The Tyrosine Phosphatase PTP1C Associates with Vav, Grb2, and mSos1 in Hematopoietic Cells*

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The association of the murine motheaten phenotype of severe hematopoietic dysregulation with loss of PTP1C tyrosine phosphatase activity indicates a critical role for this SH2 domain-containing phosphotyrosine phosphatase in the regulation of hematopoietic cell growth and differentiation. To explore the molecular basis for PTP1C effects on hematopoiesis, we have investigated the possibility that this enzyme interacts with the product of the Vav proto-oncogene, a putative guanine nucleotide exchange factor expressed exclusively in hematopoietic cells. Our data indicate that PTP1C physically associates with Vav in murine spleen cells and in EL4 T lymphoma and P815 mastocytoma cells, and that this interaction is increased following mitogenic stimulation and the induction of both PTP1C and Vav tyrosine phosphorylation. The results also reveal tyrosine phosphatase activity to be present in Vav immunoprecipitates from stimulated splenic and P815 cells and suggest that a major portion of total cellular PTP1C catalytic activity is associated with Vav. As Vav-associated tyrosine phosphatase activity was not detected in PTP1C-deficient motheaten splenocytes, it appears that PTP1C accounts for most, if not all, Vav-co-precipitable tyrosine phosphatase activity in normal cells. The data also demonstrate the capacity of the Vav SH2 domain alone to bind to PTP1C in activated P815 cells, but suggest a role for the two Vav SH3 domains in enhancing this interaction. In addition, the results reveal PTP1C association with two other molecules implicated in Ras activation, the Grb2 adaptor protein and mSos1, a GTP/GDP exchanger for Ras. PTP1C therefore has the capacity to bind and potentially modulate various signaling effectors involved in activation of Ras or Ras-related proteins, and, accordingly, regulation of Ras activation represents a possible mechanism whereby PTP1C influences hematopoietic cellular responses.

Among the phosphotyrosine phosphatases (PTP) identified to date, the cystolic enzyme PTP1C is distinguished by its predominant expression in hematopoietic cells and the presence of two N-terminal located Src homology 2 (SH2) domains, a motif found in only two other PTPs, Syp (PTP1D/SHPTP2) and the Drosophila csw protein (1–6). These properties, together with the recent data linking PTP1C gene mutations to the profound hematopoietic dysregulation manifested by motheaten (me) and viable motheaten (mev) mice (7–9), reveal a critical role for PTP1C in modulating hematopoietic cell differentiation and growth. As this PTP has been shown to associate with the activated c-kit, erythropoietin, and IL-3 receptors (10–12) and, more recently, with the B cell antigen receptor complex and the CD22 and FcyRIIB1 receptors on lymphocytes (13–15), PTP1C appears to subserve its regulatory role, at least in part, by modulating the signaling capacities of membrane growth factor/antigen/cytokine receptors. As is consistent with the marked overexpansion of multiple hematopoietic cell types observed in PTP1C-deficient motheaten mice, the data concerning PTP1C effects on the B cell antigen (13–16) and IL-3 (11) receptors suggest that this phosphatase down-regulates signaling cascades elicited by receptor engagement, presumably by dephosphorylating and deactivating receptor components or receptor-associated cytosolic protein tyrosine kinases. In conjunction with the increased susceptibility of mev heterozygous mice to development of lymphoid malignancies (8, 17) and the implicit possibility that PTP1C has tumor suppressor activity, these data suggest that the major influence of PTP1C activity on hematopoiesis may be realized through the suppression of signaling pathways that normally promote cell activation.

In contrast to PTP1C association with specific cell surface receptors, its interactions with downstream cytoplasmic signaling effectors have not been defined. In this regard, one molecule of potential interest is the 95-kDa product of the Vav proto-oncogene, another SH2 domain-containing protein which, like PTP1C, has been identified in all hematopoietic lineages and implicated by several lines of evidence in the control of hematopoietic cell growth and differentiation (18–20). Inhibition of Vav expression, for example, interferes with development of hematopoietic cells from embryonic stem cells (21), and, as is consistent with its participation in a broad range of hematopoietic cell signaling pathways, Vav has been shown to become tyrosine-phosphorylated following cross-linking of antigen receptors on lymphocytes (22–24), Fcy and e receptors on monocytes and mast cells, respectively (24, 25), and c-kit receptors on multiple hematopoietic lineages (26, 27). Vav contains a number of structural motifs found in many signaling effectors, including an SH2, a pleckstrin homology, and two SH3 do-

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PTP1C Associates with Vav in Hematopoietic Cells

PTP1C activity appears to be contained in Vav-PTP1C complexes, the results of this study demonstrate that PTP1C also binds to both Grb2 adaptor and mSos1 GEF proteins. Together, these results suggest that PTP1C effects on hematopoietic cell growth and development may be realized at least in part through modulation of the signaling events linking receptor stimulation to the activation of Ras or Ras-related proteins.

