Integrins Regulate the Association and Phosphorylation of Paxillin by c-Abl*

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The c-Abl proto-oncogene is a non-receptor tyrosine kinase whose activity and localization are regulated by integrins. Cell adhesion to fibronectin triggers the transient recruitment of c-Abl from the nucleus to focal adhesions and activation of its tyrosine kinase. To investigate the integrin regulation of c-Abl, proteins that interact with c-Abl following cell adhesion were assayed. Several proteins that were phosphorylated on tyrosine were found to transiently co-precipitate with c-Abl during cell adhesion, and one was identified as the focal adhesion protein paxillin. Abl also became transiently phosphorylated in response to cell adhesion. In addition, paxillin was found to serve as substrate for the adhesion-activated c-Abl kinase. These results suggest that c-Abl may mediate effects of integrins on cell functions by phosphorylating paxillin.

Integrins mediate adhesion of cells to extracellular matrix proteins and to other cells. During adhesion, integrins form linkages to the actin cytoskeleton and regulate intracellular signaling pathways, thereby coordinating cell attachment to cell architecture and gene expression (1–4). Integrins themselves have no enzymatic activity, transmitting signals by recruiting a number of signaling and cytoskeletal proteins to sites of cell contact with the matrix known as focal adhesions (1, 3). Proteins recruited include α-actinin, talin, actin, focal adhesion kinase, PLC-γ, p130Cas, tensin, paxillin, and c-Abl. Cell adhesion also stimulates the phosphorylation on tyrosine of focal adhesion kinase, paxillin, tensin, and p130Cas and the association of focal adhesion kinase with phosphatidylinositol 3-kinase, Grb2, and Nck (5, 6). While numerous individual interactions have been demonstrated, their roles in integrin-mediated cell spreading, migration, growth, or differentiation are largely unknown.

Integrins are important regulators of gene expression and cell cycle progression. Little is known, however, about how integrin signals might be transmitted to the nucleus. One candidate protein that may mediate effects of cell adhesion on nuclear events is the Abl non-receptor tyrosine kinase. We recently showed that c-Abl kinase activity is regulated by integrin-mediated cell adhesion. Adhesion also triggers movement of c-Abl from the nucleus to focal adhesions, followed by translocation back to the nucleus (7). Thus, c-Abl most likely undergoes transient associations with one or more focal adhesion proteins.

In addition to its tyrosine kinase domain, Abl contains an SH2 domain, which can bind to phosphotyrosine residues, and an SH3 domain that binds proline-rich regions and is required for nuclear localization (8). Abl also contains binding sites for F- and G-actin (9). In the nucleus, Abl binds to DNA and to the transcription factor Elf-1 and phosphorylates RNA polymerase II (10–13). Abl also binds to the tumor suppressor retinoblastoma protein in a cell cycle-dependent fashion, suggesting a role for Abl in cell cycle events (14). Overexpression of wild-type c-Abl does not cause cell transformation (15, 16) but instead inhibits progression of cells from G1 into S phase (17), suggesting its role in the control of cell growth is inhibitory.

In contrast to c-Abl, the oncogenic variant Bcr-Abl resides primarily in the cytoplasm. Bcr-Abl kinase is constitutively activated and is not regulated by cell adhesion (8, 15, 16). Thus, Bcr-Abl might be considered a c-Abl variant that is permanently “on” and uncoupled from any integrin signals.

Bcr-Abl stably interacts with a number of signaling proteins, in particular paxillin, Crk, Grb2, Nck, Chl, and mitogen-activated protein kinase-activated protein (18–22). There is some evidence that c-Abl can also interact in vitro with some of these same cytoplasmic proteins through its SH3 domains (23). However, the interactions of c-Abl with cytoplasmic proteins in vivo have been elusive, primarily because the regulation of c-Abl was poorly understood. Thus, the finding that integrins induce c-Abl activation and translocation to the cytoplasm enabled us to investigate cytoplasmic interactions of c-Abl in vivo.

