Integrin-associated intracellular Ca\(^{2+}\) oscillations modulate cell migration, probably by controlling integrin-mediated release of the cell rear during migration. Focal adhesion kinase (FAK), via its tyrosine phosphorylation activity, plays a key role in integrin signaling. In human U87 astrocytoma cells, expression of the dominant negative FAK-related non-kinase domain (FRNK) inhibits the Ca\(^{2+}\)-sensitive component of serum-dependent migration. We investigated how integrin-associated Ca\(^{2+}\) signaling might be coupled to focal adhesion (FA) dynamics by visualizing the effects of Ca\(^{2+}\) spikes on FAs using green fluorescent protein (GFP)-tagged FAK and FRNK. We report that Ca\(^{2+}\) spikes are temporally correlated with movement and disassembly of FAs, but not their formation. FRNK transfection did not affect generation of Ca\(^{2+}\) spikes, although cell morphology was altered, with fewer FAs of larger size and having a more peripheral localization being observed. Larger sized FAs in FRNK-transfected cells were not disassembled by Ca\(^{2+}\) spikes, providing a possible explanation for impaired Ca\(^{2+}\)-dependent migration in these cells. Stress fiber end movements initiated by Ca\(^{2+}\) spikes were visualized using GFP-tagged myosin light chain kinase (MLCK). Ca\(^{2+}\)-associated movements of stress fiber ends and FAs had similar kinetics, suggesting that stress fibers and FAs move in a coordinated fashion. This indicates that increases in Ca\(^{2+}\) likely trigger disassembly of adhesive structures that involves disruption of integrin-extracellular matrix interactions, supporting a key role for Ca\(^{2+}\)-sensitive inside-out signaling in cell migration. A rapid increase in tyrosine phosphorylation of FAK was found in response to an elevation in Ca\(^{2+}\) induced by thapsigargin, and we propose that this represents the initial triggering event linking Ca\(^{2+}\) signaling and FA dynamics to cell motility.

Cell migration is a cyclic process involving initial protrusion of the leading edge, formation of adhesive sites, contraction of the cell body, and release of adhesive sites at the cell rear (1). Adhesive sites are dynamic membrane structures that vary in size and composition during migration. Integrins, actin stress fibers (SFs)\(^1\) and other structural proteins, and regulatory signaling molecules cluster at focal adhesions (2). Focal adhesions (FAs) serve as points of traction for contractile forces underlying forward cell movement and their dynamics are finely regulated. For example, FAs are highly motile in stationary fibroblasts but are largely stationary in migrating fibroblasts, thereby transducing contractile forces into movement (3). This suggests the existence of a molecular clutch that couples cytoskeleton-mediated traction and cell contraction.

Focal adhesion kinase (FAK) is activated and localized at FAs upon cell adhesion to the extracellular matrix (ECM; Refs. 4 and 5). Given the abundance of FAs and the reduced migration of fibroblasts from FAK null mice (6), FAK is likely involved in FA remodeling during migration. FAK-related non-Kinase (FRNK), the non-catalytic C-terminal portion of FAK containing the FA targeting sequence, is also expressed as a separate dominant negative protein (7). The differential expression of FAK and FRNK is transcriptionally regulated, each of these proteins having distinct promoters within the FAK gene (8). Although the function of endogenous FRNK is not clear, FRNK has been used to alter signaling via endogenous FAK. When overexpressed in cells, FRNK acts as a negative regulator of FAK activity, inhibiting phosphorylation of FAK and different FAK-related processes, including cell cycle progression (9, 10), cell spreading on fibronectin (7, 11), and migration (12, 13). This suggests that the inhibitory effects of FRNK in migration might arise from altered FAK localization and phosphorylation.

We and others reported that migration is dependent on Ca\(^{2+}\) signaling in astrocytoma (14), smooth muscle cells (15), neutrophils (16), and neurons (17). In cerebellar granule cells, Ca\(^{2+}\)-dependent migration is correlated with the amplitude and frequency of Ca\(^{2+}\) spikes (17), which may regulate different steps during migration. Disruption of integrin-mediated adhesion involves Ca\(^{2+}\)-sensitive proteins, including calpain (18, 19), myosin light chain kinase (MLCK; Ref. 20), and calcineurin (21). These data indicate that Ca\(^{2+}\) signaling may be a component of the molecular clutch regulating transitions between stationary and non-stationary FAs.

