The coordinated and dynamic regulation of adhesions is required for cell migration. We demonstrated previously that limited proteolysis of talin1 by the calcium-dependent protease calpain 2 plays a critical role in adhesion disassembly in fibroblasts (Franco, S. J., Rodgers, M. A., Perrin, B. J., Han, J., Bennin, D. A., Critchley, D. R., and Huttenlocher, A. (2004) Nat. Cell Biol. 6, 977–983). However, little is known about the contribution of other calpain substrates to the regulation of adhesion dynamics. We now provide evidence that calpain 2-mediated proteolysis of focal adhesion kinase (FAK) regulates adhesion dynamics in motile cells. We mapped the preferred calpain cleavage site between the two C-terminal proline-rich regions after Ser-745, resulting in a C-terminal fragment similar in size to the FAK-related non-kinase (FRNK). We generated mutant FAK with a point mutation (V744G) that renders FAK resistant to calpain proteolysis but retains other biochemical properties of FAK. Using time-lapse microscopy, we show that the dynamics of green fluorescent protein-talin1 are impaired in FAK-deficient cells. Expression of wild-type but not calpain-resistant FAK rescues talin dynamics in FAK-deficient cells. Taken together, our findings suggest a novel role for calpain proteolysis of FAK in regulating adhesion dynamics in motile cells.

The involvement of both FAK and calpain in regulating the turnover of adhesions prompted us to investigate the cleavage of FAK by calpain as a possible mechanism by which FAK affects adhesion dynamics. We demonstrated previously that calpain-mediated proteolysis of talin regulates adhesion dynamics (17). Here, we show that FAK also regulates talin dynamics. We have identified the calpain cleavage site of FAK and have generated a mutant form of FAK that is resistant to calpain-mediated proteolysis. Expression of wild-type but not calpain-resistant FAK restores the adhesion dynamics of talin in FAK-deficient cells. Taken together, our findings suggest a novel role for calpain-mediated cleavage of FAK in regulating adhesion dynamics.

**EXPERIMENTAL PROCEDURES**

Reagents and Antibodies—Fibronectin was purified from human plasma by affinity chromatography as described previously (18). Mouse anti-FAK (clone 77), anti-Pyk2 (clone 11), anti-p130Cas (clone 21), and anti-paxillin (clone 349) monoclonal antibodies were purchased from BD Transduction Laboratories. Mouse anti-actin (clone AC-15), anti-talin (clone 8d4), anti-hemagglutinin (HA; clone HA-7), and anti-FLAG (clone M2) monoclonal antibodies were purchased from Sigma-Aldrich. Rabbit small interfering RNA; PMSF, phenylmethylsulfonyl fluoride; FRNK, FAK-related non-kinase.
anti-FAK polyclonal antibody (clone C-20) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-FAK polyclonal antibody was purchased from Triple Point Biologics (Forest Grove, OR). Rabbit anti-phospho–Tyr-397 FAK polyclonal antibody was obtained from Invitrogen. Rhodamine-phallolidin, rabbit anti-green fluorescent protein (GFP) antibody, Rhodamine Red-X goat anti-mouse IgG and Alexa Fluor 680 goat anti-mouse IgG were purchased from Invitrogen. IRDye 800CW goat anti-rabbit IgG was obtained from Rockland Immunochemicals (Gilbertsville, PA). Calpain inhibitor (N-acetyl-Leu-Leu-Met) and recombinant rat calpain 2 were purchased from Calbiochem. Ionomycin was purchased from Sigma-Aldrich.

