Requirement of Phosphatidylinositol 3-Kinase in Focal Adhesion Kinase-promoted Cell Migration*

(Received for publication, October 14, 1998, and in revised form, January 20, 1999)

Heinz R. Reiske‡§, Shu-Chen Kao¶,**, Leslie A. Cary‡, Jun-Lin Guan‡§, Jui-Fen Lai¶,** and Hong-Chen Chen¶**‡‡

From the ‡Department of Molecular Medicine, Cornell University, Ithaca, New York 14853 and the **Department of Zoology and the ‡‡Institute of Biochemistry, National Chung Hsing University, Taichung 402, Taiwan, Republic of China

We have previously shown that overexpression of focal adhesion kinase (FAK) in Chinese hamster ovary (CHO) cells promoted their migration on fibronectin. This effect was dependent on the phosphorylation of FAK at Tyr-397. This residue was known to serve as a binding site for both Src and phosphatidylinositol 3-kinase (PI3K), implying that either one or both are required for FAK to promote cell migration. In this study, we have examined the role of PI3K in FAK-promoted cell migration. We have demonstrated that the PI3K inhibitors, wortmannin and LY294002, were able to inhibit FAK-promoted migration in a dose-dependent manner. Furthermore, a FAK mutant capable of binding Src but not PI3K was generated by a substitution of Asp residue 395 with Ala. When overexpressed in CHO cells, this differential binding mutant failed to promote cell migration although its association with Src was retained. Together, these results strongly suggest that PI3K binding is required for FAK to promote cell migration and that the binding of Src and p130Cas to FAK may not be sufficient for this event.

Focal adhesion kinase (FAK)\(^\ast\) is a key component of integrin-mediated signal transduction (1–3). This 125-kDa nonreceptor protein-tyrosine kinase (PTK) is localized to focal contacts in fibroblasts and represents the prototype of a distinct family of nonreceptor PTKs (4, 5). It rapidly becomes phosphorylated following cell adhesion to extracellular matrix (ECM) proteins or integrin clustering by antibodies (6–9). In addition to its role in integrin signaling, FAK has also been suggested to be a point of convergence of signaling by other extracellular stimuli (10). The ability of FAK to transmit signals to downstream targets is dependent on its ability to interact with several intracellular signaling molecules including Src family kinases (11, 12), phosphatidylinositol 3-kinase (PI3K; Ref. 13), Grb2 (14), and p130Cas (15). Tyr-397 has been identified as the major binding site for both Src and phosphatidylinositol 3-kinase (PI3K; Ref. 13), Grb2 (14), and signaling molecules including Src family kinases (11, 12), phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (25). These lipid products are believed to act as second messengers in a variety of signaling processes including cell survival (26, 27) and migration (28–30). We have shown that PI3K associates with FAK in response to cell adhesion (13) or platelet-derived growth factor (PDGF) stimulation (31) in NIH 3T3 cells. The significance of this interaction in cellular processes is unknown.

In this report, we have found that a FAK mutant capable of binding to Src, but not to PI3K, failed to stimulate cell migration and that inhibition of PI3K decreases cell migration. Taken together, these results strongly implicate a role for PI3K in the facilitation of FAK-promoted cell migration and suggest that the binding of Src and p130Cas to FAK may not be sufficient for this process.

EXPERIMENTAL PROCEDURES

Antibodies—The mouse mAb KT3 (21) and the mouse mAb 12CA5 (anti-HA) (17), which recognize an epitope (KPPTPPPEPET) of the SV40 large T antigen and an epitope (YPYDVPDYA) of the hemagglutinin (HA) protein of the influenza virus, respectively, have been described previously. The rabbit polyclonal anti-FAK (11) and anti-p85 (17) sera have also been described previously. The anti-Src mAb 327 was purchased from Calbiochem (San Diego, CA). The anti-Src mAb 2–17 was purchased from Quality Biotech (Camber, NJ). The rabbit polyclonal anti-p130Cas (C-20) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The anti-phosphotyrosine (anti-PY) mAb PY20 was purchased from Transduction Laboratories (Lexington, KY).

