Current Biology, Vol. 13, 1442–1450, August 19, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00544-X

# A Novel Role for FAK as a Protease-Targeting Adaptor Protein: Regulation by p42 ERK and Src

**Neil O. Carragher,[1,*] M. Andrew Westhoff,[1] Valerie J. Fincham,[1] Michael D. Schaller,[3] and Margaret C. Frame[1,2,*]**
[1]The Beatson Institute for Cancer Research
Cancer Research United Kingdom
Beatson Laboratories
Glasgow G61 1BD
Scotland
United Kingdom
[2]Institute of Biological and Life Sciences
University of Glasgow
Glasgow G12 8QQ
Scotland
United Kingdom
[3]Department of Cell and Developmental Biology
Lineberger Comprehensive Cancer Center
Center for Thrombosis and Hemostasis
University of North Carolina
Chapel Hill, North Carolina 27599

## Summary

**Cell migration on extracellular matrix requires the turnover of integrin-dependent adhesions. The nonreceptor tyrosine kinases Src and FAK regulate focal-adhesion turnover by poorly understood mechanisms [1, 2, 3]. ERK/MAP kinase-mediated activation of the protease Calpain 2 also promotes focal-adhesion turnover [4, 5, 6]; however, it is not known if this is linked to the activities of Src and FAK. Calpain 2 has previously been demonstrated to colocalize with focal-adhesion structures [7] and can cleave several focal-adhesion complex components, including FAK [8–13]. Studies utilizing Calpain inhibitors or Calpain-deficient cells confirm that Calpain's role in regulating focal-adhesion turnover is necessary for cell migration [10, 11, 14]. We have identified a novel and kinase-independent function for FAK as an adaptor molecule that mediates the assembly of a complex consisting of at least Calpain 2 and p42ERK. Mutation of proline residues (Pro2) in the amino-terminal region of FAK blocks direct binding with Calpain 2 and also prevents formation of the Calpain 2/p42ERK complex in cells. We show that both complex formation and MEK/ERK activity are associated with Calpain-mediated proteolysis of FAK and focal adhesion turnover during transformation and migration. Furthermore, FAK is necessary for recruiting both Calpain 2 and p42ERK/MAPK to peripheral adhesion sites facilitating maximal Calpain activity.**

## Results and Discussion

### FAK and p42ERK/MAP Kinase Physically Complex with Calpain 2 in Src-Transformed Cells

We have previously demonstrated that activation of the v-Src oncoprotein induces Calpain-mediated proteolysis of FAK and focal-adhesion turnover [11, 15]. To probe the mechanism by which Src and FAK promote focal-adhesion turnover during transformation and migration, we examined whether there is a physical association between FAK and Calpain 2 in chick embryo fibroblasts. By immunoprecipitating exogenously expressed *myc*-tagged FAK and immunoblotting with anti-Calpain 2 as a probe, we found that FAK can associate with Calpain 2 (Figure 1A). In addition, we found that in cells expressing both exogenous wild-type FAK (FAK-wt) and a temperature-sensitive (*ts*) mutant of v-Src, there was coassociation of FAK and p42ERK with Calpain 2, and that the association was enhanced in v-Src-transformed cells (Figure 1B, lane 2). Although only visible in longer exposures, some FAK and p42ERK did coimmunoprecipitate with Calpain 2 even in nontransformed cells (not shown). In cells coexpressing a *myc*-tagged kinase-deficient FAK mutant (FAK-kd) with *ts* v-Src, we found that FAK-kd can readily associate with Calpain 2 and that assembly of the Calpain 2/FAK/p42ERK complex occurs to a similar extent as in FAK-wt cells (Figure 1B, lane 4 and densitometry data, Figure S1 in the supplemental data available with this article online). This data indicates that FAK's kinase activity is not required for the assembly of the Calpain 2/FAK/p42ERK complex.

To further address the determinants of assembly of the Calpain 2/FAK/p42ERK complex, we expressed a mutant FAK in which the amino-terminal proline-rich sequence at residues 368–378 was mutated (FAK-Pro2); alanine (*A*) was substituted for critical prolines (RAL*A*SI*A*) [16]. Expression of the FAK-Pro2 mutant inhibited coimmunoprecipitation of both FAK and p42ERK with Calpain 2 (Figure 1C, lane 4). Analysis of complex formation in cells expressing FAK-Pro2 was carried out in parallel with that of cells expressing FAK-wt (Figure 1C, lanes 1 and 2).

The absence of p42ERK from Calpain 2 immunoprecipitates in cells expressing FAK-Pro2 implies that the association of p42ERK with Calpain 2 requires FAK as an intermediate. This adaptor function of FAK is critical for formation of a protein complex containing the protease Calpain 2 and its upstream activator p42ERK. To demonstrate that Calpain 2 directly binds with FAK and that mutation of the Pro2 site abolishes this binding, we have performed far-Western blot analysis. In contrast to immunoprecipitated FAK-wt, recombinant Calpain 2 does not directly associate with immunoprecipitated FAK-Pro2 (Figure 2A, on left). Equal levels of immunopreciptated FAK-wt and FAK-Pro2 were separated by SDS-PAGE as determined by immunoblotting with anti-FAK (Figure 2A, on right). These data suggest that mutation of the Pro2 site on FAK abolishes its direct interaction with Calpain 2. In addition to exogenous *myc*-FAK, we also show that endogenous FAK and p42 ERK were present in anti-Calpain 2 immunoprecipitates (Figure 2B), demonstrating that formation of the complex does not require FAK overexpression such as we used in the FAK mutant analysis above.

*Correspondence: n.carragher@beatson.gla.ac.uk (N.O.C.); m.frame@beatson.gla.ac.uk (M.C.F.)