**DNA Constructs**—Murine GFP-FAK (pEGFP-C1-FAK-HA) was provided by D. Schlaeffer (University of California San Diego). GFP-FAK-V744G was generated from GFP-FAK using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the following primers: forward, 5′-CAGACCTGTGTTATCTACGCGCATGGAATCCCAGCCATG-3′; and reverse, 5′-CGGTGTAGGAGGACGCCTGTAGTTGATGTG-3′. GFP-FAKΔ724–750 was also generated by site-directed mutagenesis using the following primers: forward, 5′-CAGACCTGTGTTATCTACGCGCATGGAATCCCAGCCATG-3′; and reverse, 5′-CATGGTGGATATCTACGCGCATGGAATCCCAGCCATG-3′. FAK-FLAG was subcloned into the BamHI and KpnI sites of the pMX vector using the following primers: forward, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′; and reverse, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′. mCherry-FAK (pmCherry-C1-FAK-HA) and mCherry-FAK-V744G were generated by PCR subcloning from the pMX-mCherry-FAK-HA plasmid and pMX-mCherry-FAK-V744G vectors (a gift from Clive Svendsen) from which internal ribosome entry site-GFP was excised. pMX-mCherry-FAK was generated by PCR amplification of the FAK with the following primers: forward, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′; and reverse, 5′-CATGGTGGATATCTACGCGCATGGAATCCCAGCCATG-3′. The PCR product was subsequently digested with BglII and Sall and ligated into the BamH1 and XhoI sites of pcDNA3.1(+)(Invitrogen), which contained a C-terminal FLAG tag. FAK-FLAG was subcloned into the BamH1 and KpnI sites of pFastBac (Invitrogen) with the following primers: forward, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′; and reverse, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′. mCherry-FAK (pmCherry-C1-FAK-HA) and mCherry-FAK-V744G (pmCherry-C1-FAK-HA-V744G) were generated by PCR subcloning into the BglII and KpnI sites of the pmCherry-C1 vector (19) using the following primers: forward, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′; and reverse, 5′-GACGGATCCGCCACCATGGCAGCTGCTTATC-3′. mCherry-FAK was expressed in HEK 293 cells.

**Expression and Purification of Recombinant FAK**—The pFastBac-FLAG-FAK construct was transformed into DH10Bac Escherichia coli cells (Invitrogen) for recombinant bacmid DNA. High-titer viral stocks were used to infect SF9 insect cells. Seventy-two hours after infection, cells were lysed in phosphate-buffered saline (10 mM sodium phosphate, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) with 1% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml E-64 (Sigma-Aldrich). Lysate was incubated in cleavage buffer (50 mM Tris-HCl pH 7.4, 137 mM KCl, and 1 mM MgCl2) with indicated concentrations of purified calpain 2 at 37 °C for 30 min in the absence or presence of CaCl2. Cleavage reactions were stopped by the addition of 6X SDS sample buffer.

**Calpain Cleavage Assay**—Baculovirus-purified FAK (5 μg) was incubated in cleavage buffer (50 mM Tris–HCl pH 7.4, 137 mM KCl, and 1 mM MgCl2) with indicated concentrations of purified calpain 2 at 37 °C for 30 min in the absence or presence of CaCl2. Cleavage reactions were stopped by the addition of 6X SDS sample buffer.

**Calpain Cleavage of FAK in Adhesion Dynamics**

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**Calpain Cleavage Site Mapping**—FPGV-v-Src was provided by M. Frame (Edinburgh Facility). siRNA—HEK 293 control and calpain 2 siRNA cells were generated by retroviral infection of pSUPER.retro (Oligoengine, Seattle, WA) containing sequence encoding short hairpin RNA against a non-targeting sequence or human calpain 2 as described previously (21). The sense sequences for the Stealth siRNA oligonucleotides (Invitrogen) used are as follows: Controli, 5′-GAAUCUCAGAUAUUUGAACCAG-GAC-3′; FAKsi-203, 5′-UGACAGAUAACUAUGACUGAAC-AAA-3′; FAKsi-385, 5′-GGCCGCAUGUACAGGCAUG-AAG-3′; and FAKsi-2225, 5′-GGCCCGACCGUGUAACUGA-ACUAAA-3′. Transfections of siRNA into HEK 293 cells were performed by calcium phosphate precipitation using 180 pmol of siRNA/6-cm dish containing 1.1 × 106 cells.

**Expression and Purification of Recombinant FAK**—The pFastBac-FLAG-FAK construct was transformed into DH10Bac Escherichia coli cells (Invitrogen) for recombinant bacmid DNA. High-titer viral stocks were used to infect SF9 insect cells. Seventy-two hours after infection, cells were lysed in phosphate-buffered saline (10 mM sodium phosphate, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) with 1% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml E-64 (Sigma-Aldrich). Lysate was incubated in cleavage buffer (50 mM Tris–HCl pH 7.4, 137 mM KCl, and 1 mM MgCl2) with indicated concentrations of purified calpain 2 at 37 °C for 30 min in the absence or presence of CaCl2. Cleavage reactions were stopped by the addition of 6X SDS sample buffer.