\(^1\) The abbreviations used are: SF, stress fiber; FA, focal adhesion; FAK, focal adhesion kinase; FRNK, FAK focal adhesion-related non kinase domain; ECM, extracellular matrix; MLCK, myosin light chain kinase; Ca\(^{2+}\), calcium; Ab, antibody; mAb, monoclonal antibody; GFP, green fluorescent protein; YFP, yellow fluorescent protein; EMEM, Eagle’s minimum essential medium; BAPTA-AM, 1,2-bis(2-aminoethoxy)-N,N,N’,N’-tetraacetic acid tetrakis(acetoxymethyl ester); TRITC, tetramethylrhodamine isothiocyanate; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; ANOVA, analysis of variance.
To test whether Ca2+ signaling affects FA organization, confocal microscopy was used to visualize simultaneously Ca2+ levels and dynamics of FAs or actin SFs in U87 cells expressing FA (FAK, FRNK) or cytoskeletal (MLCK) proteins tagged with GFP. We report that Ca2+ spikes trigger movement and disassembly of FAs. Although FRNK expression did not suppress Ca2+-dependent FA disassembly, more Ca2+-insensitive FAs having a larger surface were observed in FRNK cells. Our results provide an explanation linking FA disassembly to a temporally accurate cellular signal.

EXPERIMENTAL PROCEDURES

Reagents and Cells—Cell culture media (EMEM), fetal calf serum, HEPES, L-glutamine, penicillin, streptomycin, gentamicin, and trypsin-EDTA were from Invitrogen; Fura-Red-AM, BAPTA-AM, and pluronie acid were from Molecular Probes; Matrigel and the monoclonal antibody (mAb) against FAK kinase domain were from Interchim; phallloidin-TRITC, mAb against the FAK C-terminal region (aminos 1039–1052), and anti-MLCK mAb were from Sigma; the anti-Tyr397-phosphorylated FAK Ab was from BIOSOURCE; secondary horseradish peroxidase-conjugated Abs were from Promega; the FITC-labeled goat anti-mouse (GAM-FITC) Ab was from Zymed Laboratories Inc. The human astrocytoma U87 cell line was obtained from the ATCC. Cells were maintained at 37 °C in a humidified incubator gassed with 5% CO2 in air on type 1 collagen (0.06 mg/mL-coated plastic dishes in EMEM supplemented with 10% heat-inactivated fetal calf serum, 0.6 mg/ml glutamine, 200 IU/ml penicillin, 200 IU/ml streptomycin, and 0.1 mg/ml gentamicin).

Plasmids and Transfection—Fluorescent FA-targeted protein was made by fusion of FAK (human T lymphocyte pCDM8-FAK plasmid; Ref. 40) next to the 5′-end of “yellow Cameleon-2” (pCDNA3-Yem2 plasmid; Ref. 22). To allow fusion of FAK in continuity with the Yem2 reading frame, the stop codon next to Yem2 was replaced by a tyrosine (QuickChange, Stratagene). FAK cDNA was amplified by PCR using a forward primer with an XbaI site and a 3′ primer containing a NheI site. The PCR product was digested with MfeI and NheI and cloned in-frame with the NheI site and corresponding compatible sites, EcoRI and XbaI, located in the multiple cloning site of the newly mutated pCDNA3-Yem2 vector, adjacent to EYFP, to give FAK-Ycam. To create FRNK-Ycam, the FRNK domain was amplified by PCR using pCDM8-FAK as template, a forward primer with an EcoRI site at the 5′ end of the glutamic acid codon 681 relative to the FAK start codon, and the same reverse primer as for FAK amplification, adding a NheI site. The PCR product was digested with EcoRI and NheI and cloned in-frame with the EYFP coding sequence in the EcoRI/XbaI compatible sites of the mutated pCDNA3-Yem2 vector. The MLCK-210-GFP construct (24) was in pEGFP-N1 (Clontech). The MLCK vector was verified by sequencing. The plasmids were isolated (JetStar, Genomed) before transfection by electroporation. Cells (5 × 105) were resuspended at 106 cell/mL in EP buffer (in mM: 50 K2HPO4, 20 KH2PO4, 20 KOH, pH 7.4). Plasmidic DNA (2 μg of construct-encoding plasmid, 8 μg of pBluescript) was diluted in 100 μl of EP buffer; 4 μl of 1 M MgSO4 were added and incubated with 50 μl of cell suspension for 20 min at room temperature. The cell/DNA mixture was electroporated in a 0.4-cm cuvette (Bio-Rad) using a flow cytometer (FACStar, Becton-Dickinson) before use. The cell/DNA mixture was electroporated in a 0.4-cm cuvette (Bio-Rad) using a flow cytometer (FACStar, Becton-Dickinson) before use.