In the present study, we sought to identify proteins that interact with the c-Abl tyrosine kinase during its brief transit to focal adhesions. We show that the focal adhesion protein paxillin transiently interacts with c-Abl following cell attachment to fibronectin and that paxillin can serve as substrate for the adhesion-activated c-Abl kinase.

MATERIALS AND METHODS

Cells and Cell Lines—Abl−/− mouse fibroblasts, either untransfected or stably transfected with hemagglutinin (HA)-tagged c-Abl, Flag-tagged c-Abl, or a kinase-inactive mutant of c-Abl (His−/−) were kindly provided by Dr. Jean Wang (Department of Biology, University of California at San Diego, La Jolla, CA). These cells and mouse NIH 3T3 fibroblasts were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum; mouse 10T1/2 fibroblasts were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Life Technologies, Inc.). Antibodies and Reagents—Fibronectin was prepared from human plasma by affinity chromatography on gelatin-Sepharose (24). Monoclonal anti-phosphotyrosine antibody was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Rabbit anti-c-Abl antibody was purchased from Santa Cruz Biologicals (Santa Cruz, CA), and the

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1 The abbreviations used are: SH2, Src homology 2; SH3, Src homology 3; HA, hemagglutinin; GST, glutathione S-transferase; FN, fibronectin; CTD, C-terminal domain; GST-CTD, GST C-terminal domain of RNA polymerase II.
mouse anti-c-Abl antibody 8E9 was provided by Dr. Jean Wang (Department of Biology, University of California at San Diego, La Jolla, CA). Monoclonal anti-paxillin antibody was purchased from Zymed (San Francisco, CA), anti-FLAG M2 antibody was from Eastman Kodak Co. (N. Haven, CT), and anti-phosphotyrosine antibody 4G10 was from Upstate Biotechnology Inc. (Lake Placid, NY). Antibodies directed against HA were affinity-purified from 12CA5 ascites (generously provided by Dr. Mark Ginsberg, Scripps Research Institute), using a PYD-DVPDYAC peptide-coupled thiold-Sepharose 4B column. GST-paxillin was purified from a plasmid that was a generous gift from Dr. James Griffin (Division of Hematologic Malignancies, Dana Farber Cancer Institute, Boston, MA). GST-terminal domain of RNA polymerase II (GST-CTD) was a gift from Dr. Mark Ginsberg (Scripps Research Institute). All other chemicals, unless otherwise noted, were obtained from Sigma.

Preparation of Cell Lysates—Cells were detached with trypsin, washed in Dulbecco's modified Eagle's medium containing 0.1% nuclease- and protease-free bovine serum albumin (DB) with 250 μg/ml soybean trypsin inhibitor and either allowed to attach in DB to plates coated with fibronectin (FN) or maintained in suspension on bovine serum albumin-coated plates, as described previously (25). Cells were incubated at 37 °C. Cells were harvested at 20 and 60 min for attached cells by first placing all dishes on ice and then washing each three times with ice-cold phosphate-buffered saline. Suspended cells were harvested at 30 min by first placing the ice and then collecting and washing cells by centrifugation. Each 15-cm plate of attached cells was lysed with 0.5 ml of Nonidet P-40 buffer containing 1% Nonidet P-40, 50 mM Tris-HCl, pH 7.4, 10 mM NaF, 2 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of aprotinin and leupeptin. Cell material was scraped off of the plates and extracted for 30 min on ice. Samples were centrifuged for 30 min at 14,000 × g at 4 °C, and the supernatants were removed. Protein concentrations were determined using the Pierce BCA assay.

For samples lysed in SDS, 1% SDS was substituted for Nonidet P-40 buffer and boiled for 5 min. Incorporation of phosphotyrosine into proteins was assessed by Western blotting. Panel A, 4G10 anti-phosphotyrosine antibody. Panel B, blots were stripped and reprobed with anti-paxillin.

remove unincorporated [γ-32P]ATP. Incorporation of 32P into proteins was visualized by autoradiography.

