The N-formyl peptide receptor is a G protein-coupled transmembrane receptor involved in stimulating a variety of differential responses in neutrophils including chemotaxis, degranulation, superoxide production, transcriptional activation, and actin reorganization. Although it is known that N-formyl-Met-Leu-Phe induces actin reorganization, the sequence of events from the receptor to the actin cytoskeleton is not well characterized. To study the signaling pathway from the N-formyl peptide receptor to the actin cytoskeleton, we developed a model system utilizing microinjection techniques with a nonhematopoietic cell line. An expression vector coding for the N-formyl peptide receptor was microinjected into porcine aortic endothelial cells and stimulated with N-formyl-Met-Leu-Phe to induce actin reorganization and membrane ruffling. The receptor-mediated signal was blocked by pertussis toxin and by a dominant negative Rac-N17, indicating the involvement of G\(\alpha\) subunit and the small guanosine triphosphatase Rac, respectively. Moreover, G\(\beta\gamma\) subunits and membrane targeted forms of phosphatidylinositol (PI) 3-kinase \(\alpha\) were sufficient to induce similar actin reorganization, and coexpression of various mutants of PI 3-kinase with the N-formyl peptide receptor identified a link to class Ia PI-3 kinase-mediated actin reorganization.

N-Formyl peptide receptors (FPR) are seven-transmembrane-domain rhodopsin-like G protein-coupled receptors (GPCR) commonly found in phagocytic leukocytes such as neutrophils and monocytes (1). These receptors play a key role in classical leukocyte chemoattraction leading to leukocyte migration from the blood to sites of inflammation. In phagocytes, the chemoattractant N-formyl-methionyl-leucyl-phenylalanine (fMLP) activates the FPR to stimulate diverse biological processes including chemotaxis, transcriptional activation, and actin reorganization (2). Stimulation of the FPR is pertussis toxin (PTX)-sensitive, indicating that the FPR most likely couples with G\(\alpha\) subunits of G proteins (3, 4) to initiate distinct and divergent signaling pathways.

In granulocytes a short exposure to fMLP induces actin polymerization, membrane ruffling, and cell polarization leading to cell migration toward a concentration gradient (2). Although the FPR activates proteins that are implicated in actin reorganization such as Rho GTPases and PI 3-kinase, the sequence of events initiated at the FPR and leading to the actin cytoskeleton is not well understood (5–7). PI 3-kinase activity is induced during leukocyte motility by GPCR and tyrosine kinase receptors. At least four catalytic subunits of PI 3-kinase have been described in mammalian tissues, including p110\(\alpha\), \(\beta\), \(\gamma\), and \(\delta\). The class Ia subunits (p110\(\alpha\), \(\beta\), and \(\delta\)) form a heterodimeric complex with the adaptor molecules p85, p55, and p50 (\(\alpha\) or \(\beta\) isoforms) (8). Unlike the class Ia PI 3-kinases, class Ib p110\(\gamma\) forms a heterodimer with the adaptor protein p101 and is directly activated by G\(\beta\gamma\) subunits (9). Although p110\(\alpha\) and p110\(\beta\) are ubiquitously expressed, p110\(\gamma\) and p110\(\delta\) expression is restricted to cells of hematopoietic origin.

Recent studies involving selective inhibition of specific isoforms using antibodies suggest the mammalian isoforms are not redundant in their regulatory effects on cellular responses (8). In these studies, p110\(\beta\) isoforms were found to mediate cytoskeletal changes leading to polarization and migration in the macrophage cell line Bac1.2F5, whereas p110\(\alpha\) appeared to mediate effects on cell survival and proliferation. In contrast, studies with PI 3-kinase \(\gamma\) knock out mice demonstrated a key role for this isoform in macrophage chemotaxis (10–13). Although it is generally accepted that PI 3-kinase \(\gamma\) is the only isoform that is directly activated by G\(\beta\gamma\), a recent study demonstrated the direct activation of p110\(\beta\) with G\(\beta\gamma\) (14). This finding suggests that class Ia PI 3-kinase isoforms can also be activated by GPCR.

