Attenuation of Adhesion-dependent Signaling and Cell Spreading in Transformed Fibroblasts Lacking Protein Tyrosine Phosphatase-1B

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Alan Cheng§§, Gurjeet S. Bal§, Brian P. Kennedy†, and Michel L. Tremblay§§**

From the §McGill Cancer Center and §Department of Biochemistry, McGill University, 3655 Promenade Sir William Osler, Room 715, Montreal, QC, H3G 1Y6 Canada and the |Department of Biochemistry and Molecular Biology, Merck Frosst Center for Therapeutic Research, Post Office Box 1005, Pointe Claire-Dorval, Quebec, H9R 4P8 Canada

Previous biochemical evidence has yielded conflicting models for the role of protein tyrosine phosphatase-1B (PTP-1B) in the regulation of integrin signaling. Thus, to establish the physiological relevance for such a role, we employed a genetic approach by generating embryonic fibroblasts from PTP-1B knockout mice. Both primary fibroblasts and their derived cell lines were used in this study. Immortalization of wild-type primary cells with the SV40 Large T antigen resulted in a dramatic increase in the endogenous expression of PTP-1B, suggesting a role during transformation. Moreover, the absence of PTP-1B in the transformed cell lines led to a more pronounced effect on different pathways of fibronectin-mediated signaling compared with the untransformed state. Specifically, p130Cas phosphorylation, Erk activation as well as cell spreading were delayed in PTP-1B-deficient cells, compared with their wild-type counterparts. Interestingly, this attenuation in integrin-mediated events closely resembles that of Src-deficient fibroblasts. Indeed, PTP-1B deficient, transformed fibroblasts held in suspension do exhibit a hyperphosphorylation of the inhibitory site (Tyr-527) of Src, compared with their wild-type counterparts. These results establish PTP-1B as a positive physiological regulator of integrin signaling in transformed cells, acting upstream of Src Tyr-527 dephosphorylation that leads to several adhesion-dependent events.

Adhesion to an extracellular matrix (ECM) has long been known to be critical for normal adherent cells to function efficiently. Engagement of cell surface integrins with the ECM generates intracellular signals that promote a variety of processes such as gene expression, cell survival, and cell-cycle progression (1). Furthermore, adhesion per se is insufficient to generate survival signals such that cells that attach onto a matrix but are not allowed to effectively spread may undergo apoptosis (2).

It is now well established that adhesion-dependent signaling involves changes in tyrosyl phosphorylation of several proteins that is regulated by two large protein superfamilies: protein-tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Among the PTKs that have long been recognized as critical mediators of integrin signaling, the Src family of kinases (3), as well as the FAK subgroup of kinases (4), are well characterized. Indeed genetic ablation of either FAK or Src has resulted in multiple defects in integrin signaling that include alterations in cell motility, cell spreading, and focal adhesion formation (5–7). Together and independently, Src and p125FAK phosphorylate many other proteins, leading to the activation of multiple adhesion-dependent pathways, most notably the Erk (8, 9). Independent of Src/p125FAK signaling, the Src-like kinase Fyn has been shown to be critical in adhesion-dependent Erk activation (10, 11). Furthermore, recent genetic studies have suggested that the Src/p125FAK and Fyn pathways may act in parallel to obtain optimal activation of Erk (12).

Upon integrin stimulation, the adapter protein p130Cas also becomes tyrosine phosphorylated by Src and/or p125FAK (8, 13, 14). Tyrosine-phosphorylated p130Cas acts as a large scaffolding molecule to recruit additional signaling complexes and has recently been shown to be involved in the activation of the JNK subgroup of MAP kinases (15, 16).

At the other end, the evidence for specific PTPs in the regulation of integrin signaling has been lagging from that of PTKs, although recent advances have been made in understanding the roles of several PTPs during adhesion (17). Indeed, using knockout fibroblasts, SHP-2, PTP-PEST, and RPTPα have definitively been shown to modulate integrin-mediated responses (18–21). Interestingly, each of these PTPs has been postulated to act through different mechanisms, either through dephosphorylation of p125FAK, the inhibitory tyrosine of Src kinases, or p130Cas, suggesting that each of these PTPs possess unique roles during adhesion signaling.

