Tyrosine Phosphorylation of Focal Adhesion Kinase (p125FAK): Regulation by cAMP and Thrombin in Mesangial Cells

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ABSTRACT
Stress fibers, composed of actin filaments, converge upon and associate with a number of proteins, including focal adhesion kinase (p125FAK), and integrin receptors to form areas of close contact between cells and the extracellular matrix referred to as focal adhesions. Treatment of mesangial cells with cAMP-elevating agents causes a loss of focal adhesions, fragmentation of stress fibers, and decreased tyrosine phosphorylation of p125FAK. Thrombin reverses these effects of cAMP, and this model can be used to address some of the cellular mechanisms involved in regulating the loss and formation of focal adhesions. This study reports the effects of cAMP and thrombin on mesangial cell shape, distribution of actin, formation of stress fibers, and tyrosine phosphorylation of p125FAK. cAMP-treated cells display a condensed cell body with slender processes that traverse the area formerly covered by the cell. Addition of thrombin to these cells restores actin filaments (stress fibers) and increases tyrosine phosphorylation of p125FAK, and the cells resume a flattened morphology, even in the continued presence of cAMP-elevating agents. Peptides that mimic the tethered ligand portion of the thrombin receptor have the same effects on cell morphology and stress fiber formation as thrombin. In selected experiments, agents that disrupt either stress fibers (cytochalasin D) or microtubules (nocodazole; Sigma Chemical, St. Louis, MO) were used to examine the role of these cytoskeletal elements in thrombin-induced restoration of focal adhesions. Cytochalasin D blocked the ability of thrombin to restore focal adhesions and phosphorylate p125FAK. The effects of nocodazole, an agent that destabilizes microtubules (but which has no known receptor), are very similar to those of thrombin. The findings discussed in this study indicate that thrombin can modulate the formation of focal adhesions. The organization of stress fibers and microtubules is apparently intimately related to the phosphorylation of p125FAK and can be modulated by soluble receptor agonists such as thrombin or via altered polymerization of microtubules.

Key Words: Mesangial cell, focal adhesion kinase, cell shape

α-thrombin (thrombin), in addition to its effects on coagulation, has hormone-like effects on cells, which include alterations of cell shape and stimulation of cell proliferation (1–3). Cellular proliferation (4,5) and accumulation of extracellular matrix (6,7) are frequently observed in glomerular diseases, and alterations in cell-matrix interactions, including those mediated by integrins, are likely involved in these pathological changes as well (8,9). The cytoplasmic domains of integrins have no enzymatic activity, yet integrin-receptor occupancy promotes the formation of focal adhesions, increases tyrosine phosphorylation of p125FAK (10,11), alkalinizes pH (12), and stimulates phosphatidylinositol 5-kinase (13). Thus, proposed candidate signaling molecules that may regulate the integrated formation of focal adhesions and actin filaments. Soluble receptor agonists can modulate the affinity of integrin receptors for their ligands (14), and vasopressin, bombesin, and src-mediated pathways may converge on p125FAK to influence focal adhesions and thereby regulate diverse cellular functions, including cell shape, growth, adhesion, and motility (10,15,16). Convergence of these pathways may involve autophosphorylation of p125FAK and binding to pp60src, or to its normal counterpart, pp52src-lyn, to direct these proteins to sites of cellular adhesion (17,18).

The studies presented here examine a model in which mesangial cell focal adhesions are rapidly reformed by thrombin after treatment of cells with cAMP-elevating agents. We conclude that thrombin promotes the integrated formation of actin filaments and focal adhesions and stimulates tyrosine phosphorylation of p125FAK. The effects of nocodazole (Sigma Chemical, St. Louis, MO), a microtubule toxin without known receptor-mediated effects, are similar to those of thrombin, and suggest that microtubules are intimately involved in the regulation of stress fibers and focal adhesions.
METHODS

Cell Culture

Human mesangial cells were cultured from glomeruli isolated from human kidneys immediately after surgical nephrectomy, as described previously (19). Cells were grown in Waymouth’s MB752/1 medium (Gibco) supplemented with ITS (insulin/transferrin, selenium; Collaborative Research) and 20% fetal calf serum (Irvine Scientific). Before use, cells were serum starved for 48 h by incubation in RPMI supplemented with 10 mM N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES).

