of tyrosine kinases is rather large and comprises more than 100 genes that are expressed either ubiquitously or in specific tissues (1). Putative antagonists of kinases are protein-tyrosine phosphatases (PTPs), and more than 80 members of this family have been identified (2). Although initially the assumed role of phosphatases was to deactivate cellular signaling, examples are emerging where the phosphatase is necessary for activation of signaling pathways. This was documented in experiments where overexpression of a catalytically inactive form of the SH2 domain containing phosphatase SHP-2 interfered with signal transduction from the insulin receptor (3, 4). Similarly, the leukocyte-specific transmembrane PTP CD45 dephosphorylates the carboxyl-terminal tyrosine residue of the Lck kinase and thereby mediates the activation of the T-cell receptor (5); and the transmembrane phosphatase PTPα has been shown to regulate the activity of the Src kinase by dephosphorylating its carboxy-terminal tyrosine (6, 7). The detailed mechanisms of these regulatory events, however, have not been identified.

PTPα is a ubiquitously expressed phosphatase with a small extracellular domain and two intracellular phosphatase domains. The extracellular domain consists of 123 or, in a splice variant with 9 additional amino acids next to the transmembrane sequence, 132 amino acids that are heavily glycosylated (8, 9). No soluble or cell surface-anchored ligands are known to bind to the PTPα extracellular domain. For the intracellular domain a splice variant with a 36-amino acid insert in the amino-terminal phosphatase domain was found in mouse (10). This phosphatase domain harbors the main activity of the phosphatase, whereas the carboxyl-terminal domain probably has a more regulatory role (11, 12). PTPα can be phosphorylated by protein kinase C in the juxtamembrane domain, and this will enhance its activity (13, 14). Phosphorylation on tyrosine residues occurs at the very carboxyl terminus of PTPα and leads to the binding of the adaptor protein grb2. The kinase probably responsible for this phosphorylation was identified as c-Src in in vitro and in vivo assays (15). No insight, however, has been gained into the physiological consequences of tyrosine phosphorylation and grb2 binding.

We have shown recently that, in addition to the activation of c-Src, PTPα is capable of down-regulating the insulin signal in baby hamster kidney cells (16). In the system used in that study, stimulation of the overexpressed insulin receptor led to growth reduction, rounding of cells, and finally detachment from the dish surface. Cooverexpression of PTPα but not an activated form of c-Src reestablished the normal phenotype, indicating that the effect of PTPα was not mediated by activation of c-Src but probably was directly at the level of the insulin receptor.

Obviously, the physiological effects of PTPα are rather complex and can involve different signaling pathways. We therefore decided to investigate the role of PTPα in more detail by establishing NIH3T3 cell lines overexpressing PTPα and PTPα mutants. We found that PTPα concentrates at focal cell substratum adhesion sites and that this association is abolished by mutation of the carboxy-terminal tyrosine residue or treatment of cells with vanadate.

EXPERIMENTAL PROCEDURES

Plasmids, Cell Lines, and Antibodies—PTPαs and its mutant forms have been described before (17); the kinases csk and hyl were of human origin, whereas c-src was of murine origin and was generously provided.
by T. Hunter, San Diego. NIH3T3 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and 2 mM l-glutamine. BOSC 23 cells in Dulbecco’s modified Eagle’s medium/F12 medium with the same supplements. To establish the overexpressing NIH3T3 cell lines, all cDNAs were cloned into the retroviral expression vector pLXSN, and chloramphenicol-resistant DNA was transfected into BOSC 23 cells, retrovirus-containing supernatant harvested, and used to infect NIH3T3 cells as described by Pear et al. (18). G418-resistant cells were tested for expression of the donor genes, and positive clones were expanded and used for experiments. Antibodies used to detect PTPa were a rabbit polyclonal serum a-CT, directed against a keyhole limpet hemocyanin-coupled peptide representing the carboxy-terminal 13 amino acids, or D2, a mouse monoclonal IgG2 directed against an epitope in the intracellular domain of PTPa which is not affected by grb2 binding to the phosphatase. Antibodies for detection of c-Src, Csk, and Hyl were also generated against keyhole limpet hemocyanin-coupled peptides representing the carboxyl-terminal 15 amino acids of the proteins, whereas antibodies against paxillin and vinculin were obtained from Transduction Laboratories and Sigma, respectively. Phosphotyrosine was detected by mouse monoclonal antibody 5E2. Horseradish peroxidase-coupled secondary antibodies for use in Western blotting experiments were from Bio-Rad.