Because leukocytes are very difficult to manipulate, the use of nonleukocyte cells to study the pathways involved in fMLP stimulation of actin cytoskeletal rearrangements may provide valuable models in delineating those pathways. We initiated a study utilizing porcine aortic endothelial (PAE) cells microinjected with cDNA expressing the FPR and various cDNA constructs encoding constitutively active or dominant negative proteins that have been previously shown to play a role in actin cytoskeletal signaling pathways. With this approach we were able to determine the sequence of events from the FPR to the actin cytoskeleton and to identify a link between the FPR, G\(\beta\gamma\) subunits, and class Ia PI 3-kinases leading to Rac-induced actin reorganization.

**EXPERIMENTAL PROCEDURES**

Microinjections and Immunofluorescence Microscopy

Microinjections and immunofluorescence microscopy were carried out as described previously (15). PAE cells grown in Dulbecco's modified Eagle's medium/Ham's F-12 medium containing 10% fetal bovine serum were plated on coverslips. Expression vectors encoding FLAG-tagged FPR and/or various tagged cDNAs known to be involved in G protein
receptor-coupled pathways were diluted to a concentration of 50 ng/ml in injection buffer (5 mM potassium glutamate, 130 mM KCl) and microinjected into the nucleus of >100 subconfluent PAE cells. Injected cells were incubated for 4–6 h at 37 °C. Cells were washed once with PBS, incubated for 5–8 min in 2.3 μM fMLP in PBS, and fixed in 4% formaldehyde in PBS. Cells were permeabilized with PBS containing 0.1% Triton X-100 and incubated in the presence of primary anti-tag monoclonal antibodies for 60 min. Coverslips were washed with PBS containing 0.1% Tween 20 and incubated for 30 min with secondary fluorescein isothiocyanate-conjugated anti-mouse antibody. To visualize F-actin cells were incubated with Texas red-conjugated phalloidin. Fluorescence microscopy was carried out on a Zeiss Axiophot with appropriate filters for fluorescence detection.

**Treatment with Inhibitors**

**Pertussis Toxin Treatment**—Cells were microinjected with expression vectors encoding the FPR as described above. Approximately 2 h prior to fMLP stimulation cells were treated with PTX ranging in concentration from 0 to 10 μg/ml. Following treatment with PTX cells were stimulated with fMLP, fixed, and processed as described.

**Treatment with Tyrosine Kinase Inhibitors**—PAE cells were microinjected with expression vectors encoding the FPR as described above. Approximately 2 h prior to fMLP stimulation cells were treated with Wortmannin for 8 min. Fixation and processing was as described. Control cells were treated with Me2SO alone.

**Wortmannin Treatment**—Cells were microinjected with an expression vector encoding the FPR as described and incubated for 4–6 h. Coverslips were then washed with PBS, and the cells were treated for 10 min with concentrations of Wortmannin ranging from 10 to 200 nM. fMLP was added in the presence of Wortmannin for 8 min, followed by fixation and processing as described.

**Western Blots**—PAE cell lysates were made from semi-confluent cultures of PAE cells by lysis in TG lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1 mM EGTA, 1.5 mM MgCl₂, 1% Triton X-100, 10% glycerol, 50 mM NaF, Rocke Molecular Biologicals inhibitor mixture tablet). Cell lysates from Jurkat T-cells, WI-38, and activated and unactivated HL-60 cells were also prepared as controls. Lysates were clarified by spinning for 10 min at 14,000 × g. The supernatants were mixed with SDS sample buffer, and the proteins were separated in 8% Tris-glycine polyacrylamide gels at 100 V. Proteins were blotted onto polyvinylidine difluoride membranes using a semi-dry blotting method. Blots were probed with an affinity purified anti-porcine rabbit polyclonal antibody to PI 3-kinase γ (1:2000 dilution), reacted with a secondary horseradish peroxidase-conjugated goat anti-rabbit IgG (1:20,000 dilution), and detected with the ECL luminescence system.