Reagents and Experimental Conditions

The experimental medium was RPMI supplemented with 10 mM HEPES. Methyl-(5-[2-thienylcarbonyl]-1H-Benzoimidazol-2-yl) carbamate (nocodazole) was prepared as a 10-mg/mL stock in dimethyl sulfoxide (DMSO). For experiments, the stock was diluted 1:100 into RPMI and used at 10 μL/mL for a final concentration of 3.3 μM. Thrombin (courtesy of Dr. John Fenton, Wadsworth Center Labs, Albany, NY) was used at a final concentration of 10 nM. SFLL-Tyr-Ile-Thr (Sigma) was diluted into DMSO to make a 1-mg/mL stock. For experiments, cytochalasin D (Sigma) was diluted into DMSO to make a 1-mg/mL stock. The standard experimental protocol was to treat serum-starved cells for 45 min with a 1-mM concentration of the cyclic nucleotide analog, chlor-AMP [8-(4-chlorophenylthio)adenosine-3'-5'-cyclic monophosphate (chlor-AMP) and isobutylmethylxanthine] were purchased from Sigma. The standard experimental protocol was to treat serum-starved cells for 45 min with a 1-mM concentration of the cyclic nucleotide analog, chlor-AMP [8-(4-chlorophenylthio)adenosine-3'-5'-cyclic monophosphate] and 1 mM MIX (isobutylmethylxanthine), a potent phosphodiesterase inhibitor (20, 21). Thrombin (Sigma) was diluted into DMSO to make a 1-mg/mL stock.

Assay of Actin Distribution

Cellular actin can be separated into three pools: (1) a Triton-insoluble, gelsolin-poor pool of filamentous F-actin; (2) a Triton-soluble, gelsolin-rich pool of F-actin, and (3) G-actin (23). The Triton-insoluble F-actin is a minority of F-actin, is sedimented by 15,000 × g centrifugation, and is associated with α-actinin, but not gelsolin. Triton-insoluble actin likely has a cross-linked supramolecular structure (24). By contrast, gelsolin-associated F-actin accounts for the majority of cellular F-actin and requires high g forces (366,000 × g, 5 min) to sediment. Gelsolin-associated actin is likely present as short actin filaments (23). Finally, G-actin is monomeric, accounts for the majority of actin in unstimulated cells, and fails to sediment even at high g forces (23). Cells cultured in 100-mm dishes were washed once with PBS at room temperature after experiments. Imidazole buffer (1.5 mL) was added (10 mM imidazole, 10 mM EDTA, 40 mM KCl, 1 mg/mL aprotinin, 1 mM polymethylsulfonyl fluoride (PMSF), and 1% Triton X-100). Aprotinin was added fresh each time buffer was prepared, and PMSF was added from a 100 mM stock in DMSO that was maintained frozen in aliquots. Cells were lysed for exactly 15 min at room temperature. The imidazole buffer that contained Triton-soluble actin was removed earlier from the monolayer) was sedimented at 15,000 × g for 2 min at room temperature to pellet the Triton-insoluble actin. The supernatant was discarded, and the pellet resuspended in 250 μL of 2 × sample buffer.

The Triton-soluble actin (present in the stored buffer that was removed earlier from the monolayer) was sedimented at 366,000 × g for 7 min at 4°C. The resulting pellet was resuspended in 200 μL of 1 × sample buffer while the supernatant was used to precipitate G-actin by the addition of 15 μL of 0.15% deoxycholate (DOC) to each sample. After being mixed, samples were incubated for 10 min at room temperature, followed by an addition of 15 μL of 72% trichloroacetic acid. Samples were then mixed and centrifuged for 10 min at 15,000 × g. The resulting supernatant was discarded, and the pellet resuspended in 250 μL of 1 × sample buffer. Before being loaded onto electrophoresis gels, the samples were boiled for 5 to 10 min in sample buffer that contained 0.05% mercaptoethanol.

Equal sample volumes were then loaded onto a 4% stacking sodium dodecyl sulfate-polyacrylamide gel over a 7.5% resolving gel according to manufacturers specifications (Bio-Rad). Initial protein determinations (BCA Protein Assay Kit; Bio-Rad) indicated that total protein content for the Triton-insoluble fraction was 175 to 225 μg and 80 to 100 μg for the 366,000 × g fraction. We did not attempt to assay the G-actin fraction for protein because of the presence of DOC. Subse-
After electrophoresis for 1 h at 200 V (Bio-Rad Mini-Protein II apparatus), samples were blotted onto nitrocellulose overnight at 4°C at 400 milliamps. Actin was detected using anti-actin antibody, clone C4 (Boehringer) at 1 μg/mL. Bound primary antibody was detected using the Vectastain ABC kit (Vector, Burlingame, CA).

