Growth Factor-dependent Phosphorylation of the Actin-binding Protein Cortactin Is Mediated by the Cytoplasmic Tyrosine Kinase FER

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The cytoplasmic tyrosine kinase FER belongs to a relatively small family of nonreceptor tyrosine kinases that consist of only one other member, FES (1, 2). The FES gene product is expressed only in cells of myeloid and endothelial lineages and has been implicated in cytokine signaling (3–9). In contrast, FER is expressed ubiquitously and its function remains poorly understood. FER is structurally distinct from members of the other cytoplasmic tyrosine kinase families; its amino acid sequence contains a catalytic domain adjacent to a single Src homology 2 (SH2) domain and an extensive coiled coil sequence in the amino terminus (2, 10). We previously showed that FER is tightly associated with the novel catenin pp120 and that the FER-pp120 complex is phosphorylated on tyrosines upon treatment of cells with growth factors (10). However, the significance of the interaction remains to be elucidated, because the function of pp120 is also not well understood.

Tyrosine phosphorylation has been demonstrated to be an important mechanism for regulating cell-cell and cell-substrate adhesions. For example, inhibition of tyrosine phosphatases in Madin-Darby canine kidney cells results in the deterioration of adherens junctions and dramatic changes in cell morphology (11). Treatment of epithelial cells with epidermal growth factor leads to the tyrosine phosphorylation of β-catenin, plakoglobin and pp120, as well as changes in cell morphology (12, 13). More importantly, the major targets of v-src kinase activity have been shown to be proteins that are directly implicated in cell adhesion or cytoskeletal function. These include focal adhesion kinase, cortactin, p130CAS, and pp120 (14). Results of subcellular localization and functional characterization have implicated these proteins in regulating cell motility or membrane cytoskeleton dynamics. Focal adhesion kinase and p130CAS are localized to focal adhesion complexes and focal adhesion kinase has been demonstrated to be required for the turnover of the cellular structures (15–19). Cortactin has been shown to bind F-actin in vitro and is localized to the cortical cytoskeleton (20, 21). pp120 is a component of the cadherin-catenin complexes and, as such, may be involved in membrane-cytoskeleton interactions (22, 23).

The association of FER and pp120, and their phosphorylation in cells treated with growth factors, led us to speculate that FER may be involved in a signaling pathway linking growth factor receptors with cell adhesion or the cytoskeleton. In this report, we describe studies aimed at further characterization of the interaction of FER with other cellular components. We also report on the results of experiments, using a dominant-negative FER mutant, that seek to analyze the function of the FER kinase.

MATERIALS AND METHODS

Cells and Antibodies—NIH3T3 and human embryonic kidney 293 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum and 10% fetal bovine serum, respectively. Glutathione S-Sepharose was from Amersham Pharmacia Biotech. Monoclonal anti-phosphotyrosine and anti-pp120 antibodies were from Transduction Laboratories. Monoclonal anti-cortactin antibody (4F11) was from Upstate Biotechnology, Inc. Anti-hemagglutinin (12CA5) antibodies were from Boehringer Mannheim, Texas Red-conjugated goat anti-mouse IgG and fluorescein-conjugated goat anti-rabbit IgG were from Jackson ImmunoResearch Laboratory. Polyclonal anti-dynamin antibodies were provided by Dr. Mark McNiven of the Mayo Clinic. Polyclonal FER1 and FER5 antisera were described previously (10). They were generated by immunizing rabbits with fusion proteins that contain codons 502–675 and codons 451–564 of FER, respectively. Antiserum FER2 was prepared also using a TrypE fusion protein containing codons 502–675.

Expression Plasmid Construction—Full-length FER cDNA was isolated from a human foreskin fibroblast cDNA library (CLONTECH).
using as probe a polymerase chain reaction fragment that encompasses codons 149–332. A single lysine to arginine mutation was introduced at codon 591, using the Sculptor™ in vitro mutagenesis kit (Amersham Pharmacia Biotech). Both the wild-type and K591R mutant cDNA sequences were subcloned in a CMV-based eukaryotic expression vector to yield pCMV-HA-FER(WT) and pCMV-HA-FER(K591R). The subcloning was designed to introduce an influenza virus hemagglutinin (HA) epitope at the amino terminus of the FER sequences. The human CSF-1 receptor cDNA was kindly provided by Dr. C. Sherr of the Howard Hughes Medical Institute at St. Jude’s Medical Center and was subcloned into the CMV expression vector to yield pCMV-CSF1R. Details of the subcloning procedures will be provided upon request.

