Neutrophils stimulated with the chemoattractant fMet-Leu-Phe (fMLP) are known to exhibit rapid activation of four protein kinases with molecular masses of ~69, ~63, ~49, and ~40-kDa. Activation of these kinases is blocked by antagonists of phosphatidylinositol 3-kinase and type 1 or type 2A protein phosphatases. These enzymes can be detected by their ability to undergo renaturation and catalyze the phosphorylation of a peptide substrate that corresponds to amino acid residues 297–331 of the 47-kDa subunit of the NADPH-oxidase complex fixed within a gel. In this report, we demonstrate that an antibody generated to a fusion protein containing amino acid residues 175–306 of p21-activated protein kinase 1 (Pak1) reacts with three proteins in guinea pig neutrophils with molecular masses in the 60–70-kDa range during Western blotting. This antibody immunoprecipitates both the 69- and 63-kDa renaturable kinases from lysates of stimulated cells along with a minor 60-kDa kinase. No activities were observed for any of these enzymes in immunoprecipitates from unstimulated neutrophils. However, addition of ATP and activated Rac 1 or Cdc42 to immunoprecipitates from unstimulated cells resulted in the stimulation of two renaturable kinases with molecular masses in the 69- and 63-kDa range. These immunoprecipitates also contained two novel protein kinases with masses of ~49 and 40 kDa that were selectively activated by Cdc42. In contrast, the 69- and 63-kDa kinases were not immunoprecipitated from lysates of stimulated neutrophils with an antibody to Pak2 or with nonimmune serum. These data indicate that the renaturable 69- and 63-kDa kinases are Pakks and reveal some of the upstream events that are necessary for the rapid activation of this family of protein kinases in neutrophils.

Neutrophils stimulated with the chemoattractant fMet-Leu-Phe (fMLP) exhibit a rapid and transient activation of a group of unidentified protein kinases with molecular masses in the 60–70-kDa range (1–5). These kinases can be detected by procedures based on their ability to undergo renaturation and autophosphorylation in gels (3, 5) or to catalyze the phosphorylation of an endogenous substrate fixed in a gel (3–5). The substrates utilized include histone H4, myelin basic protein, and a peptide that corresponds to residues 297–331 of the 47-kDa subunit of the NADPH-oxidase complex (p47phox) (1–5). Previous studies have shown that all of these unidentified kinases are under the control of a novel stimulatory pathway that does not involve Ca2+, CAMP, or a diacylglycerol/tumor promotor-sensitive isofrom of protein kinase C (1–5). However, activation of the 60–70-kDa group of kinases can be blocked by pertussis toxin (2, 4, 5), antagonists of protein tyrosine kinases (6), inhibitors of type 1 or type 2A protein phosphatases (3, 4, 6), and antagonists of phosphatidylinositol 3-kinase (7). The latter compounds are known to block superoxide release, degranulation, and phagocytosis in neutrophils (e.g., Refs. 7–9). Thus, the renaturable 60–70-kDa protein kinases may participate in a variety of important functional responses of neutrophils.

Neutrophils (10–12) and other cell types (13–16) have recently been shown to contain a number of p21-activated protein kinases (Pakks) that are responsive to cell stimulation (10, 16). These enzymes also exhibit molecular masses in the 60–70-kDa range and undergo autophosphorylation/activation upon interacting with the GTP-bound forms of the small GTP-binding proteins Rac or Cdc42 (p21) (10–16). Recent studies indicate that Pakks are critical components in various kinase signaling cascades (e.g., Refs. 17–19; for review see Ref. 20) and may associate with proteins that contain SH3 domains (14). In this article, we report that the renaturable 69- and 63-kDa kinases that undergo rapid activation in fMLP-stimulated neutrophils are Pakks. Important implications of these observations to the regulation of Pakks in rapidly stimulatable cells are discussed.

