Activation of Mitogen-activated Protein Kinases by Formyl Peptide Receptors Is Regulated by the Cytoplasmic Tail*

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Wild type formyl peptide receptors (FPRwt) and receptors deleted of the carboxyterminal 45 amino acids (FPRdel) were stably expressed in undifferentiated HL-60 promyelocytes. Expression of FPRwt reconstituted N-formylmethionyl-leucyl-phenylalanine (FMLP)-stimulated extracellular signal-regulated kinase (ERK) and p38 kinase activity. Expression of FPRdel resulted in a 2–5-fold increase in basal ERK and p38 kinase activity, whereas FMLP failed to stimulate either mitogen-activated protein kinase (MAPK). Pertussis toxin abolished FMLP stimulation of both MAPKs in FPRwt cells but had no effect on either basal or FMLP-stimulated MAPK activity in FPRdel cells. FMLP stimulated a concentration-dependent increase in guanosine 5’-3-O-(thio)triphosphate (GTPγS) binding in membranes from FPRwt but not FPRdel cells. GTPγS inhibited FMLP binding to FPRwt but not FPRdel membranes. Photoaffinity labeling with azidoanilide-[γ-^32P]GTP in the presence of GTPγS showed no increase labeling only in FPRwt membranes. Immunoprecipitation of α2 and α3 of solubilized, photolabeled membranes showed that FPRwt were coupled to α2 but not to α3. FPRwt cells demonstrated calcium mobilization following stimulation with FMLP, whereas FPRdel cells showed no increase in intracellular calcium. We conclude that the carboxyterminal tail of FPRs is necessary for ligand-mediated activation of Gα1 proteins and MAPK cascades. Deletion of the carboxyterminal tail results in constitutive activation of ERK and p38 kinase through a Gi2-independent pathway.

Chemoattractants, including formylated peptides, C5a, leukotriene B4, platelet-activating factor, and CXC chemokines (e.g. interleukin 8), are proinflammatory agents that recruit polymorphonuclear leukocytes (PMNs) to a site of infection or inflammation, stimulate respiratory burst activity, and induce release of lysosomal enzymes (1–3). Genes for chemoattractant receptors have been cloned and sequenced (1, 4), and all are members of the superfamily of G protein-coupled receptors (GPCRs) containing seven transmembrane domains with an extracellular amino-terminal domain and an intracellular carboxy-terminal tail separated by three intracellular loops and three extracellular loops (5). Formyl peptide receptors (FPRs), as well as C5a and leukotriene B4 receptors, couple to pertussis toxin-sensitive Gi proteins (6–8), whereas platelet-activating factor receptors activate Gi and/or Go, in addition to Gq proteins (9, 10). Transient co-transfection of chemoattractant receptors and Go16 permits activation of phospholipase C in Cos cells (11–13).

The domains of GPCRs that interact with G proteins have been examined in studies using mutant and chimeric receptors or synthetic peptides corresponding to specific receptor domains. These studies indicate that the third intracellular loop and the amino-terminal region of the carboxy-terminal tail are essential for coupling of adrenergic and muscarinic receptors and rhodopsin to G proteins (14–17). Chemoattractant receptors differ structurally from other GPCRs in that they have a relatively short third intracellular loop (1), suggesting that chemoattractant receptors may interact with G proteins using domains different from those used by other GPCRs. Studies using site-directed replacement mutants of FPRs failed to demonstrate a role for the third cytoplasmic loop in G protein activation (18). Additionally, synthetic peptides consisting of the entire third cytoplasmic loop failed to inhibit G protein-dependent, high affinity ligand binding and physical coupling of formyl peptide receptor to G proteins (19). On the other hand, studies using synthetic peptides corresponding to the second intracellular loop and the proximal portion of the carboxyterminal tail of human FPRs disrupt the physical interaction with G proteins (19–21). Additionally, phosphorylation of the carboxy-terminal tail during desensitization of FPRs results in uncoupling of the receptor from G proteins (22). These studies indicate that the second intracellular loop and carboxy-terminal tail contribute to the physical interaction of FPRs with G proteins; however, the role of these domains in FPR activation of G proteins and effectors has not been examined.