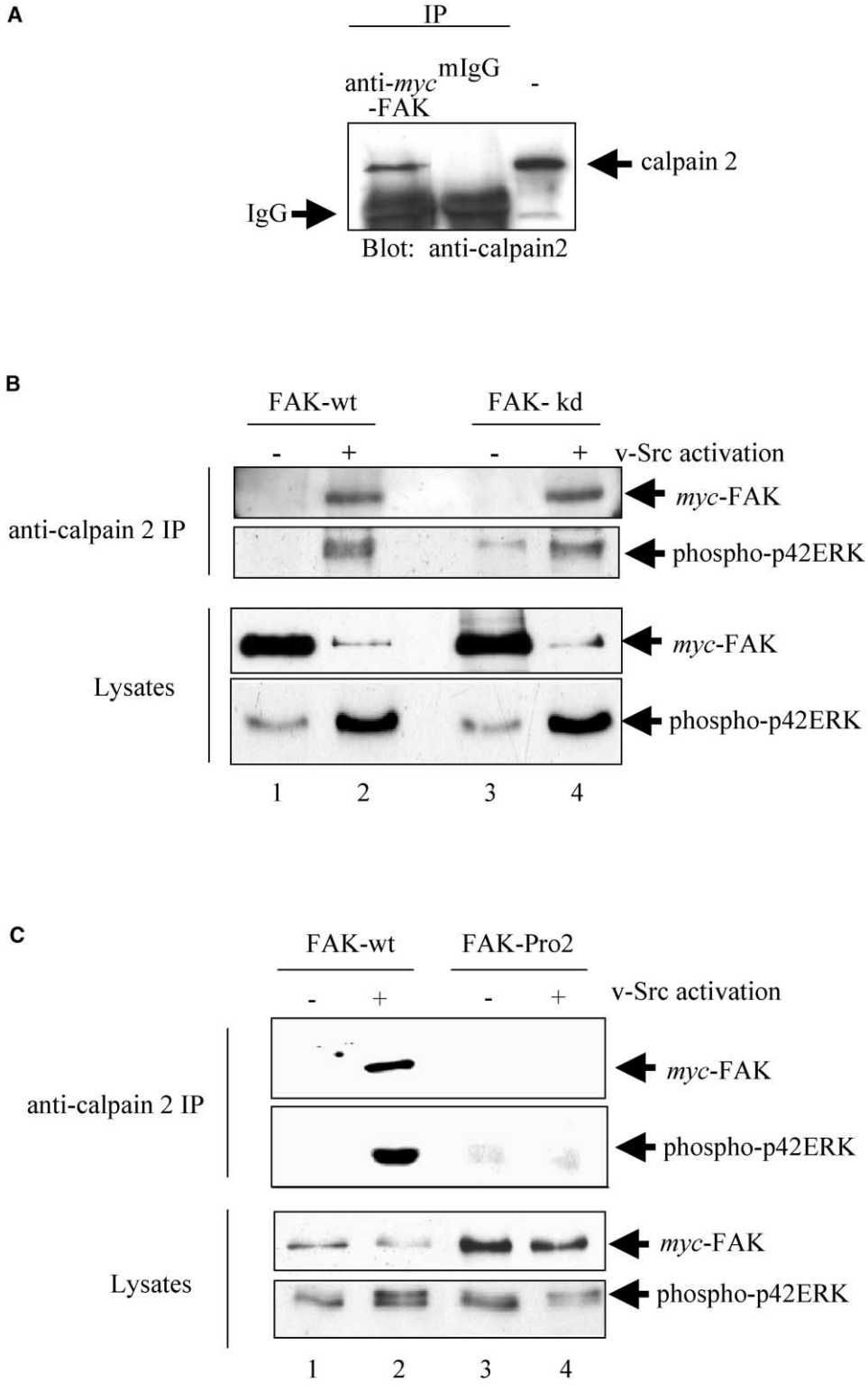


Figure 1. Calpain 2 Forms a Complex with FAK and Phospho-ERK

(A) *myc*-tagged wild-type FAK (FAK-wt) was immunoprecipitated (IP) from CEFs. Anti-*myc* IP's were separated by SDS-PAGE and immunoblotted with an antibody against Calpain 2. Control IP reactions were performed with nonspecific anti-mouse IgG (mIgG). The molecular weight of coimmunoprecipitating Calpain 2 was compared with purified Calpain 2 (-).

(B) Total cell lysates were prepared, and Calpain 2 was immunoprecipitated from CEFs coexpressing *ts LA*29 v-Src with either *myc*-tagged FAK-wt or kinase-defective FAK (FAK-kd) after culture at restrictive (-) and 24 hours at permissive (+) temperatures for v-Src activation. Calpain 2 association with exogenously expressed FAK and active p42ERK in these cells was detected by SDS-PAGE and immunoblotting with anti-phospho-p42ERK and -*myc*. Direct Western blots of the same total cell lysates used for immunoprecipitation experiments are shown