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boiled in nonreducing sample buffer to elute proteins, which were separated on SDS-polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membrane (Bio-Rad) and stained with Coomassie Blue, and an individual band was subjected to N-terminal sequencing (performed at the Baylor College of Medicine Protein Chemistry/Proteomics Core).

**Immunocytochemistry**—Glass coverslips were acid-washed, silanized, and coated with 10 μg/ml fibronectin. Cells were plated on coverslips in Dulbecco’s modified Eagle’s medium supplemented with 1% fetal bovine serum and nonessential amino acids and allowed to adhere for 4 h. Cells were then fixed, permeabilized, and stained as described previously (22).

**Immunoblot Analysis**—Cells were scraped into lysis buffer on ice and clarified by centrifugation. Protein concentrations were determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions. Equal amounts of total protein were loaded onto 6–20% gradient SDS-polyacrylamide gels and transferred to nitrocellulose. Western blots were imaged with an Odyssey infrared imaging system (LI-COR Biosciences, Omaha, NE).

**Immunoprecipitation**—For p130Cas co-immunoprecipitations, HEK 293 cells were transfected and lysed 24–48 h later in 50 mM Tris, pH 8.0, 1% Nonidet P-40, 150 mM NaCl, 0.5% sodium deoxycholate, 0.05% SDS, 0.2 mM PMSF, 1 μg/ml peptatin, 2 μg/ml aprotinin, 1 μg/ml leupeptin, 10 mM sodium fluoride, and 1 mM sodium orthovanadate. For paxillin co-immunoprecipitations, cells were lysed in 50 mM HEPES, pH 7.4, 1% Nonidet P-40, 75 mM NaCl, 0.2 mM PMSF, 1 μg/ml peptatin, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 mM sodium orthovanadate. Cleared lysates (0.5–1 mg) were incubated with 2 μg of anti-GFP antibody (Invitrogen) for 1–2 h. Immune complexes were captured with GammaBind G-Sepharose beads (GE Healthcare), washed with lysis buffer, and analyzed by immunoblotting.

**Live Fluorescence Microscopy**—Fluorescence imaging of live cells was performed using a 60× objective on an Olympus IX-70 inverted microscope housed in a 37 °C closed system. Glass-bottom dishes were acid-washed and coated with 10 μg/ml fibronectin. Cells were plated in Dulbecco’s modified Eagle’s medium containing 1% fetal bovine serum, nonessential amino acids, and 25 mM HEPES, pH 7.4, and allowed to adhere for 1 h, after which the medium was replaced with Ham’s F-12 supplemented with 1% fetal bovine serum, nonessential amino acids, and 25 mM HEPES, pH 7.4. Fluorescent images were then captured every 2 min for 2 h using MetaVue imaging software (Molecular Devices, Downingtown, PA).

**Quantification of Adhesion Dynamics**—Quantification of adhesion dynamics was performed as described previously (4, 17). Time-lapse sequences from live fluorescence imaging of GFP-talin1 were first subjected to high-pass filtration based on the “water” algorithm (23) to remove diffuse background fluorescence. Fluorescence intensities of individual adhesions from background-subtracted images were measured over time using MetaVue imaging software. Of the adhesions that formed and disassembled within the period of the movie, 10–15 randomly selected adhesions in six individual cells/condition over 3 separate days were analyzed. Rate constant measurements for assembly (increasing fluorescence intensity) and disassembly (decreasing fluorescence intensity) of individual adhesions were determined from the slopes of trend lines fitted to semilogarithmic plots of fluorescence intensity ratios over time as described previously (4, 17). The duration of an adhesion was determined by the time elapsed between the first and last frames in which an adhesion was observed.

**RESULTS**

**FAK Regulates the Adhesion Dynamics of Talin**—Previous studies have demonstrated that FAK and its phosphorylation at
Calpain Cleavage of FAK in Adhesion Dynamics