MATERIALS AND METHODS

Reagents—Polyclonal anti-PTP1C and anti-Syp antibodies were generated in rabbits immunized with GST-PTP1C SH2 domain fusion proteins as described previously (5, 9). The monoclonal anti-phosphotyrosine 4G10 and anti-Vav antibodies as well as rabbit polyclonal antibody specific to Grb2 and a synthetic peptide corresponding to residues 577–590 of the mouse Vav protein were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Steel factor (SF) was obtained from Genzyme (Cambridge, MA), and concanavalin A (Con A) as well as all chemicals for immunoblotting/immunoprecipitation analyses were obtained from Sigma.

Cells and Cell Lines—Fresh splenic cell suspensions used in this work were prepared by standard procedures from C57BL/6J- or C3HeB/FeJ- derived mice. P815 mastocytoma cells were obtained from Dr. C. Paige and ATCC (TIB64), respectively. P815 mastocytoma cells were maintained in Opti-MEM (Life Technologies, Inc.) containing 100 μg/ml penicillin/streptomycin and 10% fetal calf serum (Life Technologies, Inc.). As controls for some experiments, we also used

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A, cell lysates were prepared from 10^6 unstimulated P815 cells and 1500 μg of lysate protein, then immunoprecipitated with anti-Vav antibody plus Vav peptide (VAV + PEP), anti-Vav antibody alone, or anti-PTP1C antibody. Lysates were also prepared from 10^6 C57BL1 +/- splenic cells (lane 1) and 10^6 EL4 lymphoma cells (lane 2), and the lysate proteins (~1000 μg) were resolved on SDS-PAGE and blotted with anti-PTP1C antibody and 125I-protein A. B, cell lysates were prepared from 10^6 unstimulated P815 cells, and 800 μg (lane 1) or 1500 μg (lane 2) of lysate protein were immunoprecipitated using anti-PTP1C antibody (left panel) or anti-Vav (right panel) antibodies. Lysates (500 μg) prepared from P815 cells (L) were also resolved on SDS-PAGE and immunoblotted with anti-PTP1C antibody and 125I-protein A. C, cell lysates were prepared from unseparated splenic cells of C3HeBFeJ +/- and mSos1 mice (577–590 of the mouse Vav protein were purchased from Santa Cruz Biotechnology Inc. (Lake Placid, NY). Rabbit polyclonal antibody specific to Grb2 and a synthetic peptide corresponding to residues 577–590 of the mouse Vav protein were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Steel factor (SF) was obtained from Genzyme (Cambridge, MA), and concanavalin A (Con A) as well as all chemicals for immunoblotting/immunoprecipitation analyses were obtained from Sigma.

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**Fig. 2.** Increases in PTP1C-Vav association and tyrosine phosphorylation following cell stimulation. A, cell lysates were prepared from unstimulated (−) or ConA (20 μg/ml)-treated (+) EL4 cells and unstimulated (−) or steel factor (100 ng/ml)-treated P815 cells, and 800 μg of lysate proteins were immunoprecipitated with anti-Vav antibodies. Lysates were also prepared from 10^6 B16 melanoma cells stably transfected with either pCMV-4neo vector alone (far left lane) or pCMV-4neo ligated to the full-length PTP1C cDNA (second lane from the left, and the lysates and immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotting with anti-PTP1C antibody. B, cell lysates were prepared from unstimulated (−) and Con A-treated (−) EL4 cells and 800 μg of lysate proteins were immunoprecipitated with anti-PTP1C antibody, resolved over SDS-PAGE, and immunoblotted with the 4G10 anti-phosphotyrosine (pY) antibody. C, cell lysate proteins (500 μg) prepared from unstimulated (−) and steel factor-treated (+) P815 cells were immunoprecipitated with anti-Vav (left panel) or anti-phosphotyrosine (right panel) antibodies, resolved over SDS-PAGE, and immunoblotted with anti-phosphotyrosine (left panel) or anti-Vav (right panel) antibodies. As a control (C), Vav immunoprecipitates prepared from P815 cell lysates (800 μg) were immunoblotted with anti-Vav antibody. D, cell lysates were prepared from unstimulated (−) and steel factor-treated (+) P815 cells, and 800 μg of lysate proteins were immunoprecipitated with anti-PTP1C (left panel) or anti-phosphotyrosine (right panel) antibodies, resolved over SDS-PAGE, and immunoblotted with anti-phosphotyrosine (left panel) or anti-PTP1C (right panel) antibodies. The positions of molecular mass standards are indicated in all four panels; arrows indicate the positions of PTP1C and Vav.

