A Protein Kinase from Neutrophils That Specifically Recognizes Ser-3 in Cofilin*

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Cofilin promotes the depolymerization of actin filament, which is required for a variety of cellular responses such as the formation of lamellipodia and chemotaxis. Phosphorylation of cofilin on serine residue 3 is known to block these activities. We now report that neutrophils contain a protein kinase that selectively catalyzes the phosphorylation of cofilin on serine 3 (70%) and a nonspecific kinase that recognizes multiple sites in this protein. The selective serine 3 cofilin kinase binds to a deoxyribonuclease I affinity column, whereas the nonspecific cofilin kinase does not. Deoxyribonuclease I forms a very tight complex with actin, and deoxyribonuclease affinity columns have been utilized to identify a variety of proteins that interact with the cytoskeleton. The serine 3 cofilin kinase did not react with antibodies to LIM kinase 1 or 2, which can catalyze the phosphorylation of cofilin in other cell types. The activity of the serine 3 cofilin kinase was insensitive to a variety of selective antagonists of protein kinases but was blocked by staurosporine. This pattern of inhibition is similar to that observed for the kinase that is active with cofilin in intact neutrophils. Thus, neutrophils contain a protein kinase distinct from LIM kinase-1/2 that selectively recognizes serine 3 in cofilin.

Continuous polymerization and depolymerization of actin filaments is required for various cell responses such as the formation of lamellipodia, filopodia, and chemotaxis (e.g. Refs. 1–3). Cofilin is an essential actin-binding protein that promotes the depolymerization of older actin filaments (F-actin), thereby facilitating the rate of filament turnover (e.g. Ref. 4; for review, see Refs. 2 and 3). A recent study has shown that a simple actin-based motility system (e.g. Ref. 4; for review, see Ref. 3). The activity of the serine 3 cofilin kinase was insensitive to a variety of selective antagonists of protein kinases but was blocked by staurosporine. This pattern of inhibition is similar to that observed for the kinase that is active with cofilin in intact neutrophils. Thus, neutrophils contain a protein kinase distinct from LIM kinase-1/2 that selectively recognizes serine 3 in cofilin.

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LIMK. Some properties of this Ser-3 cofilin kinase (S3ck) are reported.

**EXPERIMENTAL PROCEDURES**

**Materials**

Affi-Gel 10 (N-hydroxyxycinamide active ester-agarose) was purchased from Bio-Rad. 1,4-Phenylenediisothiocyanate (DITC), cyanogen bromide (CNBr), myelin basic protein (MBP), and CAPS were obtained from Sigma. Phenyl isothiocyanate (PITC) was a product of Pierce. DNaSE I, HA1004, H-7, ML-7, KN-62, and staurosporine were purchased from Calbiochem. Immobilon-P transfer membranes (0.45 μm) were obtained from Millipore Corp. A goat polyclonal antibody to LIMK-2 (LIMK-2 (C-19) Ab) was obtained from Santa Cruz Biotechnology Co., Santa Cruz, CA. Sources of all other materials are described elsewhere (29–31).

**Methods**

**Preparation of Neutrophils and Cellular Fractions—**Guinea pig peripheral neutrophils were prepared as described previously (32). These preparations contained >90% neutrophils with viabilities always >90%.

Cells were suspended at a concentration of 2 × 10^7/ml in freshly prepared, ice-cold extraction buffer (20 mM Hepes (pH 7.5), 0.40 mM EGTA, 0.40 mM EDTA, 10 mM NaF, 0.40 mM NAD, 0.08 mM PMSE, 10 μg/ml leupeptin, 20 μg/ml pepstatin, and 15% (v/v) 2-mercaptoethanol) and disrupted by freeze-thawing three times in a mixture of dry ice and ethanol. The lysate was centrifuged at 200,000 × g (30 min) for 30 min. The supernatant was saved, and the pellet was resuspended to the original volume in extraction buffer. Both fractions were used immediately.

