Differential Signaling after β1 Integrin Ligation Is Mediated Through Binding of CRKL to p120<sub>CBL</sub> and p110<sub>HEF1</sub>*

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CRKL is an SH2-SH3-SH3 adapter protein that is a major substrate of the BCR/ABL oncogene. The function of CRKL in normal cells is unknown. In cells transformed by BCR/ABL we have previously shown that CRKL is associated with two focal adhesion proteins, tensin and paxillin, suggesting that CRKL could be involved in integrin signaling. In two hematopoietic cell lines, MO7e and H9, we found that CRKL rapidly associates with tyrosine-phosphorylated proteins after cross-linking of β1 integrins with fibronectin or anti-β1 integrin monoclonal antibodies. The major tyrosine-phosphorylated CRKL-binding protein in the megakaryocytic MO7e cells was identified as p120<sub>CBL</sub>, the cellular homolog of the v-Chl oncprotein. However, in the lymphoid H9 cell line, the major tyrosine-phosphorylated CRKL-binding protein was p110<sub>HEF1</sub>. In both cases, this binding was mediated by the CRKL SH2 domain. Interestingly, although both MO7e and H9 cells express p120<sub>CBL</sub> and p110<sub>HEF1</sub>, β1 integrin cross-linking induces tyrosine phosphorylation of p120<sub>CBL</sub> (but not p110<sub>HEF1</sub>) in MO7e cells and of p110<sub>HEF1</sub> (but not p120<sub>CBL</sub>) in H9 cells. In both cell types, CRKL is constitutively complexed with C3G, SOS, and c-ABL through its SH3 domains, and the stoichiometry of these complexes does not change upon integrin ligation. Thus, in different cell types CRKL and its SH3-associated proteins may form different multimeric complexes depending on whether p120<sub>CBL</sub> or p110<sub>HEF1</sub> is tyrosine-phosphorylated after integrin ligation. The shift in association of CRKL and its SH3-associated proteins from p120<sub>CBL</sub> to p110<sub>HEF1</sub> could contribute to different functional outcomes of “outside-in” integrin signaling in different cells.

Integrins play a role in cell movement and apoptosis and also act as costimulatory molecules. The integrin receptors are α/β heterodimeric transmembrane proteins that mediate cell-cell or cell-extracellular matrix interactions. Activation of integrin receptors leads to the formation of focal adhesions where integrin cytoplasmic domains are connected with actin-containing cytoskeleton components, thereby providing a link between the extracellular environment and intracellular elements. Tyrosine phosphorylation of cellular proteins is an early event after integrin receptor stimulation and is believed to initiate a series of signaling events involving protein-protein interactions leading to changes in viability, proliferation, or other functions in various cells (1, 2). One tyrosine kinase that is localized to the focal adhesion and is activated after integrin ligation has been identified as p125<sub>FAK</sub> (3). This kinase may have a negative regulatory role in the formation of focal adhesions (4). Also, another non-receptor tyrosine kinase (related adhesion focal tyrosine kinase) has been found to be partially associated with the actin cytoskeleton and is activated by integrins (5, 6).

Recently, investigators have begun to identify the major cellular proteins that are tyrosine-phosphorylated after cross-linking of integrins by ligands. For example, p120<sub>CBL</sub> is tyrosine-phosphorylated after β1 integrin ligation in the human B cell line Nalm-6 and after β1 and β2 integrin ligation in the megakaryoblastic cell line MO7e (7, 8).