**Eukaryotic Expression Vectors and Reagents**—A plasmid encoding the human FPR was provided by Dr. N. P. Gerard; plasmids coding for β-ARK, GᵣαQL, G₂, and Gy were obtained from Drs. G. Servant and S. Gutkind; a plasmid coding for pκB was provided by Dr. Len Stephens; plasmids coding for p110α-CAA and p110γ-CAA were obtained from Drs. S. Braselmann and M. Wymann; plasmids coding for p110ζ-948–981, Vav-Y174F, and Vav342–348 were provided by Dr. C. Abrams; plasmids coding for Pak₁L83,L86,R299, and Pak₁ 83–149 were provided by Dr. A. Weiss. Porcine rabbit polyclonal antibodies against p110γ were obtained from Dr. A. Weiss. Porcine rabbit polyclonal antibody against p110ζ was obtained from Dr. S. Braselmann. 

FIG. 1. Microinjection of FPR plasmid induces actin reorganization in PAE cells. A, cells expressing FPR either were stimulated for 8 min with fMLP or were left untreated, and polymerized actin was stained with phalloidin. FPR-expressing cells exhibit dramatic membrane ruffles and cortical actin staining only when stimulated with fMLP. Cells expressing the constitutively active form of Rac1 (Rac-V12) also have dramatic membrane ruffles and cortical actin staining. B, cells were visually scored for expression, and the percentage of expressing cells with membrane ruffling was plotted against the presence or absence of fMLP. In multiple experiments, expressing stimulated cells consistently formed extensive membrane ruffles.
RESULTS AND DISCUSSION

The N-Formyl Peptide Receptor Induces Actin Reorganization in PAE Cells—FPR transduces multiple signaling cascades that are essential for chemotaxis, actin reorganization, and transcriptional activation. To better understand the signaling pathway required for actin reorganization, we developed a model cell system that can be easily manipulated by co-expressing the FPR with various signaling proteins using microinjection techniques. We chose PAE cells because these cells are large with prominent nuclei, contain elaborate actin cytoskeletal structures, and have a well characterized PDGF-induced ruffling response (16, 17). In addition, these cells are easy to microinject with high efficiency and have been used extensively to study the role of Rho GTPases in regulation of the actin cytoskeleton (15, 17, 18).

To determine whether fMLP treated cells can initiate the signaling required for actin reorganization, PAE cells were microinjected with a mammalian expression plasmid coding for the human FPR protein fused with a FLAG tag, incubated for 4–6 h, and then stimulated for 5–8 min with the agonist peptide fMLP. FPR expressing cells were detected with a primary anti-FLAG tag monoclonal antibody, and F-actin was visualized with Texas red-conjugated phalloidin. (Fig. 1A).

Cells expressing the FPR but untreated with fMLP had morphologies identical to nonexpressing cells and exhibited little or no ruffling of the plasma membrane. F-actin filaments (stress fibers) are clearly visible in organized bundles in these cells. Cells expressing the FPR and stimulated with fMLP showed cell spreading and dramatic increases in lamellipodia formation and membrane ruffling that is characteristic of ruffling observed when PAE cells are stimulated with constitutively active forms of the GTPase Rac. In multiple experiments, stimulated PAE cells expressing the FPR consistently showed a ruffling response that is statistically significant over unstimulated expressing cells (Fig. 1B). These data suggest that PAE cells contain all the molecular elements required for transducing signals from a membrane integrated chemotactic receptor to the actin cytoskeleton. In time course experiments, the fMLP-induced actin reorganization was detected as early as 30 s and peaked by 2 min after treatment. This early response indicates that the stimulation of actin reorganization by FPR is

FIG. 2. fMLP-induced membrane ruffling is independent of Cdc42 and requires Rac. A and B, phalloidin-stained PAE cells expressing FPR alone or coexpressing FPR and Cdc42-N17 show typical fMLP-induced membrane ruffles. In contrast, fMLP stimulation of cells coinjected with FPR and Rac-N17 did not induce membrane ruffles.