EXPERIMENTAL PROCEDURES

Materials

Antibodies to Paks were raised in rabbits against GST fusion proteins corresponding to amino acids 175–306 of rat Pak1 (GST/Pak1175–306) and amino acids 1–252 of rat Pak2 (GST/Pak21–252) (10). These antibody preparations are referred to as Pak1 and Pak2 antibodies, respectively. Affinity purified, rabbit polyclonal antibodies raised against peptides corresponding to amino acid residues 2–21 (Pak (N-20) ab) and 325–544 PMA, 4β-phorbol 12-myristate 13-acetate; GST, glutathione S-transferase; Pak1 ab, rabbit antiserum raised against a GST fusion protein containing amino acid residues 175–306 of rat Pak1; Pak2 ab, rabbit antiserum raised against a GST fusion protein containing amino acid residues 1–252 of rat Pak2; PAGE, polyacrylamide gel electrophoresis; Me2SO, dimethyl sulfoxide; ab, antibody.
(Pak (C-19) ab) of rat Pak1 were purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. Recombinant Rac 1 and Cdc42 were prepared as outlined in Refs. 21 and 22. These proteins were loaded with GTPγS as described in Refs. 21 and 22. Nonidet P-40, GTPγS, sodium orthovanadate, and leupeptin were obtained from Sigma. ImmunoPure immobilized protein A was purchased from Pierce. Sources of all other materials are described elsewhere (3, 4).

Methods

Preparation of Neutrophils—Guinea pig peritoneal neutrophils were prepared as described previously (23).

Preparation of Samples, Immunoprecipitation of Kinases, and SDS-PAGE—Neutrophils (1.0 × 10^6/ml) were stimulated with 1.0 μM fMLP as described (3). At the appropriate time, 0.50 ml of the reaction mixture was transferred to a microcentrifuge tube containing 0.50 ml of 2× SDS buffer (2× concentrated “immunoprecipitation buffer” (ip-B)) and rapidly mixed. The final composition of SDS-B after mixing was 2.3% (w/v) SDS, 62.5 mM Tris-HCl (pH 6.8), 5.0 mM EDTA, 10.0% (w/v) glycerol, 5.0% (w/v) 2-mercaptoethanol, and 0.002% (w/v) bromphenol blue. The composition of SDS-B was 50 mM NaCl, 5.0 mM MgCl2, 1.0 mM EGTA, 50 mM NaHCO3, 10 mM sodium pyrophosphate, 1.0% (w/v) Nonidet P-40, 2.5% (v/v) glycerol, 1.0 mM Na2VO4, and 0.20 mM phenylmethylsulfonyl fluoride. The renaturable kinases in cells lysed with these different buffers are compared in Fig. 1.

Immunoprecipitation reactions were performed on lysates of neutrophils prepared only with ip-B (5 × 10^6 cells/1.0 ml) as described (10). After the final wash, the protein A-coated beads containing the bound kinases were resuspended in 1.0 ml of SDS-B. The supernatant fraction (1.0 ml) that remained after removal of the protein A-coated beads was mixed with 0.25 ml of 5 × concentrated SDS-B. All samples were immersed in a boiling water bath for 3 min and then subjected to SDS-PAGE (0.07 ml/lane) on 9.0% (w/v) polyacrylamide slab gels as described (3).

Detection of Renaturable Protein Kinases in Polyacrylamide Gels—Protein kinases were detected directly in gels by their ability to undergo renaturation and catalyze the phosphorylation of a peptide substrate “fixed” within the gel that corresponds to residues 297–331 of p47phox as described previously (4).

In Vitro Activation of Protein Kinases—Latent protein kinases from unstimulated neutrophils were immunoprecipitated (5 × 10^6 cells/1.0 ml) as noted above (10). The protein A-coated beads containing the bound enzymes were washed two times with ip-buffer (1.0 ml) followed by three washes (1.0 ml) with “kinase buffer” (50 mM Heps (pH 7.5), 10 mM MgCl2, 10 mM MnCl2, and 0.20 mM dithiothreitol). The immobilized kinases were subsequently incubated at 30 °C in 0.1 ml of kinase buffer containing 5 μM ATP, 45 μM GTPγS with or without 1.4 μg of Rac 1-GTPγS or Cdc42-GTPγS. After 60 min, the beads were isolated by centrifugation, resuspended in 0.10 ml of solubilization buffer, and immersed in a boiling water bath for 3 min. Samples (0.07 ml) were subjected to SDS-PAGE on 9.0% gels containing the p47phox peptide, and the renaturable kinases were assayed (4).

Immunoblotting—Paks were detected by Western blotting with antisera to these enzymes (1:1000 dilution) and alkaline phosphatase-conjugated anti-IgG as the secondary antibody. The desired color was achieved with Promega’s alkaline phosphatase 5-bromo-4-chloro-3-indoly phosphate/nitroblue tetrazolium kit. The conditions utilized for this assay are described (3).