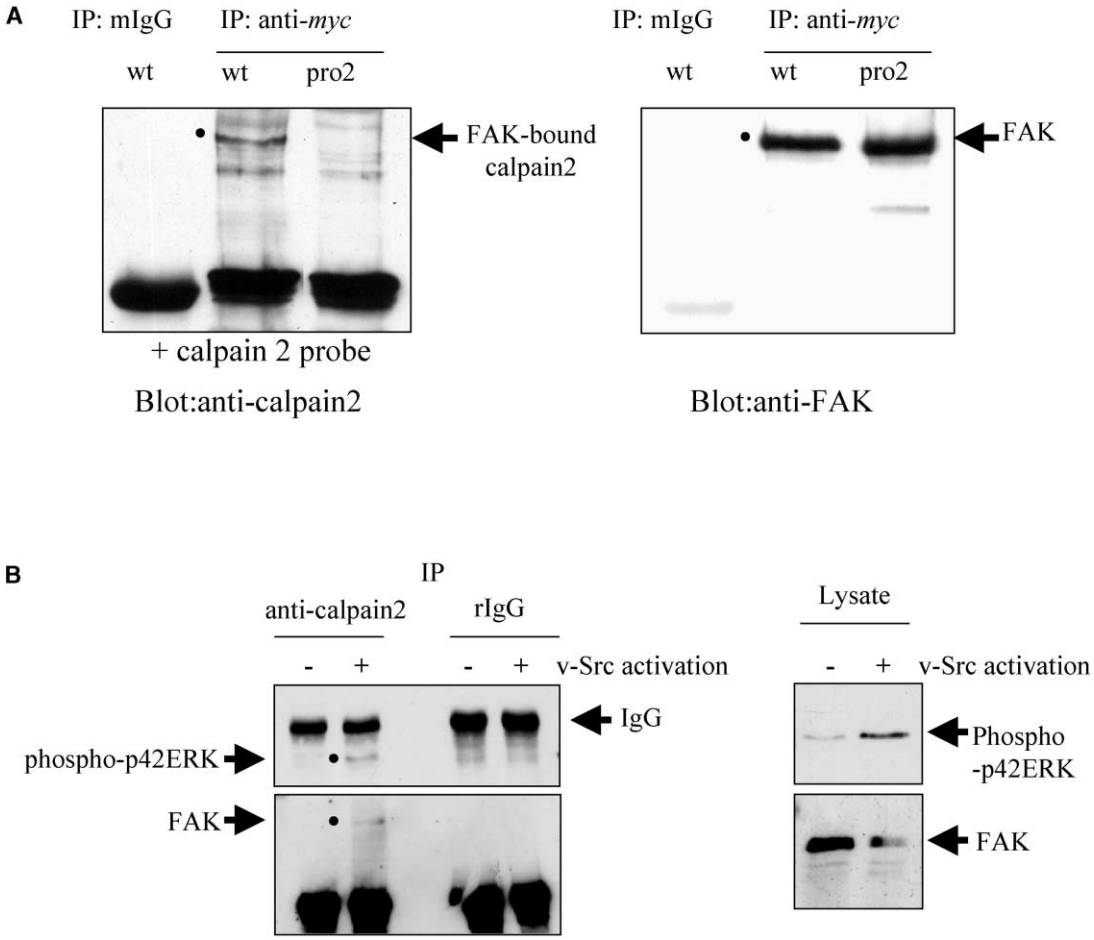


Figure 2. Calpain 2 Directly Interacts with Wild-Type FAK

(A) Wild-type (wt) and Pro2 FAK mutant (*myc* tagged) were immunoprecipitated from CEFs with anti-*myc*, separated by SDS-PAGE and incubated with recombinant Calpain 2. Calpain 2 associated with FAK-wt and FAK pro2 was detected by subsequent immunoblotting with anti-Calpain 2. Control IP reactions from FAK-wt expressing cells were performed with non-specific anti-mouse IgG (mIgG). Equal levels of immunoprecipitated FAK-wt and FAK-Pro2 were determined by immunoblotting with anti-FAK (on right).

(B) Calpain 2 IP's from CEFs expressing only endogenous FAK also demonstrate v-Src-induced association among Calpain 2, endogenous FAK, and p42ERK. Control IP's with nonspecific anti-rabbit IgG (rIgG) are shown. In these experiments we have used a phospho-specific antibody against p42ERK to monitor both the binding of ERK and its activation state within the complex. Total protein levels in cell lysates are shown on the right. Detailed methods of all techniques can be found in the Supplemental Data.

### The Amino-Terminal Proline Sequence Is Required for FAK Degradation, Focal Adhesion Turnover, and Cell Migration

Transformation induced by v-Src in primary embryo fibroblasts (but not in established cell lines) induces an extreme transformed phenotype whereby the normal balance of focal adhesion assembly and disassembly is dramatically skewed toward complete disassembly, cell rounding, and detachment. This extreme phenotype induced by strongly transforming v-Src allows us to monitor the mechanisms controlling Calpain-dependent proteolysis of FAK and focal adhesion turnover, providing an ideal model system for studying the biological consequences of the Calpain 2/FAK/p42ERK complex. To determine whether formation of the Calpain 2/FAK/p42ERK complex was required for Calpain-mediated proteolysis of FAK, we monitored the stability of wild-type FAK(-wt) and FAK-Pro2 in transformed cells, in which focal adhesions are turning over rapidly. Consistent with the degradation of endogenous FAK that we have previously reported [11, 17], exogenous FAK-wt levels were decreased by 3 hr after v-Src activation, and FAK-wt was not detectable in fully transformed cells at 24 hr (Figure 3A, upper panel). In contrast, the FAK-Pro2

below and demonstrate degradation of FAK in Src-transformed cells, as previously described [11, 17].

(C) Nontransformed and v-Src-transformed CEFs expressing either wild-type (wt) or Pro2 FAK mutant (*myc* tagged) were developed as described above. Total cell lysates were prepared, and Calpain 2 was immunoprecipitated. Calpain 2-associated active phosphorylated p42ERK (phospho-p42ERK) and exogenous FAK were detected after SDS-PAGE, transfer to membrane, and immunoblotting. Direct Western blots of the same total cell lysates used for immunoprecipitation experiments are shown below.