Lysis of Cells and Blotting—Cells were grown to confluence, made quiescent by changing the serum content to 0.5% for 24 h, and treated with 1–2 μM pyrophosphate for 1–2 h. They were lysed in 50 mM lysis buffer (50 mM HEPES, pH 7.2, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 100 mM sodium fluoride, 10 mM sodium pyrophosphate, and 1 mM sodium orthovanadate) per 10-cm dish, the lysate centrifuged for 2 min at 12,500 × g, and proteins immunoprecipitated from the supernatant for 4 h at 4 °C on a rotating wheel in the presence of 30 μl of a 1:1 slurry of protein A-Sepharose (Amersham Pharmacia Biotech) and the respective antibodies. Precipitates were washed, Laemmli buffer added, boiled for 5 min, size separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose filters, and analyzed by immunoblotting.

Focus Formation Assay—NIH3T3 cells (75,000) were seeded into a six-well culture plate the day before. They were infected with virus in the presence of 6 μg/ml Polybrene. 36 h later, cells were trypsinized and seeded into a 10-cm dish in Dulbecco’s modified Eagle’s medium containing 4% fetal calf serum. The medium was changed every other day for 3 weeks, and the cells were then stained with crystal violet.

Immunofluorescence—NIH3T3 cells were grown on uncoated glass coverslips and fixed for 30 min with 2% paraformaldehyde in phosphate-buffered saline, pH 7.4, with 0.12 μM sodium orthovanadate. Autofluorescence was quenched with phosphate-buffered saline containing 100 μM glycin and 26 mM borohydride. Cells were permeabilized with 0.5% saponin in phosphate-buffered saline for 5 min and unsppecific antibody binding blocked for 1 h with phosphate-buffered saline containing 0.5% bovine serum albumin and 0.45% fish gelatin. Incubation with primary antibody was done at room temperature for 2 h after dilution in 0.45% fish gelatin (1:50 for the polyclonal and 1:200 for the monoclonal anti-PTPa, 1:200 for anti-vinculin and anti-paxillin antibody). For double labeling experiments antibody decoration was done consecutively. After three washes with 0.45% fish gelatin, the primary antibody was detected with an isotype-specific secondary antibody fluorescein isothiocyanate (5:14,6-dichlorotriazin-2-yl]amino)fluorescein)-conjugated donkey anti-rabbit IgG (1:200) or Cy3-conjugated goat-anti-mouse IgG (1:300, Jackson Laboratories). Controls were incubated with either species-specific nonimmune serum or without primary antibody under otherwise identical conditions. Glass coverslips were mounted on microsco pe slides under glycerol with 2.5% 1,4-diazabicyclo[2,2,2]octane and were viewed with appropriate filter blocks for fluorescein and rhodamine on an LSM 410 laser scanning microscope (Zeiss) using a 40 × oil immersion objective of aperture 1.3. Images were recorded with a voxel size of 0.083 μm. In some experiments a gray scale transmission image (pseudoephase contrast) and the two individual laser confocal images were superimposed to visualize the localization of antibody and cellular morphology simultaneously. Coinmunolabeled cells were recorded at the identical exposure, brightness, and contrast settings.

RESULTS

Phosphatase Activity of PTPa Mutants in Vitro and in Intact Cells—Previous reports showed a role for PTPα in the regulation of Src kinase and found PTPα to be constitutively tyrosine-phosphorylated and bound to the adaptor protein grb2. In addition, overexpression in rat embryo fibroblasts led to cell transformation. We wanted to investigate the role of PTPα in the transformation of NIH3T3 cells using a cell line that was selected to exhibit a flat nontransformed morphology. Our approach involved initially the mutation of those amino acid residues in PTPα which may be important for phosphatase function or regulation. Exchange of amino acid 442 from cysteine to serine leads to inactivation of the amino-terminal phosphatase domain where the main activity is localized, whereas mutation of cysteine 732 to serine inactivates the second phosphatase domain (the numbering of residues is according to Kaplan et al. (8)). By homology to a similar sequence motif found in CD45 (20) tyrosine 781 represents a putative target for the kinase Csk and was exchanged to phenylalanine, as was tyrosine 798, which is phosphorylated constitutively and binds grb2. The phosphatase activity of the different mutants has been analyzed previously (17) and was comparable to wild type activity in vitro and in intact cells for the mutants C732S, Y781F, and Y798Y whereas the mutation C442S abolished phosphatase activity.