For nonradioactive tyrosine kinase assays using GST-paxillin, reactions were initiated by the addition of 10 μM unlabeled ATP. Following incubation for 30 min at room temperature, reactions were put on ice. Soluble GST-paxillin and c-Abl bound to beads were separated by centrifugation for 1 min at 3,000 rpm. Samples were boiled in SDS buffer for 5 min. Incorporation of phosphotyrosine into proteins was assessed by blotting with anti-phosphotyrosine antibodies and visualized with chemiluminescence.

RESULTS

Adhesion Induces Co-precipitation of Proteins with c-Abl—We previously reported that adhesion of cells to FN or anti-integrin antibody induced a transient translocation of c-Abl to focal adhesions (7). To investigate possible protein interactions of c-Abl during this process, we assessed the co-precipitation of c-Abl with other proteins that occurred transiently during cell adhesion. Cells expressing an HA-tagged c-Abl were lysed and immunoprecipitated with anti-HA. Because c-Abl is a tyrosine kinase, the presence of phosphotyrosine-containing proteins in the precipitates was assessed. Fig. 1A demonstrates that the association of c-Abl with phosphotyrosine-containing proteins was low in suspended cells but increased greatly when cells were plated for 20 min on FN, the time at which c-Abl localizes maximally to focal adhesions (7). Several protein bands were observed, with one of the most prominent species migrating at 70–80 kDa. These bands were decreased by 60 min, by which time c-Abl is again predominately nuclear (7). Thus, either the phosphorylation or association of several proteins with c-Abl correlates with its presence in focal adhesions.

To identify the prominent 70–80-kDa bands, the blot was stripped and reprobed with an antibody specific for paxillin (Fig. 1B). Paxillin is a focal adhesion protein with a reported molecular mass of 68–81 kDa, depending on its phosphorylation state (26, 27). Fig. 1B shows that the doublet at 70–80 kDa soluble GST-paxillin and c-Abl bound to beads was separated by centrifugation for 1 min at 3,000 rpm. Samples were boiled in SDS buffer for 5 min. Incorporation of phosphotyrosine into proteins was assessed by blotting with anti-phosphotyrosine antibodies and visualized with chemiluminescence.

C-Abl and Paxillin Co-Immunoprecipitated Transiently during Cell Spreading—To further investigate whether c-Abl and
paxillin interact transiently during cell adhesion and spreading on FN, FLAG-tagged c-Abl was immunoprecipitated from lysates of stably transfected mouse Abl−/− fibroblasts (Fig. 2A). Equivalent amounts of c-Abl were precipitated from each lysate. When immunoblots were analyzed using the antibody specific for paxillin, it was again found to co-precipitate with c-Abl and to show a maximum at 20 min. The fraction of total paxillin in the cell associating with c-Abl was found to be significant; comparison of paxillin in lysates of total cell lysates with that in immunoprecipitates indicated that 5–10% of the total paxillin was associated with c-Abl (not shown).

The reciprocal experiment was also performed (Fig. 2B). When paxillin was precipitated from lysates of mouse NIH 3T3 fibroblasts, bands that were recognized by anti-c-Abl co-precipitated at 20 min after plating on FN, with less at 60 min, and little detectable co-precipitation from suspended cells. Thus, endogenous c-Abl was also associated with paxillin, and the adhesion-dependent co-precipitation of paxillin with c-Abl could be achieved using at least four different antibodies (anti-HA, anti-FLAG, and anti-paxillin as well as an antibody specific for c-Abl (not shown)). In addition, the co-precipitation of paxillin with c-Abl occurred using multiple cell types, including reconstituted Abl−/− fibroblasts, normal NIH 3T3 fibroblasts, and mouse 10T fibroblasts (not shown).

