Integrin-linked Kinase (ILK) Binding to Paxillin LD1 Motif Regulates ILK Localization to Focal Adhesions*

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Paxillin is a focal adhesion adapter protein involved in integrin signaling. Paxillin LD motifs bind several focal adhesion proteins including the focal adhesion kinase, vinculin, the Arf-GTPase-activating protein paxillin-kinase linker, and the newly identified actin-binding protein actopaxin. Microsequencing of peptides derived from a 50-kDa paxillin LD1 motif-binding protein revealed 100% identity with integrin-linked kinase (ILK)-1, a serine/threonine kinase that has been implicated in integrin, growth factor, and Wnt signaling pathways. Cloning of ILK from rat smooth muscle cells generated a cDNA that exhibited 99.6% identity at the amino acid level with human ILK-1. A monoclonal antibody raised against a region of the carboxyl terminus of ILK, which is identical in rat and human ILK-1 protein, recognized a 50-kDa protein in all cultured cells and tissues examined. Binding experiments showed that ILK binds directly to the paxillin LD1 motif in vitro. Co-immunoprecipitation from fibroblasts confirmed that the association between paxillin and ILK occurs in vivo in both adherent cells and cells in suspension. Immunofluorescence microscopy of fibroblasts demonstrated that endogenous ILK as well as transfected green fluorescent protein-ILK co-localizes with paxillin in focal adhesions. Analysis of the deduced amino acid sequence of ILK identified a paxillin-binding subdomain in the carboxyl terminus of ILK. In contrast to wild-type ILK, paxillin-binding subdomain mutants of ILK were unable to bind to the paxillin LD1 motif in vitro and failed to localize to focal adhesions. Thus, paxillin binding is necessary for efficient focal adhesion targeting of ILK and may therefore impact the role of ILK in integrin-mediated signal transduction events.

The interaction of cells with the extracellular matrix regulates many physiological and pathological processes. Such interactions are accompanied by the recruitment of multiple cytoskeletal and regulatory proteins to focal adhesions involved in coordinating integrin-mediated signal transduction associated with cell motility, gene expression, and cell proliferation (1–3). Paxillin is a multidomain protein that localizes to focal adhesions and functions as a cytoskeletal scaffold protein for many of these proteins (4–6). The carboxyl terminus of paxillin is comprised of four LIM domains that mediate paxillin targeting to focal adhesions (7–9) and supports the binding of PTTP (10) and tubulin (11). The amino terminus of paxillin contains five leucine-rich sequences (LDXLLXXL) named LD motifs, which are highly conserved between species and between other paxillin family members such as hic-5 and Dicyostelium discoideum PaxB (7, 9). Paxillin LD motifs exhibit differential binding to several molecules important in the regulation of actin cytoskeleton such as FAK, vinculin (5, 7, 12), the Arf-GTPase-activating protein paxillin-kinase linker (PKL) (12), and the newly identified actin-binding protein actopaxin (13).

The integrin-linked kinase (ILK) is a serine/threonine kinase that was originally identified in a yeast two-hybrid screen for β1 integrin cytoplasmic tail-binding proteins (14). ILK is comprised of three domains: the amino terminus, which contains four ankyrin repeats that have been shown to interact with the LIM-only adapter protein PINCH (15, 16); a central pleckstrin homology-like domain reported to bind to phosphatidylinositol 3,4,5-triphosphate (17); and the carboxyl terminus, which contains a protein kinase catalytic domain and the binding site for β1 integrin (14). ILK activity has been implicated in growth factor and Wnt signaling pathways (14, 17, 18), as well as in integrin-mediated cellular processes including regulation of cell adhesion (14), fibronectin matrix assembly (19), and cell adhesion-dependent cell cycle progression (20–22). A second human ILK isoform (ILK-2) that is selectively up-regulated in metastatic melanoma cells has recently been described (23).

In this study, we identify ILK as a 50-kDa paxillin LD1 motif-binding protein, and we have cloned the rat homologue of human ILK-1. We demonstrate that ILK associates directly with paxillin via an interaction with the paxillin LD1 motif and co-localizes with paxillin in focal adhesions. The rat homologue of ILK and the two human ILK isoforms contain a sequence within their carboxyl terminus with similarity to the paxillin-binding subdomain (PBS) sequences of other paxillin LD-binding proteins such as vinculin, FAK, and actopaxin (7, 13). ILK PBS mutants found to be defective for paxillin binding in vitro were unable to localize to focal adhesions, indicating that the paxillin-ILK interaction is required for efficient focal adhesion targeting of ILK. Taken together, these data suggest that ILK binding to paxillin may be an important factor in the regulation of ILK signaling function.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF329194.