**Tyrosine Phosphorylation**

Tyrosine phosphorylation of focal adhesion kinase (p125FAK) was assayed by immunoblotting with or without prior immunoprecipitation of p125FAK. Cells were lysed with modified RIPA lysis buffer (1% Nonidet P40, 20 mM Tris, 150 mM NaCl, 1 mM Na3VO4, 5 mM EDTA, 0.1 mg/mL aprotinin, and 1 mM PMSF). Aprotinin was stored as a 10-mM stock in frozen aliquots, and added fresh to a final concentration of 1 mM. Cells grown in 100-mm tissue culture dishes were used for experiments. Cells were washed 2 × with cold PBS before the addition of RIPA lysis buffer for 10 min. Cells were scraped and pipetted into pestle-eyepipette tubes (VWR Scientific), and subjected to 20 twists with the pestle while on ice. After centrifugation of cells for 20 min, supernatants were transferred to fresh eyepipette tubes and protein determined by use of the BCA protein assay kit (Bio-Rad). Twenty μg of this initial lysate was loaded onto gels for electrophoresis and blotting if no immunoprecipitation was done. After immunoprecipitation with the antibody 2A7, followed by Western blotting with the BC3 antibody, and in collaboration with Drs. Michael Schaller and Amy Bouton, the amount of p125FAK that was extracted from control, cAMP, and cAMP+thrombin-treated cells was the same (not shown). We therefore proceeded with the experiments by assaying tyrosine phosphorylation directly or after immunoprecipitation with the monoclonal antibody 2A7.

For use in immunoprecipitation, protein A Sepharose (Sigma) was prepared as follows. One g of protein A Sepharose was added to 15 mL of PBS + 0.02% sodium azide and allowed to mix overnight at 4°C. After settling for 30 min, the PBS was aspirated to obtain a 50% suspension of protein A Sepharose. One hundred μL of protein A Sepharose per sample was then aliquoted into an eyepipette tube, centrifuged for 5 min at 4°C, and the pellet then resuspended in 10% BSA (Sigma) made up in RIPA lysis buffer containing 10% glycerol (BSA buffer). The ratio of sepharose to BSA buffer was 1:3 (wt/vol). After three washes in the 10% BSA-containing buffer, the protein A Sepharose was incubated with rabbit antimmune immunoglobulin G (Sigma) overnight at 4°C. The protein A Sepharose was washed three times with 10% BSA buffer and resuspended in an amount of 10% BSA buffer sufficient to aliquot 100 μL to each immunoprecipitate.

For immunoprecipitation, 500 μg of cell lysate was incubated with 4 μL of anti-p125FAK antibody, MAb 2A7 (courtesy of Dr. Thomas Parsons) overnight at 4°C. One hundred μL of protein A sepharose, prepared as described above, was then added with gentle mixing for 1½ h. Immunoprecipitates were then centrifuged and washed with RIPA/glycerol buffer. The washed pellet was resuspended in 95 μL of 2 × sample buffer. After reduction, samples were loaded onto a 7% resolving SDS-PAGE gel with a 4.5% stacking gel. Samples were then electrophoresed at constant current (35 milliamps) for 4 to 5 h at 4°C. The samples were then electroblotted onto nitrocellulose overnight at 4°C at constant current of 400 milliamps.

Phosphotyrosine-containing proteins were detected by immunoblotting. Blots were blocked with blocking buffer (10 mM Triis base, 150 mM NaCl, and 3% BSA) for 2 to 3 h followed by incubation with antiphosphotyrosine antibody (PY20; ICN) in 10 mL of wash buffer (10 mM Triis base and 150 mM NaCl) for 2 h at room temperature. After being washed with wash buffer twice, the blot was washed twice with detergent buffer (wash buffer supplemented with 0.5% Tween 20 and 0.5% Nonidet P40). The blot was then incubated with 3 μCi of 125I protein A (Amersham) in 10 mL of blocking buffer for 1 h, followed by two washes each in wash buffer and detergent buffer. The blot was then developed by autoradiography to detect radiolabelled proteins.