Construction of Expression Plasmids—The pKH3 plasmids encoding HA epitope-tagged FAK and Y397F mutants were described previously (17). The cDNA encoding other FAK mutants (D395A, D396A, D395/D396, P712/T715A) used in this study were generated using site-directed mutagenesis by overlap extension using the polymerase chain reaction. The polymerase chain reaction products containing the desired mutations were treated with restriction enzymes KpnI and NdeI and then digested with restriction enzyme EcoRI.

\(*\) This research was supported by National Science Council, Taiwan, Grant NSC88-2311-B-005-027 (to H.-C. C.) and grants from the National Institutes of Health (to J.-L. G). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ These authors contributed equally to this work.

‡‡ Is an established investigator of the American Heart Association.

¶ To whom correspondence should be addressed. Tel.: 886-4-2854922; Fax: 886-4-2851797; E-mail: hcchen@nchu.edu.tw.

1 The abbreviations used are: FAK, focal adhesion kinase; PTK, protein-tyrosine kinase; ECM, extracellular matrix; PI3K, phosphatidylinositol 3-kinase; SH, Src-homology; CHO, Chinese hamster ovary; PDGF, platelet-derived growth factor; HA, hemagglutinin; HEK, human embryonic kidney; anti-PY, anti-phosphotyrosine; WT, wild-type; GST, glutathione S-transferase; mAb, monoclonal antibody.
used to replace the corresponding fragments in the plasmid pGEX-FAK. The desired mutations were confirmed by dideoxy DNA sequencing. The cDNAs encoding FAK mutants in pGEX2T vectors were in-frame and transferred to pKH3 vectors using the BamHI and EcoRI sites. The pKH3 plasmids encoding FAK and its mutants were used to transiently express FAK proteins in human embryonic kidney (HEK) 293 cells.

The expression plasmid pCDM8-FAK and pCDM8-FAK P712/715A have been described previously (21, 22), pKH3-FAK D395A was digested with MscI and NheI to generate a 1.8-kilobase fragment containing the D395A substitution. This was then subcloned into pCDM8-FAK from which the corresponding MscI-NheI fragment had been excised. The resulting plasmid was designated pCDM8-FAK D395A and used to transfect CHO cells.

The cDNA encoding the p85 subunit of PI3K that was kindly provided by Dr. L. C. Cantley (Harvard University) was inserted into pKH3 at the BamHI and EcoRI sites. The pEVX plasmid encoding c-Src was kindly provided by Dr. D. Shalloway (Cornell University).

Cell Culture and Transfections—HEK 293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Life Technologies, Inc.). One day after plating 5 × 10⁶ cells on 60-mm dishes, HEK 293 cells were transiently transfected with 1 μg of pKH3 plasmids encoding FAK or its mutants with 10 μl of LipofectAMINE (Life Technologies, Inc.). In some cases, HEK 293 cells were co-transfected with pKH3-FAK or its mutants, pKH3- p85, and pEVX-Src. Two days after transfection, the cells were lysed in 1% Nonidet P-40 lysis buffer containing protease inhibitors as described previously (8).

CHO cells expressing wild type (WT) FAK, Y397F, and P712/715A have been described previously (21, 22) and were maintained in F-12 medium with 10% fetal bovine serum and 0.5 mg/ml G418 (Life Technologies, Inc.). To generate cells stably expressing the D395A FAK mutant, CHO cells were grown on 10-cm dishes and transfected essentially as described (21) using LipofectAMINE following the manufacturer instructions. Clones were selected in G418-containing medium and screened by immunoblotting using anti-FAK and KT3.

In Vitro Binding Assays—The plasmids pGEX-Src.SH2 and pGEX-p85.NSH2 were described previously (17). The cDNA encoding the SH3 domain of p130 Cas (22) was cloned into pGEX2T at the BamHI and EcoRI sites to generate pGEX-Cas.SH3. GST fusion proteins were immobilized on glutathione-agarose beads and then incubated with HEK 293 cell lysates (∼10 μg) containing various HA epitope-tagged FAK proteins in 1% Nonidet P-40 lysis buffer for 1 h at 4 °C. The complexes were washed four times with 1% Nonidet P-40 lysis buffer, resolved by SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting with anti-HA.