Migration Assay—A wound-healing migration model was used, as described previously (15). FAK-Ycam or FRNK-Ycam cells (2 × 106 cells/mL) were grown to confluence in Matrigel-coated (178 μg/mL) plates. A wound was made using a 2-mm wide, square, sterile plastic template (Bio-Rad Gene Pulser; 500 microfarads, 240 V). Cells were then placed in 10 mL of EMEM with 10% fetal calf serum in 80-mm2 dishes. Cells were selected 24 h later using 800 μg/mL G418 (Sigma) and maintained with 400 μg/mL G418. Cells were sorted to obtain >80% expressing cells using a flow cytometer (FACSsort, Becton-Dickinson) before use.

RESULTS

Human U87 Astrocytoma Cells Expressing FRNK-Ycam Have Impaired Calcium-Dependent Migration—We report that migration of U87 cells is associated with Ca2+-oscillations (14), as intracellular Ca2+ buffering by BAPTA partly inhibits FA-dependent migration. To analyze the role of FAK in Ca2+-dependent migration, U87 cells were transfected with FAK or the dominant negative FRNK, fused to the fluorescent yellow Cameleon-2 (Yem) tag, a fluorescence resonance energy transfer-based Ca2+ sensor containing CFP and EYFP (22). Expression of FAK-Ycam or FRNK-Ycam did not alter endogenous FAK levels compared with controls (Fig. 1A, left). FRNK-Ycam expression (Fig. 1A) was deduced by sub-
Ca\textsuperscript{2+} Oscillations Trigger Focal Adhesion Disassembly

![Image](http://www.jbc.org/content/early/2018/07/13/jbc.M118.007443/Figure1.large.jpg)

**Fig. 1.** Effects of FRNK-Ycam and FAK-Ycam expression in U87 astrocytoma cells on endogenous FAK expression, migration, and generation of calcium spikes. A, immunoblots of FAK-Ycam (199 kDa), FRNK-Ycam (117 kDa), and endogenous FAK (125 kDa) using Abs against kinase (left) or C-terminal (right) FAK domains. The right-most panel shows endogenous FAK plus FRNK-Ycam. B, migration measured 24 h after lesion in a wound-healing model (left). Serum-dependent migration in FRNK-Ycam cells was inhibited (*) compared with control, while BAPTA caused inhibition of serum-dependent migration (△) only in FAK-Ycam cells (n = 6, >180 cells/condition; p < 0.05, Student's unpaired t test). The number of Ca\textsuperscript{2+} spikes/15 min (right) was the same (one-way ANOVA) for FAK-Ycam (389 cells from four separate dishes) and FRNK-Ycam (505 cells from four separate dishes) cells. C, distribution of endogenous FAK at FAs was not altered in FRNK-Ycam cells. Endogenous FAK localization in a FRNK-Ycam cell with an anti-FAK Ab/TRITC-labeled secondary Ab (left); FRNK-Ycam was detected by EYFP fluorescence (right).

Intracellular buffering of Ca\textsuperscript{2+} with BAPTA inhibited serum-dependent migration by 33% in FAK-Ycam cells, but had no effect on serum-independent migration. Similarly, the frequency of Ca\textsuperscript{2+} spikes over 15 min in cells expressing FAK-Ycam or FRNK-Ycam was similar (Fig. 1B, right) and not different compared with controls (not shown). Among FRNK-Ycam cells, the distribution of endogenous FAK at FAs, evaluated using an Ab against the FAK kinase domain, was unaltered (Fig. 1C, left), as found previously (7). This suggests that sufficient endogenous FAK is expressed in most FRNK-transfected cells (Fig. 1C), perhaps accounting for the unaltered generation of Ca\textsuperscript{2+} spikes (23). Thus, the Ca\textsuperscript{2+}-dependent migration defect of FRNK-Ycam cells is not due to decreased Ca\textsuperscript{2+} signaling.

