Cyclic Guanosine Monophosphate–Dependent Protein Kinase Is Targeted to Intermediate Filaments and Phosphorylates Vimentin in A23187-Stimulated Human Neutrophils

By Katherine B. Pryzwansky, Todd A. Wyatt, and Thomas M. Lincoln

The effects of the calcium ionophore, A23187, on human neutrophil activation were studied in relation to the signaling mechanism of cyclic guanosine monophosphate (cGMP)-dependent protein kinase (G-kinase). Immunocytochemistry demonstrated that G-kinase translocated from a diffuse localization in the cytoplasm to the cytoskeleton after stimulation with A23187. Over a period of 5 minutes, G-kinase was transiently colocalized with the intermediate filament protein, vimentin. At 3 minutes' stimulation with A23187, colocalization with A23187, colocalization of G-kinase and vimentin was predominantly confined to filaments that extended into the uropod. The time of colocalization of G-kinase and vimentin was reduced in the A23187-stimulated cell from 3 minutes to 1 minute by 8-Br-cGMP. Coincident with colocalization was an increase in cGMP levels and transient phosphorylation of vimentin in adhered A23187-stimulated cells. Phosphorylation of vimentin was maximal after 3 minutes with A23187, and was essentially over at 5 minutes. The time of phosphorylation of vimentin was also reduced from 5 minutes to 1 minute when cells were preincubated with 8-Br-cGMP and then stimulated with A23187, which suggests that cyclic adenosine monophosphate (cAMP)-dependent protein kinase does not phosphorylate vimentin in A23187-treated neutrophils. Phosphorylation of vimentin was not observed in nonactivated cells treated only with 8-Br-cGMP. The presence of the protein kinase C inhibitors, staurosporine or H-7, did not inhibit vimentin phosphorylation in A23187-treated cells, which provides supportive data that protein kinase C is not the phosphorylating enzyme. These results suggest that vimentin and G-kinase are colocalized in a Ca2+-dependent manner in neutrophils, and that vimentin is transiently phosphorylated by G-kinase in response to the colocalization of the two proteins. The transient redistribution of compartmentalized G-kinase represents one type of neutrophil activation mechanism.

© 1995 by The American Society of Hematology.
formyl peptide, FMLP, G-kinase transiently colocalized with the intermediate filament protein vimentin, and that vimentin was phosphorylated by G-kinase at the time when the two proteins were colocalized. We suggested that Ca\textsuperscript{2+} mobilizing agents such as FMLP lead to production of NO and cGMP, which activate G-kinase to colocalize with intermediate filaments and phosphorylate vimentin. More recent studies suggest that G-kinase may regulate neutrophil secretion of FMLP and A23187-stimulated neutrophils.

In this report, we investigated the effect of A23187 on G-kinase activation to determine if both Ca\textsuperscript{2+} and cGMP are important for the targeting of G-kinase to intermediate filaments and phosphorylation of vimentin. The results of these studies support the hypothesis that human neutrophil vimentin phosphorylation is dependent on both a Ca\textsuperscript{2+}-dependent localization of G-kinase and substrate and a cGMP-producing stimulus.

MATERIALS AND METHODS

Reagents. Mono-Poly Resolving media was obtained from Flow-ICN (Costa Mesa, CA). Fluorescein isothiocyanate (FITC)- goat antirabbit IgG and tetramethylrhodamine isothiocyanate (TRITC)-sheep antimouse IgG were from Organon Teknika-Cappel (Malvern, PA). Mouse antiporcine lens vimentin was obtained from Dako (Santa Barbara, CA). Rabbit antivim. bina. aorta G-kinase was characterized for specificity in smooth muscle cells, and is cross-reactive with human neutrophil G-kinase, as determined by the immunoprecipitation of a single protein band (M_\text{r} = 77 kDa) shown by Western blot analysis of neutrophil extracts. Rabbit antihuman fibroblast vimentin was obtained from Chemicon (Temecula, CA). Purified bovine lens vimentin and staurosporine were purchased from Boehringer Mannheim (Indianapolis, IN). Bovine lung G-kinase was purified as previously described using cyclic adenosine monophosphate (cAMP)-agarose affinity chromatography. Adenosine triphosphate (ATP) ([\textsuperscript{32}P]) and [\textsuperscript{35}S]-orthophosphate were obtained from ICN Radiochemicals (Irvine, CA). Scintillation proximity assay (SPA) kit was supplied by Amersham (Amersham, UK). A23187 and H-7 [1-({5-isoquinolinesulfonyl)-2-methylpiperazine, diHCl] were purchased from Calbiotech (San Diego, CA). \beta-Mercaptoethanol was obtained from Bio-Rad (Richmond, CA). Nonidet P-40 was obtained from US Biochemicals (Cleveland, OH). The bicinechonic acid/protein assay kit was purchased from Pierce (Rockford, IL). Okadaic acid was a gift from Dr. W W. Dickey, Dauphin Island Sea Laboratory, Dauphin Island, AL. All other reagents were purchased from Sigma (St Louis, MO).