Subcellular Fractionation—Confluent cultures of NIH3T3 cells were serum-starved and stimulated with platelet-derived growth factor (PDGF) as described before (10). Cells were rinsed twice with cold PBS and scraped into ice-cold hypotonic buffer (20 mm Tris-HCl, pH 7.7, 15 mm NaCl, 2 mm MgCl₂, 1 mm sodium orthovanadate, and 40 μm ammonium molybdate) (1 ml/10-cm plate). The cell suspension was allowed to swell on ice for 15 minutes, and cell lysis was achieved by homogenization in a tight-fitting glass homogenizer. The cell lysates were centrifuged for 10 min at 2000 rpm in a tabletop centrifuge to remove nuclei and unbroken cells. The postnuclear supernatants were centrifuged for 90 min at 100,000 × g to yield an S₁₀₀₀₀ supernatant. The pellet material was resuspended by homogenization in 3 ml of buffer that contained 5% glycerol, 20 mm Tris-HCl, pH 7.7, 1 mm EDTA, 150 mm NaCl, 1 mm sodium orthovanadate, and 40 mm ammonium molybdate. To that suspension was added 10% Triton X-100 to a final concentration of 1%. The mixture was clarified by centrifugation (10,000 × g, 10 min) to yield a solubilized membrane (P₁₀₀) fraction. Protein concentrations were determined using the microBCA reagents (Pierce). Whole-cell lysates were prepared as described previously (10).

Immunoprecipitation and Protein Binding Assays—Immunoprecipitations were performed as described before, using typically 300 μg of S₁₀₀₀₀ and 50 μg of P₁₀₀₀₀ fractions and 5 μg of purified antibodies or 5 μl of antisera (10). Immunoblotting was performed using standard techniques, and binding was detected with enhanced chemiluminescence reagents (Amersham Pharmacia Biotech). For immune complex kinase assays, whole-cell lysates were immunoprecipitated with FER5 antiserum, and kinase assays were performed as described before (10).

Preparation of GST fusion proteins and in vitro binding assays were performed as described before (10). In binding assays that involved GST-CCD, the detection of cortactin required an intermediate immunoprecipitation, because the GST-CCD polypeptide comigrates with cortactin on SDS gels and prohibits the unambiguous detection of cortactin. In those experiments, proteins bound to immobilized GST fusion proteins were eluted by boiling in 1% SDS. The samples were then treated with PDGF for 5 min. Whole-cell lysates were prepared from confluent cultures of NIH3T3 cells and 0.3-mg aliquots of lysates were immunoprecipitated with nonimmune serum (NI) (lanes 1 and 2), FER1 (lanes 3 and 4), FER2 (lanes 5 and 6), or FER5 antiserum (lanes 7 and 8). The immunoprecipitates were analyzed by Western blotting with anti-phosphotyrosine antibody (PY20). In B and C, the filter was stripped of antibodies and reprobed sequentially with antibodies specific for FER or pp120. Numbers on the right side show the locations of size markers, with their molecular masses in kilodaltons.

RESULTS

Identification of a Novel Interaction between FER and Cortactin—We previously used an immunological approach to identify the association of FER with pp120 (10). To further identify other components that may interact with FER, we prepared additional FER-specific antisera and analyzed their ability to immunoprecipitate FER-containing complexes from lysates of NIH3T3 cells. As shown previously, antiserum FER1 immunoprecipitated a complex of FER and pp120 (Fig. 1, lanes 3 and 4). PDGF treatment resulted in an increase in the phosphorylation of both FER and pp120. Two other FER-specific antisera, FER2 and FER5, also immunoprecipitated FER, as well as a small amount of the 180-kDa activated PDGF receptor (Fig. 1, lanes 5–8). However, little or no pp120 was co-immunoprecipitated by either of these two antisera. FER5 immunoprecipitated additional polypeptides of 125 kDa and >205 kDa. The identity of these polypeptides has yet to be determined. These anti-FER antisera may therefore recognize distinct subpopulations or conformations of FER.