**Single Calcium Spikes Trigger FA Movement and Disassembly**—Since differences in Ca\textsuperscript{2+} signaling do not underlie impaired migration of FRNK-Ycam cells, possible effects of Ca\textsuperscript{2+} spikes on FAs were investigated by simultaneously measuring Ca\textsuperscript{2+} variations (using Fura Red) and FA dynamics (using EYFP fluorescence of FAK-Ycam and FRNK-Ycam) in migrating cells. Color overlays of three sequential images (Fig. 2A) distinguish immobile FAs (black) versus motile FAs (rainbow). Most FAs were immobile in FAK-Ycam cells irrespective of Ca\textsuperscript{2+} oscillations. Motile FAs were present in oscillatory and non-oscillatory cells, being often localized at one edge of a migrating cell (Fig. 2A). FA movements were linear and usually resulted in FA disassembly, with EYFP fluorescence decreasing or disappearing (Fig. 2B), while immobile FAs never disassembled. In many cases, FA movement and subsequent disassembly was triggered by a Ca\textsuperscript{2+} spike (Fig. 2B). In oscillatory FRNK-Ycam cells, such FA disassembly was temporally correlated with Ca\textsuperscript{2+} spikes for 64% (37/58) of motile FAs. For 19% of motile FAs (11/58), the Ca\textsuperscript{2+} oscillation frequency was too high to determine clearly a correlation, and for the remaining 17% (10/58), no correlation was found. This indicates that Ca\textsuperscript{2+} spikes were responsible for the dynamics of a subset of FAs.

Somewhat surprisingly, FA disassembly in oscillatory FRNK-Ycam cells (Fig. 2, C and D) was also temporally correlated with a Ca\textsuperscript{2+} spike for 50% (35/70) of motile FAs; the Ca\textsuperscript{2+} oscillation frequency was too high to establish a correlation for 30% of FAs (21/70), and there was no correlation for the remaining 20% (14/70).

In FAK-Ycam (Fig. 3; 45 FAs, n = 19 cells) and FRNK-Ycam cells (not shown; 30 FAs, n = 10 cells), we found no clear link between oscillatory (Fig. 3, A and B) versus non-oscillatory (Fig. 3, C and D) Ca\textsuperscript{2+} behavior and formation of FAs (evaluated as EYFP fluorescence increases). For FAK-Ycam cells, 49% (22/45) of newly formed FAs were seen in oscillatory cells, while 51% (23/45) were not, as was also the case for FRNK-Ycam cells (not shown). Thus, unlike FA disassembly, Ca\textsuperscript{2+} spikes and FA formation were not obviously correlated.

**Preferential Calcium-dependent Disassembly of Small FAs in U87 Cells Expressing FAK-Ycam**—Strikingly, the number, size, and localization of FAs were different in FRNK-Ycam compared with FAK-Ycam cells (Fig. 4, A and B). FRNK-Ycam cells had 3-fold fewer FAs (25 ± 3/cell, n = 4 cells), which were larger (Fig. 4, C and D) and more peripherally located com-

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2 P. Rondé, G. Giannone, A. Scherberich, A. Beretz, J. Haiech, and K. Takeda, submitted for publication.
**FIG. 2. Calcium spikes trigger movement and disassembly of FAs.** A and C, overlay of three sequential images (blue, red, and green, taken at times i, ii, and iii indicated in B and D) of EYFP emission in a FAK-Ycam cell (A and B) and a FRNK-Ycam cell (C and D) having Ca\(^{2+}\) oscillations (red traces in B and D). Immobile FAs are black and motile FAs appear as rainbows (blue to green). The boxed areas (left panels, A and C) are shown enlarged on the right. B and D, simultaneous measurement of Ca\(^{2+}\) spikes (red traces, Fura Red at 585 nm) and FA dynamics (green trace, EYFP at 522 nm), from the encircled region of interests in A and C.

**FIG. 3. FA formation in FAK-Ycam cells is not temporally correlated with calcium spikes.** Sequential, three-color overlay from typical cells with (A) or without (C) Ca\(^{2+}\) spikes. Immobile FAs appear white, moving FAs appear as rainbows (blue to green), and newly forming FAs appear as a single color (yellow or green). The boxed area (left panels) is enlarged in the right panels. B and D, simultaneous measurement of Ca\(^{2+}\) spikes (red trace, Fura Red at 585 nm) and FA dynamics (green trace, EYFP at 522 nm, from encircled regions of interest in A and C; i, ii, and iii indicate when the three-color images were taken).
pared with FAK-Ycam cells (74 ± 10 FAs/cell, n = 4). Morphometric analysis (ratio of longest to shortest cell lengths) showed that FRNK-Ycam cells were more elongated (3.0 ± 0.2, n = 51) than FAK-Ycam cells (2.3 ± 0.1, n = 69), suggesting that they are more strongly held to the ECM. Comparison of the Ca^{2+} dependence of FA dynamics revealed that the size distribution of motile Ca^{2+}-sensitive FAs in FAK-Ycam (F, n = 30 FAs, 10 cells) and FRNK-Ycam (F, n = 44 FAs, 19 cells) is shown. The distribution in D is different compared with the three other distributions (tested by ANOVA).