B16 melanoma cells transfected with the vector pCMV-4Neo or alternatively with a construct containing the full-length PTP1C cDNA subcloned into the pCMV-4Neo vector (provided by Dr. B. Chan). These latter lines were maintained under the same culture conditions as described above except for the addition of 2 mg/ml Geneticin (Life Technologies, Inc.) to the culture medium. For cell stimulations, cells were cultured in Opti-MEM containing 0.5% fetal calf serum for 17 h, washed, and then cultured for 10 min in the presence of 20 μg/ml ConA (EL4 and splenic cells) or for 5 min in the presence of 100 ng/ml steel factor (P815 cells).

Generation of GST-Vav Fusion Proteins—Glutathione S-transferase (GST)-Vav fusion proteins were generated by subcloning polymerase chain reaction-amplified murine Vav sequences into pGEX-2T. The amplified fragments (illustrated in Fig. 3A) subcloned into this expression plasmid include the Vav SH2 domain alone (amino acids 670–765), the Vav SH2 and C-terminal SH3 domains (amino acids 670–942), and the Vav N-terminal SH3, SH2, and C-terminal SH3 domains (amino acids 611–942). GST-Vav expression plasmids were transfected into Escherichia coli, and the fusion proteins were purified from isopropl-1-thio-

β-o-galactopyranoside-induced bacteria with glutathione-conjugated Sepharose beads (Pharmacia, Baie d’Urfé, Quebec).

Immunoblotting Analysis—Protein lysates were prepared by resuspending 10^7–10^8 resting or mitogen-treated splenic, EL4, and P815 cells in 1 ml of lysis buffer (phosphate-buffered saline containing 1% Triton X-100, 1% Tween, 1 mM sodium orthovanadate, 1 μM leupeptin, 1 μM aprotinin, and 0.001 mM dithiothreitol). 100 μg of cell lysate protein was electrophoresed through 10% SDS-polyacrylamide gels and electrophrozed onto nitrocellulose, and the blot was then incubated overnight at 4°C in 10 ml Tris, pH 8.0, 150 mM NaCl, and 0.05% Tween 20 (TBST) containing 5% skim milk. The proteins were then detected by incubating the blots for 2 h at room temperature with primary antibodies in TBST followed by 125I-protein A (DuPont, Canada). Blots were then washed with TBST and exposed to Kodak XAR film at −70°C.

To evaluate PTP1C binding to GST-Vav fusion proteins, protein lysates prepared from 10^6 steel factor-treated P815 cell lysates were incubated at 4°C for 2 h with 5 μg of fusion protein immobilized on glutathione-Sepharose beads (Pharmacia, Baie d’Urfé, Quebec). Immune complexes were collected by centrifugation, washed three times with lysis buffer, boiled for 5 min in SDS-sample buffer, and then subjected to electrophoresis and immunoblotting as described above.

In Vitro Binding Assays—To evaluate PTP1C binding to GST-Vav fusion proteins, protein lysates prepared from 10^6 steel factor-treated P815 cell lysates were incubated at 4°C for 2 h with 5 μg of fusion protein immobilized on glutathione-Sepharose beads. After several washes in lysis buffer, complexes were resuspended in sample buffer, boiled, and analyzed by SDS-PAGE and immunoblotting with anti-PTP1C antibody and 125I-protein A.

Assays of Phosphatase Activity—To assay PTP1C and Vav-associated phosphatase activities, PTP1C and Vav were immunoprecipitated as described above from 300, 600, or 900 μg of cell lysate proteins prepared from 10^7–10^8 P815 cells incubated for 5 min in medium containing 100 ng/ml steel factor. Immunoprecipitates were washed twice in lysis buffer and then incubated at 37°C for 4 h in 200 μl of phosphatase buffer (62 mM Heps, pH 7.5, 6.25 mM EDTA, 12.5 mM dithiothreitol) containing 2 mM p-nitrophenyl phosphate (Sigma). Reactions were terminated by addition of 1 ml of 0.2 M NaOH, and absorbance was measured at 410 nm. Alternatively, tryosine phosphatase activity was measured in Vav and Syp immunoprecipitates prepared from cell lye-
sates of 10⁸ motheaten or congenic wild-type ConA (20 ng/ml)-treated
splenic cells. For this assay, the immunoprecipitated proteins were
incubated at 37°C for 2 h in 25 μl of 10 mM Tris HCl, pH 7.4, containing
7.5 mM tyrosine phosphopeptide (RLIEDEpYAARG), and the reac-
tion was terminated by addition of Malachite Green solution (UBI) as
described previously (36). Phosphate release was measured after 15
min by evaluating absorbance at 605 nm.