Immunoprecipitations, Immunoblotting, and in Vitro Kinase Assays—Attached cells were lysed in Nonidet P-40 buffer containing protease inhibitors. To detect the association of FAK with Src and p85 in HEK 293 cells, lysates (50 μg) from cells co-transfected with pKH3-FAK, pKH3-p85, and pEVX-Src were incubated with anti-Src (327) or anti-p85. The co-immunoprecipitation of transfected WT FAK or mutants with endogenous c-Src in CHO cells was carried out as described previously using the anti-Src (2–17) (21). To determine the extent of p130 Cas phosphorylation in CHO cells overexpressing WT FAK or mutants, lysates (800 μg) were incubated with anti-p130 Cas as described (22). The immunocomplexes were washed, resolved by SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting with anti-HA (1:1,000), KT3 (1:3,000), anti-FAK (1:3,000), anti-Src (327) (1:500), anti-p130 Cas (1:1,000), or anti-PY (1:1,000) using the Amer- sham Pharmacia Biotech chemiluminescence system for detection. In some experiments, equal amounts of lysates were analyzed directly by immunoblotting.

To measure the tyrosine phosphorylation and in vitro autophosphorylation of ectopically expressed FAK proteins, HA epitope-tagged FAK proteins were immunoprecipitated with anti-HA from HEK 293 cell lysates (50 μg) and subjected to immunoblotting with anti-PY or to an in vitro kinase assay as described earlier (8).

To measure the PI3K activity associated with ectopically expressed FAK proteins in CHO cells, epitope-tagged FAK proteins were immunoprecipitated with KT3 from CHO cell lysates (750 μg) and subjected to an in vitro PI3K assay as described previously (13).

Cell Migration Assays—CHO cell migration assays in 48-well chemotaxis chambers were carried out as described previously (21). For experiments with the PI3K inhibitors wortmannin and LY294002 (Sigma) (reconstituted in Me2SO and stored at −20 °C), cells were harvested and washed as described earlier and then resuspended in F-12 medium containing the indicated concentrations of inhibitor or Me2SO such that the final concentration of Me2SO was constant. The cells were pretreated with the inhibitor at 37 °C and 5% CO2 for 30 min before loading them onto the chemotaxis chamber. They were allowed to migrate on 12 μg/ml fibronectin for 6 h in the presence of the inhibitor and were then fixed and stained as described previously. For all cell migration assays, migrated cells were enumerated under a light microscope at ×200 magnification using the Image-Pro Plus software, Version 3.0.

RESULTS

It is known that PI3K plays an important role in growth factor-induced cell migration (28–30). To examine the putative role of PI3K in FAK-promoted cell migration, CHO cells overexpressing WT FAK and control cells (Neo) were subjected to cell migration assays in the presence of specific PI3K inhibitors (Fig. 1). Treatment of WT cells with either PI3K inhibitor resulted in an inhibition of cell migration in a dose-dependent manner. Furthermore, in the presence of either 100 nM wortmannin or 75 μM LY294002, the majority (∼80%) of cell migration promoted by FAK overexpression in WT cells was inhibited, with little effect on control cell migration. These results
requirement of PI3K in FAK-promoted Cell Migration

FIG. 2. A mutation in Asp-395 of FAK abolishes its binding to p85 but not Src. A, the lysates were prepared from HEK 293 cells that had been transfected with pKH3 plasmids encoding HA epitope-tagged FAK or its mutants as indicated. An aliquot of cell lysates was analyzed by immunoblotting with anti-HA to verify a similar expression of epitope-tagged FAK proteins in all samples (input). The lysates were incubated with immobilized GST-Src.SH2 or GST-p85.NSH2. After washing, the bound proteins were resolved by SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-HA. B, an equal amount of lysates from HEK 293 cells that had been co-transfected with expression plasmids encoding HA epitope-tagged FAK or its mutants, c-Src, and p85 was incubated with anti-Src (297) or anti-p85. The immunocomplexes were washed and analyzed by immunoblotting with anti-HA.

demonstrate that PI3K plays a role downstream of FAK in promoting CHO cell migration on fibronectin. However, from these results, it was not clear whether FAK-promoted cell migration is dependent on PI3K, either through direct binding at Tyr-397 of FAK or through another FAK-promoted signaling pathway.