In many signal transduction pathways activated by tyrosine kinases, adapter molecules have been shown to play a key role in mediating transient protein-protein interactions. We have previously shown that the adapter protein CRKL is associated with the focal adhesion protein paxillin in cells transformed by the oncogenic tyrosine kinase BCR/ABL (9). CRKL is a 39-kDa protein with one SH2 and two SH3 domains (10). CRKL has a high homology to c-CRK-II and belongs to the CRK family of adapter proteins, which includes v-CRK, c-CRK-II, and c-CRK-I (11–13). The CRK and CRKL SH3 domains have been shown to specifically bind to c-ABL, SOS, or C3G (14–19). The SH2 domain of CRKL has been shown to bind to p120<sub>CBL</sub> in cells transformed by oncogenic tyrosine kinases (19, 20), and CRKL binds p120<sub>CBL</sub> indubitably after epidermal growth factor receptor stimulation (21) or after T cell receptor stimulation (22).

In this study, we examined the involvement of CRKL in signal transduction pathways activated after cross-linking of β1 integrins in two hematopoietic cell lines, the megakaryoblastic cell line MO7e and a T cell line, H9. In both cell lines, β1 integrin stimulation resulted in the rapid association of CRKL with a single major tyrosine-phosphorylated cellular protein. Surprisingly, however, this protein was of a different apparent molecular mass in the two cell lines. We found that p120<sub>CBL</sub> was the major tyrosine-phosphorylated CRKL-binding protein in MO7e cells, and p110<sub>HEF1</sub> was the major tyrosine-phosphorylated CRKL-binding protein in H9 cells. In both cases the binding was mediated through the CRKL SH2 domain, while proteins constitutively associated with the CRKL SH3 domain, including C3G, SOS, and c-ABL, did not appear to be affected by β1 integrin stimulation. These results indicate that CRKL and its associated signaling proteins can interact with more than one signaling pathway activated by β1 integrin ligation.

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β1 Integrin Ligation Links CRKL to p120<sup>CBL</sup> and p110<sup>HEF1</sup>

MATERIALS AND METHODS

Cells—The human megakaryoblastic cell line M07e (obtained from Dr. Steve Clark, Genetics Institute, Cambridge, MA) was maintained in Dulbecco’s modified Eagle’s medium (Mediatech, Washington, D.C.), 10 ng/ml granulocyte-macrophage colony-stimulating factor (Genetics Institute), and 20% (v/v) fetal calf serum (PAA Laboratories Inc., Newport Beach, CA) at 37 °C with 10% CO<sub>2</sub>. The BCRI/ABL-expressing M07e cell line MO7e/p210 was generated by transfection with the p2D vector containing the sequence for the p210<sup>BCR/ABL</sup> DNA (obtained from Dr. George Daley, MIT, Cambridge, MA). For stimulation studies, M07e cells were washed with Dulbecco’s phosphate-buffered saline (DPBS)<sup>1</sup> and deprived of growth factors for 20 h at 37 °C in serum-free medium with 1% (v/v) bovine serum albumin (Sigma). The human T cell line H9 (obtained from Dr. Jerome Ritz, Dana-Farber Cancer Institute) was maintained in RPMI 1640 (Mediatech) and 10% (v/v) fetal calf serum (PAA Laboratories Inc.) at 37 °C with 5% CO<sub>2</sub>. Starved H9 cells were prepared by washing with DPBS and were deprived of serum for 2 h at 37 °C in serum-free medium.

Stimulation of Cells and Preparation of Cellular Lysates—Starved M07e or H9 cells were first incubated for 15 min on ice with antibodies against CD29/β1 integrin (4B4, obtained from Dr. C. Morimoto, Dana-Farber Cancer Institute), CD3 (OKT3, Coulter Corp., Miami, FL), or an irrelevant antibody (3C11C8, an anti-interferon-γ monomolecular antibody) and then stimulated by cross-linking using affinity-purified rabbit anti-mouse Ig (Dako Corp., Carpenteria, CA) at 37 °C for 10 min. For a integrin subunit cross-linking, starved M07e and H9 cells were incubated for 20 min on ice with antibodies against α4 integrins (82P2 from Dr. C. Morimoto and B5G10 from Dr. M. Hemler, Dana-Farber Cancer Institute) or against α5 integrins (2H6 from Dr. C. Morimoto and A5-PUJ2 from Dr. M. Hemler) and then stimulated by cross-linking for 20 min as described above. Either starved H9 cells or M07e cells (washed three times in DPBS after starvation and resuspended in Dulbecco’s modified Eagle’s medium) were used for stimulation with fibronectin (Life Technologies, Inc.) in the same fashion. Cell lysates were prepared as described (23).