RESULTS

Certain protein kinases in neutrophils can be detected directly in gels by their ability to undergo renaturation and catalyze the phosphorylation of a peptide substrate fixed uniformly within the gel. The positions of the kinases are visualized by autoradiography after incubation of the gel with [γ-32P]ATP (4, 5). Utilizing this technique, neutrophils stimulated with fMLP are known to exhibit rapid activation of two protein kinases in the 60–70-kDa region with molecular masses of ~60 and ~63 kDa. A third minor band with a molecular mass of ~60 kDa was also observed in some experiments (e.g. Fig. 1A; Refs. 4, 5, 7). Interestingly, three immunoreactive bands in the 60–70-kDa region were also observed when lysates from guinea pig neutrophils were blotted with a polyclonal antibody to a GST-fusion protein containing amino acid residues 175–306 of rat Pak1 (Fig. 2, lane a). Three prominent immunoreactive bands in the 60–70-kDa area were also observed with the Pak (N-20) and Pak (C-19) antibodies that are directed toward different regions of Pak (data not shown). In contrast, an antibody raised to a fusion protein containing amino acid residues 1–252 of rat Pak2 recognized only one single band in the 60–70-kDa area. The band recognized by the Pak2 antibody (ab) exhibited a molecular mass very similar to that of the lowest band recognized by the Pak1 ab (~60 kDa) (Fig. 2, lane b, broken arrow). This was confirmed by the appearance of only three immunoreactive bands in the 60–70-kDa area during Western blotting with a mixture of Pak1 and Pak2 ab (Fig. 2, lane c). Previous studies have shown that these antibodies specifically recognize protein kinases in neutrophils with molecular masses of 60–70 kDa that are activated by Rac-GTP or Cdc42-GTP (10). None of these bands were observed with nonimmune serum, even at a concentration 5-fold greater than that employed with Pak1 or Pak2 ab (Fig. 2, lane d). At least three highly homologous members of the Pak family are known to exist in various mammalian cells (e.g. Refs. 14, 16). Neutrophils are known to contain three Rac/Cdc42-GTP binding proteins and at least 2 Paks in the 60–70-kDa range (10–12). Immunoprecipitation experiments were therefore undertaken to determine if the renaturable 69- and 63-kDa kinases were Paks (Fig. 1). The lysis buffer previously employed to rapidly disrupt neutrophils during renaturation studies contained 2.3% (w/v) SDS (SDS-B) that interferes with the antibody-antigen reaction. This buffer was therefore replaced in these studies with the lysis buffer previously employed to immunoprecipitate Pak (ip-buffer) (10). Activation of the renaturation process was achieved with GTPγS as described in Refs. 21 and 22. Nonidet P-40, GTPγS, sodium orthovanadate, and leupeptin were obtained from Sigma. ImmunoPure immobilized protein A was purchased from Pierce. Sources of all other materials are described elsewhere (3, 4).
rable 69- and 63-kDa kinases was readily detectable in lysates of fMLP-stimulated neutrophils with the ip-buffer, although considerable differences were observed for kinases in other regions of the gel (Fig. 1A). These differences may reflect the ability of SDS to rapidly inactivate proteases and phosphatases and to solubilize membrane proteins.

Treatment of lysed fMLP-stimulated neutrophils with Pak1 ab resulted in the immunoprecipitation of the 69- and 63-kDa kinases (Fig. 1B, lane a) along with the removal of these enzymes from the whole cell lysate (Fig. 1B, lane f). This anti-serum also appeared to precipitate the minor 60-kDa kinase (Fig. 1, lane a). No activities were observed for any of these kinases when the immunoprecipitates were obtained from unstimulated cells (Fig. 1B, lane d). Thus, the three immunoreactive bands observed with the Pak1 ab in the Western blots of Fig. 2 correspond to three renaturable protein kinases that are activated during cell stimulation. The 69- and 63-kDa kinases were also immunoprecipitated with the Pak (N-20) and Pak (C-19) antibodies (n = 2, data not shown). These reactions are highly specific since the 69- and 63-kDa kinases were not precipitated by Pak2 ab or nonimmune serum (Fig. 1B, lanes b and c). Earlier studies have reported that the 69- and 63-kDa kinases do not react with antibodies to various isoforms of protein kinase C, Raf-1, p70-S6 kinase, or mitogen-activated protein kinase kinase 2 (MAPKK2) (4–6). Pak2 ab did precipitate the minor 60-kDa kinase (Fig. 1B, lane b). This observation is also consistent with the data in Fig. 2 where this serum reacted with a single band in the 60–70-kDa area that corresponded to the lowest band observed with the Pak1 ab. In contrast to the results presented in Fig. 1B, the Pak 2 ab is known to immunoprecipitate an amount of activity from stimulated human neutrophils similar to that observed with the Pak 1 ab when assayed by autophosphorylation (10). Whether these results reflect differences in the species employed or differences in the abilities of Pak 1 and Pak 2 to undergo renaturation and catalyze the phosphorylation of the p47phox peptide fixed within a gel is not known.