**Preparation of Recombinant Cofilin—**A full-length cDNA clone for human non-muscle cofilin was constitutively expressed in bacteria using the plasmid vector pMW172. For large scale purification, the bacterial pellet was resuspended in 20 mM Tris, 10 mM EDTA, pH 8.0 (purification buffer), sonicated and centrifuged at 10,000 × g for 20 min. The supernatant was applied to a DE52 column equilibrated with purification buffer. After washing with purification buffer, cofilin was eluted with 50 mM NaCl in purification buffer (pH adjusted to 8.0). All steps were performed at 4 °C. Recombinant cofilin purified in this manner was greater than 95% pure, as judged by SDS-PAGE analysis. Typical yields of cofilin from 1 liter of bacteria were between 25 and 50 mg. The protein as eluted from the column was divided into small aliquots and stored at 4 °C for periods of up to 1 month. Cofilin stored in this manner for periods greater than 1 month exhibited substantially less activity than freshly prepared cofilin when utilized as a substrate for S3ck.

DNaSE I Affinity Chromatography for Cofilin Kinase—DNaSE I was covalently linked to Affi-Gel 10 according to the manufacturer's instructions. After coupling, the gel was washed with 50 mM Tris (pH 8.0) to eliminate unreacted N-hydroxyxycinamide esters. The beads were then washed with extraction buffer containing 0.40 mM NaCl, followed by extraction buffer alone. The resulting DNaSE-agarose beads (1.0 ml of swollen beads) were mixed with 3.0 ml of the soluble fraction of neutrophils in a rotating shaker for 30 min at 4 °C. The beads were then packed into a small column and extensively washed with extraction buffer (−50 ml), followed by extraction buffer containing 0.40 mM NaCl (−10 ml). The DNaSE I-agarose beads containing the bound proteins were resuspended (1:1) in extraction buffer, and 20 μl of swollen beads were employed in the kinase assays.

Cofilin Kinase Assay—Cofilin kinase activity was measured as the transfer of ^32P from [γ-^32P]ATP into recombinant cofilin. In addition to the bound kinase or neutrophil fractions, the final assay mixture (0.10 ml) contained recombinant cofilin (7.5 μM), 20 mM Hepes (pH 7.2), 10.0 mM MgCl2, 20 μM [γ-^32P]ATP (10 μCi), 0.10 mM EGTA, 2.0 mM p-nitrophenyl phosphate, 2.0 mM NaF, and 20 μM NaVO3. The reactions were run for 20 min at 37 °C. The reaction mixtures were then mixed with 12.5 μl of 5X sample buffer, immersed in a boiling water bath for 3.0 min, and subjected to SDS-PAGE in 15.0% (w/v) polyacrylamide slab gels (0.075 × 12 × 14 cm). The buffer system of Laemmli was employed (33). The final concentration of sample buffer after mixing was 1.0% (w/v) sodium dodecyl sulfate, 5.0 mM Tris (pH 6.8), 10.0% (v/v) glycerol, 10.0% (v/v) EGTA, 0.05% (v/v) 2-mercaptoethanol, and 0.002% (w/v) bromphenol blue. ^32P-labeled cofilin was visualized by autoradiography. In a number of experiments, the ^32P-labeled cofilin band was excised from the gel after staining with Coomassie Blue and the radioactivity quantified by scintillation counting. The stoichiometry of phosphorylation was calculated from the amount of radioactivity incorporated into cofilin, the specific activity of the [γ-^32P]ATP utilized, and the percentage of radioactivity in Ser-3 as determined by Edman degradation (see below). Activity was linear with regard to reaction time and protein concentration in the range employed in these studies.

**Manual Edman Degradation Technique for Sequencing and Identifying Phosphorylated Amino Acids—**Cofilin was phosphorylated in vitro in SDS-PAGE gels described above. The ^32P-labeled cofilin was transferred to an Immobilon-P-membrane as described in Ref. 29, except that the transfer buffer consisted of 10.0 mM CAPS, 10.0 mM mercaptoethanol, and 15% (v/v) methanol. The ^32P-labeled cofilin was “fixed” to these membranes with DITC (34) and subjected to manual Edman degradation with PITC as described in Ref. 35. The amount of ^32P released in each cycle was determined by liquid scintillation counting.

CNBr Digestion of Cofilin—^32P-Labeled cofilin was solubilized in a 15% SDS-acrylamide “low cross-linked” gel (100:1 (w/v) acrylamide/bisacrylamide). After separation, the cofilin band was cut from the gel and subjected to CNBr digestion as described in Ref. 36. The resulting peptide fragments were separated on a 16.5% Tricine/SDS-polyacrylamide gel (37) and stained with Ponceau Red. Phosphopeptides were visualized by autoradiography. Peptides were identified by sequencing the first 7–8 N-terminal amino acids on an automated gas phase protein sequenator (Applied Biosystems). Mass spectrometry of the peptides was performed on a Perspective Biosystems Elite MALDI-TOF mass spectrometer.