Differential Expression and Tyrosine Phosphorylation of PTPα and Its Mutants—For expression in 3T3 cells the cDNAs were cloned into a retroviral expression vector. Subsequently, recombinant retroviruses were generated and used to infect 3T3 cells. To our surprise we were not able to overexpress the native PTPα in 3T3 cells although we could demonstrate that the expression construct and the retroviruses were functional in a baby hamster kidney-insulin receptor cell rescue experiment (16). Transfection into 3T3 cells of a cytomegalovirus promoter-based PTPα expression plasmid used in the transient expression system also was not successful. Similar results were obtained with the closely related PTPε. The completely inactive PTPαC442S,C732S was overexpressed only weakly, and expression decreased over time. PTPαY781F was expressed, but the level was reduced with increasing passage number. Only PTPαC442S containing the inactive amino-terminal phosphatase domain and PTPαY798F lacking the grb2 binding site were present in the cells permanently and at high level.

The reason for this differential expression is unclear, but it probably reflects growth-inhibiting or toxic effects of PTPα in this cell line which are not exerted by the mutant PTPαs. Interestingly, other transmembrane PTPs could also not be overexpressed in this system (PTPα μ, κ, LAR, and CD45), whereas several intracellular PTPs were expressed well (PTPαs 1B, TC, SHP-1, and STEP; data not shown).

The cell lines were grown confluent, made quiescent, and left untreated or stimulated for 2 h with 1 mM vanadate. The cells were then lysed, and PTPα was immunoprecipitated with monoclonal antibody D2, directed against the intracellular domain. In the upper panel of Fig. 1 the phosphotyrosine content of PTPα mutants was evaluated and showed a strong increase of tyrosine phosphorylation in PTPα upon vanadate treatment in all cell lines. The different expression levels found for the PTPα mutants in the different cell lines are shown in the lower panel of Fig. 1. When comparing the expression levels of PTPαY798F versus C442S or Y781F the phosphorylation of Y798F was clearly reduced. This indicates that tyrosine 798 represents the major tyrosine phosphorylation site in PTPα, but it also reveals that other sites are phosphorylated in 3T3 cells, as has been shown previously by den-Hertog et al. (15). Cooverexpression of PTPαC442S with c-Src, Csk, or Hyl led to an increased phosphorylation of the phosphatase in the absence of vanadate. The candidate sites for these kinases would be tyrosine 798 for c-Src and tyrosine 781 for Csk and Hyl. Tyrosine phosphorylation of PTPα by Csk has also been shown in transient expression experiments (data not shown).

PTPαY798F Enhances Transformation of NIH3T3 Cells—

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Because activation of endogenous c-Src by overexpressed PTPα has been found in rat embryo fibroblasts and P19 embryonal carcinoma cells (6, 7) we wanted to investigate whether overexpression of PTPα or its mutant forms activates c-Src in the established cell lines. However, none of the cell lines formed foci or grew in soft agar so that activation of endogenous c-Src seemed not to be sufficient for transformation of the host cell. We therefore generated an NIH3T3 cell line overexpressing a moderate amount of murine c-Src. This cell line did not form foci or grow in soft agar either. When these cells were infected with equal amounts of retroviruses encoding PTPα, PTPαY781F, or PTPαY798F, foci were formed only upon infection with retrovirus encoding PTPαY798F (Fig. 2). The reason for this differential behavior is unclear. As discussed before, native PTPα could not be overexpressed in 3T3 cells, and this would be a simple explanation for the failure to form foci. On the other hand, PTPαY781F is catalytically active and can be overexpressed in 3T3 cells at least for several passages, therefore the phosphatase activity itself cannot be toxic, and the mutant should behave like the native phosphatase with respect to c-Src activation. We conclude that different from results obtained in REF or P19 cells, the mitogenic role of PTPα in 3T3 cells apparently is tightly controlled, and this control is reduced upon mutation of tyrosine 798.