The effects of activated Rac 1 or Cdc42 on the latent renaturable protein kinases are presented in Fig. 3. As noted above, no activity was observed for the 69- and 63-kDa kinases in Pak1 ab immunoprecipitates from unstimulated neutrophils (lane a). However, the addition of 200 μM ATP and Rac 1-GTPγS (lane b) or Cdc42-GTPγS (lane c) to these immunoprecipitates resulted in the activation of a 63-kDa kinase along with a broad, diffuse band of kinase activity that originated in the 69-kDa region of the gel. Similar results were observed in four separate experiments. The exact reason for this broad band of kinase activity is not known. However, purified Pak1 activated in vitro is known to exhibit a slower mobility and a considerably broader band on SDS-PAGE due to significant size heterogeneity that results from hyperphosphorylation (e.g. Refs. 13, 15). The highly basic nature of the peptide substrate fixed in the gel could exacerbate this situation (10 of the 35 residues are positively charged). There is evidence that Pak1 can undergo autophosphorylation on at least six serine residues in the N-terminal region (15), whereas phosphorylation on only two sites may be necessary for activation (25). Phosphorylation of Pak on only these two sites in vivo would provide an explanation for the sharp bands of kinase activity observed in stimulated neutrophils (Fig. 1) versus the more diffuse bands observed in Fig. 3 (see “Discussion”). Treatment of immunoprecipitates from unstimulated neutrophils with ATP and Cdc42-GTPγS, but not Rac-GTPγS, also resulted in a selective activation of two additional kinases with molecular masses of ~49 and 40 kDa (Fig. 3, lane c, asterisks) (see “Discussion”). The kinases observed in Fig. 3 were not activated by GTPγS alone (lane a) or if ATP was omitted from the reaction mixture (data not shown).

Experiments were performed to establish that the 69- and 63-kDa kinases observed in cells disrupted with ip-buffer were the same enzymes as those observed when the SDS-solubilization medium was employed. These studies were undertaken because of the marked differences observed for protein kinases in other regions of the gel when various lysis conditions were utilized (Fig. 1A). A variety of structurally distinct antagonists of PI 3-K (e.g. wortmannin) and type 1 and/or type 2 protein phosphatases (e.g. calyculin A) are known to prevent activation of the 69- and 63-kDa kinases in fMLP-stimulated neutrophils disrupted with the SDS-solubilization medium (3, 4, 6). Wortmannin (200 nM) and calyculin A (20 nM) also blocked activation of these kinases when the ip-buffer was employed (Fig. 4). As reported previously (3, 4), neutrophils treated with calyculin A also exhibited activation of several uncharacterized protein kinases (Fig. 4, lane d, open arrowheads). Finally, Fig. 5 compares the activities of the renaturable 69- and 63-kDa kinases in whole cell lysates prepared with SDS-B (part A) and in Pak1