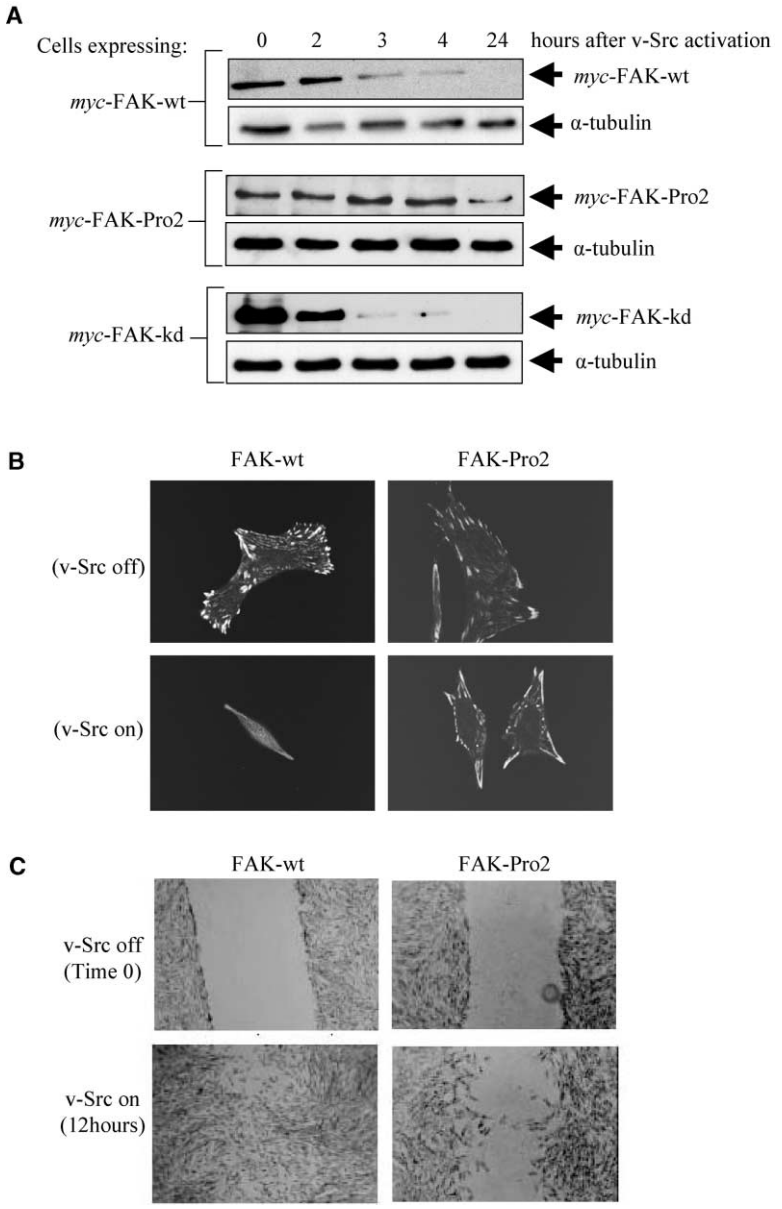


Figure 3. FAK Pro2 Sequence Is Necessary for FAK Degradation and Focal Adhesion Turnover

(A) Cell lysates were prepared at sequential time points after v-Src activation from CEFs coexpressing *ts* v-Src with *myc*-tagged wild-type FAK, pro2 FAK, and kinase-defective (kd) FAK. Protein levels of wild-type FAK, Pro2 FAK, and kd FAK were detected by SDS-PAGE and immunoblotting with anti-*myc* antibody. Protein loading was analysed by immunoblotting with anti-α-tubulin antibody.

(B) *myc*-tagged wild-type FAK and Pro2 FAK mutant retain the ability to target to focal adhesion sites in nontransformed cells (v-Src off), as determined by immunostaining with anti-*myc* antibody. Focal adhesion structures and cell morphology of CEFs expressing Pro2 FAK are not significantly altered under normal conditions (v-Src off) when these cells are compared with cells expressing wild-type FAK. However, upon v-Src activation, wild-type FAK redistributes from focal adhesion sites to a more-diffuse cytoplasmic staining pattern, and cells undergo morphological transformation. In contrast, the Pro2 FAK mutant is retained within focal adhesions at the cell periphery, and Src-induced focal adhesion disassembly and morphological transformation are impaired.

(C) CEFs coexpressing *ts* v-Src with either wild-type FAK or Pro2 FAK mutant were analyzed for their ability to invade a wounded monolayer 12 hr after v-Src activation.

mutant protein that impaired formation of the Calpain 2/FAK/ERK complex was relatively stable after v-Src activation (Figure 3A, middle panel). A kinase-defective FAK (FAK-kd) is degraded to a similar extent as FAK-wt, indicating that the kinase activity of FAK is not required for FAK degradation (Figure 3A, lower panel). Because we have previously shown that v-Src-induced proteolytic cleavage of FAK was mediated by Calpain 2 [11, 15], the above results imply that v-Src-induced formation of the Calpain 2/FAK/p42ERK complex is required for Calpain 2-mediated proteolysis of FAK.

To examine the biological consequences of expressing *myc*-tagged FAK-wt or FAK-Pro2 mutants in normal or transformed cells, we examined adherent cells by immunofluorescence and confocal microscopy by using an anti-*myc* antibody to detect exogenous FAK protein (Figure 3B). In nontransformed cells (v-Src off), cell morphology and distribution of focal adhesions were similar between cells expressing FAK-wt or FAK-Pro2 proteins (both FAK-wt and FAK-Pro2 proteins localized to focal adhesions normally in nontransformed cells). Six hours after v-Src activation (v-Src on), cells expressing FAK-wt underwent morphological transformation with few residual focal adhesions. In contrast, FAK-Pro2 mutant proteins remained in peripheral focal adhesions after v-Src activation, and morphological transformation was prevented in these short-term transformation assays (Figure 3B; similar staining was observed with an anti-vinculin antibody; results not shown).

In addition to morphological transformation, we also monitored cell migration by wound repair assays. We found that cells coexpressing *ts* v-Src with FAK-wt were efficiently able to migrate into the denuded area of cell monolayers as transformed cells (Figure 3C). In contrast, cells coexpressing *ts* v-Src and FAK-Pro2 were impaired in their ability to migrate (Figure 3C). Overexpression

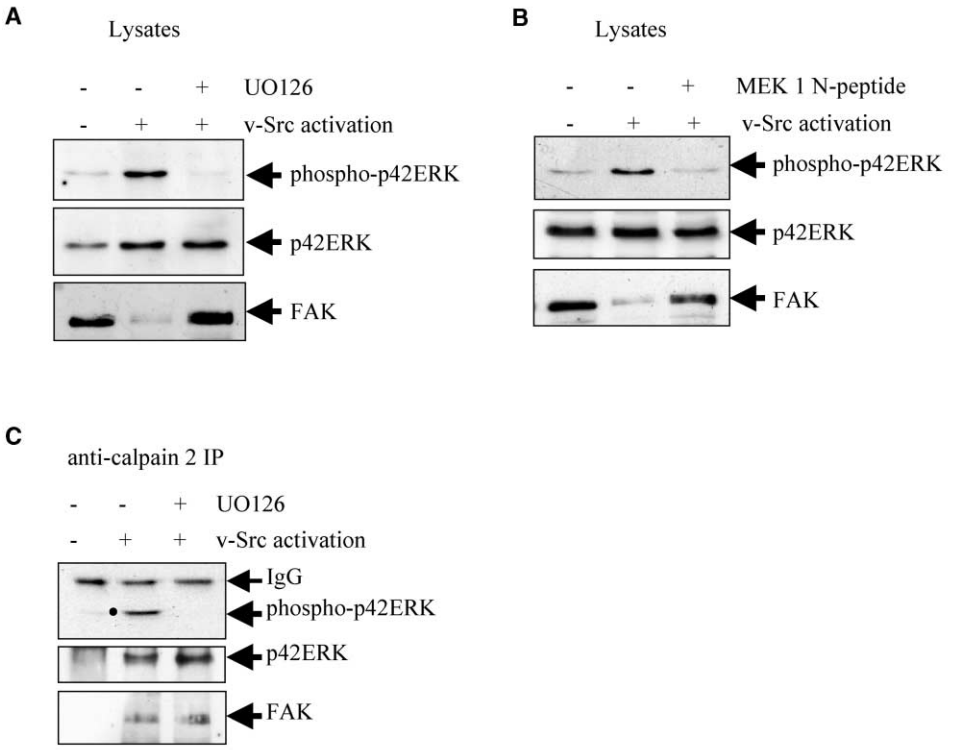


Figure 4. ERK/MAP Kinase Activity Is Required for Src-Induced FAK Proteolysis and Focal Adhesion Turnover