Cell preparation. Neutrophils were isolated from human peripheral blood with Ficol-Hypaque Mono-Poly Resolving media. The cells were resuspended at 3 x 10\textsuperscript{6} cells/mL in Gey's balanced salts buffered with 10 mM Hepes, pH 7.3, and supplemented with 1.5 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 0.3 mM MgSO\textsubscript{4}, and 10% human type AB serum. Neutrophils were adhered to glass coverslips for 15 minutes or to 60-mm Petri culture dishes for 30 minutes at 37°C. Unattached cells were removed by washing with GBS. Cells consisted of greater than 95% neutrophils as determined by Wright's stain, and were greater than 98% viable by trypan blue exclusion. All reagents were checked for endotoxin by the limulus test and contained less than 0.07 ng/mL of endotoxin.

Immunofluorescence localization. Neutrophil monolayers were stimulated with 20 \muM A23187 in GBS plus 1% human type AB serum from 30 seconds to 5 minutes at 37°C. The culture media was removed and the cells were quickly fixed at room temperature in 1% paraformaldehyde in 0.075 M cacodylate buffer containing 0.72% sucrose, pH 7.5, for 10 minutes, followed by 3.7% formaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 10 minutes, -20°C methanol for 4 minutes and -20°C acetone for 1 minute. Cells were washed in PBS after formaldehyde and acetone. In some instances, cells were preincubated with 1 \muM 8-Br-cGMP for 15 minutes, followed by incubation with 20 \muM 8-Br-cGMP for 10 minutes, fixed and viewed on a Leitz Orthoplan fluorescence microscope (E. Leitz Inc, Rockleigh, NJ). Immunofluorescence photomicrographs were taken on a Leitz Orthomat Camera with Kodak Tri-X Pan film. ASA 400 (Eastman Kodak, Rochester, NY). Each experiment was performed at least three times.

Phosphorylation of vimentin in situ. Neutrophils were adhered to 60-mm polystyrene Petri dishes for 2 hours with rocking at 37°C with 150 mM NaCl, 5 mM Tris (pH 7.4), and 10% human type AB serum, washed free of serum, and then stimulated with FMLP. Cells were preincubated with 1 \muM 8-Br-cGMP, or/and the protein kinase C inhibitors staurosporine (100 \muM) or H-7 (100 \muM). Cells were then treated with 20 \muM A23187 for 1 to 5 minutes. To demonstrate that both staurosporine and H-7 inhibited protein kinase C, at these concentrations, superoxide ion generation was measured in adherent neutrophils stimulated for 30 minutes with 20 ng/mL phorbol myristate acetate (PMA), as described. Superoxide production was inhibited by 93% and 100% by staurosporine and H-7, respectively.

The reactions were immediately terminated in a postreaction mixture consisting of 0.1% sodium docetyl sulfate (SDS), 100 \mug/mL DNase I, 100 \mug/mL RNase A, 1.2 mM p-amrinobenzamidine, 1 \muM okadaic acid, and 1 mM EGTA. The cell samples were rapidly frozen in dry ice with ethanol and thawed six times, and then sonicated at 4°C for 3 minutes. Samples were then heated to 100°C for 5 minutes, and vimentin immunoprecipitated with 5 \muL of undiluted rabbit antihuman fibroblast vimentin overnight at 4°C, and 20 \muL Protein-A-Sepharose for 4 hours at 4°C. The immunoprecipitated protein was washed three times in a wash buffer containing 50 mM Tris-Cl, 0.2 mM NaCl, 1 mM EGTA, 0.05% Triton X-100, and 0.05% SDS, reduced in SDS-reducing buffer, resolved by polyacrylamide gel electrophoresis (PAGE), and exposed to x-ray film for 72 hours at -70°C. Each experiment was performed at least three times.