![Fig. 2. Pak-related protein kinases in neutrophils. Western blots show the presence of three proteins in guinea pig neutrophils that react with antibodies generated to sequences present in various Pakks. Proteins (40 μg/lane) from unstimulated neutrophils were separated by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with Pak1 ab (GSTPak1175–306) (a), Pak2 ab (GST/Pak25–282) (b), Pak1 ab plus Pak2 ab (c), and nonimmune rabbit serum (d). Antibodies to Pakks were diluted 1:1000, whereas the nonimmune serum was diluted 1:200.](image-url)
Minor 60-kDa kinase (Fig. 1B). All three of these enzymes underwent renaturation and catalyzed the phosphorylation of the p47phox peptide (Fig. 1B). The kinetics of activation for the precipitated kinases are virtually identical to that displayed by the renaturable 69- and 63-kDa kinases in lysates of fMLP-stimulated cells (Fig. 5). In contrast, no activities were observed for these enzymes in immunoprecipitates prepared from unstimulated neutrophils. However, these immunoprecipitates contained latent kinases in the 69- and 63-kDa range that were activated by Rac- or Cdc42-GTPyS in a reaction that required ATP (Fig. 3). These in vitro activated kinases were also capable of undergoing renaturation and catalyzing the phosphorylation of the p47phox peptide substrate. The complete p47phox protein is a substrate for Pak (10). These data strongly support the recent proposal (10) that the renaturable 69- and 63-kDa kinases are Pakks. Moreover, the identification of these kinases extends our knowledge of how Pakks are activated in neutrophils by illuminating some of the upstream events in this process previously uncovered for the renaturable kinases (see below).

There are several noteworthy implications of this work that pertain to the regulation of Pakks in stimulatable cells. As noted above, Pakks undergo autophosphorylation/activation upon interacting with the active (GTP-bound) form of p21 (Rac and/or Cdc42) (10, 13). This reaction requires several minutes to 1 h for maximal activation to occur in vitro (11, 15). In contrast, optimal activity of the 69- and 63-kDa Pakks is achieved within 15 s in stimulated neutrophils (Fig. 5; Ref. 10). Moreover, fMLP and the tumor promoter PMA stimulate activation of Rac to the same extent in neutrophils as measured by the dissociation of this protein from Rho GDP-dissociation inhibitor and translocation to the plasma membrane (27, 28). However, fMLP stimulates marked activation of the 69- and 63-kDa Pakks in neutrophils, whereas PMA does not (1–5, 10). These data indicate that the rapid activation of Pakks in stimulated cells is likely to require signals in addition to the binding of p21-GTP.

Antagonists of PI 3-K and types 1 and 2A protein phosphatases block activation of the renaturable 69, 63, 49, and 40-kDa protein kinases in fMLP-stimulated neutrophils (3–7). With regard to the discussion above, it is noteworthy that PI 3-K is activated in neutrophils treated with fMLP but not PMA (29).

Moreover, the time course for the activation of PI 3-K in these cells (30) is very similar to those described for the activation of the 69- and 63-kDa Pakks (Fig. 5; Refs. 4, 5, 7, 10). This is consistent with phosphatidylinositol 3,4,5-P3 or another 3-phosphorylated inositide serving as a second messenger in this response. Products of PI 3-K are known to activate various isoforms of protein kinase C (e.g. Refs. 31, 32) and other protein kinases (e.g. Akt) (33). Alternatively, PI 3-K itself possesses protein kinase activity (34). A ubiquitous serine/threonine kinase has also been described that is activated by a type-2A protein phosphatase (35). Thus, one or more of these kinases may function as upstream activators of Pak. It is possible that antagonists that affect Pakks do so by blocking the activation of Rac or Cdc42 (cf. Ref. 36). However, neutrophils treated with calycin A exhibit an enhanced translocation of Rac to the membrane upon stimulation with fMLP (37) that indicates that at least some of these effects are likely to be independent of p21. In contrast, antagonists of protein tyrosine kinases block both translocation of Rac (37) and activation of the 60–70-kDa kinases (6). Thus, this group of inhibitors may block stimulation of Pakks by preventing the activation of p21. Interestingly, platelets stimulated with thrombin exhibit rapid activation of γ-Pak (16, 38). This Pak was also activated in platelets treated with both PMA and a Ca2+ ionophore but not when either agent was added separately (38). In contrast, treating neutro-