Because many proteins that regulate cell-cell or cell-substrate adhesion are found in detergent-insoluble cytoskeletal structures, the use of detergent lysates may preclude the detection of some FER complexes. We therefore prepared subcellular fractions by hypotonic lysis and verified the fractionation of subcellular components by Western blot analyses. The membrane (P₁₀₀₀₀) fraction showed the expected enrichment of the PDGF receptor, which is the major tyrosine phosphorylated protein in cells treated with PDGF (Fig. 2A, lanes 3 and 4). As shown previously, pp120 is enriched in the P₁₀₀₀₀ fraction (25).
Conversely, FER is localized mostly in the cytosolic (S100) fraction, but a detectable amount is also recovered in the membrane pellet. Other signaling molecules, such as cortactin and dynamin, showed predominantly cytosolic localization but with significant extent of membrane association as well.

From the S100 fractions, both FER1 and FER2 antisera immunoprecipitated a doublet of 97 and 102 kDa (Fig. 2B), of which only the 97-kDa polypeptide cross-reacted with FER antibodies in Western blotting (Fig. 2C). Antiserum FER2 also immunoprecipitated an 85-kDa phosphorylated polypeptide, which cross-reacted with antibodies to the actin-binding protein cortactin (Fig. 2, B and D). PDGF treatment resulted in an increase in tyrosine phosphorylation of FER, the 102-kDa polypeptide, as well as cortactin. In the case of cortactin, there is substantial basal phosphorylation, and the extent of association with FER appears to be insensitive to growth factor treatment. The identity of the 102-kDa polypeptide that co-immunoprecipitated with FER was analyzed by Western blotting with antibodies to signaling molecules of this size range. Antibodies to dynamin cross-reacted with a 102-kDa polypeptide that was specifically co-immunoprecipitated by the FER antisera, but not by nonimmune antibodies (Fig. 2E). However, the abundance of the dynamin in the FER immunoprecipitates did not correlate with the reactivity to the anti-phosphotyrosine antibodies (compare lanes 5–8 of B with those in E). As expected from the known localization of pp120, the FER-pp120 complex was enriched in the P100 fraction and was immunoprecipitated by antiserum FER1 (Fig. 2F). Immunoprecipitates prepared with antiserum FER2 contained a significant amount of cortactin, but only a trace of pp120 (Fig. 2, G and H). In contrast to the immunoprecipitates prepared with the S100 fractions, the P100 fraction immunoprecipitates did not contain any detectable amount of the 102-kDa phosphoprotein.

To further ascertain the FER-cortactin association, the subcellular localization of the two proteins were analyzed by double immunofluorescence staining. In unsynchronized fibroblasts, cortactin was previously shown to colocalize with cortical actin filaments (20, 21). We found that, in quiescent fibroblasts, cortactin is in the cortex, but a significant amount is also detected in the cytoplasm (Fig. 3). In these cells, FER is localized primarily in the cytoplasm, although a detectable extent of colocalization with cortactin in the cell cortex can also be observed (A and B). The staining obtained with the FER antiserum was specific, because it was not observed with preimmune serum or if the antiserum was preincubated with the immunogen. In PDGF-treated cells, cortactin becomes enriched in the cell cortex, and colocalization with FER is clearly visible in the membrane ruffles (Fig. 3, C and D).
Cortactin Binds the SH2 Sequence of FER

The molecular basis of the FER-cortactin association was further examined in in vitro binding assays. A GST fusion protein containing the SH2 sequence of FER specifically precipitated cortactin from a cytosolic fraction (Fig. 4, A and B). The CCD of FER, which was previously shown to mediate interaction with pp120, did not interact with cortactin. Cortactin is one of the major cytosolic proteins that are subject to PDGF-regulated tyrosine phosphorylation and which bind the FER SH2 sequence in vitro (Fig. 4, C and D). We also tested the ability of the FER SH2 sequence to directly interact with cortactin. Cytosolic proteins were immunoprecipitated with anti-phosphotyrosine antibodies and denatured. Cortactin was the predominant component that, following denaturation and renaturation, retained the ability to bind the FER SH2 sequence (Fig. 4, E and F). This suggests that the FER SH2 sequence has the capacity to directly interact with cortactin.