Measurement of cGMP levels. The levels of cGMP were measured in neutrophil monolayers and cell suspensions in triplicates. Cells were adhered to 60-mm Petri dishes in the presence of 10% human type AB serum, washed free of serum, and then stimulated with A23187 in the absence of serum. The culture supernatants were discarded and cGMP levels were determined on the adhered cells or cell pellets from cells stimulated in suspension. The media was quickly removed and the reaction was stopped by the addition of 1 mL ice-cold 50 mM acetate buffer, pH 4.0, containing 0.1% Triton X-100 and 1.2 mM p-aminobenzamidine. The dishes were then flash-frozen with liquid nitrogen and stored at -70°C. The cells were thawed at room temperature, scraped from the dishes, and a small portion (50 \muL) assayed for total protein. Protein concentration was determined for each sample using the BCA protein microplate assay kit. The remaining of the cellular protein was heated to 90°C for 5 minutes, microfuged for 15 minutes at 4°C, and the pellets discarded. The sample supernatants were then concentrated by a speed vac concentrator, reconstituted with 250 \muL H\textsubscript{2}O, and acetylated with 5 \muL of a reaction mixture consisting of one part acetic acid/one part 3 M H\textsubscript{3}PO\textsubscript{4}.
anhydride and two parts triethylamine. The amount of cGMP was determined in duplicates using an \(^{125}\text{I}\)-cGMP scintillation proximity assay (SPA) kit (Amersham).

**RESULTS**

*Colocalization of G-kinase and vimentin in A23187-stimulated cells.* To determine if vimentin colocalizes with G-kinase during A23187 treatment, neutrophils were stimulated from 30 seconds to 5 minutes with 20 \(\mu\text{mol/L}\) A23187 and stained by double-label immunofluorescence microscopy for G-kinase and vimentin. In unstimulated neutrophils, G-kinase was diffusely localized in the cytoplasm, with some staining at the microtubule organizing center. Colocalization of G-kinase and vimentin was not observed in unstimulated cells (Fig 1a and b), as only a diffuse localization of G-kinase was observed in the cytoplasm without any filamentous colocalization with vimentin. Similarly, no notable changes in localization were observed after 30 seconds of treatment with A23187 (data not shown). After 1 minute of stimulation with A23187, the cells began to polarize and...
Fig 2. Double-label immunofluorescence microscopy of FITC-anti-G-kinase (a,c,e,g) and TRITC antivimentin (b,d,f,h) of neutrophils preincubated with 1 μmol/L 8-Br-cGMP for 15 minutes followed by stimulation with 20 μmol/L A23187 (a,b—8-Br-cGMP alone; c,d—1 minute; e,f—3 minutes; g,h—5 minutes). Prominent colocalization was observed at 1 and 3 minutes (arrows). There is coincident staining for G-kinase and vimentin in some cells at 5 minutes (g,h, arrows). (Original magnification × 800.)
there was diffuse staining of G-kinase in the uropod and cytoplasm of some cells, while other polarized cells displayed colocalization of G-kinase and vimentin (Fig 1c and d). Maximal colocalization was observed at 3 minutes of treatment with A23187, as G-kinase and vimentin colocalized in the uropod of polarized cells and in bidirectional locomoting cells (Fig 1e and f). After 5 minutes of treatment with A23187, colocalization of G-kinase and vimentin was no longer apparent. At this time, the microtubule organizing center and the nucleus were the only identifiable structures staining for G-kinase, as staining for vimentin remained around the nucleus, albeit further condensed (Fig 1g and h). Control cells did not stain when antibody was replaced with preimmune rabbit sera or normal mouse IgG (not shown). These studies show that G-kinase is transiently colocalized with vimentin during A23187 stimulation.