**Immunolocalization of PTPα in NIH3T3 Cells—** PTPα binds with its phosphorylated carboxyl-terminal tyrosine to the SH2 domain of the adaptor protein grb2 (21, 22). It has been shown earlier that the SH3 domains of grb2 mediate association to intracellular structures (23). We therefore wanted to know whether PTPα could localize to distinct structures at the cell surface and undertook immunolocalization studies using laser confocal microscopy. Subconfluent 3T3 cells overexpressing PTPαC442S, PTPαY798F, or PTPαY781F were grown on glass discs and stained with rabbit polyclonal antisera and PTPαC442S and PTPαY781F cells were identified. The staining in the cell periphery suggested a localization of PTPα to focal cell-substratum adhesions. In none of the cells overexpressing PTPαY798F were the streak-like structures detected, indicating a different cellular localization of the phosphatase with a mutated grb2 binding site. The use of the monoclonal antibody D2 directed against a different intracellular epitope of PTPα stained the same structures in the cell. To confirm the localization of PTPα to focal adhesion sites, cells were double stained using a mouse monoclonal antibody against paxillin, a typical focal adhesion

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**Fig. 1. Tyrosine phosphorylation and expression of PTPα in NIH3T3 cells.** Parental NIH3T3 cells and cells overexpressing the indicated proteins were grown to confluence, treated with vanadate (VO4) as indicated, and lysed. PTPαs were immunoprecipitated (IP) using the monoclonal antibody D2, and precipitates were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting against phosphotyrosine (PY) antibody 5E2 (upper panel). The antibodies were then stripped off and PTPα content determined by reprobing the filter with the polyclonal antibody α-CT.

**Fig. 2. PTPαY798F mutant enhances c-Src-dependent focus formation.** NIH3T3 cells overexpressing the murine c-Src were seeded in six-well dishes, infected with retroviruses encoding the indicated PTPα form, and then tested in a focus formation assay. After 18 days the cells were stained with crystal violet and the dishes photographed.

**Fig. 3. Determination of intracellular localization of PTPα by immunofluorescence.** A, NIH3T3 cell lines overexpressing the PTPα mutant C442S, Y798F, or Y781F were grown subconfluent on glass discs, fixed with paraformaldehyde, and immunolabeled with rabbit polyclonal antibodies directed against PTPα, and analyzed with fluorescence laser confocal microscopy. B, NIH3T3 cell lines overexpressing PTPα mutant C442S or Y798F were grown subconfluent on glass discs, fixed with paraformaldehyde, and immunolabeled with rabbit polyclonal antisera directed against PTPα (upper panel) and mouse monoclonal antibodies directed against the focal adhesion-associated protein paxillin (lower panel). Staining was analyzed with fluorescence laser confocal microscopy.
protein, in addition to the rabbit polyclonal PTPα antiserum (Fig. 3B). Secondary antibodies coupled to different fluorescent tags allowed monitoring of PTPα and paxillin at the same time and revealed the staining of identical structures in the cell. Again, PTPαY798F did not localize to focal adhesions although focal adhesions were still found in the cell. A similar staining pattern for antibodies directed against PTPα was also found when A431 cells were investigated (data not shown). These data suggest that PTPα localizes preferentially to focal adhesion complexes in the basal plasmalemma and that an intact carboxyl-terminal tyrosine phosphorylation site but not phosphatase activity is required for this localization. We have, however, not been able in immunoprecipitation experiments to identify a protein that interacts with PTPα and mediates its association with the focal adhesion complex (data not shown).

**Vanadate Treatment Releases PTPα from Focal Adhesion Sites and Leads to Proteolytic Cleavage**—Treatment of cells with the phosphatase inhibitor peroxovanadate (POV) strongly increases the cellular phosphotyrosine content. Because POV also causes an increase in the phosphotyrosine content of PTPα and an increase in size and number of focal adhesions (24) we wanted to investigate its effect on the distribution of PTPα. The cell line overexpressing PTPαC442S was grown subconfluently on glass discs, treated with POV as indicated, and stained with antibodies against PTPα and vinculin, another protein typically found in focal adhesion sites. As can be seen in the top panel of Fig. 4, POV treatment abolishes the streak-like staining in the cell periphery found with the antibody specific for PTPα. The staining of the focal cell-substratum adhesions with the antibody against vinculin, however, was not reduced by POV treatment (Fig. 4, middle panel). This is also illustrated in the lower panel where pictures from the staining with both antibodies (vinculin, red; PTPα, green) are superimposed, and the yellow streaks in the cell periphery document the colocalization of PTPα and vinculin at focal contacts. After POV treatment, only the red staining of the focal adhesion protein vinculin was detected, indicating the dissociation of PTPα from these structures.