The Novel 102-kDa Polypeptide Is Closely Related to FER

In addition to cortactin, a 102-kDa polypeptide was found to co-immunoprecipitate with FER from the cytosolic fraction. Although dynamin can be detected in the FER immunoprecipitates, its abundance in the immune complexes suggests that it is distinct from the 102-kDa component detected in the anti-phosphotyrosine Western blots. The identity of the 102-kDa polypeptide was further investigated by dissecting the interactions among the components of the immune complex. The immune complex was isolated using FER2 antiserum and was denatured. The immunoprecipitated polypeptides were then subjected to a second round of immunoprecipitation using antiserum FER5, which we found to be able to recognize dena-
The 102-kDa polypeptide that co-immunoprecipitates with FER is not dynamin. Immunoprecipitates of S100 fractions were prepared with FER2 antisera and are shown in lanes 1 and 2. Samples of the immunoprecipitates were also analyzed further by denaturation in SDS. The materials recovered were immunoprecipitated with FER5 antisera, and the bound polypeptides were analyzed (lanes 3 and 4). The supernatants from the FER5 immunoprecipitations were immunoprecipitated with anti-phosphotyrosine antibody (PY20), and the polypeptides recovered were shown in lanes 5 and 6. The FER2 immunoprecipitates were also denatured and re-immunoprecipitated with anti-phosphotyrosine antibody (lanes 7 and 8). A shows the results of anti-phosphotyrosine Western blotting of the immunoprecipitates, whereas B–D show results of sequential reprobing of the filter with antibodies to dynamin, FER, and cortactin.

Fig. 5. The 102-kDa polypeptide that co-immunoprecipitates with FER is not dynamin. Immunoprecipitates of S100 fractions were prepared with FER2 antisera and are shown in lanes 1 and 2. Samples of the immunoprecipitates were also analyzed further by denaturation in SDS. The materials recovered were immunoprecipitated with FER5 antisera, and the bound polypeptides were analyzed (lanes 3 and 4). The supernatants from the FER5 immunoprecipitations were immunoprecipitated with anti-phosphotyrosine antibody (PY20), and the polypeptides recovered were shown in lanes 5 and 6. The FER2 immunoprecipitates were also denatured and re-immunoprecipitated with anti-phosphotyrosine antibody (lanes 7 and 8). A shows the results of anti-phosphotyrosine Western blotting of the immunoprecipitates, whereas B–D show results of sequential reprobing of the filter with antibodies to dynamin, FER, and cortactin.

Fig. 6. A point mutation in the ATP binding domain of FER results in a dominant-negative mutant. A, 293 cells were transfected with an empty expression vector (CMV), wild-type (WT) FER cDNA, or the K591R FER mutant cDNA. Transfected cell lysates were analyzed by Western blotting with anti-FER antibodies. B, the filter from A was reprobed with antibody to the hemagglutinin epitope (HA). C, transfected cell lysates were immunoprecipitated with nonimmune (lanes 1–3) or with FER5 (lanes 4–6) antiserum. An immune complex kinase assay was performed using [γ-32P]ATP, and the products of FER autophosphorylation are shown after SDS-polyacrylamide gel electrophoresis, alkaline treatment, and autoradiography. Results shown are representative of three independent experiments.

Cortactin Is a Direct Substrate of FER Kinase—To help analyze the functional implication of the FER-cortactin interaction, we prepared an enzymatically inactive FER by introducing a point mutation in the conserved lysozyme (Lys901) of the predicted ATP binding site of the kinase. Transient expression of the K591R FER mutant cDNA showed that it encodes a polypeptide that is slightly larger than the endogenous FER kinase, as a result of the addition of an HA epitope tag to the amino terminus (Fig. 6, A and B). In an immune complex kinase assay, the lysate of cells transfected with the K591R mutant showed no autophosphorylation of the mutant FER protein and reduced autophosphorylation of the endogenous kinase (C, lane 6). In contrast, transfection with an analogous wild-type FER cDNA resulted in a significant autophosphorylation of the tagged kinase (C, lane 5). These results suggest that the K591R FER protein may function as a dominant interfering mutant.