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1 The abbreviations used are: aa, amino acid(s); ILK, integrin-linked kinase; GFP, green fluorescent protein; CHO, Chinese hamster ovary; PBS, paxillin-binding subdomain; FAK, focal adhesion kinase; GST, glutathione S-transferase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; PKL, paxillin-kinase linker.

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Experiment 1

**Antibodies**—Mouse monoclonal ILK antibody (clone 3) was generated in collaboration with Transduction Laboratories (Lexington, KY). Paxillin (clone 349) antibody was also from Transduction Laboratories. A goat polyclonal ILK antibody (clone C19) was obtained from Santa Cruz Biotechnology, α-actinin and α-tubulin antibodies were from Sigma; actin antibody (clone C4) was from Roche (Indianapolis, IN). Xpress monocalonal antibody was from Invitrogen (Carlsbad, CA), and GFP antibody was a generous gift of Dr. P. Silver (Dana Farber Cancer Institute, Boston, MA).

**Cell Culture and Transfection**—Rat smooth muscle cells, rat embryonic fibroblasts (REF-52), and COS-7 cells were maintained in Dulbecco’s Modified Eagle’s medium (Mediatech, Washington, D.C.) supplemented with 10% (v/v) fetal bovine serum (Atlanta Biologicals), 1 mM glutamine, and 50 units/ml penicillin/50 μg/ml streptomycin (Sigma). Chinese hamster ovary (CHO)-K1 cells were cultured in modified Ham’s F-12 medium (Mediatech) supplemented with 10% (v/v) heat-inactivated, certified fetal bovine serum and 50 units/ml penicillin/50 μg/ml streptomycin at 37°C in a humidified chamber with 5% CO2. Rat intestinal epithelial IEC-18 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% (v/v) fetal bovine serum, 4 mM t-glutamine, 0.1 unit/ml insulin, and 1% penicillin/streptomycin. LipofectAMINE (Life Technologies, Inc.)-mediated transfection of HeLa cells was performed as described elsewhere (7).

**Microsequencing and Cloning of p53^-Specific Paxillin GST-LD1-Binding Proteins**—p53^-specific smooth muscle tissue lysate were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane as described previously (12). Protein bands were visualized by Ponceau S staining and excised. Proteolytic digestion, high pressure liquid chromatography fractionation, and internal peptide sequencing was performed by Dr. John Leszzyk (Worcester Foundation for Biomedical Research, Worcester, MA). Peptide sequencing derived from a 50-kDa band revealed peptides with sequences identical to those of the human integrin-linked kinase-1 sequence. The full coding sequence for ILK (aa 1–452) was isolated by reverse transcription-polymerase chain reaction from rat smooth muscle cells using forward oligonucleotide primer 5’-CGCTTAGAATGGCCGATCCACCCAG-3’ and reverse oligonucleotide primer 5’-CGCTTAGATGACTCCATCCATTCCGG-3’. A nucleotide tail coding for the EcoRI restriction site (underlined nucleotides) was engineered at the 5’ end of each oligonucleotide to facilitate in-frame cloning into the EcoRI site of pcDNA3 or pcDNA3.HisC (Invitrogen) or pEGF-P-C2 (CLONTECH) vectors. All constructs were sequenced on both strands (BioResource Center, Cornell University, Ithaca, NY).

**Preparation of Fusion Proteins and Binding Assays**—Individual GST fusion proteins of paxillin LD1 (aa 1–20), LD2 (aa 141–160), LD3 (aa 212–231), LD4 (aa 263–282), and LD5 (aa 299–317) were expressed in Escherichia coli (DH5α) and purified on glutathione-agarose beads as described previously (12).