**Image Analysis**

 Autoradiographs and Western blots were analyzed by using IMAGE, an image-processing and analysis computer program for the Macintosh computer, as previously described (25). Relative band densities are displayed in the accompanying graphs and figures. For the studies of actin distribution that were assayed biochemically, data was accumulated in six to ten separate experiments, with duplicates for each variable for each actin fraction. For statistical analysis, either t test or two-way analysis of variance (ANOVA) (experiment by treatment) was used. All assumptions underlying the ANOVA were well satisfied. Comparison of means was by Duncan’s new multiple range test.

**RESULTS**

**Morphological Changes Induced by Elevation of cAMP and Thrombin**

cAMP was elevated by incubating cells for 45 min with the cyclic nucleotide analog, chloro-AMP (8-[4-chloropheny]thio)iodenosine-3'5'-cyclic monophosphate) (1 mM) and the phosphodiesterase inhibitor, isobutylmethylxanthine (also 1 mM). During this period of time, the flat well-spread cells developed a rounded cell body with thin processes that traverse the area formerly covered by the flatter (non-cAMP-treated) cells. Photomicrographs of untreated control cells stained for actin (Figures 1A and 1B), can be compared with Figures 1C and 1D, which demonstrate the fragmentation of stress fibers in cAMP-treated cells in which actin stains as clumps or short segments. As shown in Figure 2, cAMP also causes loss of vinculin-containing focal adhesions. When added to cAMP-treated cells, thrombin restores a flattened cellular morphology and reestablishes stress fibers (Figures 1E and 1F). This effect of thrombin is duplicated by the peptide SPLL (25 μM), which mimics the tethered ligand portion of the thrombin receptor (19) (not shown). There are qualitative morphological differences between the control focal adhesions and those formed after thrombin treatment, but areas of concentrated staining for vinculin reemerge, as shown in Figure 2C. This difference between control and cAMP/thrombin focal adhesions may occur because of the fact that focal adhesions were present for a longer period of time in

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control cells than for those treated with cAMP-elevating agents followed by treatment with thrombin. In a separate study, we demonstrated that the same protocol as utilized for standard microscopic studies of vinculin localization caused loss and reformation of focal adhesions as detected by confocal microscopy (not shown). Images taken at 0.25-μM intervals showed loss and re-formation of concentrated areas of staining for p125FAK at the ventral surface of the cell. This suggests that although standard microscopy does indicate that focal adhesions formed after treatment with thrombin are morphologically smaller than those of controls, the concentrated areas of staining (as proteins aggregate to re-form focal adhesions) are on the ventral cell surface as one would expect. Nocodazole has effects on cAMP-treated cells that are essentially identical to those of thrombin (not shown).

### Actin Distribution

As confirmation and extension of the morphological studies, we examined the effects of cAMP and thrombin on the cellular distribution of actin. Three cellular pools of actin can be distinguished (23). The first is a cytoskeleton-associated pool present in Triton-X-100 lysates centrifuged at 15,000 × g that contains α-actinin but not gelsolin (also referred to as the Triton-insoluble F-actin). This 15,000 × g (15K) fraction is thought to have a cross-linked supramolecular structure (24). The second actin pool contains gelsolin and is pelleted after centrifugation at 366,000 × g (366K), and consists of...
short actin filaments associated with gelsolin. The third actin pool contains monomeric G-actin, which is precipitated by use of trichloroacetic acid.

As shown in Figure 3, elevation of cAMP decreases the relative amount of actin in the 15K fraction while increasing the amount of G-actin. The addition of thrombin to cells treated with cAMP-elevating agents reverses these changes, increasing the relative amount of actin in the 15K fraction and decreasing the G-actin fraction. For the 366K actin, there was a significant decline induced by thrombin treatment alone, and a trend toward an increase with cAMP treatment. In separate experiments during which we looked only at the 366K fraction, we demonstrated that elevation of cAMP increased the content of 366K actin relative to controls (control, 61 ± 7; cAMP, 79 ± 5; N = 3, P < 0.05) and that addition of thrombin to cells with elevated cAMP decreased the relative content of 366K actin (cAMP, 68 ± 4; cAMP + thrombin, 54 ± 6; N = 3, P < 0.05). These experiments confirmed the trends for 366K actin, as shown in Figure 3. Thus, the assays of Triton extracts for actin tend to confirm the morphological observations that cAMP leads to fragmentation of actin filaments and thrombin reverses this effect.