**Immunoprecipitation of LIM Kinase-1 and Immunoblotting—**An immunoprecipitating antibody to LIMK1 was produced in rabbits to the synthetic peptide α-acetyl-KETYRGGESLPAHPEVPD and purified by affinity chromatography and described in Ref. 38. Procedures for immunoprecipitating LIMK1 from neutrophil lysates with the LIMK1 Ab (15 μg of Ab/200 μl of lysate) and for assaying the activity of the precipitated kinase using recombinant cofilin as the substrate were identical to methods previously utilized for the p21-activated kinases (30, 39). Western blotting for LIMK1 was performed as described in Ref. 31. The primary Ab (1.5 mg/ml) was diluted 1:2,000, and antigen was visualized with a lumino-enhanced chemiluminescence detection system (Pierce) followed by autoradiography for 10–30 s (40).

**Analysis of Data—**Unless otherwise noted, all of the autoradiographic observations were confirmed in at least three separate experiments performed on different preparations of enzyme. The number of observations (n) indicates the number of different preparations of enzyme tested.

**RESULTS**

**Protein Kinases in Neutrophils That Catalyze Phosphorylation of Cofilin—**Recombinant cofilin and [γ-^32P]ATP served as substrates to search for protein kinases in neutrophils that were capable of catalyzing the phosphorylation of cofilin. The phosphorylated proteins were separated by SDS-PAGE and examined by autoradiography. Both the 200,000 × g soluble and the particulate fractions from neutrophils contained kinases that were active with cofilin (Fig. 1A). The ^32P-labeled cofilin band frequently appeared as a doublet (e.g. Fig. 3B).

To determine if the kinases in these cellular fractions recognized Ser-3 in cofilin, the ^32P-labeled cofilin bands were transferred to Immobilon-P-membranes, fixed to the membranes with DITC (34), and subjected to manual Edman degradation with PITC (35) (Fig. 1B). The amounts of ^32P released in cycle-2 from cofilin phosphorylated in vitro with the soluble and particulate fractions were 32 ± 5.0% and 2.0 ± 1.2% (S.D., n = 3), respectively. Cycle 2 releases Ser-3 from recombinant cofilin because the initial methionine residue (amino acid 1) was removed during the expression of this protein in bacteria. Thus, the soluble fraction of neutrophil protein kinase activated by LIM kinase that catalyzes the phosphorylation of cofilin on Ser-3.

Additional information on the sites in cofilin that undergo phosphorylation in vitro was obtained by digesting the ^32P-labeled cofilin bands with CNBr and subjecting the resulting peptides to one-dimensional phosphopeptide mapping (Fig. 2A). The first 7–8 N-terminal amino acids of these peptides were sequenced on an automated gas phase protein sequenator (Fig. 2A), sequenced amino acids provided on the right). All of the identified amino acids could be aligned completely with the predicted sequence of human non-muscle cofilin (41) and were preceded by methionine in accordance with the known cleavage

LIMK. Some properties of this Ser-3 cofilin kinase (S3ck) are reported.
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Isolation of a Protein Kinase from Neutrophils That Recognizes Ser-3 in Cofilin—Actin forms a stable complex with cofilin (7.5 μM). Reactions were run for 20 min at 37 °C, and phosphorylated ([32P]-labeled) cofilin was separated by SDS-PAGE and monitored by autoradiography as described under “Methods.” The autoradiograms shown are for: soluble fraction (a), soluble fraction plus cofilin (b), particulate fraction (c), and particulate fraction plus cofilin (d). The amounts of 32P in the [32P]-labeled cofilin bands in lanes b and d were approximately 40,000 and 120,000 cpm, respectively. The position of cofilin is indicated by a solid arrow. Panels B and C compare the release by manual Edman degradation of [32P]-labeled amino acids from the phosphorylated cofilin bands shown in lanes b and d of panel A. Cycle 2 of the Edman degradation method releases serine 3 from recombinant cofilin because the initial methionine residue (amino acid 1) was removed during the expression of cofilin in bacteria. Data represent mean values ± S.D. from three separate experiments.