Several fusion proteins, particularly growing NIH-3T3 cells, were metabolically labeled with [35S]methionine (in vitro translation grade; ICN) were lysed in lysis binding buffer (10 mM Tris-HCl pH 7.6, 50 mM NaCl, 1% Nonidet P-40, and 10% glycerol) containing protease inhibitors (Complete(TM) EDTA-free; Roche Molecular Biochemicals), followed by centrifugation for 15 min at 15,000 × g. Lysates (0.5–1 mg of protein) were incubated for 2 h at 4°C with GST-paxillin LD proteins (50 μg) bound to glutathione-Sepharose 4B beads in fusion protein lysis/binding buffer in a total volume of 500 μl. After incubation, the beads were washed three times with the same buffer, and bound proteins were separated by 12% SDS-PAGE and analyzed by enhanced fluorography as described previously (12). In similar experiments, GST-paxillin LD fusion proteins were incubated with [35S]methionine-labeled ILK generated by in vitro translation into a cell-free reticulocyte lysate system (TNT; Promega, Madison, WI) following the instructions of the manufacturer. The reaction mixture containing the [35S]methionine-labeled ILK was clarified by centrifugation, and before incubation with GST-fusion protein proteins we’re incubated (1 h) in fusion protein binding buffer as described above. Binding proteins were analyzed on SDS-PAGE gels followed by enhanced fluorography using Amplify (Amerham Pharmacia Biotech).

**For in vivo binding studies** association of endogenous ILK with paxillin was demonstrated by immunoprecipitation of ILK from rat smooth muscle cells or REF-52 lysates prepared in lysis binding buffer with ILK antibody and protein A/G-Sepharose for 2 h at 4°C or by immunoprecipitation of paxillin from CHO-K1/chicken paxillin stable transfectants with the chicken-specific PAX1 antibody (7), fractionation of the immunoprecipitates by SDS-PAGE, transfer to nitrocellulose membranes, and probing of the membranes with paxillin or ILK antibody, respectively.

**RNA Isolation, Reverse Transcription-Polymerase Chain Reaction, and Mutagenesis**—Total RNA from rat smooth muscle cells was prepared using the Trizol reagent (Life Technologies, Inc.) according to the manufacturer’s protocol. cDNAs were prepared from total RNA using SuperScriptII (Life Technologies, Inc.). Reverse transcription-polymerase chain reaction was performed using Pfu polymerase (Stratagene, La Jolla, CA). For the generation of ILK-PBS mutants, we used the Quick-Change Mutagenesis kit (Stratagene) according to the instructions of the manufacturer. The presence of mutations was confirmed by sequencing.

**Immunofluorescence Microscopy**—Indirect immunofluorescence analysis was performed as described previously (7) with the modification that antibody dilutions were made in Western block buffer (20 mM Tris-Cl pH 7.6, 100 mM NaCl, 0.2% Tween 20, and 3% w/v bovine serum albumin). Photographs were taken on a Zeiss Axiohot photomicroscope equipped with epifluorescence illumination using Kodak Tmax 400 film or a digital camera (SPOT(TM); Diagnostic Instruments Inc, Sterling Heights, MI). Images were processed using Adobe Photoshop 3.0.5.

**RESULTS**

Identification of ILK as a 50-kDa Paxillin LD Motif-Binding Protein—The cytoskeletal adapter protein paxillin contains five LD motifs that function as discrete protein recognition domains originally identified as vinculin- and FAK-binding sites (7). Subsequent studies, in which GST-paxillin LD motif fusion proteins were utilized in precipitation binding experiments, identified the LD motif-binding proteins PKL and actopaxin (12, 13). In addition, a prominent 50-kDa LD1 motif-binding protein was reported (denoted by an arrow in Fig. 1). To identify this LD1-binding protein, GST-LD1 fusion protein and GST control were incubated with smooth muscle tissue lysate. The bound proteins were subjected to SDS-PAGE, transferred to polyvinylidene difluoride membrane, and stained with Ponceau S. The GST-LD1 motif-bound 50-kDa protein band was excised, digested, and subjected to microsequencing as described previously (12). Two peptide sequences (GDDTPHLAASHGHR and EVPFADLSNMEIGMK) were obtained (Fig. 2B). Surprisingly, both peptides exhibited 100% identity with ILK-1, a protein previously described as having a molecular mass of 59 kDa (14).

Cloning of ILK from Rat Smooth Muscle Cells and Identification of a PBS in the Carboxyl Terminus of ILK—The ILK...
cDNA containing the entire open reading frame for this protein was cloned by reverse transcription-polymerase chain reaction from rat smooth muscle cells as described under “Experimental Procedures.” Nucleotide sequence analysis of the ILK cDNA from rat smooth muscle cells revealed a deduced amino acid sequence of 452 aa with a predicted molecular mass of 51.4 kDa. Comparison between the deduced amino acid sequences of rat and human ILK-1 (14) revealed 99.6% identity, diverging by only 2 amino acids at positions 197 (A/T) and 259 (A/S) (Fig. 2B). An alignment of the amino acid sequences of rat ILK, mouse ILK, human ILK-1, and ILK-2 is presented in Fig. 2B for comparison. The nucleotide and deduced amino acid sequences for rat ILK have been deposited in the GenBank™/European Molecular Biology Laboratory/DDBJ data bases under accession number AF329194.