Tyr-397 are important for regulating focal adhesion turnover (4, 5). However, the contribution of calpain proteolysis of FAK to the regulation of adhesion dynamics has not been defined. To begin to address this, we first tested the effects of FAK depletion on the dynamics of the focal adhesion protein talin. We generated FAK-deficient HEK 293 cells by using siRNA to deplete endogenous levels of FAK by ~80% without affecting the expression of the proline-rich tyrosine kinase Pyk2 (Fig. 1A) (24). Immunoblotting of endogenous FAK (~115 kDa) with an antibody specific for the C terminus showed an additional band of ~35 kDa in size. We visualized the dynamics of GFP-talin1 in control and FAK-deficient cells by time-lapse microscopy (Fig. 1B and supplemental Movies 1 and 2). Live fluorescence imaging demonstrated that talin-containing adhesions in FAK-depleted cells were extended in duration by ~2-fold compared with control cells (Fig. 1C). From plots of GFP-talin1 fluorescence intensities over time, we generated rate constants for net adhesion assembly and disassembly rates. Interestingly, both adhesion assembly and disassembly rates were impaired in FAK-deficient cells. Thus, the extended duration of talin-containing adhesions in FAK-deficient cells resulted from the impaired ability of talin both to incorporate into adhesions and to disassemble from adhesions. Our results are consistent with findings in FAK-null bone marrow macrophages, which exhibit impaired adhesion turnover due to decreased assembly and disassembly rates (25). Taken together, these data indicate that FAK regulates talin dynamics in HEK 293 cells.

FAK Is Cleaved by Calpain 2 in Vitro and in Vivo—On the basis of previous studies (16, 26, 27), we hypothesized that the 35-kDa band present upon FAK immunoblotting was due to calpain proteolysis. However, the specific calpain-mediated FAK proteolytic site had not yet been characterized. We first wanted to confirm the ability of calpain 2 to cleave purified FAK using an in vitro cleavage assay (Fig. 2A). As expected, in the absence of calpain 2, FAK was not cleaved. The addition of calpain 2 without calcium also did not result in cleavage of FAK. In the presence of 1 mM calcium and low concentrations of calpain 2, immunoblot analysis with N and C terminus-specific antibodies showed a single predominant cleavage band at 80 kDa (Fig. 2A, left panel) and 35 kDa (right panel), respectively, indicating that calpain likely cleaves FAK in the C-terminal region of the protein. Higher concentrations of calpain resulted in limited cleavage and subsequent degradation of FAK. Therefore...
fore, these data demonstrate that FAK is a direct substrate of calpain 2 in vitro.

We next tested the capacity of several cell lines to generate the proteolytic fragment of FAK. Control mouse embryonic fibroblasts, v-Src-transformed NIH 3T3 fibroblasts, and HEK 293 cells were able to produce a 35-kDa C-terminal cleavage fragment, which was diminished in calpain 2-deficient cell lines (Fig. 2, B–D). In addition, upon exogenous expression of FAK-FLAG (Fig. 2E) or GFP-FAK (Fig. 2F), calpain 2-deficient HEK 293 cells displayed a reduction in the single proteolytic fragment compared with control cells. Similar to our results from in vitro calpain cleavage of FAK (Fig. 2A), a predominant single band was detected, although we did not observe FAK degradation, indicating that there are likely mechanisms in vivo that limit calpain activity and FAK degradation (reviewed in Ref. 14). Intriguingly, in calpain-deficient mouse embryonic fibroblasts and v-Src-transformed NIH 3T3 fibroblasts, immunoblot analysis revealed an increase in an additional band of higher molecular mass, which was similar in size to FRNK-related non-kinase (FRNK). Protein isoforms were immunoprecipitated (IP) with anti-HA antibody, followed by immunoblotting with anti-HA and anti-phospho-Tyr-397 (pY397) FAK antibodies. Immunoblot shown are from one of three independent experiments.
Mapping the Calpain Cleavage Site of FAK—The site of calpain proteolysis of FAK had not been previously determined likely due to the lack of a consensus calpain cleavage site (30) and to the fact that only a small percentage (5–10%) of FAK is cleaved. To enhance calpain-mediated generation of the FAK cleavage fragment, we treated HEK 293 cells with the calcium ionophore ionomycin (Fig. 3A). The ionomycin-induced proteolysis of FAK was diminished in calpain 2-deficient cells. Likewise, treatment of HEK 293 cells ectopically expressing FAK-FLAG with ionomycin resulted in the enhanced generation of the proteolytic fragment. We were able to isolate the in vivo cleavage fragment, which was further analyzed by N-terminal sequencing (Fig. 3B). The cleavage site of FAK occurs after Ser-745, located between the two C-terminal proline-rich regions (Fig. 3C).