Cell transformation was initiated by v-Src activation in the absence (-) or presence (+) of (A) MEK inhibitor (UO126, 50 μM) or (B) the N-terminal dominant-negative MEK1 peptide (MEK1-N, 100 μM). Cell lysates were separated by SDS-PAGE and immunoblotted with antibodies against total and active (phospho) p42ERK and FAK. Cells expressing *ts* v-Src were preincubated with UO126 or MEK1-N 1 hr prior to v-Src activation, then for 24 hr after v-Src activation. (C) Calpain 2 was immunoprecipitated from transformed cells (+ v-Src activation) in the absence (-) or presence (+) of UO126 (50 μM). Calpain 2-associated, total and active (phospho) p42ERK and FAK was determined by SDS-PAGE and immunoblotting with specific antibodies. (D) v-Src-transformed cells were incubated in the absence (control) or presence of UO126 (50 μM) or MEK1-N (100 μM). Cell morphology and focal adhesion structures were analyzed by immunostaining with anti-vinculin antibody.

of kinase-defective FAK did not impair morphological transformation or cell migration of CEFs after v-Src activation (results not shown), further indicating that FAK regulates these events in primary fibroblasts via its protein adaptor function. Together with our previous data showing that Calpain inhibition suppresses v-Src-transformed cell migration [11], our results imply that assembly of the Calpain 2/FAK/p42ERK complex is required for Calpain 2-mediated proteolysis of FAK and focal adhesion turnover during cell migration and Src-mediated transformation.

### ERK/MAPK Activity Is Required for FAK Proteolysis, Transformation, and Migration

Because p42ERK/MAP kinase is recruited to the Calpain 2/FAK/p42ERK complex, we addressed whether activity of MEK1/ERK is required for effective proteolysis of FAK and focal adhesion turnover. Cells were treated with the MEK inhibitor U0126 (50 μM) [18] or the dominant-negative MEK1 N-terminal peptide (MEK1-N, 100 μM) [19]. Treatment with UO126 or MEK1-N effectively inhibited activation of p42ERK after v-Src activation, as shown by immunoblotting with an antibody against the

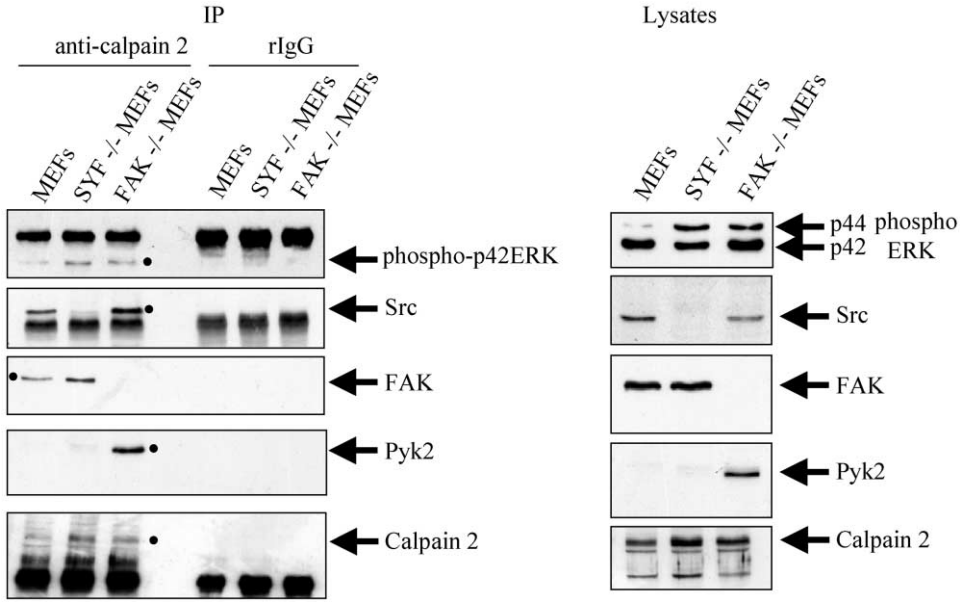


Figure 5. Pyk2 Substitutes for FAK in Calpain 2-ERK/MAPK Complex

Cell lysates were prepared, and Calpain 2 was immunoprecipitated from wild-type, FAK knock-out ($FAK^{-/-}$), and Src, Yes, Fyn knock-out ($SYF^{-/-}$) MEFs. Cell lysates and Calpain 2 IP's were separated by SDS-PAGE, transferred to membrane, and immunoblotted with antibodies against active ERK (phospho-p42ERK), FAK, Pyk2, Src, and Calpain 2. We confirmed that none of the Calpain 2 coimmunoprecipitating proteins (ERK, FAK, Src, and Pyk2) were present in normal rabbit IgG control immunoprecipitations from MEFs. Total protein levels were monitored in cell lysates by direct Western blot on the right.

phosphorylated activated form of p42ERK (phospho-p42ERK) (Figures 4A and 4B). However, inhibition of ERK activation by UO126 did not stop the Calpain 2/FAK/ERK complex from forming (Figure 4C). Both UO126 and MEK1-N treatment suppressed the proteolytic degradation of FAK (Figures 4A and 4B) and focal adhesion disassembly during v-Src-induced transformation (Figure 4D). In addition, UO126 and MEK1-N treatment substantially reduced Calpain activity as early as 6 hr after v-Src activation (data are shown in Figures S2A and S2B in the supplemental data) and suppressed migration of both normal and Src-transformed cells in wound repair assays (data are shown in Figures S3A and S3B). These results indicate that ERK (p42ERK in the case of chicken embryo fibroblasts used here), which is recruited into the Calpain 2/FAK complex (Figures 1 and 2) and to focal adhesion sites after v-Src activation [20], is needed for Calpain 2-dependent proteolysis of FAK and focal adhesion turnover.