Calcium Elevation Rapidly Increases FAK Tyrosine Phosphorylation—Since FAK regulates FA dynamics (5, 6), and Ca^{2+} spikes trigger FA disassembly, this may be related to FAK activity. Therefore, FAK tyrosine phosphorylation was evaluated after thapsigargin-induced Ca^{2+} elevation (14). Increases in Tyr^{397} phosphorylation of endogenous FAK and FAK-Ycam were detected within 30 s after 1 μM thapsigargin treatment (Fig. 5A). After 1 min, phosphorylation was maximal (73% increase above control; Fig. 5B). Thus, rapid phosphorylation of FAK in response to Ca^{2+} increases provides a possible link between FA movements and Ca^{2+} spikes, supporting that these are regulatory events in migration.

Calcium-induced FA Disassembly Correlates with Retraction of Stress Fibers—As FA disassembly involves disruption of ECM-integrin and/or SF-integrin interactions, we simultaneously followed the dynamics of SF ends and Ca^{2+} signals using MLCK-GFP-transfected cells (Fig. 6). No differences were found in the localization, distribution, and expression of MLCK in MLCK-GFP cells compared with controls (Fig. 6, A and B), as reported previously (24). Moreover, the MLCK-GFP construct used here has the same activity in phosphorylating the 20-kDa regulatory light chain kinase when compared with endogenous MLCK (25). Evaluation of SF end dynamics (using MLCK-GFP fluorescence; Fig. 6C) and Ca^{2+} variations (using Fura Red) revealed that SF end movements were triggered by a Ca^{2+} spike (Fig. 6D).

The average latency between a Ca^{2+} spike and SF end movement was 28 ± 6 s, with a rate of movement of 0.25 ± 0.04 μm/min (n = 21 SFs, 6 cells; Fig. 7). These SF kinetic parameters were compared with those for motile Ca^{2+}-sensitive FAs in FAK-Ycam and FRNK-Ycam cells (Fig. 7C). The latency between Ca^{2+} spikes and the onset of FA movement was 33 ± 5 s in FAK-Ycam cells (n = 21 FAs, 8 cells) and 31 ± 5 s in FRNK-Ycam cells (n = 35 FAs; 14 cells), with a FA speed of 0.18 ± 0.02 and 0.22 ± 0.02 μm/min, respectively. These kinetic parameters were not different, suggesting that Ca^{2+} spikes trigger coordinated movement of SF-associated FA complexes. This implies that during cell migration, Ca^{2+} signaling results in disruption of ECM-integrin interactions rather than a decrease in FA-cytoskeletal interactions.

**DISCUSSION**

We investigated the implication of intracellular Ca^{2+} elevations in FA dynamics during migration of U87 astrocytoma cells. During oscillatory Ca^{2+} signaling, single Ca^{2+} spikes triggered FA disassembly and subsequent cell edge retraction.
In FRNK-Ycam cells, smaller sized FAs were more sensitive to Ca\textsuperscript{2+} triggered disruption compared with large FAs, consistent with FA stability being a limiting factor in motility. FAK phosphorylation was rapidly induced by a Ca\textsuperscript{2+} increase, indicating that FAK is a Ca\textsuperscript{2+} target during migration. Analysis of FA and SF kinetic parameters suggests that Ca\textsuperscript{2+} signaling coordinates disruption of ECM-integrin interactions at FAs.