Another PTP that has been implicated in integrin signaling is protein tyrosine phosphatase 1B (PTP-1B), the first PTP that was identified and cloned (22–26). Its structure consists of an N-terminal catalytic domain followed by two tandem proline-rich motifs that possess a consensus for binding to proteins with SH3 domains (27). At the extreme C terminus, a small hydrophobic stretch has been shown to be sufficient to localize the enzyme to the endoplasmic reticulum (28). Biochemical analyses and PTP “substrate-trapping methods” have suggested that PTP-1B may dephosphorylate the insulin, IGF-I, and EGF receptors, as well as antagonize bcr-abl signaling (29–33). In contrast, the role for PTP-1B in integrin signaling is less clear.

Originally, overexpression studies have shown that PTP-1B negatively regulates integrin signaling, which was thought to depend on its association with and dephosphorylation of
Antibodies and Reagents—The p130Cas rabbit antiserum was generously given by Jean François Côté and has been previously described (18). TC-PTP monoclonal antibodies (36) were kindly provided by Maria tartanty, these effects were abrogated when a proline mutant that did not bind p130Cas was used.

Independently, experiments using a catalytically inactive mutant indicated that PTP-1B had a positive role in integrin signaling (35). In comparison with the above study, expression of this mutant in L cells decreased FN-mediated cell spreading and p125FAK phosphorylation, whereas wild-type PTP-1B had no effect. In this study, PTP-1B is suggested to dephosphorylate the inhibitory site on Src kinases and thus activate them, thereby promoting integrin-mediated events. Consistent with this notion, L cells expressing the catalytically inactive mutant displayed decreased Src activity. It has been argued that the opposite roles of PTP-1B on integrin signaling may reflect the different cell systems employed, although in both cases a fibroblast cell type was used. Alternatively, the promiscuous nature of overexpressed PTP-1B in L cells may reflect its dephosphorylation of non-physiological substrates and affect signaling in an artificial manner.

Herein we have used a genetic loss-of-function approach to ascertain the physiological role of PTP-1B in adhesion-depend-ent signaling. We provide evidence that PTP-1B is required for the efficient transduction of integrin-mediated signals and that loss of PTP-1B delays fibronectin-induced cell signaling and cell spreading. In PTP-1B-deficient fibroblasts, a transient hyperphosphorylation of Tyr-527 on Src was observed, suggesting that PTP-1B is involved in Src activation during adhesion.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—The p130Cas rabbit antiserum was generously given by Jean François Côté and has been previously described (18). TC-PTP monoclonal antibodies (36) were kindly provided by Maria de Jesus Ibarrasanchez. Polyclonal rabbit antibodies against PTP-1B have been previously described (37). Pan Src and phosphospecific (Tyr-527) Src antibodies were purchased from BIOSOURCE. Antibodies against Erk and phospho-Erk were obtained from New England Biolabs. Anti-β1 integrin and monoclonal p130Cas antibodies were purchased from Transduction Laboratories. To detect phosphotyrosine-containing proteins, mAb 4G10 (Upstate Biotechnology) conjugated to horseradish peroxidase was used (kind gift of J. F. Cote). Fibronectin was purchased from BD Biosciences.

Cells and Cell Culture—All cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies) supplemented with 1% fetal bovine serum, 0.2% bovine serum albumin, 1% goat serum in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20) for 2 h. Incubation with the primary antibodies took place at room temperature for 1.5 h (or overnight at 4 °C for Src and Erk antibodies), washed five times in TBST, and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 30 min. After five washes with TBST, proteins were detected using an enhanced chemiluminescence kit (PerkinElmer Life Sciences).

Cell Spreading Assays—Cells were treated similarly to that for the cell signaling studies. Approximately 2 × 105 cells in a volume of 1 ml were added to 35-mm FN-coated dishes for the indicated times at 37 °C. Afterward the dishes were chilled on ice for 10 min, placed on an eclipse TE300 (inverted Nikon microscope, and photographed with a CCD camera) using Metamorph software (version 4.1). Single cells that were phase-bright with rounded morphology were scored as non-spread, whereas those that possessed a flattened shape and were phase-dark were scored as spread. Three different fields for each sample were averaged. At least 100 cells were counted in each field. Error bars represent S.E. from these counts.