To determine whether the direct binding of PI3K to FAK is a prerequisite for its function in FAK-promoted cell migration, a FAK mutant deficient only in PI3K binding was generated by a substitution of Asp-395 with Ala (see below). Songyang et al. (32) suggested that residues both N- and C-terminal to the phosphotyrosine may contribute to the specific binding of the SH2 domain to its cognate phosphotyrosine and showed that the optimal binding sequence for the p85 N-terminal SH2 domain is EEDpYVEM. An examination of FAK sequences revealed that sequences flanking Tyr-397 (TDDpYAEI) conformed well with the binding motif for the p85 N-terminal SH2 domain. Based on these observations, two Asp residues upstream of Tyr-397 were mutated either singly or in combination to generate a PI3K binding-defective FAK mutant. These FAK mutants were transiently expressed in HEK 293 cells, and their ability to bind the SH2 domains of Src and p85 was examined in vitro (Fig. 2A). Similar to the mutation in Tyr-397, the mutation converting Asp-396 (or in combination with Asp-395) to Ala abolished FAK binding to the SH2 domains of Src and p85. Interestingly, a mutation in Asp-395 inhibited FAK binding to the SH2 domain of p85 but not Src. To examine if the FAK D395A mutant also selectively binds to Src but not p85 in intact cells, HEK 293 cells were transiently co-transfected with expression plasmids encoding HA epitope-tagged FAK or its mutants, c-Src, and p85. Two days after transfection, cells were lysed, and the association of FAK with Src or p85 was detected by co-immunoprecipitation (Fig. 2B). Consistent with the results from in vitro binding experiments, the mutation in Asp-396 abolished FAK association with both Src and p85, and importantly, the mutation in Asp-395 only prevented FAK association with p85 but not Src.

To examine the effects of FAK mutations in Asp-395 and Asp-396 on its tyrosine phosphorylation and in vitro autophosphorylation, HA epitope-tagged WT FAK or its mutants were transiently expressed in HEK 293 cells, immunoprecipitated with anti-HA, and subjected to immunoblotting with anti-PY or to an in vitro kinase assay (Fig. 3A). The tyrosine phosphorylation and in vitro autophosphorylation of FAK mutants containing a substitution of Asp-396 with Ala were as low as those of the Y397F mutant, indicating that Asp-396 is essential for the phosphorylation event upon Tyr-397. This also reflects the inability of the FAK D395A mutant to bind the SH2 domains of Src and p85. In contrast, the tyrosine phosphorylation and in vitro autophosphorylation of FAK D395A mutant remained comparable with the WT. These results indicated that the inability of the D395A mutant to bind PI3K was most likely not because of an overall conformational change. To exclude the possibility that the mutation in Asp-395 causes a subtle change in protein conformation and thereby affects the binding of FAK to other proteins, the capacity of FAK D395A to bind to the SH2 domain of p130Cas was not affected by mutating Asp-395. Together, these data suggest that the inability of FAK D395A to bind p85 is indeed because of the preference of the p85 SH2 domain for an aspartic acid at residue 395 in FAK.

To determine whether PI3K binding is required for FAK-
promoted cell migration, stable CHO cell lines overexpressing FAK D395A were established. The expression of FAK D395A was examined by immunoblotting with anti-FAK or KT3 (Fig. 4A). The expression levels of ectopic WT FAK and D395A mutant in CHO cells were comparable and severalfold higher than that of endogenous FAK. To examine the association of endogenous PI3K with WT and mutant FAK in CHO cells, ectopically expressed FAK proteins were immunoprecipitated by mAb KT3, and the immunocomplexes were subjected to an in vitro PI3K activity assay (Fig. 4B). Approximately 10% of total cellular PI3K activity were found to associate with ectopic WT FAK (data not shown). As expected, the levels of PI3K activity associated with WT FAK and P712/715A mutant were similar. Surprisingly, approximately 15% of PI3K activity that WT FAKs had were co-precipitated with Y397F and D395A mutants. The cell migration assays indicated a marked increase (~2.5-fold) in cell migration for WT cells compared with both Y397F and control (Neo) cells, which were similar in their rates of migration. Interestingly, both D395A clones failed to promote cell migration (Fig. 4C). These results together suggest that the residual binding of PI3K to FAK Y397F or D395A mutant in CHO cells is not sufficient for promoting migration. Moreover, adhesion assays indicated that all clones had similar adhesive strength to fibronectin (data not shown), indicating that the decrease in migration observed in D395A clones was not because of an alteration in cell adhesion.