Two mitogen-activated protein kinase (MAPK) cascades, the extracellular signal-regulated kinases (ERKs) and p38 kinases, are stimulated in PMNs by chemoattractants (23–31). ERKs are reported to participate in PMN adherence and respiratory burst activation (23, 29, 30), whereas p38 kinases participate in PMN adherence, chemotaxis, and respiratory burst activity (27, 28, 30). Nick et al. (28) reported that pertussis toxin inhibited ERK activation but not p38 kinase activation by FMLP in human PMNs. Additionally, chemoattractants stimulate differ-
ent levels of MAPK activity. For example, interleukin 8 and platelet-activating factor stimulate a weaker ERK response than C5a and FMLP (25, 28). These findings suggest that specific domains of chemoattractant receptors regulate MAPK activity by stimulating different G protein-coupled pathways or by disparate rates of activation of the same pathway. The present study was designed to determine the role of the carboxyl-terminal tail in FPR-mediated activation of ERK and p38 MAPKs. A deletion mutant of the carboxyl-terminal tail of FPRs was constructed by site-directed mutagenesis, and both mutant and wild type receptors were stably expressed in undifferentiated HL-60 cells. Activation of MAPK cascades and G proteins by wild type and mutant receptors was examined. Our results indicate that the carboxyl-terminal tail of FPRs plays a significant role in control of basal activity and ligand-stimulated G protein-dependent activation of both ERK and p38 kinases.

EXPERIMENTAL PROCEDURES

Materials—FMLP, hygromycin, and genetin (G418) were obtained from Sigma. GDP, GTP and GTPγS were obtained from Boehringer Mannheim. GDPγS was obtained from Amersham Pharmacia Biotech (Mannheim, Germany). Pertussis toxin was from List Biological Laboratories (Campbell, CA). Fluoro-3 was from Molecular Probes (Eugene, OR).

A polyclonal anti-p38 antisera was raised in rabbits using the 14-amino acid peptide CFYPFFPLDEQAMES corresponding to the carboxyl terminus. The specificity of the p38 antisera was determined by immunoblotting of recombinant p38 from bacterial lysates. A polyclonal anti-Gαi2 antibody was obtained from Chemicon (Temecula, CA). Goi2. Goi3, and Goi4 antisera were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Pertussis toxin was from LIST Biological Laboratories (Campbell, CA). Fluoro-3 was from Molecular Probes (Eugene, OR).

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A polyclonal anti-p38 antisera was raised in rabbits using the 14-amino acid peptide CFYPFFPLDEQAMES corresponding to the carboxyl terminus. The specificity of the p38 antisera was determined by immunoblotting of recombinant p38 from bacterial lysates. A polyclonal anti-Gαi2 antibody was obtained from Chemicon (Temecula, CA). Goi2. Goi3, and Goi4 antisera were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Pertussis toxin was from LIST Biological Laboratories (Campbell, CA). Fluoro-3 was from Molecular Probes (Eugene, OR).
autoradiography and densitometry. To determine the AA-GTP binding to specific G proteins, the remaining supernatant was precleared with protein A-Sepharose beads for 15 min. Precleared solubilizate was incubated overnight with specific antisera for Gα12 or Gα13 at a dilution of 1:50. G protein antibody complexes were recovered by the addition of 25 μl of protein A-Sepharose beads. Following incubation for 30 min, the beads were washed with cold phosphate-buffered saline and resuspended in 60 μl of Laemmli buffer. Labeled G protein subunits were separated by 10% SDS-PAGE under reducing conditions and identified by autoradiography. Relative densities of the G protein bands were determined with a Personal Densitometer SI (Molecular Dynamics).

GTPγS Binding Assay—GTPγS binding was performed as described previously (7). Briefly, assays were performed in a reaction mixture (100 μl) containing 50 mM triethanolamine/HCl, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, 1 mM MgCl2, 150 mM NaCl, 1.0 mM GDP, and 0.02–0.04 μCi/tube of [35S]GTPγS. Reactions were initiated by the addition of 2–10 μg of membrane protein/tube. Each reaction was allowed to proceed at 30 °C for 20 min and terminated by rapid filtration through Whatman GF/C filters. Filters were counted by liquid scintillation spectrometry. Specific binding was calculated by subtracting the amount of [35S]GTPγS bound in the presence of 10 μM GTPγS from total [35S]GTPγS bound and expressed as fmol of GTPγS bound per mg of membrane protein.

