Steel Factor Induces Tyrosine Phosphorylation of CRKL and Binding of CRKL to a Complex Containing c-Kit, Phosphatidylinositol 3-Kinase, and p120CBL

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Steel factor (SF) is a growth and survival factor for hematopoietic cells. The receptor for SF, c-Kit, contains intrinsic tyrosine kinase activity, and binding of SF induces rapid tyrosine phosphorylation of several cellular proteins, including c-Kit itself. Activation of c-Kit is shown here to induce tyrosine phosphorylation of CRKL, and CRKL coprecipitated with c-Kit through an interaction that required the CRKL SH3 domains and not the SH2 domain. CRKL associated with c-Kit indirectly as part of a larger complex of proteins. Two proteins in this complex were identified as the p85 regulatory subunit of phosphatidylinositol 3-kinase (p85PI3K) and the proto-oncoprotein p120CBL. Because p85PI3K is known to bind to the activated c-Kit receptor, the possibility that CRKL interacted with c-Kit indirectly through p85PI3K was investigated. Far Western blotting with a CRKL-SH3 glutathione S-transferase fusion protein showed that CRKL binds directly to p85PI3K in vitro. However, although a small amount of CRKL was preassociated with p85PI3K, the interaction was increased after SF stimulation, suggesting that the interactions of these three proteins are complex. We conclude that SF induces the formation of a signaling complex potentially containing CRKL and p120CBL, both of which bind to c-Kit through p85PI3K. These data suggest that one function of CRKL in normal cells might be to recruit signaling molecules such as CBL into a complex with PI3K. Such complexes could be important in propagating signals involving PI3K such as gene expression and adhesion.

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¶ The abbreviations used are: SF, steel factor; PI3K, phosphatidylinositol 3-kinase; GST, glutathione S-transferase.
Using a human megakaryoblastic cell line, MO7e, SF stimulation was found to result in the rapid tyrosine phosphorylation of CRKL and coprecipitation of CRKL with c-Kit. However, although this interaction was inducible, it was mediated through the CRKL SH3 domain. This suggested the possibility that CRKL was not binding to c-Kit directly, but associated with c-Kit as part of a larger complex of proteins. We found that the coprecipitation of CRKL with c-Kit is, at least in part, likely to be mediated through binding of p85<sub>PI3K</sub> to c-Kit as well as to CRKL. Other proteins are likely to be involved in this complex, with the most prominent identified as the proto-oncoprotein p120<sub>CBL</sub>. These data demonstrate that CRKL is involved in the formation of a complex of signaling proteins that bind to c-Kit through PI3K and further suggest that CRKL, and its SH2-binding partners such as p120<sub>CBL</sub>, may function downstream of PI3K.

MATERIALS AND METHODS

Cells—The human promegakaryoblastic cell line MO7e (obtained from Dr. Steve Clark, Genetics Institute, Cambridge, MA) was maintained in Dulbecco’s modified Eagle’s medium (Mediatech, Washington, DC), 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>. For stimulation studies, MO7e cells were deprived of growth factors for 18 h at 37 °C in serum-free medium containing 1% (w/v) bovine serum albumin (Sigma).

Stimulation of Cells and Preparation of Cellular Lysates—Starved MO7e cells were left untreated or stimulated with recombinant human SF (Amgen, Thousand Oaks, CA) or D-galactopyranoside induction and isolated from sonicated bacterial lysates. The membranes were loaded with cold Dulbecco’s phosphate-buffered saline, and cell lysates were prepared as described (33).

Immunoprecipitation and Immunoblotting—Immunoprecipitation and immunoblotting using a chemiluminescence technique was performed as described (34). Immunochemical detection of tyrosine phosphorylated proteins in Western blots utilized monoclonal antibody 4G10 (kindly provided by Dr. B. Druker, Oregon Health Science University, Portland, OR). Polyclonal rabbit antisera were obtained against c-Kit (Santa Cruz Biotechnology, Santa Cruz, CA), p120<sub>CBL</sub> (Santa Cruz Biotechnology, CRKL (Santa Cruz Biotechnology), CRKL (Santa Cruz Biotechnology), and p85<sub>PI3K</sub> (Upstate Biotechnology Inc., Lake Placid, NY). A murine monoclonal antibody was generated against the N-terminal SH3 domain of CRKL and used for immunoblotting in this study. The pGEX vectors containing the N-terminal or C-terminal SH2 domain and the SH3 domain of p85<sub>PI3K</sub> were kindly provided by Dr. L. Cantley (Beth Israel Hospital, Harvard Medical School, Boston, MA). pGEX vectors containing the SH2 and the SH3-SH3 domains of CRKL were kindly provided by Dr. J. Groffen (Children’s Hospital, UCLA, Los Angeles, CA). The GST fusion proteins were expressed in Escherichia coli (DH-5α) 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.