**FIG. 4. Effects of various antagonists on the activation of the 69- and 63-kDa protein kinases.** Autoradiograms demonstrate the ability of wortmannin or calycin A to block activation of the 69- and 63-kDa protein kinases in fMLP-stimulated neutrophils. Cells were lysed with the buffer employed in the immunoprecipitation experiments (ip-B). The following conditions are shown: lane a, cells treated with 0.25% (v/v) Me2SO vehicle (negative control); lane b, cells treated with 0.25% (v/v) Me2SO for 5 min followed by 1.0 μM fMLP for 30 s; lane c, cells treated with 0.25% (v/v) Me2SO for 5 min followed by 1.0 μM fMLP for 30 s; lane d, cells treated with 200 nM wortmannin for 5 min followed by 1.0 μM fMLP for 30 s; lane e, cells treated with 20 nM calycin A for 10 min followed by 1.0 μM fMLP for 30 s. The positions of the 69- and 63-kDa kinases are designated by an arrowhead and arrow, respectively, whereas the broken arrow denotes the 60-kDa kinase. Uncharacterized protein kinases that undergo marked activation in cells treated with calycin A are denoted with open arrowheads.

**FIG. 5. Activation of the 69- and 63-kDa protein kinases in neutrophils stimulated with fMLP for various times.** The 69- and 63-kDa renaturable protein kinases were measured in lysates of whole neutrophils disrupted with solubilization buffer containing SDS (SDS-B) (part A) and in complexes from lysed cells immunoprecipitated with Pak1 ab (part B). The autoradiograms shown are from cells treated with 0.25% (v/v) Me2SO for 15 s (unstimulated cells) (a), 1.0 μM fMLP for 15 s (b), 1.0 μM fMLP for 30 s (c), 1.0 μM fMLP for 3 min (d), 1.0 μM fMLP for 5 min (e), and 0.25% (v/v) Me2SO for 5 min (f). The positions of the 69- and 63-kDa kinases are designated by an arrowhead and arrow, respectively. The open arrowhead denotes a protein kinase that undergoes activation at time points greater than 30 s.

**DISCUSSION**

In this paper, we report that an antisera to a fusion protein containing residues 175–306 of rat Pak1 reacts with three proteins in guinea pig neutrophils with molecular masses in the 60–70-kDa range (Fig. 2). This antibody immunoprecipitated two active protein kinases from fMLP-stimulated neutrophils with molecular masses of ~69 and 69 kDa along with a major 60-kDa kinase (Fig. 1B). All three of these enzymes underwent renaturation and catalyzed the phosphorylation of the p47phox peptide (Fig. 1B). The kinetics of activation for the precipitated kinases are virtually identical to that displayed by the renaturable 69- and 63-kDa kinases in lysates of fMLP-stimulated cells (Fig. 5). In contrast, no activities were observed for these enzymes in immunoprecipitates prepared from unstimulated neutrophils. However, these immunoprecipitates contained latent kinases in the 69- and 63-kDa range that were activated by Rac- or Cdc42-GTPyS in a reaction that required ATP (Fig. 3). These in vitro activated kinases were also capable of undergoing renaturation and catalyzing the phosphorylation of the p47phox peptide substrate. The complete p47phox protein is a substrate for Pak (10). These data strongly support the recent proposal (10) that the renaturable 69- and 63-kDa kinases are Pakks. Moreover, the identification of these kinases extends our knowledge of how Pakks are activated in neutrophils by illuminating some of the upstream events in this process previously uncovered for the renaturable kinases (see below).

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phils with the combination of a Ca\textsuperscript{2+} ionophore and PMA did not result in the activation of the 69- and 63-kDa kinases but, in fact, reduced the basal levels of these enzymes (3). Thus, different regulatory pathways may be responsible for the rapid activation of specific Paks and/or modulate Paks in various stimulatory cells.

In contrast to Pak that may participate in the phosphorylation of p47\textsuperscript{phox}, the NADPH oxidase is also stimulated directly by activated Rac but not Cdc42 in a cell-free system (22, 39). The cell-free system generally employed bypasses the necessity for phosphorylation of p47\textsuperscript{phox} (e.g. Ref. 40). Direct binding of Rac to the 67-kDa subunit of the oxidase has been demonstrated both in vitro (12, 41) and in vivo (42). Finally, recent studies suggest that small GTP-binding proteins (e.g. Ras and Rac) can serve as components of various signaling complexes that consist of two or more physically associated protein kinases (16, 43). It will be of interest to determine if the Cdc42-activated protein kinases that coprecipitated with Pak (Fig. 3, lane c) are involved in such a complex and if these enzymes are identical to the renaturable 49- and 40-kDa kinases that also undergo rapid activation in chemotaxtractant-stimulated neutrophils (3–6).

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