To investigate the effect of POV on the PTPα protein we grew PTPαC442S-overexpressing cells to confluence, made the cells quiescent, and left them untreated or treated with POV. PTPα was immunoprecipitated from cell lysates with monoclonal antibody D2 directed against the cytoplasmic domain that does not interfere with the association of proteins to phosphorylated tyrosine 798. Alternatively, the polyclonal antiserum generated against the carboxyl-terminal 13 amino acids of PTPα was used which can bind only when no protein is associated with the phosphorylated tyrosine 798. The immunoprecipitates

**Fig. 4.** POV treatment abolishes localization of PTPα to focal adhesion sites. NIH3T3 cells overexpressing PTPαC442S were grown subconfluent on glass discs and treated with POV or left untreated. They were then immunolabeled with antibodies directed against PTPα (top panels) or vinculin (middle panels), another focal adhesion-associated protein, and analyzed by fluorescence laser confocal microscopy. The bottom panels show an overlay of stainings for PTPα (coded to pseudocolor green), and vinculin (coded to pseudocolor red). In untreated cells the double labeling overlay results in the color yellow, indicating that PTPα and vinculin are present at focal adhesions, whereas in vanadate-treated cells only vinculin (red) is detected.
were separated on an SDS-polyacrylamide gel, and the proteins were transferred to a nitrocellulose membrane and blotted with antibody D2. As shown in Fig. 5, the staining pattern of PTPα was dependent on the antibody used for immunoprecipitation. Antibody D2 detected the 130-kDa form of PTPα; however, POV treatment decreased the amount of the 130-kDa form and reduced its mobility, as expected when additional residues are phosphorylated. Furthermore, a second form of PTPα was detected only after POV treatment which had an apparent molecular mass of 75 kDa. By contrast, immunoprecipitation of PTPα with the polyclonal serum was possible only after POV treatment, and no reduced size form was detected. At the bottom of the figure and around 100 kDa in the lanes representing the immunoprecipitates with the polyclonal serum, IgG is decorated by the secondary antibody. We conclude that PTPα is localized to focal adhesion plaques in the cell and that after POV treatment a part of the PTPα population is proteolytically cleaved. This fragment still is phosphorylated (data not shown) and associated with a protein bound to the carboxyl terminus because it is not detected by the antisemum recognizing the carboxyl terminus. The intact form of PTPα is no longer associated with a protein bound to the carboxyl terminus, and therefore immunoprecipitation with the polyclonal serum is possible.

**DISCUSSION**

In this paper we have investigated the function of PTPα in NIH3T3 cells. Despite the previously reported positive regulatory function, we were not able to overexpress native PTPα but only mutant forms. From these mutants only PTPαY798F was able to activate Src and transform the 3T3 cells. The mutation of the carboxyl-terminal tyrosine residue also led to a different intracellular localization: the phosphatase inactive mutant and the mutant PTPαY781F were found at focal adhesion sites, and this localization was abolished by treatment of cells with POV. The Y788F mutant, however, was dislocated from focal adhesion sites.

In our hands, not only PTPα but also several other receptor-type PTPs could not be overexpressed in 3T3 cells. Recently, Weng et al. (25) described the overexpression of native PTPα in rat embryonic fibroblast cells which was only possible to a level exceeding the endogenous phosphatase 2–3-fold. Higher protein levels were found when they used an inducible expression system, and cells underwent apoptosis after induction of LAR overexpression. However, PTPα has been successfully overexpressed in several cell lines already having a detectable level of endogenous PTPα (6, 7, 26). With the antibodies available in our hands we had no problem detecting endogenous PTPα in 293, A431, or baby hamster kidney cell lines but could not do so in our NIH3T3 cells, suggesting an extremely low level of endogenous PTPα in these cells (data not shown). This could indicate a specific sensitivity of our 3T3 cells toward the activity of PTPα. However, because native PTPα and its tyrosine mutants do not show a clear difference in their phosphatase activity (17), a toxic effect of the phosphatase activity should not be the sole physiological basis for the differential expression. This is demonstrated by the overexpression of the Y798F and the Y781F mutants, although the latter could only be overexpressed for a limited number of passages. The intermediate toxicity of the Y781F mutant compared with the native phosphatase or the Y788F mutant could be caused by a change in the structure of the carboxyl terminus of PTPα which would reduce the tyrosine phosphorylation and the subsequent binding of grb2 or other proteins without having a major impact on the phosphatase activity. Another consequence could be a weaker or differently regulated association with the focal adhesion sites. This phosphorylation, protein binding, or localization mediated by the carboxyl-terminal tyrosine residue is not required for the Src activating function of PTPα because the mutant PTPαY788F is capable of generating Src-dependent foci. Moreover, the focus formation experiment even indicates that this residue may have a down-regulating function in the native PTPα.