Immunoprecipitation and Western Blotting—Western blotting using a chemiluminescence technique was performed as described (23). Immunohistochemical detection of tyrosine-phosphorylated proteins in Western blots utilized monomolecular antibody 4G10 (kindly provided by Dr. B. Druker, Oregon Health Science University, Portland, OR). Polyclonal rabbit antibody antisera against p120<sup>CBL</sup> (Santa Cruz Biotechnology, Santa Cruz, CA), CRKL (Santa Cruz Biotechnology), p110<sup>HEF1</sup> (HEF1 SB) (24), and mouse monoclonal antibodies against c-ABL (AB-3 from Oncogene Science, Manhasset, NY) and CRKL (the mouse monoclonal was generated as described elsewhere (25) and only used for Western blotting) were used for this study. The pGEX vector containing the SH2 and SH3 domains of CRKL was obtained from Dr. J. Groffen, Children’s Hospital, UCLA, Los Angeles, CA. The GST-fusion proteins were expressed in Escherichia coli (DH-5a) by isopropyl-1-thio-β-D-galactopyranoside induction and isolated from sonicated bacterial lysates using glutathione-Sepharose beads (Pharmacia Biotech Inc.) according to the manufacturer’s directions.

Flow Cytometry Analysis—M07e and H9 cells (0.5 × 10<sup>6</sup> cells/sample) were incubated with murine monoclonal antibodies against integrin receptors including α1 (TS2/7), α2 (A2–2E10), α3 (A3–2F5), α4 (B5G10), α5 (A5-PUJ2), α6 (A6-ELE) (all anti-α integrin receptor antibodies were obtained from Dr. M. Hemler, Dana-Farber Cancer Institute), β1 (4B4), or an irrelevant monoclonal antibody (3C11C8) for 20 min on ice and then washed once with DPBS. Cells were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse serum (Southern Biotechnology Assoc., Birmingham, AL) for an additional 20 min and subsequently washed twice in DPBS before analysis using a Coulter Epics XL flow cytometer (Coulter Corp.) for analysis.

Far-Western Blotting—Using previously established techniques (26), far-Western blotting was performed as described previously (19). Briefly, immunoprecipitated proteins were transferred after rehydration to Immobilon-P (polyvinylidene difluoride) membrane (Millipore) and blocked with 5% nonfat dry milk in 0.1% Tween 20 in phosphate-buffered saline, pH 7.4. The specific direct binding was evaluated by probing the membrane with GST-fusion proteins and visualized with a combination of anti-GST monomolecular antibody (Santa Cruz Biotechnology) and horseradish peroxidase-coupled anti-mouse IgG antibody by chemiluminescence.

<sup>1</sup>The abbreviations used are: DPBS, Dulbecco’s phosphate-buffered saline; GST, glutathione S-transferase.
a p120<sup>CBL</sup> antibody (data not shown). Based on its molecular mass and the presence of multiple potential CRKL SH2 binding motifs (Tyr-X-X-Pro), we examined p110<sup>HEF1</sup> for possible coprecipitation with CRKL. The blot was stripped, and the phosphoprotein was identified as p110<sup>HEF1</sup> by immunoblots (Fig. 1B, upper right panel). The lower right panel in Fig. 1B demonstrates that comparable amounts of CRKL were loaded. These results demonstrate that integrin receptor activation with fibronectin can induce the formation of a CRKL-p120<sup>CBL</sup> complex in MO7e cells and a CRKL-p110<sup>HEF1</sup> complex in H9 cells. However, we did not detect significant association of p120<sup>CBL</sup> with CRKL in H9 cells or with p110<sup>HEF1</sup> in MO7e cells at any time points tested between 0 and 60 min (data not shown).