To assess the effect of the K591R FER mutant on pp120 and cortactin phosphorylation, we cotransfected 293 cells with cDNA encoding CSF-1 receptor and mutant or wild-type FER. It was shown previously that the tyrosine phosphorylation of pp120 and cortactin is also regulated by the CSF-1 receptor (26). S100 fractions of transfected cells were immunoprecipitated with FER antisera. Antiserum FER1 immunoprecipitated a complex mixture of endogenous and tagged FER proteins, as well as a small amount of pp120 (Fig. 7). Whereas the anti-phosphotyrosine blot revealed the presence of multiple bands of 97–102 kDa in the wild-type FER immunoprecipitates, there was considerably less heterogeneity in the case of cells transfected with the K591R mutant (A and B, lanes 3 and 4). Similarly, the anti-FER2 immunoprecipitates contained a complex mixture of FER proteins and cortactin (lanes 5–8). The anti-phosphotyrosine blot revealed that expression of the K591R FER mutant resulted in a significant reduction in the tyrosine phosphorylation of cortactin (A, lanes 7 and 8).

The effect of the FER mutant on cortactin phosphorylation was further ascertained by analyzing immunoprecipitates obtained with anti-cortactin antibodies. As shown with the FER2 antibodies, immunoprecipitation with anti-cortactin antibodies clearly demonstrated the association of FER and cortactin (Fig. 8A). In cells co-expressing the CSF-1 receptor and wild-type FER, M-CSF treatment resulted in an increase in phosphorylation of both FER and cortactin (Fig. 8A, lanes 1 and 2). In contrast, expression of the K591R FER mutant resulted in a marked reduction of both basal and factor-dependent phosphorylation of cortactin (lanes 3 and 4). It is noteworthy that the mutant FER did not show any impairment in its association with cortactin. Examination of the FER polypeptides in the anti-cortactin immunoprecipitates clearly shows the mobility
differences of the wild-type and mutant FER products. We also further verified the effect of the FER mutant on pp120 phosphorylation by immunoprecipitating P100 fractions using anti-phosphotyrosine antibody. As shown previously, epithelial cells such as 293 cells express a heterogeneous mixture of pp120 isoforms (22). There was no difference in the extent of pp120 phosphorylation in cells expressing wild-type or K591R mutant FER.

**DISCUSSION**

We previously demonstrated the stable association of FER with the catenin pp120 and estimated that no more than 10–15% of the total FER protein was involved in that complex (10). As shown in the subcellular fractionation studies here, that previous estimate is consistent with the abundance of FER that is associated with the membrane fraction. The use of subcellular fractionation and the FER2 antiserum permitted the identification of a novel interaction between FER and the cytoskeletal protein cortactin. Immunoprecipitation of FER resulted in the coprecipitation of either cortactin or pp120, but not both. Thus, FER appears to interact with pp120 and cortactin in a mutually exclusive manner, although binding to the two polypeptides requires distinct FER sequences. These observations suggest that the FER-pp120 and FER-cortactin complexes may be involved in different signaling functions. The FER-cortactin complex is detectable in a cytosolic fraction, but not in a lysate prepared using detergent-containing buffers. The association was verified by functional and structural analyses using independent assays and is therefore unlikely to be an artifact of the lysis procedure. The FER-cortactin association is unambiguously demonstrated by immunofluorescence colocalization in the membrane ruffles of PDGF-treated cells. Most significantly, the effect of the FER K591R mutant on cortactin phosphorylation strongly supports a direct physical interaction between the two proteins. We therefore believe that the interaction involves cytoskeletal structures that are detergent insoluble. Our analyses suggest that between 10 and 0% of the cellular cortactin is associated with FER.

In addition to cortactin, we identified also a novel 102-kDa polypeptide that co-immunoprecipitated with FER. We recently showed that cortactin interacts with dynamin 2, which has an apparent molecular mass of ~100 kDa. Our analyses showed that dynamin could be detected in immunoprecipitates prepared with anti-FER antibodies, presumably by virtue of the interaction of FER with cortactin. However, the results also clearly showed that the 102-kDa polypeptide is distinct from dynamin. The ability of the FER5 antiserum to recover both the 97-kDa FER and the 102-kDa polypeptide under denaturing conditions suggests that the latter is a highly modified form of FER or a closely related polypeptide. As is evident in the transient expression assays, there is a pronounced size heterogeneity in the cytosolic pool of wild-type FER protein. The fact that the size heterogeneity is absent in the case of the FER mutant suggests that it is dependent on FER kinase activity. It

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**FIG. 7.** Effect of the K591R FER mutant on pp120 and cortactin phosphorylation. 293 cells were transfected with cDNA that encodes the CSF-1 receptor together with wild-type or K591R FER cDNA. Transfected cells were deprived of serum for 18 h, and some cultures were treated with M-CSF. S100 fractions were prepared from the transfected cells and immunoprecipitated with FER1 (lanes 1–4) or FER2 (lanes 5–8) antiserum. A shows results of Western blotting with anti-phosphotyrosine antibody. B and C show results of blotting the same filter with antibodies to FER and cortactin.