RESULTS AND DISCUSSION

PTP1C Interacts with Vav in Resting P815 Cells—To inves-
tigate the possible association of PTP1C with Vav in unstimu-
lated hemopoietic cells, Vav immunoprecipitates prepared from
P815 mastocytoma cell lines and from me, me₂, and wild-type
control splenic cells were examined by immunoblotting analy-
sis for the presence of PTP1C. As shown in Fig. 1, the ~70-kDa
PTP1C protein was coprecipitated with Vav from both P815
cells (Fig. 1A) and resting normal murine splenocytes (Fig. 1C).
This association was detected both by immunoblotting anti-
Vav immunoprecipitates with anti-PTP1C antibody and, con-
versely, by immunoblotting anti-PTP1C immunoprecipitates
with anti-Vav antibody (Fig. 1A and B). Moreover, the capacity
of PTP1C to interact with Vav in P815 cells was observed to be
diminished markedly by preincubation of the P815 lysates with
a synthetic peptide representing a 13-amino-acid segment of
the Vav protein (Fig. 1A). As anticipated, no PTP1C was de-
tected in Vav immunoprecipitates from me splenic cells (Fig.
1C) which have been shown to lack PTP1C protein (9). By
contrast, while the phosphatase domain mutations found in
me² PTP1C proteins severely reduce this enzyme’s catalytic
activity, these mutant proteins retain the capacity to interact
with Vav (Fig. 1C).

The data shown in Fig. 1A also reveal isolated murine
splenocytes to express two PTP1C species (~67 and 70 kDa,
respectively), only one of which (the latter species) is detectable
in P815 cells. The existence of PTP1C isoforms has been ob-
served previously in other hemopoietic cell populations (9) and,
based on sequence analysis of PTP1C transcript, ascribed to
the alternative splicing of a 39-amino-acid segment within the
PTP1C C-terminal SH2 domain (7). The functional significance
of PTP1C SH2 domain variants is not known, but the expres-
sion of only one PTP1C species in P815 cells is consistent with
previously reported data indicating the two species to be ex-
pressed variably in different hemopoietic and epithelial lin-
eages (9, 37). Moreover, based on the exclusive detection of the
higher molecular weight PTP1C species in Vav immunoprecipi-
tates from murine splenocytes (Fig. 1C), it appears that Vav
may selectively interact with this single PTP1C variant. While
further studies are required to address this issue, the data
shown here reveal the capacity of PTP1C to associate with Vav
in both mast and unseparated splenic cells and suggest that
the PTP1C sequences which mediate Vav binding in resting cells
map to regions flanking the site of the me² phosphatase domain
mutation.

PTP1C Binding to Vav Increases following Mitogenic Stim-
ulation and Appears to Be Mediated through the Vav SH2
Domain—It has been previously reported that PTP1C associa-
tion with both the c-kit and IL-3 receptors is markedly in-
creased following receptor engagement (10, 11). To determine
whether PTP1C binding to Vav is also increased following cell
stimulation, Vav immunoprecipitates were prepared from rest-
ing, ConA-treated EL4 and steel factor-treated P815 cells, and
the coprecipitation of PTP1C was assessed by immunoblotting
analysis. As shown in Fig. 2A, stimulation of both EL4 and
P815 cells induced marked increases in the association of
PTP1C with Vav. This result cannot be ascribed to ConA/steel
factor-driven increases in expression of these proteins, as levels

![Diagram](https://example.com/diagram.png)

**Fig. 3. Association of Vav SH2/SH3 domains with activated PTP1C.** A, schematic showing Vav sequences present in the three GST-Vav
fusion proteins used for in vitro binding assays. Numbers below each construct refer to amino acid positions of domain boundaries. B, cell lysates
were prepared from 10⁸ steel factor-stimulated P815 cells and incubated for 2 h at 4 °C with 5 μg of purified GST-fusion protein immobilized on
glutathione-Sepharose beads. Complexes as well as lysate (L) alone (i.e. no GST-fusion protein added) were electrophoresed through SDS-PAGE
and subjected to immunoblotting with anti-PTP1C antibody. Numbers at the top represent the GST-Vav expression protein used in duplicate
samples. Molecular size markers are indicated on the right, and the position of PTP1C is shown on the left.