Consistent with a model in which paxillin LD motifs interact with their binding partners through conserved sequences known as PBSs (8, 12, 24, 25), we have identified a PBS sequence within the carboxyl-terminal kinase domain of ILK (Fig. 2). Sequence alignment of the ILK PBS sequence with those of vinculin, FAK, and actopaxin revealed conservation among all three proteins (Fig. 2C). The ILK PBS (aa 377–396), which is invariant in all ILK isoforms, displays 33% identity with the actopaxin PBS (aa 273–290), 20% identity with the FAK PBS (aa 919–938), and 15% identity with the vinculin PBS (aa 951–970) (Fig. 2D). Val928/Leu931 of FAK and Val282/...
Leu285 of actopaxin have previously been shown to be essential for binding to paxillin (13, 25). The corresponding amino acids of ILK, Val386 and Thr387, were both mutated to glycine and used in the experiments described in Fig. 7.

Protein Expression of ILK—In an effort to resolve the discrepancy in the molecular mass for the p50ILK band observed in the paxillin LD1 motif binding experiments and the reported p59ILK protein, we generated a monoclonal antibody, in collaboration with Transduction Laboratories, raised against a GST fusion protein of the carboxyl terminus of ILK (aa 326–453). This sequence is 100% conserved between rat ILK and human ILK-1. Western blotting of total lysates from various cell lines and rat tissues indicated that the ILK monoclonal antibody specifically recognizes a band with an apparent molecular mass of 50 kDa in all cell lines and tissues examined without any evidence of a 59-kDa band (Fig. 3). To further confirm the relative molecular mass of ILK, the blots were reprobed sequentially with α-tubulin (recognizing a 58-kDa band) and actin (recognizing a 42-kDa band) antibodies (Fig. 3). Identical results were obtained with another commercially available ILK antibody (clone C19; Santa Cruz Biotechnology; data not shown). Finally, in vitro transcription translation of the rat ILK cDNA using a reticulocyte lysate system produced a 50-kDa peptide (see Fig. 5C) that was recognized by both ILK antibodies (data not shown).

ILK Subcellular Localization—To evaluate the subcellular distribution of ILK, rat smooth muscle, REF-52, and HeLa cells were processed for immunofluorescence microscopy using either the mouse monoclonal ILK antibody (clone 3) from Transduction Laboratories (Fig. 4) or the goat polyclonal ILK antibody (clone C19; Santa Cruz Biotechnology; data not shown). In all cell lines mentioned above, as well as in CHO-K1 and NIH-3T3 cells (data not shown), ILK demonstrated robust focal adhesion staining at the end of actin stress fibers (Fig. 4, A, B, E, and F) and co-localized with paxillin (Fig. 4, C and D).

To confirm the subcellular distribution of ILK, the rat ILK cDNA was subcloned in-frame with GFP as described under “Experimental Procedures.” Transfection of the GFP-ILK construct into HeLa cells, followed by replating of the transfectants on fibronectin-coated slips, revealed that GFP-ILK is efficiently targeted to focal adhesions (Fig. 4, G and H). Identical results were obtained with Xpress-tagged ILK (data not shown).
ILK binds directly to the Paxillin LD1 Motif in Vitro—Previously identified paxillin LD-binding proteins bind either a single LD motif (e.g. PKL) or multiple LD motifs (e.g. vinculin, FAK, and actopaxin) (12, 13). To determine whether ILK binds directly and exclusively to the LD1 motif, each paxillin LD motif was synthesized as a GST fusion protein (Fig. 5A) and incubated with 35S-labeled rat ILK prepared by coupled in vitro transcription/translation. Strong binding was observed only with the paxillin LD1 motif (Fig. 5B), consistent with the precipitation data presented in Fig. 1.

Paxillin Binds ILK in Vivo in Both Adherent and Suspension Cells—To evaluate the interaction between ILK and paxillin in vivo, co-immunoprecipitation experiments were performed using either REF-52 or rat smooth muscle lysates. Total lysates of these cell lines were incubated with ILK antibody or with control IgG. Immunoprecipitates were resolved on Western blots and probed sequentially with paxillin and α-actinin antibodies. Paxillin was efficiently co-immunoprecipitated with ILK antibody from lysates of adherent cells, whereas α-actinin failed to bind (Fig. 6A). The same results were obtained from lysates of cells maintained in suspension (Fig. 6A), indicating that the paxillin-ILK association is constitutive rather than adhesion-induced.