### FAK Targets Calpain 2 and ERK/MAPK to Peripheral Focal Adhesion Sites

To further define the role of endogenous Src family kinases (SFKs) and FAK in assembly of the Calpain 2/p42ERK complex, we immunoprecipitated Calpain 2 from normal mouse embryo fibroblasts (MEFs) or from MEFs that were null for all three of the ubiquitous SFKs (Src, Yes, and Fyn; [$SYF^{-/-}$]) or for FAK ($FAK^{-/-}$). We found that Calpain 2 coimmunoprecipitated with endogenous FAK and p42ERK in normal MEFs and that some endogenous Src was also present in the complex (Figure 5). Furthermore, Calpain 2 and p42ERK coimmunoprecipitated equally well from $SYF^{-/-}$ MEFs (Figure 5), suggesting that although Src was present in the complex, ubiquitous Src family kinases were not required for assembly. In addition, Calpain 2 and p42ERK coimmunoprecipitated from $FAK^{-/-}$ MEFs (Figure 5). This was surprising because our experiments in cells that overexpressed the FAK-Pro2 mutant implied that FAK's adaptor function was important for formation of the complex (Figure 1C). However, an explanation was provided by the apparent replacement of FAK with the homologous protein, Pyk2, in the Calpain 2 complex in $FAK^{-/-}$ cells (Figure 5). As previously reported, we found that Pyk2 was substantially upregulated in $FAK^{-/-}$ cells ([21]; Figure 5, right panels). Interestingly, although mutation of the amino-terminal proline sequence (Pro2) of FAK inhibited association with Calpain 2 (Figure 1C), this sequence is not conserved in Pyk 2, suggesting that the FAK Pro2 sequence may perform functions other than making direct contacts with Calpain 2. Such functions might include maintaining conformation to allow other sequences in FAK to bind to cellular partners, including Calpain 2.

As shown, Pyk2 can compensate for FAK's adaptor function in assembly of the Calpain 2/FAK/p42ERK complex in $FAK^{-/-}$ cells, yet focal adhesion turnover and migration is impaired in these cells [1]. This indicates that Pyk2 cannot compensate for FAK function in inducing focal adhesion turnover and cell migration. Reasoning that Pyk 2 differs from FAK by having a less-efficient focal adhesion targeting sequence and is unable to locate to focal adhesions in $FAK^{-/-}$ cells [21], we addressed whether localization of Calpain 2 or ERK was impaired when Pyk2 replaced FAK in the Calpain 2/FAK-Pyk2/p42ERK complex. We found that Calpain 2 partially colo-