Receptor Binding Assay—FMLP binding assays were performed in a reaction mixture (100 μl) containing 50 mM Tris, pH 7.5, 1 mM EDTA, 5 mM MgCl2, as described previously (7). Reactions were initiated by addition of 15–25 μg of membrane protein and incubated for 30 min at 25 °C. Reactions were terminated by rapid filtration through Whatman GF/C filters, which were dried, placed in 4 ml of scintillation mixture, and counted in a liquid scintillation spectrometer. Specific binding was calculated by subtracting the amount of N-formyl-Met-Leu[3H]Phe bound in the presence of excess ligand from the total N-formyl-Met-Leu[3H]Phe bound. Binding parameters were estimated using a nonlinear least squares curve fitting procedure (SCTFIT), as described previously (7).

Flow Cytometric Assay for Formyl Peptide Receptors—Binding of N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein to FPRs was performed as described previously (34). Cells were harvested by centrifugation, washed in PBS, and resuspended at 1 × 10^6 cells/ml in PBS. Binding was performed in 1 ml with 10 nM N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein (Molecular Probes) for 15 min on ice. Specificity of binding sites was determined by addition of 10 μM fMet-Leu-Phe. The cells were analyzed by flow cytometry (Epics Profile II, Coulter). Quantum 24 fluorescein microbeads (Flow Cytometry Standards Corp., San Juan, PR) containing 4 × 10^5 to 6.6 × 10^6 molecules of equivalent soluble fluorochromes were used as standards to determine the average number of FPRs per cell. Data were stored and analyzed using WinList 3.0 (Verity Software House, Inc., Topsham, ME).

Calcium Mobilization—HL-60 cells at 1 × 10^6/ml were incubated with Fluo-3 for 30 min at 37 °C followed by stimulation with 0.3 μM FMLP. The increase in fluorescence intensity attributed to the increase in intracellular calcium was monitored as a function of time with a confocal microscope (Meridian, Okemos, MI) using excitation and emis-
After addition of 3% N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein in FPRwt HL-60 cells and 1 μM N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein in FPRdel HL-60 cells. Fig. 1 shows the histogram of one flow cytometric experiment representative of three separate experiments in which binding of N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein to vector-, FPRwt-, and FPRdel-transfected cells was measured. The specific binding of N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein was used to calculate receptor numbers for both the cell types. FPRwt was expressed at an average of 15,000 receptors/cell, whereas FPRdel was expressed an average of 20,000 receptor/cell. Both FPRwt and FPRdel were expressed in 70–80% of transfected cells (results not shown). Vector-transfected undifferentiated HL-60 cells did not express receptors capable of specifically binding N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein. These results indicate that cells transfected with FPRwt and FPRdel represent a relatively homogeneous population and that deletion of the carboxy-terminal tail does not result in an altered tertiary structure of FPRs that prevents either expression or ligand binding.

Reconstitution of FMLP-Stimulated MAPK Activity in FPRwt-transfected Undifferentiated HL-60 Cells—FPRs expressed by HL-60 granulocytes following differentiation by dimethyl sulfoxide stimulate ligand-dependent activation of both ERKs and p38 kinases (30). To determine that expression of FPRwt in undifferentiated HL-60 cells reconstitutes the signal transduction pathways necessary for MAPK activation, ERK and p38 kinase activities were assayed following FMLP stimulation of FPRwt HL-60 cells. Similar to results in differentiated HL-60 granulocytes (30), maximal stimulation of ERK (mean ± S.E., 5.4 ± 0.3-fold; n = 3) and p38 (3.2 ± 0.4-fold; n = 3) activity occurred 1 min after addition of 3 × 10−7 M FMLP (Fig. 2). Neither ERK nor p38 kinase activity was stimulated by FMLP in vector-transfected, undifferentiated HL-60 cells (data not shown). Thus, signaling components necessary for FMLP stimulation of MAPK cascades are present in undifferentiated FPRwt HL-60 cells.