Cell migration includes phases of protrusion, adhesion, and retraction (1), involving, respectively, formation, strengthening, and disassembly of focal contacts. FAK plays an important role in the dynamics of cell adhesion, but is not required for FA formation beneath lamellipodia of migrating cells (26). FAK aggregates to clustered integrin receptors with or without ligand occupancy (27), consistent with FAK localization to FAs being an early or late event in the cascade of interactions, respectively, leading to formation or disruption of integrin-cytoskeletal linkages. This justifies our choice of FAK-Ycam (22) as a probe for FA dynamics, indirectly allowing detection of clustered integrins. FAK-Ycam and FRNK-Ycam were localized to newly forming, punctate structures in protrusive areas, further supporting their use to follow indirectly integrin association/dissociation in FAs. As suggested previously (28), these punctate structures very probably are focal complexes, putative precursors of FAs.

FAK tyrosine kinase activity is involved in the regulation FA turnover (29). In FAK-deficient cells, reduced motility is accompanied by an increased number of FAs (6). FRNK expression inhibits integrin-stimulated migration and phospho-
rylation of endogenous FAK and other FA components such as paxillin and tensin (7, 11, 13). Thus, FAK phosphorylation likely governs FA dynamics and, hence, motility. In FAK-Ycam cells, FA morphology was similar to controls, and as found in previous studies using FAK-transfected U87 cells (12), migration was unchanged, unlike in Chinese hamster ovary cells where migration increased after FAK overexpression (30). Expression of FRNK-Ycam induced elongated morphology and a sparse and peripheral distribution of enlarged FAs. Much evidence supports that defective FAK signaling leads to enlarged FAs. Cells expressing a kinase-deficient mutant of Src have larger FAs and reduced migration (31). The interaction and activation of Src occurs at the FAK autophosphorylation site, which is absent for FRNK (11). Src kinase activity weakens integrin-cytoskeletal linkages (32) and may stimulate FA turnover by favoring lateral diffusion of integrins away from FAs. Thus, reduced FA dynamics leading to enlarged FAs is consistent with decreased migration of FRNK-Ycam cells. The disassembly of large FAs in FRNK-Ycam cells was Ca2+-insensitive, possibly because for this subset of FAs, endogenous Ca2+ levels were small compared with FRNK levels. Our data support that FA size affects migration by also reflecting the Ca2+ sensitivity of FAs and, hence, their remodeling.

FA disassembly involves disruption of ECM-integrin and/or integrin-cytoskeletal interactions, which are regulated by several calciproteins. For instance, contractile force activation via Ca2+/calmodulin-dependent MLCK may strengthen integrin-cytoskeletal linkages (26) and is necessary for Ca2+-dependent migration of neutrophils (20). Conversely, integrin-cytoskeletal linkages are disrupted by calpain, a Ca2+-dependent protease, and calpain inhibition or disruption mimics the effects of FRNK expression on FA morphology (18, 19). Calreticulin appears to be essential for integrin affinity/avidity to both intracellular and extracellular partners. Calsequestrin, a protein phosphatase, is involved in integrin recycling to the front of migrating neutrophils (21), probably via affinity modulation. We show that Ca2+ spikes trigger FA disassembly and propose that ECM-integrin linkages are disrupted, given the identical kinetic parameters for FA and SF dynamics, which suggests coordinated FA/SF movement. In agreement, in migrating fibroblasts, FAs move with a similar speed of 0.12 ± 0.08 μm/min and remain associated with SFs in retractile edges (3). Our observation of linearly distributed FAs, illustrating this asymmetry. Since FA disassembly but not formation was temporally correlated with Ca2+ spikes, Ca2+ signaling and/or regulatory Ca2+ target proteins may be spatially restricted to discrete subcellular compartments (38, 39). We are currently investigating compartmentalized Ca2+ signaling in migrating cells using our Ycam constructs as local detectors of Ca2+ near FAs.

That a single Ca2+ spike is sufficient to trigger FA disassembly agrees with initiation of a regenerative process leading to irreversible FA disruption. The Ca2+-triggered event might transiently increase FAK signaling, perhaps followed by a Ca2+-independent process. This implies a latency between the Ca2+ spike and cell edge retraction resulting from FA disassembly. Consistent with our data, Ca2+ elevation and increased migration speed were positively correlated in neutrophils (16), but since there was a 20-s delay between Ca2+ elevation and increased motility, Ca2+ was proposed not to be the immediately causal signal. However, we show that FA disassembly begins 30 s after a Ca2+ spike, and once triggered, several minutes are required for complete disassembly. This supports that Ca2+ elevation is a proximal signal leading to increased motility. Finally, rapid thapsigargin-induced FAK Ty397 phosphorylation agrees with Ca2+-dependent FAK activation being an initial event associated with FA disassembly.

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