Generation of a Calpain-resistant Mutant of FAK—To specifically address the effects of calpain proteolysis on adhesion dynamics, we engineered a calpain-resistant mutant form of FAK. We performed site-directed mutagenesis of GFP-FAK in several residues proximal to the cleavage site (Fig. 4A). A 27-amino acid deletion (Δ724–750) surrounding the cleavage site abolished the cleavage fragment. Furthermore, analogous to the mutations introduced into talin and spectrin that rendered them calpain-resistant (17, 31), a point mutation of amino acid 744 at the P2 position from a valine to a glycine (V744G) significantly impaired calpain-mediated proteolysis of GFP-FAK. An additional point mutation (P748G) and a 6-amino acid deletion (Δ743–748) also impaired calpain proteolysis of GFP-FAK, whereas an R724L or S725F mutation did not affect the ability of FAK to be cleaved (data not shown). Furthermore, expression of GFP-FAK and calpain-resistant mutants did not affect the capacity of endogenous FAK to be cleaved. Quantification of the susceptibility to calpain proteolysis demonstrated that GFP-FAK-V744G was 10-fold resistant compared with wild-type GFP-FAK (Fig. 4B). Moreover, we confirmed that GFP-FAK-V744G retained the ability to be phosphorylated at Tyr-397 (Fig. 4C). These data demonstrate, for the first time, the site of calpain cleavage of FAK and the successful generation of a point mutant of FAK that is resistant to calpain proteolysis.

Calpain-resistant FAK Retains Localization and Biochemical Interactions—To further determine whether calpain-resistant GFP-FAK-V744G is functional, we examined the intracellular distribution of GFP-FAK-V744G. We expressed GFP-FAK and GFP-FAK-V744G in HEK 293 cells, followed by immunostaining with an antibody to the adhesion marker paxillin (Fig. 5A). We found that both GFP-FAK and GFP-FAK-V744G localized with paxillin-containing adhesions. Moreover, to determine whether wild-type and calpain-resistant GFP-FAK retained interactions with normal binding partners, we performed co-immunoprecipitation experiments and found that GFP-FAK and GFP-FAK-V744G were able to interact with p130Cas (Fig. 5B) and paxillin (Fig. 5C). Thus, these data indicate that calpain-resistant GFP-FAK-V744G is otherwise functional.

Expression of Wild-type but Not Calpain-resistant FAK Rescues Impaired Adhesion Turnover of Talin in FAK-deficient Cells—Given our findings that FAK regulates the adhesion dynamics of talin, we wanted to investigate the role of calpain cleavage of FAK in regulating talin dynamics. Accordingly, we generated mCherry fusions of wild-type and calpain-resistant FAK and were able to deplete cells of endogenous FAK without affect-
ing the expression of exogenously expressed mCherry-FAK or mCherry-FAK-V744G (Fig. 6A). Using cell lines stably expressing mCherry, mCherry-FAK, or mCherry-FAK-V744G, we transiently depleted cells of endogenous FAK and visualized the dynamics of GFP-talin1 (Fig. 6B and supplemental Movies 3–6). Live fluorescence imaging showed that talin-containing adhesions in FAK-deficient mCherry-expressing cells were extended in duration compared with those in control cells (Fig. 6C). Quantification of adhesion dynamics demonstrated that the extended durations were due to significantly decreased rates of adhesion assembly and disassembly in FAK-deficient cells. Expression of wild-type mCherry-FAK was able to restore adhesion dynamics of talin in FAK-deficient cells. However, expression of calpain-resistant mCherry-FAK-V744G failed to rescue adhesion dynamics, indicating that calpain cleavage of FAK regulates talin dynamics. Taken together, these data indicate that FAK and its cleavage by calpain contribute to the regulation of adhesion turnover.

**DISCUSSION**

We showed previously that calpain-mediated proteolysis of talin regulates adhesion dynamics (17); however, the contribution of cleavage of other adhesion proteins has not been defined. Here, we have demonstrated that FAK regulates talin dynamics. We have identified the calpain cleavage site of FAK and have generated mutant FAK that is resistant to calpain proteolysis but retains proper localization and biochemical interactions. Expression of wild-type but not calpain-resistant FAK restores the adhesion dynamics of talin in FAK-deficient cells. Collectively, our data highlight a novel function for calpain proteolysis of FAK in regulating adhesion dynamics.