10–20% of the cellular PTPα is constitutively associated with the adaptor protein grb2 and therefore tyrosine phosphorylated in NIH3T3 cells (15, 26). The level of tyrosine phosphorylation in PTPα not associated with grb2 is probably down-regulated by the autodephosphorylating activity of PTPα. It is therefore conceivable that inhibition of tyrosine phosphatases by treatment with vanadate increases the degree of phosphotyrosine in the different mutant forms, as shown in Fig. 1. The finding of enhanced tyrosine phosphorylation in 3T3 cells also overexpressing the tyrosine kinases Csk and Hyl indicates the potential role of these proteins for the regulation of PTPα activity.

By coimmunoprecipitation experiments we have tried to identify the focal adhesion protein that associates with PTPα. However, we failed to detect any association with the major focal adhesion site proteins. Therefore, PTPα must bind to an as yet unidentified protein, or the interaction with focal adhesion proteins is not stable under lysis conditions. Other transmembrane PTPs have also been shown to localize to special areas at the cell surface: PTPs PCP-2 (27), μ (28), and LAR (29, 30) were found at sites of cell-cell adhesion, whereas PTPLAR has also been described as localizing to focal adhesion sites (31). For none of these PTPs was a correlation of localization with phosphatase activity possible so far. Because PTPα can activate the tyrosine kinases of the Src family and these kinases also localize to the focal adhesion sites, a role of PTPα in the regulation of Src-like kinases is an intriguing hypothesis. Two recent reports have also suggested a recruitment of PTPα to the focal adhesion sites: in nerve growth cones plating on laminin leads to an enhanced association of PTPα to the adhesion sites (32). Interestingly, vanadate treatment did not abolish the association of PTPα with focal adhesion points. This may be caused by the different cellular systems used or by the use of vanadate instead of POV. However, because in the particular experiment the cytoskeletal fraction of the growth cones was analyzed by immunoblotting, a reallocation of PTPα cannot be excluded. Harder et al. (33) have found that in A431 cells overexpression of PTPα increases cell-substratum adhesion (33). In an in vitro assay they could show that Csk was able to...
phosphorylate PTPα, supporting our data showing enhanced tyrosine phosphorylation of PTPα in Csk overexpressing 3T3 cells. However, it is difficult to understand how a PTPα-mediated activation of the Src kinase complies with the enhanced cell-substratum adhesion.

Treatment with POV not only abolished association of PTPα with the focal adhesion sites, it also led to a proteolytic processing of the phosphatase. POV-dependent cleavage of the receptor tyrosine kinase erbB-4 has recently been described by Vecchi et al. (34). Using specific inhibitors these authors showed that metalloproteases are responsible for this cleavage. The released extracellular part of a metalloprotease-processed protein can act as a diffusible growth factor, lead to reduced adhesion, or increase migration. Whether a metalloprotease is responsible for cleavage of PTPα is currently under investigation.

In addition to the processing, POV also changed the binding of intracellular proteins to the carboxyl-terminal tyrosine of PTPα. Intact and unprocessed phosphatase was no longer associated with this protein but only the 75-kDa processing product. Because metalloproteases cleave their target in the juxtamembrane region of the extracellular domain this would mean that both populations of the phosphatase are cell surface-anchored. Both forms would be able to move to different sites on the cell surface and could therefore be involved in down-regulating tyrosine phosphorylation of cell surface proteins.

In conclusion, we have shown that PTPα lacking a grb2 binding site has an enhanced potential to activate the Src kinase and that this activated state correlates with a change in its intracellular localization. Furthermore, the change in localization can also be induced by POV treatment. It will be interesting to see what the physiological regulators of the association of PTPα with the focal adhesion plaques are.

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