**p120<sup>CBL</sup> and p110<sup>HEF1</sup> Are Differentially Tyrosine-phosphorylated after β1 Integrin Ligation in MO7e Cells and H9 Cells—** Since we observed differential association of tyrosine-phosphorylated p120<sup>CBL</sup> and p110<sup>HEF1</sup> with CRKL in MO7e cells or H9 cells, respectively, we asked if these proteins were differentially tyrosine-phosphorylated after β1 integrin ligation in these cells. Stimulation of the megakaryocytic MO7e cells or the T cell line H9 with a monoclonal antibody to cross-link β1 integrins induced rapid tyrosine phosphorylation of cellular proteins compared with unstimulated cells (Fig. 2A, left panel). Mock stimulation with an irrelevant antibody (3C11C8, an anti-interferon-γ murine monoclonal antibody) did not induce tyrosine phosphorylation (data not shown). The major tyrosine-phosphorylated proteins in MO7e cells include proteins with apparent molecular masses of 145, 120, 95, 70, and 40 kDa, whereas in H9 cells two prominent proteins of 110 and 95 kDa were tyrosine-phosphorylated. H9 cells treated with an irrelevant antibody also did not induce tyrosine phosphorylation of cellular proteins. The tyrosine phosphorylation pattern induced by fibronectin was virtually identical to the β1 integrin-induced pattern.

We identified the 120-kDa protein as p120<sup>CBL</sup> in phosphotyrosine immunoprecipitations of stimulated MO7e cells but not H9 cells (Fig. 2A, middle panel). In contrast, the 110-kDa protein in the phosphotyrosine immunoprecipitation of H9 cells was found to be the recently cloned p130<sup>CAS</sup>-related protein p110<sup>HEF1</sup> (Fig. 2A, right panel). In addition, p120<sup>CBL</sup> and p110<sup>HEF1</sup> were also inducibly (but again selectively) tyrosine-phosphorylated with fibronectin stimulation in MO7e and H9 cells, respectively (data not shown). Interestingly, p110<sup>HEF1</sup> is not tyrosine-phosphorylated in MO7e cells. The increased tyrosine phosphorylation of p120<sup>CBL</sup> and p110<sup>HEF1</sup> is likely to mediate the specific interaction with CRKL after integrin cross-linking (Fig. 1). The differences in phosphorylation of p120<sup>CBL</sup> or p110<sup>HEF1</sup> could not be attributed to differential expression of p120<sup>CBL</sup> and p110<sup>HEF1</sup> as expression of these proteins in MO7e and H9 cells by Western blotting was comparable (Fig. 2B). In addition to p110<sup>HEF1</sup>, the antiserum to p110<sup>HEF1</sup> recognized a 95-kDa protein in Western blot experiments (Fig. 2B, right panel). The identity of the 95-kDa protein is not known at this time. However, our preliminary data suggest that it may be the SH3 domain-containing p130<sup>CAS</sup>-related protein p95<sup>HEF1/SIN</sup> (27, 28) (data not shown).