As an additional control, lysates were prepared from unreconstituted Abl−/− cells. These cells do not express c-Abl, but they contain paxillin in amounts similar to that of the Abl+/− cells reconstituted with wild-type c-Abl (Fig. 3A, bottom). Anti-paxillin antibodies did not precipitate bands co-migrating with c-Abl for these cells (Fig. 3A, top), and c-Abl immunoprecipitates did not contain paxillin (Fig. 3B, top). The co-precipitation of paxillin with c-Abl therefore appears to be specific.

**Co-precipitation of Paxillin with c-Abl Is Disrupted by Treatment with 1% SDS**—As a further control, the effect of denaturing conditions was tested (Fig. 3B). Cells plated on FN were lysed in the presence of 1% SDS, followed by dilution with the standard Nonidet P-40 buffer to less than 0.2% SDS. Whereas c-Abl was efficiently immunoprecipitated from the SDS lysates (Fig. 3B, bottom), paxillin did not co-precipitate with c-Abl (Fig. 3B, top). Thus, the interaction between paxillin and c-Abl is

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**Fig. 2.** Adhesion induces co-immunoprecipitation of paxillin and c-Abl. Abl−/− cells stably transfected with FLAG-tagged c-Abl (panel A) or 3T3 fibroblasts (panel B) were detached and then plated on FN for 0, 20, or 60 min. Panel A, lysates were immunoprecipitated (IP) with anti-FLAG, and immune complexes were analyzed by Western blotting. Blots were developed with an antibody specific for paxillin (top), or with antibody 8E9 against c-Abl (bottom). Panel B, an antibody specific for paxillin was used for immunoprecipitation, and immune complexes were analyzed by Western blotting. Blots were developed with an antibody 8E9 against c-Abl (top). Blots were then stripped of antibodies and reprobed with an antibody specific for paxillin (bottom). 3T3 lysate, 20 μg of whole cell lysate from stably adherent 3T3 fibroblasts.

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**Fig. 3.** Panel A, immunoprecipitation (IP) of paxillin in Abl−/− cells. Abl−/− cells, or cells stably transfected with FLAG-tagged c-Abl (Abl+/+) were detached and plated on FN. At 0, 20, or 60 min, cells were lysed and immunoprecipitated with anti-paxillin, and immunoprecipitates were analyzed by Western blotting. Blots were probed with anti-Abl (8E9) (top). 3T3 lysate, 20 μg of whole cell lysate from stably adherent 3T3 cells. Blots were then stripped and reprobed with anti-paxillin (bottom). Panel B, co-precipitation of paxillin with c-Abl is disrupted by SDS treatment. Abl−/− cells or cells stably transfected with FLAG-tagged c-Abl (Abl+/+) were detached and plated on FN. At 20 min, cells were lysed in buffer containing either 1% Nonidet P-40 (Nonidet P-40) or 1% SDS followed by dilution with the Nonidet P-40 buffer to 0.2% SDS (SDS). An antibody specific for FLAG was used for immunoprecipitation, and immune complexes or total cell lysates (lysate) were separated by SDS-polyacrylamide gel electrophoresis and immunoblotted. Top, blots were developed with an antibody against paxillin. Bottom, as a control for immunoprecipitations, total cell lysates (pre IP) were compared with immunodepleted lysates (post IP) by blotting with antibody 8E9 against c-Abl. The results show that c-Abl was completely removed from the lysates in both cases.
were analyzed by Western blotting with antibody 8E9. min at 34 °C, either with or without added type I phosphatase. Samples of c-Abl are transiently induced by cell adhesion. By contrast, phosphorylated forms of c-Abl at 130 kDa (not shown). Thus, slower migrating species in the 20-min FN sample that were absent from suspended cells. The slowest migrating bands (Fig. 4A) are present in total cell lysates. Fig. 4A shows that bands of c-Abl from whole cell lysates exhibited one or more slowly migrating species in the 20-min FN sample that were absent from suspended cells. Stably adherent cells also show only a single band of c-Abl at 130 kDa (not shown). Thus, slower migrating species of c-Abl are transiently induced by cell adhesion. By contrast, bands of c-Abl from cells expressing a kinase-inactive c-Abl mutant exhibited only the 130-kDa species throughout the time course of plating on FN (Fig. 4A). This result indicates that the formation of lower mobility species depends on c-Abl tyrosine kinase activity.