FIG. 3. Pertussis toxin inhibits fMLP-induced membrane ruffling. A and B, phalloidin-stained PAE cells expressing FPR show typical membrane ruffling when stimulated with fMLP. Cells expressing FPR were treated with 2.5–10 μg/ml of pertussis toxin for 2 h prior to stimulation with fMLP. Membrane ruffling in all treatments reduced fMLP-stimulated ruffling to levels equal to or below background levels.
most likely a direct activation of downstream signaling pathways and not a consequence of cross-talk with a tyrosine kinase receptor.

**fMLP-induced Membrane Ruffling in PAE Cells Is Mediated by the GTPase Rac**—The small GTPase proteins Rac and Cdc42 have both been implicated in signaling cascades leading to actin reorganization. We have previously shown that activated Rac and Cdc42 mutants can induce lamellipodia formation in PAE cells (17, 18). To determine which small GTPase plays a role in fMLP-induced ruffling, PAE cells were cojected with expression vectors coding for the dominant negative form of either Cdc42 (Cdc42-N17) or Rac (Rac-N17) and the FPR. Cells coexpressing the FPR and Rac-N17 consistently showed inhibition of membrane ruffling to background levels (Fig. 2). In contrast, cells coexpressing the FPR and Cdc42-N17 had no inhibition of membrane ruffling relative to cells injected with FPR alone. These results suggest that fMLP-induced membrane ruffling in PAE cells is mediated by the GTPase Rac but not by Cdc42.

**fMLP Induces Actin Reorganization through a Pertussis Toxin-sensitive Heterotrimeric G Protein**—In neutrophils, the FPR couples to PTX-sensitive G~i~ subunits to activate downstream signaling cascades. PTX covalently modifies and subsequently inactivates G~i~ subunits. To determine whether fMLP-induced membrane ruffling in PAE cells was also mediated through PTX-sensitive G proteins, we treated injected cells with various
concentrations of PTX. Following PTX treatment, cells were stimulated with FMLP, fixed, and processed as described. In these experiments greater than 50% of FPR expressing cells treated with FMLP exhibited dramatic ruffling and spreading in the absence of PTX (Fig. 3). In contrast, only 10% (background levels) of FPR expressing cells treated with any of the indicated concentrations of PTX exhibited membrane ruffling and spreading, indicating that FPR couples to a pertussis-sensitive heterotrimeric G subunit in these cells.

The Gβγ Subunits Induce Actin Reorganization Similar to FMLP-induced Ruffling—Our PTX data indicate the FPR induces actin reorganization by activating heterotrimeric G proteins. Activation of the receptor promotes binding of GTP to the Ga subunit and subsequently leads to dissociation of the Ga from the Gβγ subunits. Both Ga and Gβγ subunits have been shown to play a role as transducer proteins (19). To assess the ability of specific subunits to stimulate cell spreading and membrane ruffling in PAE cells, we injected expression vectors coding for various G protein subunits (Fig. 4A). Injection of cells with a vector expressing a constitutively active form of Ga (GCaQL) did not induce membrane ruffles but did lead to extensive reorganization of F-actin stress fibers. Microinjection with a vector expressing Gγ2 subunit had no observable effect on cellular morphology, whereas expression of Gβ3, Gβ5, and Gβ2 subunits together induced membrane ruffling and spreading similar to that observed with FMLP stimulation or Rac-V12 injection. These data strongly suggest that actin reorganization is most likely mediated by Gβγ subunits and not by Ga subunit. In addition to Gβγ transduction activity, Gβγ can also mediate the membrane targeting of the β-ARK to phosphorylate and desensitize GPCR (20). To test whether the activation of the FPR is sensitive to β-ARK, we coexpressed the FPR expression vectors with vectors encoding β-ARK and stimulated the cells with FMLP. Cells expressing both the FPR and β-ARK exhibited a dramatic reduction in FMLP-dependent membrane ruffling (Fig. 4, B and C). This finding further suggests that the induction of membrane ruffling by FPR is directly linked to Gβγ.