Effects of cGMP on G-kinase and vimentin colocalization. G-kinase is an inactive enzyme, which requires an elevation of cGMP levels for activation. To determine whether the elevation of cGMP enhances G-kinase colocalization with vimentin, cells were preincubated with 1 µmol/L 8-Br-cGMP for 15 minutes and then stimulated with A23187. The time of colocalization of G-kinase and vimentin was reduced by 8-Br-cGMP from 3 minutes to 1 minute, but only in the presence of A23187 (Fig 2). Incubation of cells with 8-Br-cGMP alone did not induce targeting of G-kinase to intermediate filaments (Fig 2a and b). In the presence of A23187, colocalized staining of G-kinase and vimentin was observed at all time points on filaments within the uropod of polarized cells or within a focal region in the cytoplasm (Fig 2). However, the most dramatic colocalization was observed at 3 minutes (Fig 2e and f), when shape changes were most dramatic. Colocalization of G-kinase and vimentin also appeared to be transient, as colocalization was less frequent after 5 minutes of treatment with A23187 and 8-Br-cGMP (Fig 2g and h).

cGMP levels in A23187-stimulated neutrophils. Based on the immunofluorescence data suggesting that increasing exogenous cGMP enhances A23187-stimulated colocalization of G-kinase and vimentin, intracellular levels of cGMP were monitored in adhered neutrophils to determine whether cGMP levels were elevated in response to A23187. As shown in Fig 3, cGMP levels were significantly increased within 30 seconds of stimulation with A23187, and remained elevated for 5 minutes. No changes in cGMP levels were detected at any time point in unstimulated cells or in cells stimulated in suspension (data not shown). The amount of cGMP was expressed per milligram of protein to control for variability in numbers of attached cells per dish. The overall trend for each subject was identical, although the individual responses between subjects varied as previously reported.26

Phosphorylation of vimentin by G-kinase. Adherent neutrophils were labeled with 32P and stimulated with A23187 for various time points both with and without 1 µmol/L 8-Br-cGMP to determine if immunoprecipitated vimentin was phosphorylated by G-kinase. Phosphorylation of vimentin by G-kinase also appeared to be transient. In adhered neutrophils, maximal phosphorylation of vimentin was detected in cells stimulated with A23187 for 3 minutes (Fig 4, lane 2), and with some phosphorylation observed at 1 minute (Fig 4, lane 1). No phosphorylation was detected at 5 minutes. As observed with the effects of 8-Br-cGMP on G-kinase and vimentin colocalization, 8-Br-cGMP alone (ie, no A23187 treatment) had no effect on vimentin phosphorylation (Fig 4, lane 4). However, in those neutrophils preincubated with 8-Br-cGMP and then stimulated with A23187, maximal phosphorylation was detected at the earlier time of 1 minute (Fig 4, lane 5). Therefore, 8-Br-cGMP by itself was not capable of directing colocalization of G-kinase with vimentin or vimentin phosphorylation unless 8-Br-cGMP was added with A23187. These results suggest that the phosphorylation of vimentin by 8-Br-cGMP required the colocalization of kinase and substrate by A23187, and suggest that the targeting of G-kinase to vimentin in the A23187-activated neutrophil is both Ca2+ dependent and essential for vimentin phosphorylation in the intact cell.

To address the possibility that protein kinase C was contributing to vimentin phosphorylation, inhibitors of protein kinase C were added to the reaction mixture. Phosphorylation was detected only in those cells stimulated with A23187 or with A23187 and 8-Br-cGMP (Fig 5, lanes 2 and 6). The presence of the protein kinase C inhibitors, H-7 (100 µmol/L) or staurosporine (100 nmol/L), did not inhibit phosphorylation (Fig 5, lanes 9 and 10). We have noted in other studies that these concentrations have little effect on cGMP-dependent protein phosphorylation.19 No changes in phosphorylation were detected in those cells treated with H-7 alone or staurosporine alone (Fig 5, lanes 7 and 8). Each phosphorylation experiment was performed a minimum of three times, and the data were consistent. These results suggest that the activation of protein kinase C is not responsible for vimentin phosphorylation in A23187-treated neutrophils. This result is not unexpected, since A23187 does not increase diacylglycerol in intact cells.
COLOCALIZATION OF G-KINASE AND VIMENTIN

Fig 4. Phosphorylation of vimentin in intact neutrophils stimulated with 20 μmol/L A23187 in the presence or absence of 8-Br-cGMP for various times. Stimulation of neutrophils with A23187 for 1, 3, and 5 minutes (lanes 1 to 3, respectively) shows maximal phosphorylation of vimentin at 3 minutes (lane 2). Preincubation of neutrophils with 1 μmol/L 8-Br-cGMP and subsequent stimulation with A23187 for 1, 3, and 5 minutes (lanes 5 to 7, respectively) shows maximal phosphorylation of vimentin at 1 minute (lane 5). No phosphorylation was observed with 8-Br-cGMP alone (lane 4). This assay was performed at least three times, with identical results observed.