Actin Staining—Cells were seeded at a density of 5000 cells/cm2 onto Falcon chamber slides and allowed to grow for 21 h. Cells were washed once with PBS, fixed in 4% paraformaldehyde in PBS for 20 min at 4 °C, and then permeabilized with 0.1% Triton X-100. Slides were blocked overnight with 1% bovine serum albumin in PBS at 4 °C. To detect filamentous/polymerized actin, the cells were incubated with rhodamine-conjugated phalloidin (1:500 dilution) for 2 h at room temperature, washed three times with PBS, and then mounted in a 1:1 mixture of 35% 1,4-diacyclobutane [2,2,2.] octane (DABCO; Sigma). Cells were then visualized with the eclipse TE300 (inverted) Nikon microscope, and photographed with a CCD camera using Metamorph software (version 4.1).

RESULTS

Derivation of PTP-1B-null Cell Lines—The details of the targeted inactivation of PTP-1B and its role in insulin signaling have been previously described (37). However, to clarify the role of PTP-1B in integrin signaling, we have generated embryonic fibroblasts and cell lines to use as a model. Primary embryonic fibroblasts were derived from E14 wild-type (+/+) or mutant (−/−) embryos, and experiments were performed within three passages. Cell lines were also obtained by immortalization of primary cells by the SV40 Large T antigen (Tag). Western blotting analysis of PTP-1B protein confirmed the genotype of each cell line (Fig. 1A). Interestingly, the levels of PTP-1B in SV40 Tag immortalized cell lines were dramatically increased compared with levels found in primary embryonic fibroblasts (Fig. 1A, lanes 1, 3, 4, and 5), suggesting a role for PTP-1B during immortalization or transformation. Consistent with this notion, PTP-1B protein levels has been shown to be increased by the expression of the bcr-abl oncogene (30). Furthermore, this effect seems to be specific for PTP-1B, as the expression level of T-cell protein tyrosine phosphatase (TCPPT), a closely related PTP, was not significantly altered by SV40 Tag (Fig. 1B).

In embryonic fibroblasts, the αβ3 receptor is the major inte-grin involved in the recognition of the fibronectin ligand. Immuno-precipitation with antibodies against the α1 chain suggests that this was the case in all our cells tested (Fig. 1C), validating their use for subsequent cell adhesion studies.

Fibronectin-mediated Tyrosine Phosphorylation—One of the earliest events following engagement of integrins by the extra-cellular matrix includes the tyrosine phosphorylation of several proteins, most noticeably within the 120-130 kDa range that

p130Cas (27, 34). Overexpression of wild-type PTP-1B in Rat-1 fibroblasts impaired adhesion-dependent Erk activation as well as tyrosine phosphorylation of p130Cas and p125FAK. Furthermore, overexpression of PTP-1B readily reduced fibronectin (FN)-mediated cell spreading and cell migration. Importantly, these effects were abrogated when a proline mutant that did not bind p130Cas was used.
includes p130Cas and p125FAK (38, 39). In addition, previous biochemical studies suggested that PTP-1B negatively regulates integrin signaling through the binding and dephosphorylation of the adapter protein p130Cas (27, 34). Thus, to determine the physiological importance of PTP-1B toward integrin induced tyrosine phosphorylation of p130Cas, serum-starved PTP-1B wild-type and mutant cells were either kept in suspension or replated onto fibronectin-coated dishes for the indicated period of times. The levels of protein tyrosine phosphorylation were then analyzed by immunoblotting of cell lysates with antibodies. Cells held in suspension exhibited almost a complete absence of phosphotyrosine (pY)-containing proteins of around 120–130 kDa as expected (Fig. 2). Upon re-attachment to FN, the pY levels within the 120–130-kDa range were rapidly elevated. In primary fibroblasts, these pY levels were not considerably different between wild-type and mutant cells when normalized against p130Cas protein levels (Fig. 2A). Interestingly, the pY levels for the 120–130 kDa proteins were somewhat decreased in PTP-1B mutant Tag cell lines after 20 min of replating onto FN (Fig. 2B, lanes 6–10) but were restored by 40 min (Fig. 2B, lanes 11–20). In contrast, constitutive hyperphosphorylation of proteins around 180 and 80 kDa were observed in PTP-1B wild-type cells, whereas in PTP-1B mutant Tag cell lines, no appreciable differences were observed for PTP-1B wild-type or mutant primary cells. However, in the case of Tag cell lines, after 20 min of replating onto fibronectin, wild-type cell lines displayed an increase in phospho-Erk levels (Fig. 2B, lanes 11–20). In contrast, PTP-1B mutant Tag cell lines were impaired in their ability to induce adhesion-dependent Erk activation. Interestingly, PTP-1B mutant cells that were fully adherent in culture, displayed normal Erk activation in response to serum suggesting that the FN-dependent impairment is transient or rescued by other signaling pathways activated by components in serum (data not shown). Regardless, these results suggest that PTP-1B positively regulates FN-mediated Erk activation as well as p130Cas phosphorylation in transformed but not primary cells.