Site for CNBr. Peptides p1, p2, and p3 exhibited substantial amounts of [32P] (Fig. 2A). Peptide p3 contained the N-terminal sequence of recombinant cofilin and exhibited a mass of ~8,200 on mass spectrometry. The mass of p3 indicated that this peptide was a partial hydrolysis product that consisted of amino acid residues 2–74. We were unable to isolate a peptide consisting of residues 2–18 in our system. Peptide p3 generated from cofilin phosphorylated in vitro with the soluble fraction contained substantially more [32P] than peptide p3 from cofilin phosphorylated with membrane fraction (Fig. 2A, compare p3 in lanes b and c). Peptide p2 contained substantial amounts of [32P] in Ser-24 (Fig. 2B). Peptide p1 consisted of residues 116–166 and was also phosphorylated. Thus, neutrophils contain kinases that can catalyze the phosphorylation of cofilin on a variety of sites in vitro.

Isolation of a Protein Kinase from Neutrophils That Recognizes Ser-3 in Cofilin—Actin forms a stable complex with cofilin in the soluble and particulate fractions of neutrophils that catalyze phosphorylation of recombinant cofilin (7.5 μM). Reactions were run for 20 min at 37 °C, and phosphorylated ([32P]-labeled) cofilin was separated by SDS-PAGE and monitored by autoradiography as described under “Methods.” The autoradiograms shown are for: intact cofilin labeled with the soluble fraction (a), cofilin labeled with the soluble fraction and digested with CNBr (b), and cofilin labeled with the membrane fraction and digested with CNBr (c). The first 7–8 N-terminal amino acids of some of these peptides were identified on a gas phase protein sequenator (sequences are provided on the right). The numbers in parentheses refer only to the identified amino acids, not to the entire sequence of the separated peptide. Intact cofilin is designated by an arrow, and certain peptides resulting from the digestion of cofilin are labeled p1, p1.5, p2, and p3 (right margin). Panels B and C show the release by manual Edman degradation of [32P]-labeled amino acids from peptides p3 and p2 of lane b in panel A. Data represent mean values ± S.D. of three separate experiments.

As noted above, cofilin phosphorylated in vitro with the soluble fraction frequently appeared as a double band after SDS-PAGE (Fig. 3, B; and C; lane c, broken arrows). The amounts of 32P in Ser-3 of the upper and lower cofilin bands in lane c were determined by manual Edman degradation and found to be ~5% and 58%, respectively. A pronounced diminution of the lower phosphorylated cofilin band was observed when the proteins not bound to the DNase-agarose beads were tested for kinase activity (Fig. 3C, lane d). In contrast, the protein kinase that bound to the DNase-agarose beads catalyzed the phosphorylation/generation of only the lower, [32P]-labeled cofilin band.
cofilin (individual 32P-labeled cofilin bands are designated DNase. Phosphorylated cofilin frequently appeared as a doublet (present in panel B). The positions of cofilin and actin in the gel are designated by a solid arrowhead broken arrow(s) or trisfuge. The kinase bound to the beads (S3ck) was not removed by washing the column with 0.40 M NaCl (Fig. 5D). The specificity of the kinase bound to the DNase-affinity column was further investigated by digesting the product of this reaction with CNBr and examining the resulting phosphopeptides (Fig. 4). The predominant 32P-labeled peptide was p3 (Fig. 4, lane b), and the amounts of radioactivity in peptides p2 and p1 were markedly reduced when compared with those generated from cofilin phosphorylated in vitro with the kinases present in the soluble fraction. These data are in agreement with those obtained by the Edman degradation method and further establish that the bound activity selectively recognizes Ser-3 in cofilin (see “Discussion”). Since the bound S3ck has not been purified to homogeneity, we do not know if this activity represents a single enzyme or a group of enzymes.

Interactions of recombinant cofilin with the DNase affinity column were investigated (Fig. 5). Significant quantities of unphosphorylated cofilin were bound to the DNase-agarose beads under the conditions of the kinase assay (Fig. 5A). However, after phosphorylation, 32P-labeled cofilin did not associate with the beads (Fig. 5B). Phosphorylation of cofilin on Ser-3 is known to result in the dissociation of cofilin-G-actin complexes (10, 44).