FMLP Stimulation of Membrane Ruffling Is Independent of Src Kinases—Multiple tyrosine kinases have been shown to be activated by the FMLP receptor in phagocytes. In neutrophils, the protein-tyrosine kinase inhibitor genistein attenuates FMLP-induced phosphatidylinositol-3,4,5-triphosphate formation (21). To determine whether FMLP stimulation of membrane ruffling in PAE cells is dependent on Src-like tyrosine kinases, we treated FPR-expressing, FMLP-stimulated cells with the Src-specific inhibitor PD166285. There was no effect on FMLP-induced ruffling at any concentration of inhibitor tested (Fig. 5). In addition, stress fiber formation appeared normal, with bundles of actin filaments anchored in membrane ruffles. No toxic effects were detected in the cells because of the presence of inhibitors, because uninjected cells appeared morphologically normal in all respects. Treatment with the tyrosine kinase inhibitor genistein had similar effects, with little or no inhibition of membrane ruffling, although in this case treatment appeared somewhat toxic, leading to difficulties in scoring for ruffling. However, even at the most toxic levels, induction of membrane ruffling was easily detected (data not shown). Results with inhibitors of tyrosine kinases indicate that no tyrosine kinases are implicated in the FMLP-mediated actin reorganization. Although it was reported that GPCR can intracellularly transactivate tyrosine kinase receptors (22, 23), our findings along with the time course data indicate that FPR response is independent of tyrosine kinase receptors.

FMLP Stimulation of Membrane Ruffling Is Mediated by Class Ia PI 3-Kinase—In human neutrophils, activation of the FPR induces dissociation of Gβγ from Ga, leading to branching of signal into at least two transduction pathways, a calcium-dependent phospholipase C-mediated pathway and a wortmannin-sensitive PI 3-kinase pathway (24). In an effort to determine whether FMLP stimulation in our model system was operating through the PI 3-kinase transduction pathway, we treated FPR expressing cells with the fungal metabolite wortmannin, a potent PI 3-kinase inhibitor. FMLP-induced membrane ruffling was inhibited in a dose-dependent manner (Fig. 6, A and B), reducing the induction of membrane ruffling to below background levels. Maximal inhibition was apparent at doses of 100 nm. Furthermore, stress fiber disorganization as well as perinuclear clustering of F-actin occurred in these cells at the higher concentrations of 100 and 200 nm. Membrane ruffling in PAE cells injected with an expression vector encoding a constitutively active form of Rac (Rac-V12) was not inhibited by Wortmannin, suggesting that PI 3-kinase is upstream of Rac (data not shown). It therefore appears that G

![Image](https://www.jbc.org/content/2629/4/26229/F5.large.jpg)
protein-linked fMLP-induced ruffling in FPR expressing PAE cells requires the PI 3-kinase pathway.

To determine which PI 3-kinase isoforms were involved in regulating fMLP-induced ruffling in PAE cells, we injected plasmids coding for membrane targeted forms of p110α (p110α-CAA) or p110γ (p110γ-CAA). Both isoforms were capable of inducing cell spreading and membrane ruffling morphologically similar to that observed with fMLP stimulation (Fig. 7A, p110α-CAA only shown). Although these experiments indicate that activation of PI 3-kinase is critical for the induction of membrane ruffling, they do not implicate a particular isoform and suggest that membrane targeted PI 3-kinase is sufficient to initiate the signal cascade required for actin reorganization.

To further analyze whether class Ia PI 3-kinases are implicated in fMLP-induced membrane ruffling, we co-injected a plasmid encoding a dominant negative form of the p85 subunit with the FPR expression vector. Subunit p85 links class Ia PI 3-kinases to tyrosine kinase receptors. Deletion of an inter-SH2 domain within the p85 (Δ85) subunit results in loss of ability to bind p110 subunits without a concomitant loss of ability to bind upstream signals (17). The p85 subunit partially inhibited ruffling when coexpressed with the FPR (Fig. 7A), suggesting that class Ia PI 3-kinases are indeed involved in mediating fMLP-induced actin reorganization.