Tyrosine Phosphorylation of Focal Adhesion Kinase (p125FAK)

Various signaling pathways may converge on p125FAK to influence cell shape, growth, and adhesion. As shown in Figure 4, p125FAK is present in mesangial cell focal adhesions in a distribution similar to that of vinculin (compare with Figure 2). Figure 5 shows the analysis of tyrosine phosphorylation of p125FAK by the immunoblotting of cell lysates without immunoprecipitation. A protein of approximately 125 kd demonstrates decreased phosphorylation after treatment with cAMP, and increased phosphorylation after treatment with thrombin in the continued presence of cAMP (26). Additional (separate) studies were carried out after immunoprecipitation with mAb 2A7 as described in "Methods." Figure 5 shows the appearance of an autoradiograph in which tyrosine phosphorylated proteins are detected either without immunoprecipitation (left three lanes), or after immunoprecipitation (right three lanes). The most abundant tyrosine-phosphorylated protein in the Western blots of lysates is near 125 kd, and a band at this same location is immunoprecipitated by the antibody 2A7, shown in the right three lanes of Figure 5. Figure 6 shows that tyrosine phosphorylation of p125FAK immunoprecipitated with 2A7 is de-
Regulation of p125FAK by cAMP and Thrombin

Cytochalasin D also prevents the reversal of shape change by thrombin, and we examined the effects of treatment of mesangial cells with this agent on the phosphorylation of p125FAK. As can be seen in Figure 7, cytochalasin D markedly reduced tyrosine phosphorylation of p125FAK regardless of the presence of thrombin.

DISCUSSION

Cellular motility, shape, adhesion, and formation of focal adhesions are intimately related to actin polymerization (27-30), and our results indicate that cAMP and thrombin have potent effects on mesangial cell shape and on the distribution of actin. The effects of cAMP and thrombin are mediated by signaling mechanisms that regulate actin polymerization and formation of focal adhesions. Thrombin proteolytically activates the thrombin receptor (31) with downstream signaling events that include generation of diacylglycerol and inositol trisphosphate (19) from phosphatidylinositol 4,5-bisphosphate (4,5-PIP2). Formation of diacylglycerol and inositol trisphosphate are linked to the stimulation of protein kinase C and the mobilization of cellular calcium, respectively. However, modulation of cAMP-induced shape change by thrombin occurs in the absence of extracellular calcium, and is not mimicked by the calcium ionophore A23187 or by phorbol myristate acetate, an activator of protein kinase C (32). An additional role for 4,5-PIP2 itself could be to regulate the function of actin-binding proteins such as gelsolin, profilin, α-actinin, and p39CapZ (33-36). Recent studies support a role for 4,5-PIP2 in transducing integrin-mediated signaling whereby integrin receptor occupancy (adhesion) and cellular suspension are associated with increases and decreases in 4,5-PIP2 levels, respectively (37). Increased synthesis of 4,5-PIP2 is thought to occur because of PIP5-kinase (38). The integrative function of 4,5-PIP2 on the organization of stress fibers and focal adhesions is supported by data that indicates that α-actinin and

Figure 5. Photograph of an autoradiogram that shows increased tyrosine phosphorylation of p125FAK in lysates detected by Immunoblotting (without immunoprecipitation, three lanes on the left) as described in "Methods." The three lanes on the right show tyrosine phosphorylation after immunoprecipitation of cell lysates as described in "Methods." Arrows Indicate molecular weight markers for 215 (uppermost arrow), 105, and 70 kd.

Figure 7. Effects of thrombin (10 nM) and cytochalasin D (0.1 μg/mL) on the tyrosine phosphorylation of p125FAK. p125FAK was immunoprecipitated and tyrosine phosphorylation detected as described for Figure 6.

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vinculin (both present in focal adhesions) bind 4,5-
PIP2 (39).