We have previously demonstrated that phosphorylation of Tyr-397 in FAK is required for binding the SH2 domains of Src and PI3K (17). Thus, it is unclear whether the inability of FAK Y397F to promote cell migration is because of its inability to bind Src and/or PI3K. To clarify this, the association of FAK D395A with c-Src in CHO cells was examined. Both WT FAK and FAK D395A but not FAK Y397F co-precipitated with c-Src in CHO cells (Fig. 5A). We have previously demonstrated that the association of p130Cas with FAK and FAK-promoted phosphorylation of p130Cas by Src are critical for FAK-promoted migration (22). We examined the phosphorylation state of p130Cas in FAK D395A cells as an indicator of a FAK/Src-depended cell migration pathway as well as an indirect indicator of FAK/p130Cas association. Consistent with prior experiments (22), WT FAK exhibited a promotion of p130Cas phosphorylation compared with the FAK P712/715A (Fig. 5B), which is unable to bind p130Cas or promote p130Cas phosphorylation (22). FAK D395A was able to promote p130Cas phosphorylation at an estimated 2-fold level above WT FAK, indicating that the FAK/Src specific pathway is still functioning in the D395A mutant. Taken together, these results strongly suggest that PI3K binding to FAK is required for FAK to promote cell migration and that FAK:Src association alone may be insufficient for this cellular function.

**DISCUSSION**

In this report, we examined the role of PI3K in FAK-promoted cell migration. First we showed that two PI3K inhibitors, wortmannin and LY294002, were able to inhibit FAK-promoted migration in a dose-dependent manner. Our results are consistent with other work showing the importance of PI3K in increased cell motility stimulated by growth factors such as PDGF and hepatocyte growth factor (28–30). However, these data do not indicate whether PI3K association with FAK is required for increased cell migration and cannot rule out the involvement of PI3K in a FAK-independent migration pathway. To address this, we next established stable cell lines overexpressing FAK and a mutant defective in PI3K binding. Using this system, we showed that the PI3K binding-deficient mutant failed to promote cell migration toward fibronectin. These results strongly suggest that FAK:PI3K association is required for FAK-promoted cell migration.

We have previously identified that the autophosphorylation site Tyr-397 is the primary site for PI3K binding (17) and is
that thresholds of signals transmitted independently through the associations of p130Cas and PI3K with FAK have to be reached for enhanced cell migration. Lack of either one will not turn on an "intracellular machinery" for the induction of cell migration. Another possible interpretation is that the inability of FAK D395A to promote cell migration is because of a defect in p130Cas binding. This defect may result from a subtle conformational change by the D395A substitution, or a mutually dependent binding of PI3K and p130Cas to FAK. The latter possibility was excluded by our observation that the D395A mutant retained its ability to bind the SH3 domain of p130Cas 

in vitro

and promote p130Cas phosphorylation 

in vivo.

The tyrosine phosphorylation of p130Cas and its subsequent association with the adapter protein Crk have been shown to play an important role in promoting cell migration (34). In addition, the tyrosine phosphorylation of p130Cas has also been shown to be a result of binding to FAK (22). Here, we have shown that expression of FAK D395A increased p130Cas tyrosine phosphorylation at a roughly 2-fold level above WT FAK in CHO cells. Although the reason for this elevation in p130Cas phosphorylation is unclear, this result supports our conclusion that p130Cas binding and phosphorylation is necessary but not sufficient for FAK-promoted cell migration. Furthermore, several studies have shown that Src is important for phosphorylation of p130Cas (35, 36). Because FAK D395A still binds to Src, our results also suggest that FAK:Src association alone is not sufficient for the enhancement of cell migration. However, we cannot exclude the necessity of Src in FAK-promoted cell migration because of its function, at least, in phosphorylating p130Cas. Therefore, it is likely that FAK-promoted cell migration depends on the coordinate regulation of a number of these signaling events involving PI3K, p130Cas, and Src.