Far Western Blotting—Using previously established techniques (34), far Western blotting was performed as described (24). In brief, immunoprecipitated proteins were transferred after SDS-polyacrylamide gel electrophoresis to Immobilon-P (polyvinylidene difluoride) membrane (Millipore Corp., Bedford, MA) and blocked with 5% nonfat dry milk in 0.1% Tween 20 in phosphate-buffered saline, pH 7.4). The specific direct in vitro binding was evaluated by probing the membrane with GST fusion proteins and visualized with a combination of anti-GST monoclonal antibody (Santa Cruz Biotechnology) and horseradish peroxidase-coupled anti-mouse IgG antibody by chemiluminescence.

PI3K Enzyme Assay—The activity of PI3K was measured in anti-c-Kit, CRKL, p120<sub>CBL</sub>, and preimmune control immunoprecipitates of unstimulated and SF-stimulated MO7e cell lysates. The kinase assay was performed as described (35, 36).

RESULTS

SF Induces Tyrosine Phosphorylation of CRKL in MO7e Cells—Stimulation of c-Kit induces tyrosine phosphorylation of cellular proteins and the receptor itself. For initial experiments we stimulated MO7e cells with SF for different time points between 0 and 60 min. Fig. 1 shows that SF induces rapid and transient tyrosine phosphorylation of cellular proteins with apparent molecular masses of 39, 55, 70, 80, 120, and 155 kDa. The tyrosine phosphorylation of these proteins followed different kinetics. Whereas proteins with apparent molecular masses of 55, 70, 80, and 120 kDa had maximum tyrosine phosphorylation at 1 min, the tyrosine phosphorylation of proteins with apparent molecular masses of 39 and 155 kDa peaked at 10 min and decreased thereafter.

To investigate the potential role of the 39-kDa adapter protein CRKL in SF signaling, we looked for tyrosine phosphorylated proteins that coprecipitated with CRKL. SF induced tyrosine phosphorylation of CRKL itself and association of CRKL with prominent phosphotyrosine proteins with apparent molecular masses of 120 and 155 kDa (Fig. 2A). In addition, a single 39-kDa tyrosine phosphorylated band was also detected in CRKL immunoprecipitates of SF-stimulated but not of unstimulated denatured lysates, suggesting that this band is indeed CRKL (data not shown). The tyrosine phosphorylation of CRKL and association with the 120-kDa phosphotyrosine protein was maximal within 1 min, and the association with the 155-kDa phosphotyrosine protein was maximal within 10 min of SF stimulation.

CRKL Associates with the Activated c-Kit Receptor and p120<sub>CBL</sub>—The 155-kDa tyrosine phosphorylated protein that coimmunoprecipitated with CRKL comigrated with c-Kit. This membrane was therefore rebotted, and the 155-kDa protein was identified by immunoblotting as c-Kit (Fig. 2A). The lowest panel in Fig. 2A demonstrates that equal amounts of CRKL were loaded. Similarly, we found that a 39-kDa tyrosine phosphorylated protein was identified as c-Kit in CRKL immunoprecipitates. This 39-kDa protein was identified as CRKL by reblotting with anti-CRKL antibodies (Fig. 2B). We also noted inducible coprecipitation of tyrosine phosphorylated proteins in c-Kit immunoprecipitates with apparent molecular masses of 55, 70, and 120 kDa. Preimmune rabbit serum did not precipitate tyrosine phosphorylated proteins. The upper panel on the right side of Fig. 2B demonstrates that equal amounts of c-Kit were loaded. In BCR/ABL-transformed cells, we and others have previously demonstrated that CRKL can bind to tyrosine phosphorylated p120<sub>CBL</sub>. We therefore also asked if CRKL coprecipitates with p120<sub>CBL</sub> after SF stimulation. Fig. 2C shows immunoprecipitates of p120<sub>CBL</sub> after 0–60 min SF stimulation, demonstrating rapid association with CRKL within 1 min of SF stimulation, which decreased thereafter. p120<sub>CBL</sub> coimmunoprecipitated also with a major tyrosine phosphorylated protein of an apparent molecular mass of 155 kDa. Similarly, in both the CRKL and c-Kit immunoprecipitations, the 120-kDa tyrosine phosphorylated protein was identified as p120<sub>CBL</sub> (data not shown). The middle panel in Fig. 2C demonstrates that equal amounts of p120<sub>CBL</sub> were loaded. These data suggest that CRKL...
forms an inducible complex with c-Kit and p120 CBL after SF stimulation.