The failure to tyrosine-phosphorylate p110<sup>HEF1</sup> in MO7e cells and p120<sup>CBL</sup> in H9 cells could be due to defects in signaling pathways leading to tyrosine phosphorylation of these proteins. To address this issue, other pathways known to induce phosphorylation of p120<sup>CBL</sup> and p110<sup>HEF1</sup> were examined. We found p110<sup>HEF1</sup> in phosphotyrosine immunoprecipitates of MO7e cells expressing the oncogenic tyrosine kinase BCR/ABL but not in untransfected cells, demonstrating apparent phosphorylation of p110<sup>HEF1</sup> in response to BCR/ABL (Fig. 2C). Also, p120<sup>CBL</sup> was found to be inducibly tyrosine-phosphorylated after CD3 cross-linking in H9 (Fig. 2D). These data suggest that p120<sup>CBL</sup> and p110<sup>HEF1</sup> can be tyrosine-phosphorylated in both cell lines by stimuli other than β1 integrin receptor cross-linking. Tyrosine-phosphorylated p120<sup>CBL</sup> and p110<sup>HEF1</sup> were also found to be inducibly and selectively associated with CRKL after cross-linking with α4 integrin in MO7e and H9 cells, respectively (Fig. 2E). Cross-linking of α5 integrin in MO7e cells produced similar results; however, the increased association of CRKL with p110<sup>HEF1</sup> was very small in H9 cells (data not shown). We further tested if different signaling was due to differences in α or β integrin receptor expression. We found that both cell lines had comparable expression of the β1 integrin as well as α4, α5, and α6 integrins. Expression of α1, α2, and α3 integrins was lower or negligible (Fig. 2F). Overall these results demonstrate that similar integrin receptors can activate distinct signaling proteins in different cell lines.

**In Vitro Association of CRKL GST-fusion Proteins with p120<sup>CBL</sup> and p110<sup>HEF1</sup>—** The above results suggest the potential induction of one or more multimeric protein complexes containing CRKL, p120<sup>CBL</sup>, or p110<sup>HEF1</sup>. The binding of CRKL to p120<sup>CBL</sup> and p110<sup>HEF1</sup> appears to require tyrosine phosphorylation of these proteins. Since CRKL has one SH2 and two adjacent SH3 domains, we sought to determine the mechanism of CRKL binding to p120<sup>CBL</sup> and p110<sup>HEF1</sup> using GST-fusion proteins containing various segments of each protein. The SH2 domain of CRKL precipitated p120<sup>CBL</sup> from lysates of stimulated (but not unstimulated) MO7e cells (Fig. 3A). The blots were stripped and reprobed with antibodies against c-ABL demonstrating that GST-CRKL-SH3 but not GST-CRKL-SH2 constitutively precipitated c-ABL (Fig. 3A). We also found constitutive coprecipitation of C3G and SOS with the ABL-SH3 domain (data not shown). Using lysates from H9 cells, the SH2 domain of CRKL precipitated p110<sup>HEF1</sup> after fibronectin stimulation, while the GST-CRKL SH3 domain did not (Fig. 3B).

The in vitro GST-fusion protein precipitations with p120<sup>CBL</sup>, c-ABL, and CRKL do not indicate if binding of the SH2 or SH3 domains is direct or indirect. We therefore used a far-Western technique to examine possible direct in vitro interactions. Cellular lysates from unstimulated and β integrin-stimulated MO7e cells were used for immunoprecipitations with anti-p120<sup>CBL</sup> or anti-CRKL antibody. Fig. 3C shows that GST protein alone does not bind to proteins in p120<sup>CBL</sup> or CRKL immunoprecipitations. Direct binding of a single 120-kDa protein band in CRKL immunoprecipitations using the GST-CRKL SH2 protein as a probe was found only after β integrin ligation. This protein was identified as p120<sup>CBL</sup> in the p120<sup>CBL</sup> immunoprecipitation and the CRKL SH2 far-Western blot. We also observed binding of the CRKL SH3 domain to a set of proteins between 140 and 160 kDa; however, this interaction was not changed upon β integrin ligation (data not shown). The binding of SH2 domains to p120<sup>CBL</sup> is likely to require phosphotyrosine, since no binding was observed to p120<sup>CBL</sup> in lysates from unstimulated cells where tyrosine phosphorylation of p120<sup>CBL</sup> is not induced. These results indicate that in MO7e cells, CRKL is linked through its SH2 domain to a pathway involving p120<sup>CBL</sup>, whereas in H9 cells CRKL is linked to a pathway involving p110<sup>HEF1</sup>.
results were obtained when the immunoprecipitation and blotting antibodies were reversed (data not shown). We did not observe detectable induction of tyrosine phosphorylation of c-ABL after integrin ligation (data not shown). These data demonstrate that integrin cross-linking does not alter the constitutive complexes of CRKL with c-ABL, C3G, and SOS.