To study the role of p110γ in fMLP-induced actin reorganization, we co-injected an expression vector coding a catalytically inactive form of p110γ (p110γΔ948–981) with the FPR expression vector. A normal ruffling response to fMLP stimulation occurred in these cells (Fig. 7A). To further verify that effects seen in these studies were due to mediation by p110α/β and not to regulation by p110γ subunits, we prepared PAE cell lysates and probed for the presence of the p110γ isoform using a polyclonal rabbit antiserum specific for porcine p110γ. The p110γ isoform was detected only in Jurkat T-cell lysates and in promyelocytic differentiated PLB cell lysates but not in WI-38, undifferentiated PLB, or PAE cells (Fig. 7B). In phagocytes it has been demonstrated that p110γ is directly activated by the fMLP receptor, and our data suggest that class Ia PI 3-kinases are also linked to fMLP receptor activation. The exact mechanism remains unknown, although in other nonhematopoietic cell types, p110β/p85 PI 3-kinase isoforms appear to function as co-mediators in the synergistic activation of dual signaling pathways by tyrosine and G protein-coupled receptors (24). In agreement with our results, it was recently shown that expression of Δp85 (the regulatory subunit of class Ia PI3 kinase mutant lacking the SH2 domain) in a promyelocytic cell line considerably reduced interleukin-8-mediated phosphatidylinositol-3,4,5-triphosphate formation (21), suggesting that G protein-coupled receptors mediate the activation of PI 3-kinase class Ia in phagocytes. In addition, Gβγ was shown to directly activate p110β/p85 kinase activity (14). These data indicate a direct link from Gβγ to PI 3-kinase class Ia.

**The Rac Guanosine Exchange Factor, Vav, Does Not Mediate fMLP-induced Actin Reorganization**—Our data indicate a key role for Rac in FPR-mediated actin reorganization. GTPases are activated by guanosine exchange factors (GEF) that cata-
lyze the release of GDP and consequent binding of GTP. Multiple GEF for Rac have been identified, although the GEF that activates Rac during FPR stimulation is currently unknown. One likely candidate is the hematopoietic specific GEF, Vav. Vav1 is a 92-kDa protein that becomes phosphorylated in response to mitogenic or antigenic stimulation and subsequently catalyzes GDP/GTP exchange on Rac-1 (25). In Cos-7SH cells, Vav1 mutants were shown to inhibit fMLP-mediated cortical actin changes induced by a myristylated form of p110γ (26), suggesting a role for Vav in the transduction of signals from p110γ to Rac. In T cells, Vav1 is activated by phosphorylation and by PI 3-kinase (25). To determine whether PI 3-kinase mediates fMLP-induced Rac signaling via the exchange factor Vav, we co-injected expression vectors coding for various Vav mutants with the FPR expression vector and looked at the effects on cell spreading and membrane ruffling. Expression of a full-length Myc-tagged Vav construct alone was sufficient to induce extensive shape changes and membrane ruffling very similar to that observed with injection of constitutively active forms of Rac-V12 (data not shown). fMLP stimulation of PAE cells coexpressing the FPR and a VavY174F mutant deficient in tyrosine phosphorylation-induced (27) membrane ruffling similar to that seen with Vav-WT, supporting our results that the induction of membrane ruffling is not mediated by tyrosine kinases. Lastly, expression of Vav variants containing a deletion of the domains required for GEF activity, Dbl homology domain (Vav-SH (Fig. 8); VavΔ342–34 (data not shown)) did not induce cell spreading, membrane ruffling, or other cortical actin rearrangements when injected alone and also failed to inhibit fMLP-induced membrane ruffling when coexpressed with FPR. Therefore, Vav, although capable of functioning as a GEF for Rac in these cells, does not appear to be functioning downstream of FPR and PI 3-kinase to induce Rac-mediated membrane ruffling. Our data suggest that an unknown GEF is implicated in linking PI 3-kinase to Rac activation. Possible candidates include the Ras-GEF family, a member of which (Ras-GRF1) was recently shown to link Gβγ with Rac activation (28).