p85 PI3K Forms an Inducible Complex with c-Kit, CRKL, and p120 CBL

We have previously shown that the BCR/ABL oncoprotein induces formation of a complex containing p210 BCR/ABL, p120 CBL, CRKL, and p85 PI3K (24). Because p85 PI3K is known to bind to c-Kit after SF activation (37), we looked for association of PI3K with CRKL or p120 CBL after SF stimulation of MO7e cells. In lysates of SF-stimulated MO7e cells, p85 PI3K was associated with tyrosine phosphorylated proteins with apparent molecular masses of 155, 120, 70, 55, and 39 kDa (Fig. 3, left panel). The 155-kDa protein was identified as c-Kit, the 120-kDa protein was identified as p120CBL, and the 39-kDa protein was identified as CRKL by specific immunoblotting (Fig. 3, right panels). Whereas the association of p85 PI3K with c-Kit and p120 CBL was dependent on SF stimulation, a small amount of CRKL was already preassociated with p85 PI3K. The association of p85 PI3K with CRKL increased considerably upon SF stimulation of MO7e cells. These data suggest the formation of a multimeric signaling complex after SF stimulation that includes CRKL, c-Kit, p120CBL, and p85 PI3K.

Coprecipitation of PI3K in Vitro Kinase Activity with c-Kit, CRKL, and p120 CBL

The previous results suggested that c-Kit, CRKL, and p120 CBL form a complex with p85 PI3K, suggesting that the p110 PI3K enzymatic subunit is also present. To determine if enzymatically active PI3K is present in this complex, we immunoprecipitated c-Kit, CRKL, and p120 CBL from lysates of unstimulated and SF-stimulated MO7e cells. Fig. 4 demonstrates that significant PI3K activity was detected in c-Kit, CRKL, and p120 CBL immunoprecipitates of SF-stimulated MO7e cells and in CRKL immunoprecipitates of unstimulated cells. However, very little PI3K coimmunoprecipitated with anti-p120 CBL compared with anti-c-Kit and CRKL immunoprecipitates; preimmune serum did not immunoprecipitate PI3K activity. These results also demonstrate that CRKL is preassociated with active PI3K, and after SF stimulation the association of active PI3K with CRKL and c-Kit is markedly increased.

In Vitro Association of CRKL and p85 PI3K GST Fusion Proteins with c-Kit, CRKL, and p120 CBL

The above results suggest the potential induction of one or more multimeric c-Kit receptor complexes containing CRKL, p85 PI3K, and p120 CBL. The association of c-Kit with CRKL and also with p85 PI3K and p120 CBL appears to require tyrosine phosphorylation of some of these proteins. Because CRKL has one SH2 and two adjacent SH3 domains, we sought to determine the mechanism of binding of CRKL to the proteins in this complex using GST fusion proteins containing various segments of each protein. Surprisingly, the SH3 domain of CRKL precipitated c-Kit from lysates of stimulated but not unstimulated cells. Using denatured lysates of unstimulated and SF-stimulated MO7e cells, we did...
not observe precipitation of the CRKL SH2 or CRKL SH3 domains with c-Kit (data not shown). In contrast, the SH2 domain of CRKL precipitated p120<sub>CBL</sub> from cell lysates of stimulated but not unstimulated MO7e cells. However, both the SH2 and the SH3 domains precipitated p120<sub>CBL</sub> from SF-stimulated and unstimulated lysate with increased specific binding of CRKL fusion protein to p120<sub>CBL</sub> following stimulation (Fig. 5A). Because p85<sub>PI3K</sub> contains two proline-rich motifs, we tested whether the CRKL-SH2 interacts with p85<sub>PI3K</sub>. Fig. 5B demonstrates that p85<sub>PI3K</sub> binds constitutively to the CRKL SH2 domain but does not associate with the CRKL SH2 domain or the ABL-SH3 domain. We next tested whether GST fusion proteins containing the N-terminal SH2 domain, the C-terminal SH2 domain, or the SH3 domain of p85<sub>PI3K</sub> could precipitate c-Kit or p120<sub>CBL</sub>. Fig. 5C demonstrates that the C-terminal and the N-terminal SH2 domain of p85<sub>PI3K</sub> can associate with the c-Kit receptor. This is consistent with earlier findings demonstrating binding of p85<sub>PI3K</sub> to c-Kit (37). This membrane was reblotted with antibodies to p120<sub>CBL</sub> demonstrating <em>in vitro</em> association of p120<sub>CBL</sub> with the SH3 domain and both SH2 domains of PI3K.