It is well established that MAP kinase activation depends on an intact cytoskeleton and is associated with the formation of actin structures (40, 41). Because FN-mediated cell spreading is dependent on actin bundling and correlates with efficient MAP kinase activation in NIH 3T3 cells, it is possible that PTP-1B mutant cells might exhibit a defect in cell spreading. To explore this possibility, we plated PTP-1B wild-type and mutant primary cells onto FN for either 10, 20, or 60 min. Afterward, cells were chilled on ice for 10 min and then photographed. Single cells that were phase-bright with rounded morphology were scored as non-spread, whereas those that possessed a flattened shape and were phase-dark were scored as spread. Fig. 5A shows representative photographs obtained from PTP-1B wild-type and mutant primary cells. Typically, the PTP-1B wild-type cells displayed around 30% spreading efficiency after 10 min on FN, whereas PTP-1B mutant cells displayed only 15% spreading efficiency (Fig. 5B). By 20 min, PTP-1B mutant cells were still lagging behind wild-type cells in terms of spreading, although the difference became less apparent as time continued. In addition, similar results were ob-
tained using the Tag cell lines (data not shown). Thus, these results suggest that PTP-1B is required for efficient spreading on FN in mouse embryonic fibroblasts.

Cell morphology is known to be dependent on the actin cytoskeleton. Subsequently, we analyzed the actin staining in both PTP-1B wild-type and mutant cells using Rhodamine-conjugated Phalloidin, that specifically recognizes F-actin (polymerized actin). At low densities (5000 cells/cm²), most PTP-1B wild-type transformed cells exhibit a round, spread morphology (Fig. 5C, a and c); in contrast a relatively high proportion of PTP-1B mutant transformed cells displayed an elongated, spindle like shape (Fig. 5C, b and d). These results suggest that PTP-1B regulates an aspect of actin dynamics that affects cell morphology in transformed cells.

Src Inhibitory Site Phosphorylation—Previous gene-targeting studies have clearly established the physiological importance of Src during adhesion-mediated cell spreading (7). During adhesion onto FN, it has previously been shown that Tyr-527 of Src transiently becomes dephosphorylated and then quickly becomes rephosphorylated (42). In addition, Src is primarily regulated through this site (43–47), allowing the possibility to be activated and regulated by PTPs. One candidate for dephosphorylation of this inhibitory site was previously suggested to be PTP-1B (35). Indeed, expression of PTP-1B in breast cancer cells was also shown to result in decreased phosphorylation of Tyr-527, concomitant with increased Src activity (48). If PTP-1B positively regulates integrin signaling through activation of Src kinases, one would expect increased phosphorylation of Tyr-527 in PTP-1B −/− cells, similar to that seen in RPTPα −/− cells (20, 21). To address this possibility, we performed immunoblot analysis using phosphospecific antibodies toward pY527 on Src (Fig. 6). In primary cells, Tyr-527 was similar in both PTP-1B wild-type and mutant cells kept in suspension or after a 20-min plating onto FN (Fig. 6A). In our transformed SV40 Tag cell lines, Tyr-527 was hyperphosphorylated in PTP-1B mutant transformed cells compared with wild-type controls, but only when cells were kept in suspension and not when plated onto fibronectin for 20 min (Fig. 6B). These data suggest that PTP-1B regulates Src Tyr-527 phosphorylation for transformed cells in suspension and that the loss of PTP-1B results in delayed FN-mediated signaling, likely because of the attenuation in the dephosphorylation-phosphorylation cycle of Tyr-527 of Src.

DISCUSSION

Earlier biochemical studies suggested that PTP-1B could play both positive and negative roles in integrin-mediated events, presumably through dephosphorylation of the adapter p130Cas or the PTK Src (27, 34, 35). To resolve this issue and to establish the physiological role of PTP-1B in integrin signaling, we have employed a knockout approach. By doing so, we eliminated any undesired effects caused by overexpression. In the present study, our results suggest that in primary embryonic fibroblasts, PTP-1B plays a minor role in FN signaling. However, upon transformation by SV40 Tag, the dependence on PTP-1B in FN signaling is increased. In this state, PTP-1B, presumably through Src kinases, plays a positive role in FN-induced p130Cas phosphorylation and Erk activation.