Role of the Carboxyl-terminal Tail in MAPK Activation—To examine the role of the carboxyl-terminal tail in MAPK activation, FMLP-stimulated ERK and p38 kinase activities were measured in FPRwt HL-60 cells and FPRdel HL-60 cells 1 min after addition of 3 × 10−7 M FMLP. FMLP stimulated a 5.8 ± 1.9-fold (mean ± S.E.; n = 3) increase in ERK activity in FPRwt HL-60 cells, whereas FMLP failed to stimulate an increase in ERK activity (1.3 ± 0.1-fold; n = 3) in FPRdel HL-60 cells (Fig. 3A). Basal activity in FPRdel cells was 2.1 ± 1.4-fold (mean ± S.E.; n = 3) higher than in FPRwt. FMLP stimulated a 3.5 ± 0.7-fold (mean ± S.E.; n = 6) increase in p38 kinase activity in FPRwt cells, whereas only a 1.3 ± 0.1-fold (mean ± S.E.; n = 6) increase in p38 activity was stimulated in FPRdel cells (Fig. 3B). Basal activity in FPRdel HL-60 cells was 3.4 ± 0.7 (mean ± S.E., n = 6)-fold higher than in FPRwt HL-60 cells. To determine whether FPRwt and FPRdel are coupled to ERK and p38 kinases by pertussis toxin-sensitive G proteins, cells were pretreated with 100 ng/ml pertussis toxin for 24 h. This time

and concentration has been shown previously to inhibit Gαi protein activation by FMLP in HL-60 cells (6). Pertussis toxin suppressed FMLP-stimulated ERK and p38 activities in FPRwt HL-60 cells by 94 and 88%, respectively, indicating that pertussis toxin-sensitive G proteins couple FPRwt to both MAPK cascades (Fig. 3A and B). Basal ERK and p38 activities were not significantly altered by pertussis toxin pretreatment in cells expressing either FPRwt or FPRdel. Thus, deletion of the carboxy-terminal tail uncouples FPRs from ligand-stimulated MAPK activation and results in increased basal MAPK activity.

Role of the Carboxyl-terminal Tail in G Protein Activation—Because both pertussis toxin and FPRdel inhibited FMLP stim-
ulation of MAPKs, the contribution of the carboxyl-terminal tail of FPRs to the interaction with G proteins was examined by FMLP-stimulated GTP\(_{\gamma}\)S binding and GTP\(_{\gamma}\)S inhibition of FMLP binding in plasma membranes from FPRwt and FPRdel expressing cells. FPRwt membranes exhibited a concentration-dependent increase in GTP\(_{\gamma}\)S binding upon addition of FMLP.
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(Fig. 4A). On the other hand, GTP-γS binding was not increased by FMLP in FPRdel plasma membranes. Additionally, basal GTP-γS binding was reduced in FPRdel membranes, compared with FPRwt membranes, suggesting reduced affinity of FPRdel for ligand. Guanine nucleotides inhibit ligand binding to formyl peptide receptors by reducing receptor affinity (36–38). Addition of GTP-γS reduced FMLP binding to FPRwt membranes in a concentration-dependent manner, whereas GTP-γS had no effect on FMLP binding in FPRdel membranes (Fig. 4B). These data indicate that the absence of the carboxyl-terminal tail results in uncoupling of FPRs from G proteins.

The increased, pertussis toxin-independent basal ERK and p38 kinase activities in FPRdel HL-60 cells suggested that these mutant receptors were constitutively active for pathways independent of G proteins. To examine possible activation of other G proteins, FPRwt and FPRdel plasma membranes were photoaffinity labeled with AA-GTP before and after stimulation with 10⁻⁸ M FMLP. Autoradiography after SDS-PAGE separation (Fig. 5A). No increase in Gαq photolabeling was seen in either FPRwt or FPRdel membranes following addition of FMLP (data not shown). Immunoblotting of Gαq subunits in membranes from undifferentiated HL-60, FPRwt and FPRdel HL-60 membranes showed equal density of Gαq2 and Gαq11 subunits (data not shown), demonstrating that decreased photolabeling of G proteins in FPRdel HL-60 membranes was not due to decreased amounts of G proteins present. No basal or FMLP-stimulated photolabeling of α12 or α13 was detected, and neither of these α subunits was detected by immunoblotting of HL-60 plasma membranes (data not shown).