DISCUSSION

The biological effects of cross-linking integrins may vary widely from cell to cell, ranging from stimulation of proliferation to induction of apoptosis. When integrins are cross-linked through binding with a natural ligand such as fibronectin, a series of signaling events are initiated. This signaling is asso-


**Fig. 3.** Precipitation of p120<sup>CRM1</sup> and p110<sup>HEF1</sup> with CRKL GST-fusion proteins. Unstimulated (−) or β1 integrin (+)-stimulated MO7e cells or H9 cells were used for precipitations. A, lysates of 7.5 × 10<sup>5</sup> MO7e cells before (−) and after (+) β integrin stimulation (7.5 min, 37 °C) were incubated with 10 μg of GST-fusion protein and GST immobilized on glutathione beads. GST-fusion proteins of the SH2 domain (SH2) and both SH3 domains (SH3-SH3<sub>2</sub>) of CRKL were used for precipitations. Coprecipitation of p120<sup>CRM1</sup> (CBL) or c-ABL was detected by Western blotting. B, lysates of 15 × 10<sup>5</sup> H9 cells before (−) and after (+) β1 integrin stimulation (30 min, 37 °C) were incubated with 10 μg of GST-fusion protein and GST immobilized on glutathione beads. GST and GST-fusion proteins of the SH2 domain (SH2) and both SH3 domains (SH3-SH3<sub>2</sub>) of CRKL were used for precipitations. Coprecipitation of p110<sup>HEF1</sup> (HEF1) was detected by Western blotting. C, lysates of 10 × 10<sup>5</sup> unstimulated (−) or β integrin-stimulated cells (+) were incubated with antibodies against CRKL or p120<sup>CRM1</sup> (CBL) as indicated. Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane, and specific direct binding of GST-fusion proteins to p120<sup>CRM1</sup> was detected by far-Western blotting. GST and GST-fusion proteins of the CRKL SH2 domain (CRKL-SH2) were used.

Associated with the following changes in the actin cytoskeleton: formation of a cytoskeletal complex of proteins that includes actin, vinculin, talin, p125<sup>FAK</sup>, paxillin, and tensin, activation of tyrosine phosphorylation, and activation of other signal transduction pathways such as the p21<sup>Ras</sup> pathway. Overall, this outside-in signaling of integrins is likely to be an important part of the signals sent by the microenvironment to influence cell behavior (1, 2).

However, the mechanisms of outside-in signaling are not well understood. This is due in part to the complexity of studying a system with many related receptors (the integrin family) that are expressed heterogeneously on different cell types, coupled with the fact that different integrins may share the same ligand. Since the biological effects of outside-in signaling may vary widely in different cells, it is of interest to determine how integrin cross-linking in one cell may augment proliferation but induce apoptosis in another cell type. It would be anticipated that different integrins may activate different signaling pathways in the same cell and also that the same integrin could potentially activate different pathways in different cells. Despite this prediction, there are few examples of differential signaling by integrins and even fewer examples where specific integrin-activated signaling pathways have been directly linked to a biological event.