Additionally, ILK was co-precipitated in paxillin immunoprecipitates (Fig. 6B). For these experiments, CHO-K1 cells stably transfected with chicken paxillin cDNA (8) were lysed in co-immunoprecipitation buffer. Chicken paxillin was immunoprecipitated with a polyclonal chicken-specific PAX1 antibody (7), and the immunoprecipitates were prepared for Western blot analysis and probed with ILK and α-actinin antibodies. ILK was efficiently co-immunoprecipitated with the PAX1 antibody, whereas α-actinin was not (Fig. 6B), confirming the in vivo association of paxillin and ILK.

PBS Mutants of ILK Are Incapable of Paxillin Binding and of Localizing to Focal Adhesions—To investigate the importance of the paxillin-ILK association in the localization of ILK to focal adhesions, we generated ILK mutants carrying either a deletion of a portion of the PBS (aa 384–390) (ILKΔPBS) or a double point mutation of V386G/T387G within the PBS (ILK PBSpoint mut). Residues Val1229/Leu1230 of FAK and Val1229/Leu1230 of actopaxin (Fig. 2C) have previously been shown to be essential for binding to paxillin (25, 13). To test the ability of these ILK PBS mutants to bind paxillin LD1 motif, they were generated in vitro by coupled transcription translation as [35S]methionine-labeled proteins and used in binding reactions with GST or the paxillin GST-LD1 motif. Results showed that in contrast to wild-type ILK, which bound strongly to GST-LD1, neither of the ILK PBS mutants were able to bind to the paxillin LD1 motif, thus confirming the paxillin-binding site on ILK (Fig. 7A).

Analysis of the ability of either of the ILK PBS mutants defective for paxillin-binding to localize to focal adhesions was evaluated by transfection of GFP-ILK, GFP-ILKΔPBS, and GFP-ILK V386G/T387G (GFP-ILK PBSpoint mut) into HeLa cells. Western blotting analysis of total lysates from all transfectants probed with the ILK antibody confirmed expression and the correct size of the GFP-ILK mutants (Fig. 7B). Immunofluorescence analysis of the transfectants showed that, in contrast to GFP-ILK which localized efficiently to focal adhesions, GFP-ILKΔPBS and GFP-ILK PBSpoint mut were unable to localize to focal adhesions (Fig. 7C). In contrast, the localization of paxillin to focal adhesions was unaffected by ectopic expression of the GFP-ILK mutants (Fig. 7D). These results indicate that the paxillin-ILK interaction is a requirement for efficient focal adhesion targeting of ILK.

DISCUSSION

We have previously determined that individual paxillin LD motifs function as independent and selective binding interfaces for focal adhesion proteins including vinculin, the focal adhesion tyrosine kinase FAK, PKL, and actopaxin (12, 13). Such interactions are consistent with a role for paxillin as a molecular adapter protein involved in integrin-mediated cytoskeletal organization and intracellular signaling (6). In the present study, we have identified a 50-kDa paxillin LD1-binding protein as the ILK.

To further explore the observed ILK-paxillin association, an ILK cDNA was cloned from rat smooth muscle cells. This cDNA exhibited 99.6% identity at the amino acid level with the human ILK-1 (14) and ILK-2 genes (23). A similar high level of homology is exhibited by ILK genes cloned from mouse (26) and guinea pig (GenBank™ accession number AF256520). ILK has previously been reported as a 59-kDa protein (14, 19). Although the rat ILK isoform identified in this report as a 50-kDa protein represents the rat homologue of human ILK, its
predicted molecular mass of 51.4 kDa is identical to the predicted molecular mass of either human ILK-1 or ILK-2. A monoclonal antibody raised against a sequence in the carboxyl terminus of ILK that is 100% conserved between human ILK-1 and rat ILK, recognized a single 50-kDa band on Western blots of all tissues and cells examined. Thus, whereas it has been suggested that the expression of different ILK isoforms may be developmentally and spatially restricted (23, 27), the reason for the discrepancy in the reported size for ILK is unclear at this time.

Although ILK was originally identified as an integrin β subunit cytoplasmic domain-associated protein (14), its ability to co-localize with integrins in focal adhesions has only recently been demonstrated (16). In this report, we confirm the robust localization of endogenous ILK to focal adhesions, using the monoclonal antibody raised against the carboxyl terminus of ILK. Expression of epitope-tagged rat ILK cDNA in either HeLa or CHO-K1 cells confirmed that exogenously expressed ILK demonstrated a similarly restricted distribution.