To investigate the possibility that the slower migrating proteins are phosphorylated forms of c-Abl, immunoprecipitates of c-Abl from cells plated on FN for 20 min were washed to remove the phosphatase inhibitors and treated with acid phosphatase. As shown in Fig. 4B, phosphatase treatment completely removed the slower migrating bands, which appeared to merge with the 130-kDa c-Abl band. Therefore, the extra bands appear after plating cells on FN represent phosphorylated forms of c-Abl. We also observed that removal of the phosphatase inhibitors was sufficient to cause the disappearance of the slowest migrating bands (Fig. 4C), suggesting that c-Abl might co-precipitate endogenous protein phosphatases, but nonspecific associations cannot be excluded.

In Vitro Phosphorylation of Proteins Co-precipitating with c-Abl Tyrosine Kinase—To investigate whether any of the co-precipitating proteins might be substrates of c-Abl, an in vitro kinase reaction was carried out in the immunoprecipitates (Fig. 5A). Interestingly, a doublet co-migrating with paxillin was a prominent band in cells plated on FN for 25 min; this band was nearly absent in suspended cells and was diminished at 120 min. C-Abl autophosphorylation followed a similar pattern over the time course. Additional protein substrates for the adhesion-activated c-Abl kinase were evident, but their identity is not yet known. The increased intensity of phosphorylated bands in the precipitates from cells plated on FN suggests adhesion-dependent phosphorylation of substrate proteins. However, whether this increase is due to increased c-Abl kinase activity, increased levels of substrate proteins, or both cannot be determined from these data.

Adhesion-activated c-Abl Kinase Phosphorylates Exogenous GST-Paxillin—These results suggested that paxillin might be a substrate for c-Abl. To test this hypothesis, the ability of c-Abl to phosphorylate exogenous, purified GST-paxillin was assessed (Fig. 5B). The addition of GST-paxillin to immunoprecipitated c-Abl correlated with the appearance of a labeled protein at 97 kDa, which co-migrated with GST-paxillin. No kinase activity toward paxillin was precipitated from Abl−/− cells (Fig. 5C). In addition, when GST-paxillin was added to immunoprecipitated kinase-inactive c-Abl, no tyrosine kinase activity was observed (Fig. 5D). In this experiment, nonradioactive ATP was used in the kinase reaction, and phosphorylation was assayed by Western blotting with anti-phosphotyrosine antibody, confirming that phosphorylation was on tyrosine. Taken together, these results demonstrate that paxillin can serve as a substrate for the adhesion-activated c-Abl tyrosine kinase.

Phosphorylation of the cytoplasmic protein paxillin was compared with the nuclear substrate, RNA polymerase II C-terminal domain. GST-CTD, which migrates slightly below 97 kDa, was used in these assays. As observed previously, phosphorylation of GST-CTD by c-Abl was adhesion-dependent (Fig. 6) (7), with a 2.6-fold increase after 20 min on FN. Phosphorylation of GST-paxillin was also adhesion-dependent, with a 9-fold increase in c-Abl kinase activity after 20 min on FN. However, although 2-fold more paxillin than CTD was added to the c-Abl kinase, the incorporation of 32P label into paxillin was less efficient than for CTD. This result most likely reflects the number of tyrosines phosphorylated on each protein; paxillin has three known tyrosine phosphorylation consensus sequence sites, whereas CTD has 52 sites, with up to 30 sites phosphorylated by c-Abl in vitro (12, 28). The results therefore show that paxillin is a good substrate for c-Abl and that c-Abl kinase activity toward paxillin is strongly adhesion-dependent.