The <em>in vitro</em> GST fusion protein precipitations with c-Kit, p85<sub>PI3K</sub>, and p120<sub>CBL</sub> do not indicate whether binding of the SH2 or SH3 domains is direct or indirect. We therefore used a far Western technique to identify direct <em>in vitro</em> interactions. Cellular lysates from unstimulated and SF-stimulated MO7e cells were used for immunoprecipitations with c-Kit, CRKL, and anti-p120<sub>CBL</sub> antibodies. Fig. 5D shows that GST protein alone does not bind to proteins in immunoprecipitations. Direct binding of a single 120-kDa protein band in p120<sub>CBL</sub> immunoprecipitations using the GST-CRK-L SH2 protein as a probe was found after SF stimulation. We also observed <em>in vitro</em> binding of the CRKL SH3 domain to p85<sub>PI3K</sub> this interaction was not changed upon SF stimulation. However, we also observed inducible binding of the CRKL SH3 and the CRKL SH2 domains to a 155-kDa protein in anti-c-Kit and p85<sub>PI3K</sub> immunoprecipitations but not in anti-p120<sub>CBL</sub> immunoprecipitations. The identity of this protein remains unknown but we do not believe that it is c-Kit because we could not precipitate c-Kit with the CRKL SH2 domain. The p85<sub>PI3K</sub> SH3 domain was found to bind directly to p120<sub>CBL</sub> and the C-terminal SH2 domain to c-Kit. We did not observe significant binding of the N-terminal SH2 domain of p85<sub>PI3K</sub> to c-Kit, CRKL, or p120<sub>CBL</sub>. These results suggest that in MO7e cells, CRKL is linked through its SH3 domain to the P13k pathway, whereas the SH2 domain of CRKL binds to p120<sub>CBL</sub> after SF stimulation. In addition, the results also suggest direct binding of the C-terminal p85<sub>PI3K</sub> SH2 domain to c-Kit and binding of the p85<sub>PI3K</sub> SH3 domain to p120<sub>CBL</sub>.

![Fig. 4. Coprecipitation of PI3K in vitro kinase activity with c-Kit, CRKL, and p120<sub>CBL</sub>.](http://www.jbc.org/)

**Fig. 4.** Coprecipitation of PI3K <em>in vitro</em> kinase activity with c-Kit, CRKL, and p120<sub>CBL</sub>. PI3K <em>in vitro</em> kinase activity was measured in immunoprecipitates using lysates of unstimulated (−) and SF (+) stimulated MO7e cells. Proteins were immunoprecipitated (IP) with preimmune serum (NRS) and antibodies to c-Kit, CRKL, or p120<sub>CBL</sub> (CBL).

![Fig. 5. In vitro association of CRKL and p85<sub>PI3K</sub> GST fusion proteins with c-Kit, p85<sub>PI3K</sub>, and p120<sub>CBL</sub>](http://www.jbc.org/)

**Fig. 5.** <em>In vitro</em> association of CRKL and p85<sub>PI3K</sub> GST fusion proteins with c-Kit, p85<sub>PI3K</sub>, and p120<sub>CBL</sub>. Lysates of unstimulated MO7e cells (−) or cells stimulated for 10 min with 40 ng/ml SF (+) were used for precipitations. A, B, and C, lysates of 15 × 10<sup>6</sup> MO7e cells were incubated with 10 μg of GST and GST fusion protein immobilized on glutathione beads. Coprecipitation of c-Kit, p120<sub>CBL</sub> (CBL), or p85<sub>PI3K</sub> (PI3K) was detected by Western blotting (W.B.). A, GST or GST fusion proteins of the SH2 domain and both SH3 domains of CRKL. B, GST or GST fusion proteins of the SH3 domain of c-ABL (ABL), both SH3 domains, and the SH2 domain of CRKL. C, GST fusion proteins of the N-terminal SH2 domain (N-SH2), the C-terminal SH2 domain (C-SH2), and the SH3 domain of p85<sub>PI3K</sub> were used for precipitations. D, lysates of 20 × 10<sup>6</sup> MO7e cells were immunoprecipitated with antibodies against c-Kit, p120<sub>CBL</sub> (CBL), or p85<sub>PI3K</sub> (PI3K) as indicated. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane, and specific direct binding of GST fusion proteins to proteins in the immunoprecipitates was detected by far Western blotting. GST and GST fusion proteins of the SH2 domain (SH2) and both SH3 domains of CRKL or GST fusion proteins of the N-terminal SH2 domain (N-SH2), the C-terminal SH2 domain (C-SH2), and the SH3 domain (SH3) of p85<sub>PI3K</sub> were used.
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DISCUSSION