fMLP-induced Actin Reorganization Does Not Require Pak—In an attempt to delineate the pathways downstream of Rac involved in membrane ruffling and actin reorganization, we co-injected the FPR expression vector with expression vectors coding for various mutants of downstream targets of Rac. Stimulation of neutrophils with fMLP induces rapid and transient activation of the Rac effector Pak (29), and Pak1 localizes to membrane ruffles and lamellipodia in fMLP-stimulated leukocytes (30). Various mutants of Pak were analyzed including Pak1L83,L86,R299, a double mutant that is unable to bind to Cdc42 or Rac1, is catalytically inactive (31), and acts as a dominant negative for Pak function. Infection of
Pak1L83,L66,R299 induces unipolar membrane ruffling in Swiss 3T3 cells. Overexpression of this mutant in PAE cells also induces membrane ruffling and failed to inhibit fMLP-induced membrane ruffling (data not shown), suggesting that Pak1 kinase activity is not required for Rac-mediated actin rearrangements in PAE cells.

The Pak inhibitory domain mutant Pak 83–149 (32) has been shown to block actin reorganization induced by constitutively active forms of Rac and Cdc42 as well as the morphological effects of Pak itself. This mutant also induced membrane ruffling when expressed in PAE cells and failed to inhibit Rac-mediated fMLP-induced membrane ruffling (data not shown).

N-WASP WH2-C Interferes with fMLP-induced Membrane Ruffling—The hematopoietic specific WASP (Wiskott-Aldrich syndrome protein) and its ubiquitously expressed relatives N-WASP and WAVE are highly homologous multidomain proteins known to regulate the actin cytoskeleton (33, 34). The C terminus of WASP proteins contains a conserved WH2 domain and a series of acidic residues that were shown to interact with the Arp2/3 complex to activate actin nucleation and polymerization (35). Although the Arp2/3 complex is concentrated in the leading edge and membrane ruffles of neutrophils, it is not clear whether Arp2/3 is implicated in regulating the FPR-mediated membrane ruffling (36). To determine whether WASP-like proteins and Arp2/3 are involved in fMLP-induced membrane ruffling, we coexpressed expression vectors coding for various WASP domain mutants and looked for inhibition of ruffling. Neither full-length WASP-WT nor a WASP mutant lacking the last 59 amino acids at the C terminus, WASPΔC, had any effect on fMLP-induced ruffling (Fig. 9). In contrast, expression of the N-WASP C terminus (containing the WH2 domain and the C terminus) interfered with fMLP-induced ruffling, strongly suggesting that WASP/Arp2/3 are involved at some level in regulating fMLP-induced membrane ruffling. This experiment does not identify which WASP protein is implicated in this pathway but suggests the involvement of Arp2/3-mediated actin polymerization during FPR stimulation.

In summary, we propose the following model shown in Fig. 10. Activation of FPR by fMLP stimulates the dissociation of Gα from Gβγ to initiate a signaling cascade leading to actin reorganization. Although in neutrophils Gβγ can directly activate p101/p110γ, our data suggest that class Ia PI 3-kinases can also be activated by FPR and Gβγ. PI 3-kinase is essential for Rac-dependent actin reorganization and most likely plays a role in the activation of a Rac GEF other than Vav. Induction of Rac-dependent membrane ruffling is mediated by a WASP-like protein and Arp2/3 complex to nucleate actin and to form new actin filaments required for the formation of FPR stimulation.