We also considered the possibility that the interaction with paxillin could activate c-Abl when cells are plated on FN. To test this hypothesis, c-Abl immunoprecipitated from suspended cells was mixed with CTD plus a trace amount (10%) of paxillin; the ability of c-Abl to phosphorylate CTD was then assessed. Interestingly, while the addition of trace CTD to paxillin had little effect on paxillin phosphorylation (1.2-fold more 32P incorporated), the addition of trace paxillin to CTD reduced the extent of CTD phosphorylation by 50%. Thus, paxillin does not activate c-Abl. Rather, paxillin and CTD appear to compete noncovalent and is not an artifact of antibody cross-reactivity.

Adhesion Induces Slower Migrating, Phosphorylated Forms of c-Abl—During the course of the immunoprecipitation experiments, we noticed that c-Abl blots of immunoprecipitates from cells plated on FN contained one or two more slowly migrating bands, in addition to the expected 130-kDa band. Therefore, the extra bands appear after plating cells on FN represent phosphorylated forms of c-Abl. We also observed that removal of the phosphatase inhibitors was sufficient to cause the disappearance of the slowest migrating bands (Fig. 4C), suggesting that c-Abl might co-precipitate endogenous protein phosphatases, but nonspecific associations cannot be excluded.
were performed either with or without the addition of 7.5 m M FLAG. Immune complexes were split, and kinase reactions were carried out with the immune complexes, which were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Panel A, kinase reactions in c-Abl immunoprecipitates. Panel B, immunoprecipitates from the 25-min FN lysates were split in half, and kinase reactions were performed either with or without the addition of 7.5 m M of GST-paxillin. Panel C, Abl+/− cells and Abl−/− cells transfected with FLAG-tagged c-Abl (WT) were plated on FN for 25 min. Samples were immunoprecipitated with anti-FLAG, and kinase reactions were carried out with 7.5 m M of GST-paxillin. Panel D, Abl+/− cells transfected with c-Abl (WT) or a kinase-defective form of c-Abl (KD) were plated on FN for 20 min. Samples were immunoprecipitated, and kinase reactions were carried out using nonradioactive ATP, with (+) or without (−) 7.5 m M of GST-paxillin. The soluble GST-paxillin was analyzed with antibody 4G10 to detect phosphotyrosine (top), and immunoprecipitated c-Abl was blotted with antibody 8E9 (bottom). WT, wild type.

for binding to the c-Abl kinase, and paxillin may have a higher affinity for c-Abl than does CTD.

**DISCUSSION**

Integrins regulate cell survival, proliferation, and differentiation, events that require transfer of information to the nucleus regarding the attachment state of the cell. We previously found that plating cells on FN triggers exit of Abl from the nucleus, its transient localization to focal contacts, and a peak in the activity of its tyrosine kinase that correlated with its presence in focal contacts. These findings suggest that c-Abl may provide one pathway through which integrins may regulate nuclear events (7). To further explore the regulation of c-Abl by adhesion, we used an immunoprecipitation strategy to examine the proteins that interact with c-Abl after plating cells on FN. Our results showed that adhesion induces co-precipitation of c-Abl with a number of proteins that are phosphorylated on tyrosine and identified one of these as paxillin. The association with paxillin occurred on the same time scale as the previously observed residence of c-Abl in focal adhesions and the transient increase in its kinase activity. Furthermore, we found that paxillin is a good substrate for the c-Abl kinase and that kinase activity toward paxillin is adhesion-dependent.

During the course of these experiments, we also observed that c-Abl became transiently phosphorylated during cell adhesion. This phosphorylation appeared to depend on its tyrosine kinase activity, indicating either that it was due to auto-phosphorylation or that c-Abl kinase activity is required for recruitment of other protein kinases. Abl kinase activity and phosphorylation do not appear to be required for the interaction with paxillin, since kinase-defective c-Abl also co-precipitated with paxillin (data not shown). Kinase activity and thus, by inference, phosphorylation are also not required for the transient localization to focal adhesions (7). We also observed that removal of phosphatase inhibitors from the precipitates resulted in significant dephosphorylation of c-Abl. This result raises the possibility that Abl may also co-immunoprecipitate with a phosphatase, suggesting that phosphorylation of c-Abl during cell adhesion may be a regulated event; however, the specificity of this association has not been established.