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**Table:**

| GST-Vav Expression Protein | GST-Vav SH2 | GST-Vav SH3 | GST-Vav SH2/SH3 |
|---------------------------|------------|------------|-----------------|
| PTP1C                     | 67         | 70         | 67              |

**Figure 3:**

**A** schematic showing Vav sequences present in the three GST-Vav fusion proteins used for in vitro binding assays. Numbers below each construct refer to amino acid positions of domain boundaries.

**B** cell lysates were prepared from 10⁸ steel factor-stimulated P815 cells and incubated for 2 h at 4 °C with 5 μg of purified GST-fusion protein immobilized on glutathione-Sepharose beads. Complexes as well as lysate (L) alone (i.e. no GST-fusion protein added) were electrophoresed through SDS-PAGE and subjected to immunoblotting with anti-PTP1C antibody. Numbers at the top represent the GST-Vav expression protein used in duplicate samples. Molecular size markers are indicated on the right, and the position of PTP1C is shown on the left.
of PTP1C and Vav were not appreciably different in resting versus stimulated cells (data not shown). By contrast, steel factor and ConA treatment induced marked increases in Vav tyrosine phosphorylation in P815 (Fig. 2 C) and EL4 (data not shown) cells, respectively. PTP1C tyrosine phosphorylation was also increased in association with stimulation of these cells (Fig. 2, B and D). These findings are consistent with previous data revealing the induction of Vav (22–27) and PTP1C (13, 38, 39) tyrosine phosphorylation following stimulation of a variety of cell surface receptors and suggest that the enhanced association of PTP1C with Vav in activated cells is mediated through an SH2 domain-phosphotyrosine interaction.

In view of these findings, as well as previous data showing that Vav association with another signaling effector in activated T cells, the protein tyrosine kinase ZAP70, is mediated through binding of the Vav SH2 domain to phosphotyrosine site(s) on ZAP70 (40), the contribution of the Vav SH2 domain to Vav-PTP1C interaction was investigated. To this end, GST fusion proteins containing the Vav SH2 domain alone and the Vav SH2 domain combined with the carboxyl-terminal or both Vav SH3 domains (Fig. 3 A) were coupled to glutathione-Sepharose, incubated with steel factor-treated P815 cells, and evaluated for PTP1C binding by immunoblotting with anti-PTP1C antibody. As shown in Fig. 3 B, the results of this in vitro analysis revealed PTP1C binding with the fusion protein containing the Vav SH2 domain alone, but PTP1C binding was observed considerably increased with the fusion proteins containing an SH2 and SH3 domain and even more increased with the fusion protein containing both Vav SH3 domains. These results indicate the capacity of the Vav SH2 domain to interact with PTP1C in activated cells, and, as has been demonstrated previously with respect to the SH2 domain-mediated intramolecular repression of Src activity (41), the data also suggest that optimal binding of these molecules requires the Vav SH3 domains as well. However, these results do not preclude the possibility that the PTP1C SH2 domains and/or other sites within the Vav protein contribute to the interaction of these proteins.

Detection of Vav-associated Tyrosine Phosphatase Activity—To evaluate the potential biologic relevance of PTP1C association with Vav, Vav was immunoprecipitated from steel factor-treated P815 cells and the immune complexes were assessed for associated phosphatase activity. As evaluated by the dephosphorylation of p-nitrophenol phosphate substrate, phosphatase activity was clearly detected in these immunoprecipitates, the levels of activity increasing linearly in proportion to the amount of cell lysate protein (Fig. 4 A). To evaluate the extent to which PTP1C contributes to Vav-associated phosphatase activity, this experiment was repeated using ConA-treated splenic cells from mice. As shown in Fig. 4 A, the level of Vav-coprecipitated phosphatase activity in these PTP1C-deficient cells was negligible and unaltered by the use of increasing amounts of cell lysate protein. These findings suggest that PTP1C accounts for the majority of Vav-associated phosphatase activity detected in steel factor-treated P815 cells and thus imply that Vav or Vav-associated signaling molecules may represent targets for PTP1C-induced tyrosine dephosphorylation.