Although there are a number of signaling molecules known to be tyrosine phosphorylated by c-Kit, most downstream signaling pathways are not well understood. After stimulation of c-Kit with SF, previous studies have demonstrated rapid tyrosine phosphorylation of c-Kit itself and cellular proteins including SHP2, phospholipase C-γ, SHC, and p120CBL (19–21, 38, 39). These molecules in turn contribute signals to various pathways that influence growth, viability, adhesion, migration, or differentiation. PI3K has been the focus of several studies related to c-Kit signaling. For example, mutation of tyrosine 709 to phenylalanine in the Tyr-Xaa-Xaa-Met motif of the murine c-Kit receptor has been shown to reduce binding of the p85 subunit of PI3K (37). Further, this mutation also caused defects in SF-mediated adhesion and early gene expression, presumably by interfering with activation of PI3K (40). However, despite the apparent importance of PI3K signaling in this and other receptors, downstream signaling events have been difficult to identify.

In the studies reported here, we found that the adapter protein, CRKL, was tyrosine phosphorylated after c-Kit activation and coprecipitated with c-Kit. However, there was no evidence that CRKL bound to c-Kit directly, and the data presented here suggest rather that CRKL binds directly to p85 PI3K and indirectly to c-Kit through p85 PI3K. The interaction of CRKL with p85 PI3K was found to utilize the CRKL SH3 domain and could be further increased in response to factor stimulation. Also, we found that after SF stimulation, p120 CBL is tyrosine phosphorylated and coprecipitates with CRKL, suggesting the formation of a signaling complex that contains c-Kit, PI3K, CRKL, and p120 CBL. Overall, the data suggest that the possibility that CRKL and/or p120 CBL play a role in sending or modulating signals from c-Kit that require PI3K.

One of the most intriguing findings here is the specific, inducible association of CRKL with p85 PI3K through the CRKL SH3 domains. Previous studies suggested that CRK is constitutively associated with c-ABL, C3G, or SOS through the CRK SH3 domains. These proteins were first described to bind to the CRKI SH3 domain, but we and others have shown that they also bind to the CRKL SH3 domain (24, 28–32). SOS has known guanine exchange factor activity for p21Ras, whereas C3G appears to have specific guanine exchange activity for p21Rap1 (41). C3G has unique binding affinities to the CRK family proteins, because it preferentially binds to the N-terminal SH3 domain (29). The exact function of the tyrosine kinase c-ABL is unknown, although c-ABL has been shown to be involved in transcriptional activation and possibly is activated in response to certain types of DNA damage (42, 43). Recently a consensus sequence for binding to the CRK SH3 domain, Pro-Xaa-Leu-Pro-Xaa-Lys, has been described (44, 45). Consistent with our finding of CRKL SH3 binding to p85 PI3K, a proline-rich motif with this consensus sequence, Pro-Ala-Leu-Pro-Lys (amino acids 305–310, human sequence), is present in p85 PI3K (46). The SH3 domain interaction of CRKL with p85 PI3K may occur at this site, although this has not yet been directly tested. Inducible association of an adapter protein through SH3 domain interactions has been previously described for the binding of GRB2 to SOS in T lymphocytes, although most SH3 domain-mediated interactions are constitutive (47). One explanation for this phenomenon would be the presence of other proteins binding to CRKL, p85 PI3K, and c-Kit that are important for overall stability of the complex and that are brought into the complex in response to receptor activation. We believe that p120 CBL may be important in this regard, and this will be discussed in more detail below. Overall, our data strongly suggest that PI3K should be added to the list of signaling proteins known to interact with the CRKL SH3 domains but in the specific situation of c-Kit activation.