Another candidate pathway for thrombin-induced
regulation of actin filaments is the stimulation of phos-
phatidylinositol-3-kinase and activation of pp60src (40).
Phosphatidylinositol (3,4,5)P3, a phosphoinositide pro-
duct of phosphatidylinositol-3-kinase, binds gelsolin, an
important actin-regulatory protein (41,42). Activation of
phosphatidylinositol-3-kinase by pp60src would gener-
ate increased amounts of 3-phosphate-containing phos-
phoinositides that bind gelsolin and promote actin poly-
merization. Thus, thrombin may have effects on actin
polymerization via stimulation of both PI 3- and PI
5-kinases.
The effects of cAMP on actin filaments and cell
shape in our experiments are generally opposite those
of thrombin, including their contrasting effects on the
distribution of actin and phosphorylation of p125FAK.
Transient elevation of cAMP accompanies fibroblast
contraction (43) and implies that fragmentation and
reorganization of stress fibers are integrated with
other mechanisms that modulate cell shape and/or
contraction. Among the actions of cAMP that could
account for its effects on stress fibers are activation of
Type 1 phosphatase (44) and inactivation of myosin
light-chain kinase (45), which could both promote
depolymerization of the cytoskeleton and reduce the
interaction of actin with myosin. Because actin/myosin
interactions likely play a key role in formation of stress fibers (46), decreased actin/myo-
sin interactions would be expected to accompany the
fragmentation of stress fibers. It is interesting to note
that cytochalasin B, which depolymerizes actin stress
fibers, produces a change of shape similar to that
induced by cAMP (29). Therefore, it is possible that
cAMP effects changes in actin/myosin interactions
that eventuate in loss of stress fibers and initiate a
sequence of alterations that culminate in loss of focal
adhesions. In contrast, thrombin may promote actin/
myosin interactions by stimulating PI3- and 5-kin-
ases, which, in turn, increase the amounts of phos-
phoinositides to bind actin-binding proteins such as
gelsolin. Morphological restoration of stress fibers by
thrombin (implying the organization of actin into
larger units) is accompanied by decreases in the G-
actin and 366,000 x g fractions and a relative in-
crease in the 15,000 x g fraction. This may reflect the
utilization of monomers and short actin filaments for
the formation of more organized actin networks and
filaments.
The morphological effects of thrombin were indistin-
guishable from those of nocodazole, which disrupts
microtubules. The tensile strength of cell architecture
attributes rigidity to microtubules that oppose contractile forces generated by actin/myosin fila-
ments (stress fibers) (47). In contrast to the known
signaling effects of thrombin, nocodazole binds to the
colchicine-binding site on tubulin (48) without spe-
cific receptor/ligand interactions that result in sec-
ondary signals generated at the cell membrane or
within the cell. Thus, thrombin and nocodazole repres-
sent agents with very different modes of action. Anal-
ysis of results similar to ours led to the suggestion that
microtubules associate with molecules capable of
“signaling” the promotion of stress-fiber formation
(49). Displacement of such molecules and perturba-
tion of microtubule dynamics with nocodazole would
promote the formation of stress fibers. Microtubule-
associated proteins are candidates for such a role, and
at least one previous study suggests that the micro-
tubule-associated enzyme kinesin associates with
stress fibers after the experimental disruption of mi-
crotubules (50). Despite their divergent actions, both
thrombin and nocodazole promote phosphorylation of
p125FAK. The effect of thrombin actually led to tyrosine
phosphorylation in excess of controls (Figure 7). This
“hyperphosphorylation” is similar to that observed
during the adhesion of suspended cells to extracellu-
lar matrix (11). Thus, an initial period of relatively
greater tyrosine phosphorylation of p125FAK was fol-
bbed by a decline to a lower plateau. It is possible that
periods of change in cell adhesion are accompanied by
relatively increased phosphorylation of p125FAK.
Cytochalasin D, which interferes with the formation
of stress fibers, completely dephosphorylates p125FAK
and blocked thrombin-induced tyrosine phosphoryla-
tion of p125FAK. These results confirm the findings of
Sinnett-Smith et al. (51), who found that bombesin-
stimulated phosphorylation of p125FAK in Swiss 3T3
cells was blocked by cytochalasin D. Taken together,
our data suggest that the organization of actin fila-
ments into stress fibers is required for thrombin-
duced phosphorylation of p125FAK.
In summary, we find that tyrosine phosphorylation
of p125FAK is correlated with actin polymerization.
When added to cAMP-treated cells, thrombin pro-
duces dramatic alterations in cell shape that accom-
pany formation of focal adhesions, stress-fiber forma-
tion, and an increased phosphorylation of p125FAK.
The effects of nocodazole imply that microtubule or-
ganization is related to the organization of stress
fibers in a reciprocal manner, as suggested by Ingber
(52). Mechanisms that influence the dynamic behav-
ior of actin filaments may also affect microtubules (or
vice versa) and thereby regulate cell shape and func-
tion (42,53). Indeed, dysfunction or overexpression of
microtubules may interfere with the physiological
contraction of muscle cells (54), an observation that
reinforces the hypothesis that microfilaments and
microtubules may have reciprocal functions in the
promotion of cellular contraction and changes in cell
shape.

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