Immortalization of wild-type fibroblasts with SV40 Tag resulted in a substantial increase in PTP-1B levels (Fig. 1). This may reflect cell response to p53 status or oncogenic signaling. One could thus imagine that overexpression of PTP-1B might extend the cellular feedback machinery within a cell beyond its natural limit and result in artifacts. This might be one plausi-

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**Fig. 2. FN-induced tyrosine phosphorylation.** Serum-starved cells were kept in suspension and replated onto FN for various periods of time (20 min for PMEFs). Cells were briefly washed twice in PBS and then lysed in TNE buffer as depicted in the text. A, lysates (15 μg) from primary embryonic fibroblasts were analyzed by immunoblotting with anti-pY antibodies. B, similar analysis of SV40 T antigen cell lines (2 wild-type, 3 knockout) revealed a delayed tyrosine phosphorylation of proteins of about 120–130 kDa in mutant cells. In contrast, constitutive hyperphosphorylation of proteins around 80 and 180 kDa is seen in mutant cells. Lanes 1, 6, 11, and 16 represent one cell line; lanes 2, 7, 12, and 17 represent another cell line, and so on.
FIG. 3. FN-induced tyrosine phosphorylation of p130Cas. TNE lysates from cells treated as in Fig. 2 were analyzed for phosphorylation of p130Cas. Lysates were immunoprecipitated with anti-p130Cas antibodies and analyzed by immunoblotting with anti-pY antibodies. A, FN-induced tyrosine phosphorylation is not significantly altered in primary mutant cells. B, FN-induced tyrosine phosphorylation of p130Cas is delayed in PTP-1B mutant cell lines, paralleling the situation observed for proteins in the 120–130 kDa range in Fig. 2. The lanes are numbered as in Fig. 2. Lanes 1, 6, 11, and 16 represent one cell line; lanes 2, 7, 12, and 17 represent another cell line, and so on.

FIG. 4. Adhesion-dependent Erk activation is delayed in PTP-1B mutant cells. Serum-starved cells were kept in suspension and replated onto FN for 20 min. TNE lysates from cells were analyzed for Erk activation. Lysates were analyzed by immunoblotting with phosphospecific antibodies as described in the text. A, adhesion-dependent-induced Erk activation is normal in primary mutant cells. B, FN-induced Erk activation is impaired in PTP-1B mutant Tag cell lines.
endogenous levels, we believe that the physiological role of PTP-1B has been properly represented. Regardless, we have utilized both primary embryonic fibroblasts and established cell lines in the experiments to draw our conclusions.

Our phosphotyrosine profiles of primary and transformed cells revealed no major proteins that were hyperphosphorylated in mutant cells in response to FN (Fig. 2). However, hypophosphorylation of proteins around 120–130 kDa were observed in cell lines at early stage after (20 min) replating cells onto FN (Fig. 2B). This suggested that PTP-1B was indirectly responsible for phosphorylation of these proteins at an early event during FN signaling. Moreover, constitutive hyperphosphorylation of 80 and 180 kDa proteins were prominently seen in PTP-1B mutant cells, suggesting them to be physiological substrates of PTP-1B. Thus, the absence of PTP-1B in fibroblasts causes a perturbation in intracellular tyrosine phosphorylation, possibly affecting multiple pathways.

Previous work showed that p130Cas was a potential substrate of PTP-1B (27). Our results using in vitro substrate trapping techniques confirm these findings (data not shown). However, our analysis of p130Cas phosphorylation after activation by FN (Fig. 3), or cells in culture (data not shown), indicates that p130Cas is not a robust physiological substrate under these conditions. In fact, tyrosine phosphorylation of p130Cas is delayed at an early point in PTP-1B mutant cells after replating onto FN (Fig. 3B), corresponding to the delayed phosphorylation of the 130 kDa protein mentioned above (Fig. 2B). At present, we cannot exclude the possibility that p130Cas may become hyperphosphorylated in PTP-1B mutant cells under other conditions. Alternatively, other PTPs may rescue p130Cas dephosphorylation in PTP-1B mutant cells. One such possibility includes PTP-PEST, an enzyme found to be critical for cell spreading and migration (18, 49). In contrast to PTP-1B knockout cells, PTP-PEST knockout cells display hyperphosphorylation of p130Cas, likely reflecting p130Cas as a bona fide substrate of PTP-PEST (18, 50).