Paxillin is localized predominantly to focal adhesions and was originally identified as a major substrate for the v-Src tyrosine kinase (26). While the function of paxillin is not yet known, it can bind to vinculin, focal adhesion kinase, talin, Crk, c-Src, and Bcr-Abl (28–31). Paxillin contains a proline-rich region, which provides a potential binding site for SH3-containing proteins such as c-Abl. There are also three sites for tyrosine phosphorylation in paxillin that can mediate binding to the SH2 regions of other proteins, as has been shown to occur in vitro for v-Crk or CrkL (30). During cell adhesion, paxillin becomes phosphorylated on both tyrosine and serine residues;
recent evidence suggests that protein kinase C may be the serine kinase acting on paxillin during cell adhesion (32). Identification of tyrosine kinases that modify paxillin during cell adhesion has proven more complicated. In vitro, paxillin can be phosphorylated on tyrosine by focal adhesion kinase to create binding sites for the SH2 regions of Src and v-Crk (33, 34). However, paxillin can become phosphorylated on tyrosine in vivo during cell adhesion in the absence of focal adhesion kinase (35), indicating that in vivo there is at least one other kinase for paxillin. Our data raise the intriguing possibility that c-Abl may fill this role.

The association of c-Abl with paxillin could in principle fulfill several functions. Paxillin might serve as a docking site to localize c-Abl to focal adhesions; it could function as an upstream regulator that activates c-Abl; or it could be a substrate that functions downstream of the tyrosine kinase. Our data showed that paxillin is in fact a substrate for c-Abl, suggesting that it may mediate some effects of c-Abl on downstream cytoplasmic signaling pathways. Abl kinase activity toward recombinant paxillin is strongly adhesion-dependent, indicating that paxillin is not sufficient for activation; thus, a role for paxillin as an upstream activator of c-Abl seems unlikely.

The ability of integrins to regulate c-Abl kinase activity and localization may be important for proper regulation of cell growth and differentiation. This hypothesis gains strength when viewed in the context of Bcr-Abl, whose activity and localization are not regulated by integrins. Bcr-Abl is an oncogenic variant of c-Abl that has a constitutively active tyrosine kinase and that resides exclusively in the cytoplasm. When expressed in 3T3 cells, Bcr-Abl induces anchorage-independent growth without altering cells’ requirements for serum. Thus, Bcr-Abl constitutively activates an integrin-dependent growth regulatory pathway without affecting growth factor signaling pathways.

In this light, our current data regarding c-Abl support the general concept that oncogenes constitutively activate pathways normally regulated by their cognate proto-oncogenes. Cells expressing Bcr-Abl have constitutively elevated levels of phosphotyrosine on several focal adhesion proteins, most notably paxillin. Bcr-Abl also has been shown to directly phosphorylate paxillin (28). Thus, Bcr-Abl stably associates with and phosphorylates paxillin, whereas c-Abl does so in a regulated manner. Our data therefore provide molecular evidence that Bcr-Abl functions like c-Abl but constitutively transmits signals normally controlled by cell adhesion.

To summarize, our results lead to three major conclusions. First, plating cells on FN induces the association of c-Abl with the focal adhesion protein paxillin on a time scale correlating with both activation and localization of c-Abl to focal adhesions. Second, paxillin appears to be a substrate for c-Abl and may therefore mediate the effects of c-Abl on cytoplasmic signaling pathways. Third, these results suggest that Bcr-Abl represents an activated form of c-Abl that continuously stimulates pathways that are normally integrin-regulated. Further analysis of the integrin-regulated functions of c-Abl may therefore prove useful for understanding both normal integrin signaling and transformation by Bcr-Abl.

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