DISCUSSION

The findings presented in this report provide evidence that both Ca2+ mobilization and increases in cGMP levels are required for activation of G-kinase in adherent neutrophils. Previously, we reported that vimentin was transiently phosphorylated by G-kinase when adherent neutrophils were stimulated with FMLP. Since the addition of exogenous 8-Br-cGMP alone to neutrophils does not induce colocalization of G-kinase with vimentin or phosphorylation of vimentin, we proposed that a Ca2+-linked activator of neutrophils such as FMLP was required to achieve maximal phosphorylation of vimentin by G-kinase. To determine if Ca2+ mobilization was required for G-kinase phosphorylation of vimentin, neutrophils were treated with the calcium ionophore, A23187, to bypass receptor activation. In this report, we demonstrate that stimulation with A23187 alone is sufficient to induce a rapid increase in cGMP levels, leading to the targeting of G-kinase to intermediate filaments and phosphorylation of vimentin. Thus, a Ca2+-mobilizing agent appears to be required for phosphorylation of vimentin by G-kinase when the two proteins are colocalized.

A rapid increase in cGMP levels was observed in adhered neutrophils stimulated with A23187. This spike in cGMP levels at 30 seconds preceded the time of targeting of G-kinase to intermediate filaments and phosphorylation of vimentin (1 to 3 minutes). These elevated levels were sustained throughout the 5-minute period of stimulation. However, despite this sustained increase in cGMP levels, colocalization of vimentin and G-kinase and phosphorylation of vimentin was transient. This suggests that other regulators such as phosphatases may be activated and/or cGMP may be regulating another function in another cell compartment. As reported by others, we detected no significant increases in cGMP levels in neutrophils activated in suspension.

Neutrophils stimulated with Ca2+-mobilizing agents such as FMLP or A23187 undergo transient polarization followed by increased motility and secretion. These actions appear to involve an increase in cGMP levels, because (1) inhibitors of nitric oxide formation (N'-monomethyl-L-arginine) and soluble guanylate cyclase (LY83583) attenuate chemotaxis and secretion; (2) activators of nitric oxide production (L-arginine) enhance chemotaxis and secretion; and (3) analogs of cGMP enhance chemotaxis and secretion in activated neutrophils.

The direct association of G-kinase with its substrate supports the concept of compartmentalization for the purpose of effecting neutrophil functions. By immunofluorescence microscopy, we demonstrate that when neutrophils are activated with A23187, G-kinase transiently moves over a period of 5 minutes from a diffuse state in the cytoplasm to a colocalized distribution with vimentin (1 to 2.5 minutes), and then returns to a distribution resembling that of the unstimulated cell (5 minutes). Since no colocalization is observed in resting, nonactivated cells, this directed association of cell components during activation supports the concept of cellular compartmentalization. In further support of this hypothesis, G-kinase was found to phosphorylate vimentin only at the time that the enzyme and substrate were colocal-
PRYZWANSKY, WYATT, AND LINCOLN

**Fig 5.** Phosphorylation of vimentin by G-kinase in intact neutrophils. Cells were stimulated with A23187 for 3 minutes, A23187 plus 8-Br-cGMP for 1 minute, or A23187 plus 8-Br-cGMP for 1 minute in the presence or absence of staurosporine or H-7 as follows: lane 1, control cells; lane 2, A23187; lane 3, cGMP; lane 4, cGMP, H-7; lane 5, cGMP, staurosporine; lane 6, A23187, cGMP; lane 7, H-7; lane 8, staurosporine; lane 9, A23187, cGMP, H-7; lane 10, A23187, cGMP, staurosporine. Phosphorylation is achieved on stimulation with A23187 or A23187 in the presence of exogenous cGMP and/or protein kinase C inhibitors. This assay was performed at least three times, with identical results observed.

We estimate that the amount of G-kinase in neutrophils is 1,000-fold less than that of vimentin. Thus, targeting of G-kinase to a specific subcellular compartment seems to ensure that the appropriate extracellular signals will lead to a rapid and efficient phosphorylation by G-kinase.