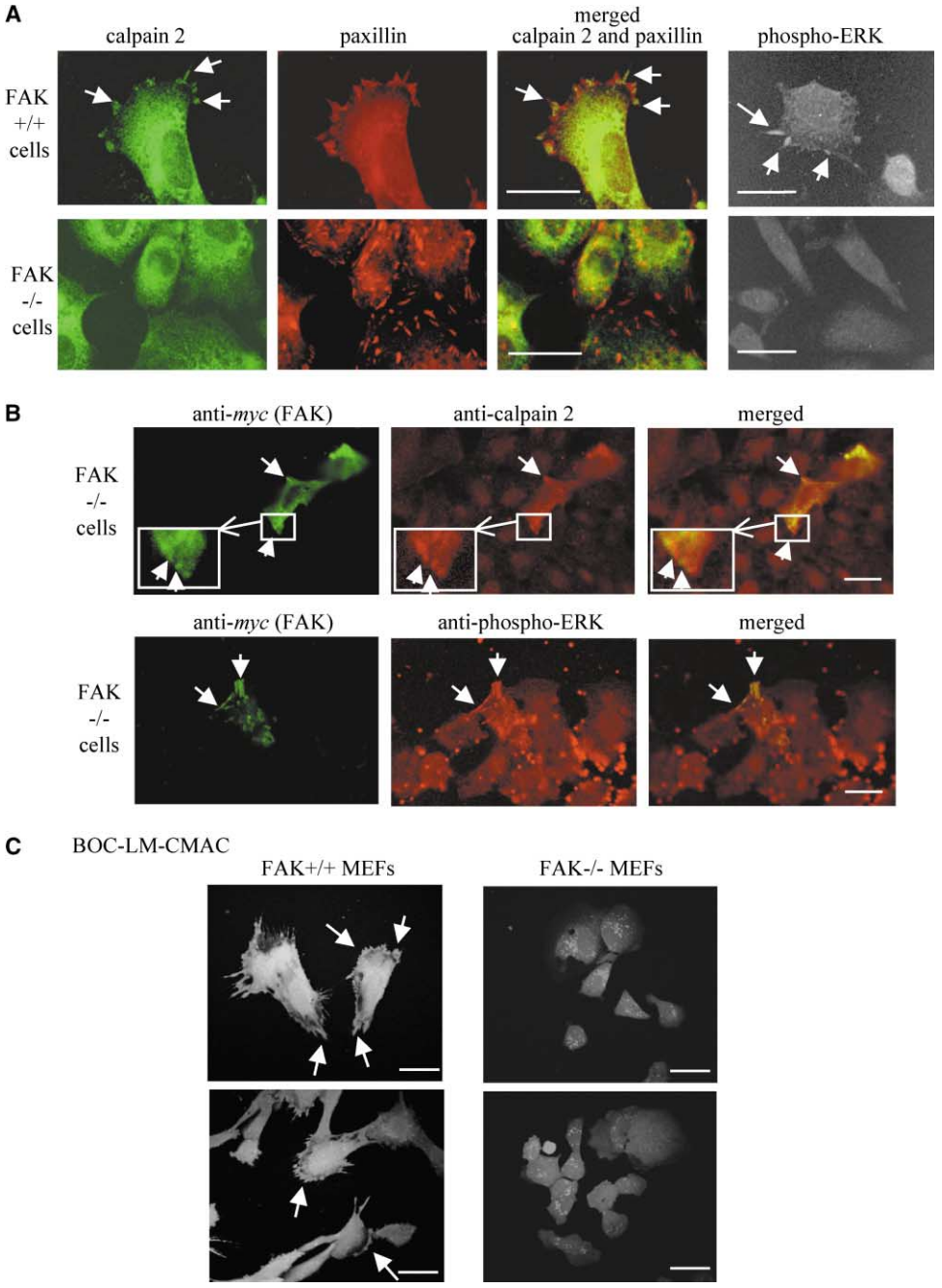


Figure 6. FAK Targets Calpain 2 and ERK/MAPK to Peripheral Focal-Adhesion Sites

(A) Wild-type ($FAK^{+/+}$) and $FAK^{-/-}$ cells were dual-labeled by immunostaining with antibodies against Calpain 2 and paxillin. Cells were also immunostained with an antibody against active phosphorylated ERK (phospho-ERK). All cells were examined by confocal microscopy. Arrows indicate localized Calpain 2 and phospho-ERK staining. The scale bars represent 25 μm.

(B) $FAK^{-/-}$ cells subjected to transient reexpression of *myc*-tagged wild-type FAK were coimmunostained with either anti-*myc* and anti-Calpain 2 antibodies or anti-*myc* and anti-phospho-ERK antibodies. Arrows indicate cells expressing FAK and localized Calpain 2 and p42ERK immunostaining. The scale bars represent 25 μm.

(C) Calpain activity was visualized in wild-type and $FAK^{-/-}$ MEFs by incubation of cells with the cell-permeable fluorogenic Calpain substrate BOC-LM-CMAC (Molecular probes Inc.) and analysis by confocal microscopy (X630 magnification). The scale bars represent 25 μm.

calized with paxillin at the cell periphery of wild-type MEFs ($FAK^{+/+}$ cells), the pattern of staining indicating that Calpain 2 was present in a subset of discrete focal adhesion regions (Figure 6A, merged images). However, Calpain 2 colocalization with focal adhesions was not evident in $FAK^{-/-}$ cells, with Calpain 2 in these cells being diffusely localized throughout the cytoplasm (Figure 6A). Similarly, antibodies recognizing the activated form of ERK (phospho-ERK) demonstrate a diffuse staining pattern in $FAK^{-/-}$ cells, whereas there was some

phospho-ERK concentrated at peripheral focal adhesion sites in $FAK^{+/+}$ cells (Figure 6A). Quantification of the proportion of MEFs in which Calpain 2 or phospho-ERK was visible at the cell periphery is shown in the Supplemental Data (Figure S4). Reexpression of a *myc*-tagged wild-type FAK in $FAK^{-/-}$ cells rescues localized staining of Calpain 2 and phospho-ERK to the cell periphery (Figure 6B). These results indicate that FAK plays a key role in the targeting of both Calpain 2 and ERK to a subset of focal adhesions, whereas Pyk2 is unable to substitute for FAK in this regard.

Because our results indicate that FAK may combine to spatially couple Calpain 2 to its upstream regulator ERK/MAP kinase, we addressed whether FAK contributed to total cellular Calpain activity. Using the cell-permeable fluorescent Calpain substrate BOC-LM-CMAC, we have shown in live cells that fluorescence intensity relating to Calpain activity is visibly reduced in $FAK^{-/-}$ cells relative to wild-type MEFs (Figure 6C). In addition, the BOC assay demonstrates that Calpain activity localizes to the cell membrane in wild-type MEFs but not in $FAK^{-/-}$ cells (Figure 6C). Furthermore, we found that total Calpain activity in extracted cell lysates was reduced in $FAK^{-/-}$ cells relative to wild-type MEFs (Figure S5). These results are consistent with our conclusion that FAK is required for assembly of a complex containing both Calpain 2 and phospho-ERK, its upstream activating kinase, and for recruitment of this complex to the plasma membrane, which is known to promote full Calpain activation [22–25].

In summary, this study describes a novel function for FAK as an adaptor molecule that permits the assembly of a Calpain 2/FAK/p42ERK complex. FAK-dependent complex assembly and localization to the cell periphery facilitates ERK/MAPK-induced activation of Calpain. This subsequently permits Calpain-mediated cleavage of FAK (and most likely other adhesion components), focal adhesion turnover, and cell migration. These findings provide a mechanistic explanation for the similar adhesion turnover and migratory defects of FAK null and Calpain-deficient cells.

### Supplemental Data

Full experimental details of methods used in this study are provided in the Supplemental Data online at http://www.current-biology.com/cgi/content.full/13/16/1442/DC1/. Additional data are also available online. Additional data includes the following: densitometry of FAK-wt and FAK-kd coimmunoprecipitating with Calpain 2; measurement of total cellular Calpain activity after v-Src activation in the absence and presence of MEK inhibitors; cell migration/wound assays of normal and v-Src-transformed cells in the absence and presence of the MEK inhibitors; quantification of the percentage of $FAK^{+/+}$ and $FAK^{-/-}$ MEFs, where Calpain 2 and phospho-ERK are localized to peripheral adhesion sites; and measurement of total Calpain activity in cell extracts from $FAK^{+/+}$ and $FAK^{-/-}$ cells.

### Acknowledgments

We thank Phillipe Soriano for SYF MEFs, Dusko Ilic for FAK null MEFs, Tom Parsons for kinase-defective FAK constructs, and John Wyke for reading this manuscript. This work was funded by Cancer Research UK. M.D.S. received support from the National Institutes of Health.