These results suggest that PTP-1B lies upstream of p130Cas phosphorylation during FN signaling. Another aspect of FN signaling involves the Erk subgroup of MAPKs. In primary cells, once again, no differences were observed (Fig. 4A). In contrast, we found that adhesion-dependent Erk activation was impaired in PTP-1B mutant cell lines (Fig. 4B). This defect in Erk activation seems transient, as fully spread PTP-1B mutant cells are able to efficiently activate Erk in response to serum (data not shown). Taken together, PTP-1B is required for FN-mediated signaling in transformed fibroblasts, that includes efficient p130Cas phosphorylation and Erk activation. Furthermore, paralleling the delay in these adhesion-dependent events, we also observed an attenuation of cell spreading in PTP-1B mutant cells.

The delay in the FN-mediated events implied that the inhibitory site of Src kinases might be regulated by PTP-1B as previously suggested (35). Indeed, Src-deficient fibroblasts display impaired adhesion-dependent p130Cas phosphorylation and Erk activation, as well as a delay in FN-mediated cell spreading (similar to PTP-1B-deficient fibroblasts) (7, 8). Furthermore, overexpression of PTP-1B in breast cancer cells has also been shown to decrease Tyr-527 phosphorylation, paralleling an increase in Src kinase activity (48). Here, using phosphospecific antibodies, we were able to observe a transient hyperphosphorylation of Tyr-527 of Src in PTP-1B-deficient transformed cells kept in suspension. It is likely that the high basal level of Tyr-527 phosphorylation in these cells leads to the attenuation of FN-mediated events. In PTP-1B mutant transformed cells growing in culture, we did not detect any significant differences in Tyr-527 phosphorylation (data not

![Fig. 5. Delayed FN-mediated cell spreading in PTP-1B mutant cells.](http://www.jbc.org/)

A. Representative photographs from PTP-1B wild-type and mutant primary fibroblasts. B. Quantitative evaluation of spreading efficiency of primary cells. Three different fields for each sample were averaged. At least 100 cells were counted in each field. Error bars represent the mean ± S.E. from these counts. C. Cell morphology and actin staining of PTP-1B wild-type- (a and c) and mutant (b and d)-transformed fibroblasts. Cells were seeded at a density of 5000 cells/cm² and grown for 21 h before staining for actin. Pictures were taken at ×100 (a and b) and ×400 (c and d) magnification.

Fig. 5. Delayed FN-mediated cell spreading in PTP-1B mutant cells.
shown), suggesting that under those conditions, other PTPs may be more crucial. Indeed, constitutive Tyr-527 hyperphosphorylation is observed in RPTPα-deficient cells, embryos, and brain tissues (20, 21).

At present, the plethora of studies on PTP-1B has mainly focused on its role during insulin signaling. In fact, genetic ablation of PTP-1B in mice supports an important physiological role in diabetes and obesity (37, 51). However, the physiological relevance of PTP-1B in other aspects of signaling has not been well established. Our results here indicate that PTP-1B is required for efficient FN signaling in fibroblasts. It is also interesting to note that FN signaling enhances insulin-mediated activation of the IRS-1/P13-kinas/Akt pathway in rat adipocytes (52), underscoring the importance of crosstalk between different signaling pathways. Furthermore, insulin-stimulated receptor phosphorylation is enhanced in muscle and liver but not fat tissues in PTP-1B-deficient mice. Thus it is intriguing to speculate that in PTP-1B-deficient adipose tissue, insulin receptor phosphorylation may not be dramatically altered because of the positive and negative effects of PTP-1B on FN and insulin signaling respectively.

Recent work by Broome and Courtneidge (53) has suggested caution in the interpretation of data utilizing cell systems employing the SV40 Large T antigen. Their results point out that the SV40 Large T antigen is able to abrogate the requirement for Src kinases during PDGF signaling and may have revealed Tyr-527 hyperphosphorylation in PTP-1B-deficient primary fibroblasts. Cells from littermate embryos were used. More specifically, a set of cell lines were repeated for this set of cell lines, as well with an additional knockout experiment for Src kinases during PDGF signaling and may have resulted because of the positive and negative effects of PTP-1B on insulin receptor phosphorylation may not be dramatically altered because of the positive and negative effects of PTP-1B on FN and insulin signaling respectively.

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