We have previously shown that FAK:PI3K association is enhanced by cell adhesion to ECM proteins (13) or PDGF stimulation (31). In this report, we have shown that this association is likely to be involved in cell migration upon cell adhesion to ECM proteins. However, it is not clear if this interaction is also involved in PDGF-stimulated cell migration. Furthermore, we do not know if FAK generally participates in growth factor-induced cell motility using a mechanism similar to that in ECM-protein-induced cell migration. It is known that the tyrosine phosphorylation of FAK is stimulated by a number of growth factors (31, 37). Although the mechanisms by which growth factor receptors and integrins regulate FAK phosphorylation may be different (38), it is possible that phosphorylated FAK recruits the same set of intracellular signaling molecules to facilitate cell migration. Answers to these questions will be interesting, and experiments are now in progress to test these possibilities.

Acknowledgments—We thank Dr. L. C. Cantley for the cDNA encoding the p85 subunit of PI3K and Dr. D. Shalloway for the expression vector encoding c-Src. We also thank Dr. D. C. Han for the construction of pGEX-Cas.SH3 and P.-C. Chan for the critical reading of this manuscript.

REFERENCES

1. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Biol. 11, 549–589
2. Juliano, R. L., and Haskell, S. (1993) J. Cell Biol. 120, 577–585
3. Guan, J.-L., and Chen, H.-C. (1996) Int. Rev. Cytol. 168, 81–108
4. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B., and Parsons, J. T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5182–5186
5. Hanks, S. K., Calabia, M. B., Harper, M. C., and Patel, S. K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8487–8489
6. Guan, J. L., Trewithick, L. E., and Hyynes, R. O. (1991) Cell Regul. 2, 951–964
7. Burridge, K., Turner, C. E., and Romer, L. H. (1992) J. Cell Biol. 119, 893–903
8. Guan, J.-L., and Shalloway, D. (1992) Nature 358, 690–692
9. Kornberg, L. J., Earp, H. S., Parsons, J. T., Schaller, M., and Juliano, R. L. (1992) J. Biol. Chem. 267, 23439–23442
10. Zachary, I., and Rozengurt, E. (1992) Cell 71, 891–894
11. Xing, Z., Chen, H.-C., Nowlen, J. K., Taylor, S., Shalloway, D., and Guan, J.-L. (1994) Mol. Biol. Cell 5, 413–421
Requirement of PI3K in FAK-promoted Cell Migration

12. Cobb, B. S., Schaller, M. D., Leu, T.-H., and Parsons, J. T. (1994) *Mol. Cell. Biol.* **14**, 147–155
13. Chen, H.-C., and Guan, J.-L. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 10148–10152
14. Schlaepfer, D. D., Hanks, S. K., Hunter, T., and van der Geer, P. (1994) *Nature* **372**, 786–791
15. Polte, T. R., and Hanks, S. K. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 10678–10682
16. Schaller, M. D., Hildebrand, J. D., Shannon, J. D., Fox, J. X., Vines, R. R., and Parsons, J. T. (1994) *Mol. Cell. Biol.* **14**, 1680–1688
17. Chen, H.-C., Appeddu, P. A., Isoda, H., and Guan, J.-L. (1996) *J. Biol. Chem.* **271**, 26329–26334
18. Schlaepfer, D. D., and Hunter, T. (1996) *Mol. Cell. Biol.* **16**, 5623–5633
19. Harte, M. T., Hildebrand, J. D., Burnham M. R., Bouton, A. H., and Parsons, J. T. (1996) *J. Biol. Chem.* **271**, 13649–13655
20. Ilc, D., Furuta, Y., Kanazawa, S., Takeda, N., Sekine, K., Nakataeji, N., Nomura, S., Fujimoto, J., Okada, M., Yamamoto, T., and Aizawa, S. (1995) *Nature* **377**, 539–543
21. Cary, L. A., Chang, J. F., and Guan, J.-L. (1995) *J. Cell Sci.* **109**, 1787–1794
22. Cary, L. A., Han, D. C., Polte, T. R., Hanks, S. K., and Guan, J.-L. (1996) *J. Cell Biol.* **140**, 211–221
23. Escobedo, J. A., Navankasattusas, S., Kavanaugh, W. M., Milfay, D., Fried, V. A., and Williams, L. T. (1991) *Cell* **65**, 75–82
24. Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ulrich, A., and Schlessinger, J. (1991) *Cell* **65**, 83–90