Received: January 30, 2003
Revised: July 4, 2003
Accepted: July 4, 2003
Published: August 19, 2003

### References

1. Ilic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995). Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature *377*, 539–544.
2. Klinghoffer, R.A., Sachsenmaier, C., Cooper, J.A., and Soriano, P. (1999). Src family kinases are required for integrin but not PDGFR signal transduction. EMBO J. *18*, 2459–2471.
3. Volberg, T., Romer, L., Zamir, E., and Geiger, B. (2001). pp60(c-src) and related tyrosine kinases: a role in the assembly and reorganization of matrix adhesions. J. Cell Sci. *114*, 2279–2289.
4. Glading, A., Chang, P., Lauffenburger, D.A., and Wells, A. (2000). Epidermal growth factor receptor activation of calpain is required for fibroblast motility and occurs via an ERK/MAP kinase signaling pathway. J. Biol. Chem. *275*, 2390–2398.
5. Glading, A., Lauffenburger, D.A., and Wells, A. (2002). Cutting to the chase: calpain proteases in cell motility. Trends Cell Biol. *12*, 46–54.
6. Xie, H., Pallero, M.A., Gupta, K., Chang, P., Ware, M.F., Witke, W., Kwiatkowski, D.J., Lauffenburger, D.A., Murphy-Ullrich, J.E., and Wells, A. (1998). EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLCgamma signaling pathway. J. Cell Sci. *111*, 615–624.
7. Beckerle, M.C., Burridge, K., DeMartino, G.N., and Croall, D.E. (1987). Colocalization of calcium-dependent protease II and one of its substrates at sites of cell adhesion. Cell *51*, 569–577.
8. Cooray, P., Yuan, Y., Schoenwaelder, S.M., Mitchell, C.A., Salem, H.H., and Jackson, S.P. (1996). Focal adhesion kinase (pp125FAK) cleavage and regulation by calpain. Biochem. J. *318*, 41–47.
9. Yuan, Y., Dopheide, S.M., Ivanidis, C., Salem, H.H., and Jackson, S.P. (1997). Calpain regulation of cytoskeletal signaling complexes in von Willebrand factor-stimulated platelets. Distinct roles for glycoprotein Ib-V-IX and glycoprotein IIb-IIIa (integrin alphaIIbbeta3) in von Willebrand factor-induced signal transduction. J. Biol. Chem. *272*, 21847–21854.
10. Carragher, N.O., Levkau, B., Ross, R., and Raines, E.W. (1999). Degraded collagen fragments promote rapid disassembly of smooth muscle focal adhesions that correlates with cleavage of pp125(FAK), paxillin, and talin. J. Cell Biol. *7*, 619–630.
11. Carragher, N.O., Fincham, V.J., Riley, D., and Frame, M.C. (2001). Cleavage of focal adhesion kinase by different proteases during SRC-regulated transformation and apoptosis. Distinct roles for calpain and caspases. J. Biol. Chem. *276*, 4270–4275.
12. Small, G.W., Chou, T.Y., Dang, C.V., and Orlowski, R.Z. (2002). Evidence for involvement of calpain in c-Myc proteolysis in vivo. Arch. Biochem. Biophys. *400*, 151–161.
13. Wang, Y.K., Wang, Y.H., Wang, C.Z., Sung, J.M., Chiu, W.T., Lin, S.H., Chang, Y.H., and Tang, M.J. (2003). Rigidity of collagen fibrils controls collagen gel-induced down-regulation of focal adhesion complex proteins mediated by alpha2beta1 integrin. J. Biol. Chem. *278*, 21886–21892.
14. Dourdin, N., Bhatt, A.K., Dutt, P., Greer, P.A., Arthur, J.S., Elce, J.S., and Huttenlocher, A. (2001). Reduced cell migration and disruption of the actin cytoskeleton in calpain-deficient embryonic fibroblasts. J. Biol. Chem. *276*, 48382–48388.
15. Carragher, N.O., Westhoff, M.A., Riley, D., Potter, D.A., Dutt, P., Elce, J.S., Greer, P.A., and Frame, M.C. (2002). v-Src-induced modulation of the calpain-calpastatin proteolytic system regulates transformation. Mol. Cell. Biol. *22*, 257–269.
16. Thomas, J.W., Ellis, B., Boerner, R.J., Knight, W.B., White, G.C., 2nd, and Schaller, M.D. (1998). SH2- and SH3-mediated interactions between focal adhesion kinase and Src. J. Biol. Chem. *273*, 577–583.
17. Fincham, V.J., Wyke, J.A., and Frame, M.C. (1995). v-Src-induced degradation of focal adhesion kinase during morphological transformation of chicken embryo fibroblasts. Oncogene *10*, 2247–2252.
18. Favata, M.F., Horiuchi, K.Y., Manos, E.J., Daulerio, A.J., Stradley, D.A., Feeser, W.S., Van Dyk, D.E., Pitts, W.J., Earl, R.A.,

Hobbs, F., et al. (1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J. Biol. Chem. *273*, 18623–18632.

19. Kelemen, B.R., Hsiao, K., and Goueli, S.A. (2002). Selective in vivo inhibition of mitogen-activated protein kinase activation using cell-permeable peptides. J. Biol. Chem. *277*, 8741–8748.
20. Fincham, V.J., James, M., Frame, M.C., and Winder, S.J. (2000). Active ERK/MAP kinase is targeted to newly forming cell-matrix adhesions by integrin engagement and v-Src. EMBO J. *19*, 2911–2923.
21. Sieg, D.J., Ilic, D., Jones, K.C., Damsky, C.H., Hunter, T., and Schlaepfer, D.D. (1998). Pyk2 and Src-family protein-tyrosine kinases compensate for the loss of FAK in fibronectin-stimulated signaling events but Pyk2 does not fully function to enhance FAK-cell migration. EMBO J. *17*, 5933–5947.
22. Arthur, J.S., and Crawford, C. (1996). Investigation of the interaction of m-calpain with phospholipids: calpain-phospholipid interactions. Biochim. Biophys. Acta *1293*, 201–206.
23. Molinari, M., Anagli, J., and Carafoli, E. (1994). Ca(2+)-activated neutral protease is active in the erythrocyte membrane in its nonautolyzed 80-kDa form. J. Biol. Chem. *269*, 27992–27995.
24. Pontremoli, S., Melloni, E., Sparatore, B., Salamino, F., Michetti, M., Sacco, O., and Horecker, B.L. (1985). Role of phospholipids in the activation of the Ca2+-dependent neutral proteinase of human erythrocytes. Biochem. Biophys. Res. Commun. *129*, 389–395.
25. Glading, A., Uberall, F., Keyse, S.M., Lauffenburger, D.A., and Wells, A. (2001). Membrane proximal ERK signaling is required for M-calpain activation downstream of epidermal growth factor receptor signaling. J. Biol. Chem. *276*, 23341–23348.