The mechanism by which ILK is localized to focal adhesions appears to be quite complex. Sequence analysis of ILK demonstrated the presence of a carboxyl-terminal paxillin-binding PBS domain with homology to the PBS domains of FAK, vinculin, and actopaxin (7, 13, 25). Mutation of this region not only confirmed its function in mediating paxillin binding but also revealed that a functional interaction between ILK and paxillin is required for the localization of ILK to focal adhesions. However, another protein contributing to ILK localization to focal adhesions is the LIM-containing adapter protein PINCH (15). Elimination of the PINCH binding site within the amino-terminal ankyrin repeat of ILK also inhibits ILK targeting to focal adhesions (16). The mechanism by which PINCH localizes to focal adhesions is currently unknown. Finally, the integrin cytoplasmic domains represent a third potential docking site for ILK. A detailed analysis of a role for integrin binding in ILK subcellular localization has not been performed, although expression of an ILK mutant comprising the first 230 amino acids and thus containing the PINCH binding site but not the carboxyl integrin and paxillin binding sites failed to target to focal adhesions (16). Thus multiple interactions utilizing regions within both the amino terminus and the carboxyl terminus of ILK likely contribute to its stable localization to focal adhesions.

Both paxillin and ILK have been implicated as important

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**Fig. 7.** PBS mutants of ILK are unable to bind paxillin or to localize to focal adhesions. A, ILK, ILK ΔPBS, and ILK V386G/T387G (ILK PBS point mut) mutants were labeled with [35S]methionine by coupled *in vitro* transcription/translation. Labeled proteins were allowed to bind with GST or LD1 paxillin fusion proteins. After washing, binding proteins were resolved by SDS-PAGE and visualized by fluorography. In contrast to ILK, neither the ILK ΔPBS nor the ILK PBS point mutants were able to bind paxillin LD1 motif. B, GFP-ILK and GFP-ILK ΔPBS and GFP-ILK V386G/T387G (GFP-ILK PBS point mut) mutants were transfected into HeLa cells. 24 h after transfection, transfectants were trypsinized, and total lysates were prepared. 30 µg of each lysate was subjected to SDS-PAGE, followed by Western blotting with ILK antibody. Lane 1, GFP-ILK transfectants; lane 2, HeLa control untransfected cells; lane 3, GFP-ILK ΔPBS; lane 4, GFP-ILK PBS point mut. C, cells from the same transfections described in B were replated on fibronectin-coated slips. 20 h after plating, transfectants were fixed and processed for immunofluorescence microscopy. In contrast to GFP-ILK, which was enriched at focal adhesions, neither of the GFP-ILK mutants was able to localize to focal adhesions. Instead, they exhibited a diffuse cytoplasmic and perinuclear distribution. Rhodamine phalloidin was used to visualize actin stress fibers. D, double staining of the same transfectants described in C with paxillin antibody showed that paxillin localization to focal adhesions was unaffected by ectopic expression of either GFP-ILK or the GFP-ILK PBS mutants. Bar, 5 µm.
downstream effectors of integrin activation, displaying roles in the regulation of cell adhesion, cell motility, and gene expression (6, 21, 28). The requirement for paxillin binding in the recruitment of ILK to focal adhesions, coupled with the close physical proximity of the paxillin and integrin binding sites on ILK, may have important implications in the efficient transduction of extracellular cues via these proteins. In this regard, it is noteworthy that several additional regulatory proteins critical to integrin function, including the tyrosine kinases FAK and Src, bind to regions within the amino terminus of paxillin adjacent to the ILK binding site (6). This may not only facilitate cross-talk between these proteins but may also facilitate the activation of shared downstream targets such as AKT/PKB, a component of the cell survival pathway that is activated in both an ILK- and FAK-dependent manner (3, 17).

Finally, ILK has also been implicated in the regulation of the Wnt/β-catenin signaling pathway in epithelial cells (18) and therefore in the control of gene expression and mesenchymal morphogenesis. A potential role for the paxillin-ILK interaction in these events is suggested by the co-precipitation from epithelial cells of a protein complex containing ILK, paxillin, and dishevelled, a cytoplasmic signaling component of the Wnt/β-catenin pathway (29). Furthermore, dishevelled, like paxillin and ILK, was found to localize to focal adhesions in these cells.

Delineating the precise role for the paxillin interaction in mediating the ability of ILK to regulate cell survival, transformation, and differentiation represents an important area for future study.

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