If the binding of S3ck to the DNase-agarose beads was due to the kinase forming a complex with endogenous cofilin associated with the beads, it was possible that S3ck could be released from the beads by exogenous cofilin. This possibility was examined by incubating the bound kinase with exogenous cofilin (7.0 μM) for 30 min in the absence of ATP and then separating the beads from the supernatant/soluble fraction. Assaying the resulting fractions either with or without additional cofilin demonstrated that the majority of the kinase activity remained associated with the beads (Fig. 5C). These data strongly suggest that the binding of S3ck to the DNase affinity column remained bound to the beads was highly selective for Ser-3 in cofilin with 78% of the 32P present in this residue after catalysis (Fig. 3D). This value for six different experiments was 74 ± 5% (n = 6, S.D.). Affi-Gel beads without covalently linked DNase did not contain this kinase activity (Fig. 3B, lane g). The stoichiometry of phosphate incorporation into Ser-3 of cofilin (7.5 μM) with S3ck bound to the DNase-agarose beads (~0.8 × 10^7 cell eq) under the standard assay conditions (37 °C, 20 min) was calculated to be about 0.4 mol of phosphate incorporated/mol of cofilin (0.42 ± 0.12; S.D., n = 4).
involves more than the formation of a simple enzyme-substrate complex.

Comparison of LIMK1 in Neutrophils to S3ck—As noted above, recent studies have reported that LIMK1 can catalyze the phosphorylation of cofilin on Ser-3 (24–26). LIMK1 is a cytosolic protein, has a predicted molecular mass of 74 kDa, and catalyzes the phosphorylation of MBP in vitro (45). An affinity-purified, polyclonal anti-LIMK1 Ab generated to a peptide corresponding to the C-terminal region of rat LIMK1 (38) was utilized to probe for this kinase in various fractions from neutrophils (Fig. 6). Two prominent immunoreactive bands with molecular masses of ~83 and 74 kDa were observed when the 200,000 × g soluble fraction was examined by Western blotting along with several other minor bands (Fig. 6B, lane a). The 83-kDa band ran at the same position as LIMK1 in a lysate from rat brain during Western blotting, and both the 83- and 74-kDa bands were completely blocked by the immunizing peptide (data not shown). Interestingly, none of these bands were observed when the proteins bound to the DNase-affinity column were analyzed (Fig. 6B, lane c), even though this fraction was highly enriched in S3ck (Fig. 6A, lane c). The 83- and 74-kDa proteins remained soluble and did not bind to the DNase-agarose beads (Fig. 6B, lane b). No bands were observed in any of the fractions from neutrophils when a commercial Ab to LIMK-2 (LIMK-2 (C-19) Ab) was utilized in these experiments (data not shown).

When lysates of neutrophils (2 × 10^8 cell eq/ml) were treated with the LIMK1 Ab (15 µg of Ab/200 µl of lysate for 3.0 h) and the resulting immune complexes subjected to SDS-PAGE and Western blotting with the same Ab, immunoreactive bands of 83 and 74 kDa were observed (data not shown). When the LIMK1 immune complexes derived from neutrophils were tested in protein kinase assays, substantial activity was observed when MBP was the substrate (Fig. 7, lane e). Considerably less activity was observed with recombinant cofilin (Fig. 7, lane c). In three separate experiments analyzing the activity of the kinase(s) immunoprecipitated from neutrophils with the LIMK1 Ab, the amounts of radioactivity in the phosphorylated MBP and cofilin bands were 6,008 versus 750 cpm, 9,030 versus 514 cpm, and not detectable versus 1,763 cpm, respectively. When the 32P-labeled cofilin bands from these experiments were subjected to manual Edman degradation, the amounts of 32P in Ser-3 were 8%, 16%, and 18%. In contrast, under the same assay conditions, the corresponding values for the reaction catalyzed by the S3ck bound to DNase-agarose (i.e. Fig. 7, lane g) were 77%, 71%, and 78%, respectively. Cofilin phosphorylated with the immunoprecipitated kinase(s) (Fig. 7, lane c) ran at a slightly slower rate during SDS-PAGE than that phosphorylated with S3ck (Fig. 7, lane g), which indicates that the immunoprecipitated enzyme(s) catalyzed the phosphoryla-
immunoprecipitating LIMK1 from neutrophil lysates are described using 0.50 ml/min with the "extraction buffer" containing 100 mM by HPLC on a Shodex KW-803 column, which separates proteins obtained with the LIM kinase 1 antibody and S3ck from neutrophils—

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phagocytosis, and chemotaxis (11–14). Cofilin kinase in unstimulated neutrophils was not affected by the antagonists H-7, HA1004, ML-7, and KN-62 but inhibited by staurosporine (11). Activated cofilin would promote rapid dephosphorylation/activation of cofilin that is observed in unstimulated neutrophils and that the dephosphorylation in unstimulated neutrophils and that the cofilin phosphatase would account for the rapid dephosphorylation/activation of cofilin on Ser-3 in vitro (data not shown) (cf. Ref. 10).