In this study we have investigated the specific role of CRKL (an adapter protein that has one SH2 domain and two SH3 domains) in integrin signaling as part of a larger effort to understand the cellular functions of CRKL. During preliminary studies in the human megakaryoblastic cell line MO7e, we had noted that after cross-linking of β1 integrins by monoclonal antibody, CRKL was induced to bind through its SH2 domain to a 120-kDa protein identified as p120<sup>CRM1</sup>. p120<sup>CRM1</sup> was shown to be one of the most prominently tyrosine-phosphorylated proteins induced after integrin activation in these cells (8) and virtually the only tyrosine phosphoprotein coprecipitating with CRKL. However, in another hematopoietic cell line, the T cell line H9, we noted that CRKL did not coprecipitate with p120<sup>CRM1</sup> after integrin cross-linking, despite the fact that the H9 cell line was found to have the same pattern of β1 integrin expression as MO7e as well as abundant expression of p120<sup>CRM1</sup> (29, 30). This unexpected result was made more interesting by the finding that CRKL was induced to coprecipitate with another tyrosine phosphoprotein in H9 cells, p110<sup>HEF1</sup>, which is a signaling protein related to p130<sup>Cas</sup>. Again, p110<sup>HEF1</sup> was virtually the only tyrosine phosphoprotein coprecipitating with CRKL after integrin stimulation, and the interaction was mediated by the CRKL SH2 domain. Like p120<sup>CRM1</sup>, p110<sup>HEF1</sup> has multiple copies of potential CRKL SH2 binding motifs (phospho Tyr-X-X-Pro) (7, 24). These combined observations suggest that CRKL is not only involved in integrin-mediated outside-in signaling, it can also participate in different pathways depending on which upstream molecule (p120<sup>CRM1</sup> or p110<sup>HEF1</sup>) is phosphorylated (probably at the phospho Tyr-X-X-Pro motifs previously shown to represent binding sites for CRK and CRKL SH2 domains). This provides for the possibility of an intracellular
signaling “switch” that could couple integrin signaling to different biological effects.

In contrast to the effects of integrin-induced tyrosine phosphorylation on the binding of the CRKL SH2 domain to signaling molecules, the proteins that were bound to the CRKL SH3 domains were not affected by integrin cross-linking. The known CRKL SH3-binding proteins include c-ABL, C3G, and SOS. These proteins were first described as binding to the CRKII SH3 domain; however, we and others have shown that they also bind to the CRKL SH3 domain (14–19). SOS has known guanine-exchange factor activity for p21RAS; in contrast, C3G appears to have specific guanine exchange activity for p21RAP1. C3G does not have substrate specificity for p21RAS (31), but its substrate p21RAP1 appears to regulate, at least in part, the signal from p21RAS to the RAF kinase. C3G also shows sequence similarity to CDC25 and SOS family proteins (17) and preferentially binds to the N-terminal SH3 domain (16). The exact function of the tyrosine kinase c-ABL is unknown, although c-ABL has been shown to be involved in transcriptional activation (32) and possibly is activated in response to certain types of DNA damage (33). Interestingly, c-ABL can interact with the actin cytoskeleton through an actin binding site in its C terminus (34). During integrin signaling, c-ABL, C3G, or SOS could be linked to either p120CBL or p110HEF1 by CRKL, although no direct evidence of such mutlipermic proteins was demonstrated in this study.

The protooncoprotein p120CBL (for Casitas B-lineage lymphoma) is a widely expressed 120-kDa protein. It is the cellular homolog of v-Cbl, the oncoprotein in the CAS NS-1 retrovirus (35, 36) that induces pre-B cell lymphomas and myelogenous leukemias in mice (37). The p120CBL homolog Sli-1 in Caenorhabditis elegans is a negative regulator of the epidermal growth factor receptor tyrosine kinase homolog Let-23 (38). p120CBL is also known to be a substrate of tyrosine kinases in response to T cell (39) and B cell (40) activation, FC-γ receptor cross-linking (41, 42), and growth factors (23, 43–45). In malignant cells, the function of p120CBL is not known, although several interactions with other signaling proteins have been reported. For example, p120CBL has been shown to associate with active phosphatidylinositol 3-kinase and p110HEF1 as well as p110HEF1. How-2 This suggests that the mechanisms that activate either pathway are not mutually exclusive. The differential activation of p120CBL and p110HEF1 could be directly mediated through a process that leads to activation of different tyrosine kinases that are specific for p120CBL or p110HEF1, and we are currently investigating this possibility.

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