Additional supportive evidence that G-kinase catalyzes the phosphorylation of vimentin in the A23187-stimulated neutrophil is that (1) vimentin is a good substrate for purified G-kinase in vitro, especially when the two proteins are preincubated together; (2) phosphorylation in the presence of A23187 is specifically enhanced by low concentrations (1 μmol/L) of 8-Br-cGMP, suggesting that A-kinase is not responsible for vimentin phosphorylation; and (3) phosphorylation occurs in the presence of protein kinase C inhibitors. However, the use of specific inhibitors of G-kinase to demonstrate kinase-specific phosphorylation presents a problem. There are few potent and selective inhibitors of G-kinase, and we have found that the putative G-kinase inhibitor, KT5823, fails to inhibit G-kinase activity in vitro or in vivo, and, in fact, activates neutrophils. Furthermore, others have suggested that the cyclic nucleotide inhibitor, H-8, is ineffective in discerning cGMP-mediated events. Thus, to provide supportive data that G-kinase is the phosphorylating enzyme, alternate approaches may be required. To that end, we recently used the quinolidone, LY-83583, to lower cGMP levels in neutrophils by inactivating guanylate cyclase, and showed that LY-83583 inhibited phosphorylation of vimentin in both FMLP- and A23187-stimulated cells. In addition, cGMP analogs, which act as antagonists for G-kinase (eg, Rp-8-Br-cGMPS), may offer another more direct approach for inhibiting G-kinase activity (personal observations).

G-kinase is an inactive enzyme that requires an elevation of cGMP levels for activation. The signaling pathway for elevating cGMP formation in neutrophils has renewed interest with the finding that activated neutrophils generate NO, a compound that is known to activate guanylate cyclase. Recently, we reported that intracellular levels of cGMP are significantly elevated when cells are preincubated with L-arginine, the precursor for NO formation, and then stimulated with FMLP or A23187. Similar to our findings with 8-Br-cGMP, L-arginine shortened the time of phosphorylation of vimentin by G-kinase in FMLP- and A23187-stimulated cells. The potentiation of increases in cGMP levels by L-arginine and phosphorylation of vimentin suggests that NO may be one pathway important in the signal transduction of A23187 and FMLP. Furthermore, this pathway may be involved in regulating secretion in A23187- and FMLP-stimulated neutrophils.

Of particular interest is the relationship between the cytoskeleton and neutrophil secretion. A23187-induced secretion is neither accompanied by increases in microtubule numbers, nor inhibited by colchicine. In addition, Ca²⁺ does not appear to be regulating microtubule numbers during neutrophil secretion, although concentrated numbers of 10-nm bipolar filaments (intermediate filaments) were observed by electron microscopy in the pericortical region of A23187-treated neutrophils. Similar filament concentrations were reported in CsA-treated neutrophils upon Ca²⁺ influx. However, we have found in preliminary in vitro studies that vimentin is not depolymerized into monomers after phosphorylation by G-kinase (data not shown). We believe this study contributes to two important con-
cept toward the understanding of neutrophil signaling: (1) G-kinase must be targeted or brought together with vimentin in order for phosphorylation to occur, and (2) this targeting is the result of the involvement of at least two specific mediators, cGMP and calcium.

ACKNOWLEDGMENT

We wish to thank Dr Jeff Wiseman and Dr Mehul Patel of Glaxo Inc for technical help with the SPA assays, Elizabeth Merricks for help with the photography, and Donald Lawrence and Dr William Sasiela for help with the phosphorylation and superoxide assays, respectively.

REFERENCES

4. Becker EL, Showell HJ, Henson PM, Hsu LS: The ability of chemotactic factors to induce lysosomal enzyme release. II. The characteristics of the release, the importance of surfaces and the relation of enzyme release to chemotactic responsiveness. J Immunol 112:2047, 1974
7. Scheib-Frederick E: Stimulation of the oxidative metabolism of polymorphonuclear leukocytes by the calcium ionophore A23187. FEBS Lett 48:37, 1974
Cyclic guanosine monophosphate-dependent protein kinase is targeted to intermediate filaments and phosphorylates vimentin in A23187-stimulated human neutrophils

KB Pryzwansky, TA Wyatt and TM Lincoln