Finally, we could not detect significant activity for S3ck after fractionation of the proteins in the 200,000×g soluble fraction by HPLC on a Shodex KW-803 column, which separates proteins on the basis of size. The column was run at a flow rate of 0.50 ml/min with the "extraction buffer" containing 100 mM KCl or NaCl used as the elution buffer. In contrast, a nonspecific cofilin kinase that was not active at Ser-3 was readily detectable in the 40–50-kDa region of the chromatograms (n = 3; data not shown). We are currently surveying a variety of tissues and techniques to enrich S3ck for sequencing experiments.

**DISCUSSION**

In this paper, we describe a protein kinase from neutrophils that selectively (>70%) catalyzes the phosphorylation of cofilin on Ser-3. As noted above, phosphorylation of cofilin on this residue blocks its ability to promote disassembly of actin-filaments (7, 8, 10). The S3ck we describe appears to be distinct from LIMK1 which recognizes cofilin in other cell types (24–26). The significance of these observations and properties of the enzyme are discussed below.

We have previously presented pharmacological evidence that cofilin is regulated by a continual cycle of phosphorylation and dephosphorylation in unstimulated neutrophils and that the phosphatase undergoes activation during cell stimulation (11). Activation of the cofilin phosphatase would account for the rapid dephosphorylation/activation of cofilin that is observed in stimulated neutrophils (11–14). Activated cofilin would promote the rapid changes in actin polymerization and depolymerization that are crucial for lamellipodia/pseudopodia formation, phagocytosis, and chemotaxis (11–14). Cofilin kinase in unstimulated neutrophils was constitutively active, insensitive to H-7, HA1004, ML-7, and KN-62 but inhibited by staurosporine (11). The cofilin kinase we describe in this paper exhibits sim-
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The reaction with 10 \( \mu \text{M} \) inhibitor for 5 min in the standard reaction mixture before initiating bound to the DNase-agarose beads. The kinase was incubated with the effects of selective inhibitors of protein kinases on the cofilin kinase from neutrophils.

The kinase was incubated with the phosphorylation of cofilin in these cells, it is not likely to be stimulated by activated Rac. As noted above, Rac undergoes a pronounced activation in stimulated neutrophils during the same time period (e.g. Refs. 27 and 28) in which cofilin undergoes massive dephosphorylation (11–14). Thus, the available data indicate that the major S3ck in neutrophils is not likely to be LIMK1 and that activated Rac does not promote net phosphorylation of cofilin in these cells.

As noted above, when \( ^{32}\text{P} \)-labeled cofilin prepared with S3ck was subjected to manual Edman degradation, about 70–80% of the \( ^{32}\text{P} \) was released with Ser-3 (Fig. 3). In fact, the actual amount of phosphorylation at this residue is likely to be even higher. The manual Edman degradation method works only on proteins/peptides in which the N-terminal amino group is free/unmodified (35).

The DNase-agarose beads used to partially purify S3ck form a very tight complex with monomeric actin, which in turn binds to a variety of actin-binding proteins (e.g. Ref. 43). Binding of this kinase to DNase-agarose beads is likely to involve (a) protein(s) other than cofilin since free cofilin alone could not elute S3ck from the beads (Fig. 5B).

The possibility exists that the phosphorylation of cofilin on Ser-3 may require proteins in addition to S3ck that are also bound to the beads (cf. Ref. 53).

In summary, we have reported that neutrophils contain a constitutively active protein kinase that selectively recognizes Ser-3 in cofilin and that this enzyme appears to be distinct from LIMK1. The sensitivity of this enzyme to a variety of kinase inhibitors in vitro parallels that observed in vivo for the physiological enzyme. This kinase can now be utilized to generate the large quantities of substrate that are needed to characterize the phosphatase that catalyzes the dephosphorylation/activation of cofilin in stimulated neutrophils.

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