The fact that the CRKL SH3 domains can bind to several different, unrelated signaling proteins suggests that CRKL may play a role in several different signaling pathways. Thus, it is possible that the biological functions of CRKL may vary widely in different cells. Alternatively, it is possible that different stimuli may activate different signaling pathways involving CRKL in the same cell. CRKL has been shown to be tyrosine phosphorylated in cells transformed by onco-proteins including BCR/ABL, v-Ab1, v-Src, or in normal signaling after EGF receptor stimulation (34, 48, 49). Binding of CRKL to p120 CBL appears to be independent from CRKL tyrosine phosphorylation, because in other systems, including T cell signaling, CRKL is not tyrosine phosphorylated, although it binds to p120 CBL (50). Surprisingly, we did not find significant association of CRK with c-Kit in SF signaling in M07e cells, despite abundant expression of CRK in this cell line (data not shown) and the reported similarity of target proteins selected by CRKL and CRK during in vitro binding studies. It may be worthwhile to look specifically for situations in which CRK and not CRKL is preferentially selected for tyrosine phosphorylation and activation in various cell lineages.

Thus, the data presented here support the notion that CRKL is involved in a signaling pathway that also involves PI3K. Our data suggest that a substantial proportion of the PI3K activity associated with an activated c-Kit receptor is also associated with CRKL, because approximately the same amount of PI3K enzymatic activity is found in anti-CRKL as in anti-c-Kit immunoprecipitates. The biological effects of activated PI3K may vary widely from cell to cell, ranging from regulation of apoptosis, viability, or early gene expression to regulation of adhesion. CRKL may be involved in signaling to any or all of these different biological events. Specifically with regard to c-Kit, mutant c-Kit receptors that fail to bind PI3K, fail to induce c-fos or junB expression, and lack the ability to induce binding of cells to fibronectin and CRKL could be involved in one or all of these events (40).

As noted above, a protein known to bind to the CRKL SH2 domain in certain transformed cells, p120 CBL, was found to coprecipitate with CRKL after c-Kit stimulation. In vitro binding studies suggested that the SH2 domains of both CRKL and p85 PI3K can bind to p120 CBL, and the SH3 domain of PI3K also interacted directly with p120 CBL. Of course, other proteins that can interact with the CRKL SH2 domain may also be brought into any such complex, and in addition to p120 CBL, the known possibilities include p130 CAS, and paxillin (25–27). However, our data in M07e cells suggest that p120 CBL is the most abundant tyrosine phosphoprotein coprecipitating with CRKL after SF stimulation in these cells. The proto-oncoprotein p120 CBL (for Casitas B-lineage lymphoma) is the cellular homolog of v-Cbl, the oncoprotein in the CAS NS-1 retrovirus (51, 52) that induces pre-B cell lymphomas and myelogenous leukemias in mice (53). p120 CBL is also known to be a substrate of tyrosine kinases in response to T cell (54) and B cell (55) activation, FC-γ receptor cross-linking (56, 57), and growth factors (33, 58–60). In mammalian cells, the function of p120 CBL is not known; however, it may act downstream of c-Src signaling for bone resorption by osteoclasts (61). Also, the p120 CBL homolog Sli-1 in Caenorhabditis elegans is a negative regulator of the epidermal growth factor receptor tyrosine kinase homologue Let-23 (62). The formation of a complex containing CRKL, p85 PI3K, and p120 CBL has been previously demonstrated by us in BCR/ABL-transformed cells (24). Although the function of the CRK family proteins is not known, a model by Feller et al., shows a hypothetical role for the tyrosine phosphorylation of...
CRK (31). In this model the N-terminal SH3 domain interacts with the proline-rich domain of c-ABL. Subsequent tyrosine phosphorylation of p85PI3K leads to dissociation of c-ABL from CRK and binding of the CRK SH2 domain to the tyrosine phosphorylated site in CRK. However, the data presented here and our previous studies in BCR/ABL-transformed cells do not suggest that CRK, unlike CRK, undergoes intramolecular binding to its own SH2 domain.

Overall, one model consistent with our data would be that a tyrosine phosphorylated c-Kit receptor first attracts the p85<sup>PI3K</sup> subunit through its SH2 domain to Tyr<sup>709</sup>. Some CRKL partially stabilizing the complex. Thereafter the complex may leave the receptor intact or disassembled, and it is not clear at this time if p120<sup>SHC</sup> and CRKL are regulators of PI3K enzymatic activity or downstream effectors that require PI3K activity for an as yet unknown signaling function. In any case, it is likely that further elucidation of the functions of this complex will be helpful in understanding the signaling of c-Kit and PI3K in particular.

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Steel Factor Induces Tyrosine Phosphorylation of CRKL and Binding of CRKL to a Complex Containing c-Kit, Phosphatidylinositol 3-Kinase, and p120 CBL

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