**FIG. 8.** Phosphorylation of cortactin, but not pp120, is downregulated in cells expressing the K591R FER mutant cDNA. A, 293 cells were cotransfected with CSF-1 receptor and wild-type (lanes 1 and 2) or K591R mutant (lanes 3 and 4) FER cDNA and were deprived of serum. S100 fractions were prepared from untreated or M-CSF-treated cultures and were immunoprecipitated with antibody to cortactin. The immunoprecipitates were analyzed by Western blotting with anti-phosphotyrosine antibody. B and C show the same filter reprobed with antibodies to FER and cortactin. D, P100 fractions were similarly immunoprecipitated with anti-pp120 antibody and analyzed. E shows the result of reprobing the filter from D with antibody to pp120.

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therefore seems plausible that the 102-kDa polypeptide may be a highly phosphorylated form of FER that is present in such low abundance as to be undetectable by Western blotting with anti-FER antibodies. The functional significance of this polypeptide, and its selective localization in the cytosol, remain to be investigated further.

In vitro binding assays demonstrated that the SH2 sequence of FER is capable of directly binding cortactin. This contrasts with our previous finding that the coiled coil sequence at the amino terminus of FER is required for binding pp120 (10). Expression of the FER K591R mutant was found to result in an inhibition of the autophosphorylation of the endogenous FER kinase, thus suggesting that the mutant was capable of functioning in a dominant interfering manner. The FER mutation has no apparent effect on its ability to interact with cortactin and pp120, and expression of the FER mutant has no effect on the expression of the two cytoskeletal proteins (data not shown). The mutation did result in an inhibition of the tyrosine phosphorylation of cortactin, but not of pp120, in response to growth factor receptor signaling. These data argue that cortactin, but not pp120, is a direct substrate of FER kinase. However, we cannot exclude the possibility that a residual amount of endogenous FER activity is sufficient to effect the phosphorylation of pp120. It should be noted that we have never been able to demonstrate a direct binding between FER and pp120.

Recently, peptide libraries were used to determine the sub- strate specificity of protein tyrosine kinases (27). Such analyses led to the conclusion that nonreceptor tyrosine kinases tend to phosphorylate peptide sequences that are recognized by their SH2 domains. Our observations on the FER-cortactin interaction and their putative enzyme-substrate relationship would be consistent with the specificity proposed on the basis of the peptide substrate analyses. Most significantly, our data suggest that the regulation of cortactin phosphorylation by growth factor receptors may be mediated in part by FER.

Although cortactin was originally identified as a target of tyrosine phosphorylation by v-Src, a role for c-Src in cortactin phosphorylation has not been established. The subcellular localization of c-Src to endosomal membranes suggests that c-Src and cortactin are localized to different cellular compartments (28). In addition to v-Src and FER, the cytoplasmic kinase FYN has also been implicated in the regulation of cortactin phosphorylation (29). It is therefore plausible that cortactin may be a substrate of multiple tyrosine kinases that serve to relay different extracellular signals. The significant basal phosphorylation of cortactin may be carried out by an unidentified tyrosine kinase. The partial phosphorylation allows cortactin to bind FER, and the FER-cortactin complex becomes a subject of regulation by growth factor signaling. The localization of cortactin to the cortical cytoskeleton, and its affinity for filamentous actin, led to the suggestion that cortactin may be involved in transmitting signals from the membrane to the actin cytoskeleton. That proposal is further substantiated by the observation that cortactin phosphorylation is induced upon integrin signaling in cell adhesion and in the cytoskeletal reorganization that accompanies bacterial entry of epithelial cells (30, 31). Recently, cortactin was shown to cross-link F-actin in vitro, and the cross-linking activity was down-regulated by the tyrosine phosphorylation of cortactin (32). These observations have consolidated a role for and FER and cortactin in the transmission of signals for reorganization of the actin cytoskeleton.

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