We found that the calpain cleavage site of FAK resides between consensus caspase cleavage sites (32), indicating that this region is particularly susceptible to proteolytic regulation. Caspases have been demonstrated to cleave FAK under conditions of apoptosis (32–35). Recent evidence has also shown the incorporation of caspase-8 into a complex containing FAK and calpain 2 to promote tumor cell migration and metastasis (36). Alternatively, it has been proposed that calpain
cleavage may play a role in terminating FAK signaling by attenuating its kinase activity (27).

Our findings suggest that calpain proteolysis of FAK regulates the dynamic turnover of talin at adhesions. This is interesting in light of our previous study (17) that demonstrated that calpain proteolysis of talin is necessary for its own disassembly from focal adhesions as well as for affecting the dynamics of other adhesion components. We now provide evidence to suggest that calpain cleavage of FAK can also affect talin dynamics, indicating that proteolysis of multiple substrates rather than one specific substrate at adhesion sites is likely important for regulating the dynamic turnover of adhesions. However, how calpain cleavage of FAK regulates the adhesion dynamics of talin has yet to be defined. Also, at present, our data cannot determine whether the simultaneous or sequential cleavage of talin and/or FAK is required for regulating adhesion dynamics. Interestingly, similar to our findings with talin proteolysis (data not shown), we did not find a significant difference in migration in FAK-deficient cells re-expressing wild-type or calpain-resistant FAK, suggesting that proteolysis of multiple adhesion proteins may be necessary to affect cell migration. Future studies should address whether the inability to cleave both FAK and talin leads to a synergistic impairment of adhesion dynamics.

We cannot exclude the possibility that the effects of calpain-mediated proteolysis may depend on cellular context. Several oncogenic programs have been shown to exhibit differential cleavage of adhesion proteins (37). For example, in Src-transformed cells, FAK is extensively cleaved compared with other adhesion complex proteins (38, 39). In addition, human tumors overexpressing cyclin E have been associated with increased proteolysis of FAK (40). By contrast, in myoblasts overexpressing calpastatin, the proteolytic patterns of talin and FAK are unchanged, whereas an accumulation of MARCKS (myristoylated alanine-rich C kinase substrate) has been observed, indicating that calpain may preferentially cleave MARCKS to affect myoblast migration (41, 42).

It is intriguing that calpain proteolysis of FAK generates an FRNK-like fragment, suggesting that calpain proteolysis can generate a functional FRNK-like protein temporally and spatially within cells. Interestingly, we detected an increase in expression of an FRNK-like protein in calpain-deficient mouse embryonic fibroblasts and v-Src-transformed NIH 3T3 fibroblasts. FRNK expression is under the control of an alternative promoter that resides within an intron of FAK (29). These findings suggest that increased FRNK may compensate for the absence of calpain-mediated generation of the 35-kDa C-terminal fragment in calpain-deficient cells. Previous studies have shown that adhesion disassembly is disrupted but not abrogated in cells lacking calpain (15, 16). It is possible that, in cells deficient in calpain activity, the enhanced generation of FRNK functions as a compensatory mechanism to regulate adhesion turnover. Indeed, exogenously expressed FRNK has been demonstrated to function as a dominant-negative inhibitor of FAK (2, 43, 44). Intriguingly, a parallel mode of regulation has been observed in hippocampal neurons, in which the expression of protein kinase Mζ is directed by an intronic promoter within the protein kinase Cζ gene (45). The calpain-dependent proteolytic fragment of protein kinase C is similar in size to the protein generated from the alternative gene transcript and may be important in memory formation (46). Thus, it is very intriguing to speculate that calpain may play a role in mediating cross-talk between transcriptional and post-translational mechanisms of signaling.

In summary, our findings demonstrate a novel role for calpain-mediated limited proteolysis of FAK in affecting talin dynamics and support the notion that proteolysis of adhesion proteins is an important mechanism for regulating the turnover of adhesions. It is interesting to speculate that FAK may modulate proteolysis-mediated effects on adhesion dynamics both by functioning as an adaptor protein through its capacity to interact with calpain 2 (47) and by its direct proteolysis by calpain 2. With our successful generation of a calpain-resistant FAK mutant, future studies should investigate the interplay between FAK and calpain 2 binding and cleavage in modulating adhesion dynamics and cell migration. A challenge will be to determine how the proteolysis of different substrates temporally and spatially within cells affects adhesion turnover and cell motility.

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