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REFERENCES

1. Murphy, P. M. (1994) Annu. Rev. Immunol. 12, 593–633
2. Panaro, M. A., and Mitolo, V. (1999) Immunopharmacol. Immunotoxicol. 21, 397–419
3. Suzuki, T., Hazeki, O., Hazeki, K., Ui, M., and Katada, T. (1996) Biochem. Biophys. Acta 1313, 72–78
4. Klinker, J. F., Wenzel-Seifert, K., and Seifert, I. (1996) Gen. Pharmacol. 27, 33–54
5. Benard, V., Bohl, B. P., and Bokoch, G. M. (1999) J. Biol. Chem. 274, 13198–13204
6. Akasaka, T., Koga, H., and Sumimoto, H. (1999) J. Biol. Chem. 274, 18053–18059
7. Hall, A. (1998) Science 279, 509–514
8. Vanhaesebroeck, B., Jones, G. E., Allen, W. E., Zicha, D., Hosshmand-Rad, R., Sawyer, C., Wells, C., Waterfield, M. D., and Ridley, A. J. (1999) Nat. Cell Biol. 1, 9–14
9. Stephens, L., Smreka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C., and Hawkins, P. T. (1994) Cell 77, 83–93
10. Sasaki, T., Irie-Sasaki, J., Jones, R. G., Oliveira-Santos, A. J., Stanford, W. L., Bolon, B., Wakeham, A., Hie, A., Bouchard, D., Krizanadk, I., Jozsa, N., Mak, T. W., Ohashi, P. S., Suzuki, A., and Penninger, J. M. (2000) Science 287, 1040–1046
11. Li, Z., Jiang, H., Xie, W., Zhang, Z., Smreka, A. V., and Wu, D. (2000) Science 287, 1046–1049
12. Dekker, L. V., and Segal, A. W. (2000) Science 287, 982–9833
13. Maier, U., Babich, A., and Nurnberg, B. (1999) J. Biol. Chem. 274, 29311–29317
14. Symons, M. (1996) Curr. Biol. 6, 723–734
15. Symons, M. (1999) Trends Biochem. Sci. 21, 178–181
16. Hawkins, P. T., Eginoza, A., Qu, R. G., Stokoe, D., Cooke, F. T., Walters, R., Wennstrom, S., Claesson-Welsh, L., Evans, T., Symons, M., and Stephens, L. (1995) Curr. Biol. 5, 393–403
17. Abo, O., Qu, J., Cammarano, M. S., Dan, C., Fritsch, A., Baud, V., Belisle, B., and Minden, A. (1999) EMBO J. 17, 6527–6540
18. Clapham, D. E., and Neer, E. J. (1993) Nature 365, 403–406
19. Koch, W. J. (1999) J. Biol. Chem. 274, 5256–5260
20. Dab, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996) Nature 379, 557–560
21. Katada, T., Kuroa, H., Okada, T., Suzuki, T., Tsujimoto, N., Takasuga, S., Kontani, K., Hazeki, O., and Ui, M. (1999) Mol. Cell. Biol. 19, 4960–4964
22. Thelen, M., Wynn, M. P., and Langen, H. (1996) Proc. Natl. Acad. Sci. U. S. A. 91, 4956–4961
23. Crespo, P., Schubel, K. E., Ostrom, A. A., Gutkind, J. S., and Bustelo, X. R. (1997) Nature 385, 169–172
24. Ma, A. D., Matsu, I., Bagrodia, S., Taylor, S., and Abrams, C. S. (1998) Mol. Cell. Biol. 18, 4744–4751
25. Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R. D., Krishna, U. M., Falck, J. R., White, M. A., and Broek, D. (1998) Science 279, 558–560
26. Kiyono, M., Satoh, T., and Kaziro, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4820–4825
27. Huang, R., Lian, J. P., Robinson, D., and Badwey, J. A. (1998) Mol. Cell. Biol. 18, 7130–7138
28. Dharmawardhane, S., Brownson, D., Lennartz, M., and Bokoch, G. M. (1999) J. Leukocyte Biol. 66, 521–527
29. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J. (1999) J. Immunol. 162, 202–210
30. Zhao, Z. S., Ma, A. D., Metjian, A., Bagrodia, S., Taylor, S., and Abrams, C. S. (1998) J. Biol. Chem. 273, 9771–9776
31. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J. (1999) J. Biol. Chem. 274, 202–210
32. Zhao, Z. S., Ma, A. D., Metjian, A., Bagrodia, S., Taylor, S., and Abrams, C. S. (1998) J. Biol. Chem. 273, 9771–9776
33. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J. (1999) J. Biol. Chem. 274, 202–210
34. Zhao, Z. S., Ma, A. D., Metjian, A., Bagrodia, S., Taylor, S., and Abrams, C. S. (1998) J. Biol. Chem. 273, 9771–9776
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