To extend these data, Vav immunoprecipitates from ConA-treated normal me and me' splenic cells were also assessed for their capacity to dephosphorylate a tyrosine-phosphorylated synthetic peptide. As is consistent with the contention that PTP1C accounts for the majority of Vav-associated phosphatase activity, levels of tyrosine phosphatase activity detected in Vav immunoprecipitates from me and me' splenic cells were dramatically reduced relative to those observed in splenic cell
that this activity is engendered by PTP1C. Based on the relative levels of phosphatase activity contained in Vav versus PTP1C immunoprecipitates from stimulated P815 cells (Fig. 4A), it also appears that a considerable proportion of cellular PTP1C activity is associated with Vav, an observation which is consistent with previous data indicating that PTP1C-ligand binding substantially enhances PTP1C catalytic function (42). By inference, these findings are highly suggestive of a critical role for PTP1C in modulating the signal transducing functions of Vav and/or Vav-associated proteins.

Association of PTP1C with Grb2 and mSos1 in P815 Cells—While the precise relationship between Ras activation and Vav-mediated transformation remains unclear, recent data suggest that Ras proteins and Vav cooperate in a synergistic fashion to induce cellular transformation (34). The potential relevance of Vav to Ras-related signaling events is also highlighted by the observed capacity of Vav to associate physically with the Grb2 protein (43), a ubiquitously expressed molecule known to interact via its SH2 domain with proline-rich motifs in the Ras GEF mSos1 and via its SH3 domain to phosphotyrosine residues in activated growth factor receptors (44–46). In view of these findings and the current data revealing the association of PTP1C with Vav, we next explored the possibility that PTP1C might also interact with the Grb2-mSos1 complex in hematopoietic cells. To this end, Grb2 and mSos1 or, alternatively, PTP1C immunoprecipitates were prepared from resting and steel factor-treated P815 cells and subjected to immunoblotting analysis with anti-PTP1C or anti-Grb2/mSos1 antibodies, respectively. As shown in Fig. 5, the results of this analysis reveal the capacity of PTP1C to bind to both Grb2 (Fig. 5A) and mSos1 (Fig. 5B) in unstimulated cells. As the interaction of Grb2 with Vav has also been detected in unstimulated T cells (43), it appears that PTP1C, Vav, and the Grb2-mSos1 molecules may associate with one another as a multimeric complex in resting hematopoietic cells. PTP1C interactions with Grb2 and mSos1 were also examined in steel factor-treated cells, and, as observed with respect to PTP1C binding to Vav, interactions between this phosphatase and both Grb2 and mSos1 were increased, following cell activation (Fig. 5C). These findings are again consistent with the contention that PTP1C and Vav interactions mediate receptor-evoked mitogenic signaling cascades and suggest that this modulatory effect also involves the association of these proteins with Grb2-mSos1. While the structural basis for the amalgamation of these four signaling effectors requires further definition, recent data revealing the capacity of the Grb2 carboxyl-terminal SH3 domain to physically associate with the Vav amino-terminal SH3 domain (47) together with data implicating the Grb2 amino-terminal SH3 domain in mSos1 binding (45) provide some indication as to the mode of Grb2-mSos1 association with PTP1C-Vav complexes. In addition, the localization of a major site for PTP1C tyrosine phosphorylation (Tyr-538) within a sequence (pYGNI) representing a consensus sequence (pYXNX) for Grb2 SH2 domain binding (38, 39, 48) suggests a role for Grb2 SH2 domain interaction with PTP1C phosphotyrosine in the genesis of this multimeric complex. In this context, PTP1C may act as an adaptor linking activated receptors to Grb2-mSos1, a role already demonstrated for the ubiquitously expressed Syk PTP in relation to the platelet-derived growth factor receptors (49, 50). Whether or not PTP1C plays this latter role in
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In summary, we have shown that PTP1C associates with Vav, Grb2, and mSos1, three cytosolic molecules expressed broadly among hematopoietic cells and implicated in the activation of Ras or Ras-related signaling pathways. The capacity of PTP1C to interact with and potentially modulate these signaling proteins strongly suggests that PTP1C effects on hematopoietic cell differentiation and growth are realized at least in part through the regulation of Ras and/or Ras-related proteins. Similarly, the association of Vav with PTP1C protein and phosphatase activity implies a role for PTP1C in modulating Vav-induced transformation events. The definition of the structural basis for and physiologic relevance of PTP1C associations with these signaling effectors thus represents a promising avenue toward elucidating the intracellular events regulating downstream transmission of receptor-evoked activation signals in hematopoietic cells.

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