**Effects of Carboxyl-terminal Tail on Calcium Mobilization—**
Ligand stimulation of FPRs also results in an increase in cytosolic calcium concentration in HL-60 cells via a G protein-mediated stimulation of phospholipase C and subsequent generation of inositol 1,4,5-trisphosphate (39). To determine whether the carboxyl-terminal tail of FPRs also contributes to signal transduction pathways leading to calcium mobilization, intracellular calcium concentrations were determined in FPRwt- and FPRdel-expressing HL-60 cells before and after the addition of 3 x 10⁻⁷ M FMLP. Calcium concentrations were determined by loading cells with Fluo-3 prior to stimulation with FMLP. Cells expressing FPRwt receptor responded to FMLP with a rapid increase in intracellular calcium concentration (Fig. 6). On the other hand, FMLP stimulation of cells expressing FPRdel showed no calcium mobilization. No differ-

**Fig. 5.** AA-GTP photolabeling of FPRwt- and FPRdel-containing HL-60 Membranes. Panel A, autoradiogram of AA-GTP-photolabeled G proteins in solubilized membranes from FPRwt and FPRdel HL-60 cells. Photolabeling of intact membranes was performed in the presence and absence of 1 x 10⁻⁸ M FMLP prior to solubilization. Results are representative of three separate experiments. Panel B, autoradiogram of AA-GTP photolabeling of Goq2 immunoprecipitated from solubilized membranes. Photolabeling of intact membranes was performed in the presence and absence of 1 x 10⁻⁵ M FMLP in FPRwt and FPRdel membranes prior to solubilization. Results are representative of three separate experiments.
ences in basal calcium levels were observed (fluorescence intensity (mean ± S.E.), 76 ± 16 versus 68 ± 18; n = 43 and n = 38, FPRwt versus FPRdel, respectively). Both cell types demonstrated a similar increase in calcium concentration following addition of ionomycin (data not shown).

DISCUSSION

Defining the structural basis for divergent PMN functional response and signal transduction pathway activation to chemotaxtants provides the opportunity to develop strategies for pharmacologic regulation of these responses. The present study was designed to determine the role of the carboxy-terminal tail of FPRs in the activation of MAPK cascades. Our data show that undifferentiated HL-60 cells provide a useful model in which to examine structure-function relationships of chemotactant receptor activation of MAPKs. Stable expression of wild type FPRs permitted FMLP to stimulate a 3–5-fold increase in ERK and p38 kinase activities. The time course, concentration response, and pertussis toxin sensitivity were all similar to those seen in differentiated HL-60 cells and human PMNs (26, 28–30). Additionally, FMLP stimulation of GTPγS binding and GTPγS inhibition of FMLP binding to membranes from FPR HL-60 cells was similar to that seen in PMNs and HL-60 granulocytes. Our findings are consistent with a previous report showing that stable expression of FPRs in undifferentiated HL-60 cells results in the ability of FMLP to stimulate pertussis toxin-sensitive calcium mobilization (34). Thus, stable expression of FPRs in undifferentiated HL-60 cells permits examination of chemotactant receptor activation of G proteins and MAPK cascades.

Various receptors use different domains to couple to G proteins, and specific rules or consensus sequences that determine G protein coupling do not exist. In PMNs and HL-60 cells, FPRs couple to G proteins containing αi2 and αi3 (21, 40, 41). Physical interaction of FPRs with Gi proteins is interrupted by synthetic peptides derived from the carboxyl-terminal tail but not those derived from the third intracellular loop (19–21). Synthetic peptides from the second intracellular loop have been reported to inhibit and to have no effect on FPR-G protein coupling (19, 20). To determine the role of the carboxyl-terminal tail in FPR activation of MAPKs, we constructed a mutant FPR in which carboxyl-terminal amino acids 301–346 were deleted and stably expressed this mutant receptor in undifferentiated HL-60 cells.

Recently, our laboratory has reported that FMLP stimulation of HL-60 granulocytes, a model for PMNs (39), activates ERK and p38 kinases (30), similar to results obtained in human PMNs (25–28, 42). To determine the role of the carboxyl-terminal tail of FPRs in receptor function, the ability of FMLP to stimulate ERK and p38 MAPK activation, calcium transients, and G protein activation was compared between undifferentiated HL-60 cells stably transfected with FPRwt and FPRdel. Deletion of the carboxyl-terminal tail prevented FMLP-stimulated ERK and p38 kinase activities and blocked FMLP-stimulated calcium transients. The observed differences in MAPK activation and calcium responses are unlikely to be due to differences in expression of FPRwt and FPRdel. Expression of both receptor types by transfected HL-60 cells was similar, as determined by fluorescein-labeled formyl peptide binding. It remains possible that the deletion mutation results in changes in the tertiary structure that impair ligand binding. However, the ability of the fluorescent formyl peptide to specifically bind to FPRdel HL-60 cells suggests that any conformational change does not significantly affect ligand binding. Taken together, our data and the previously published results with synthetic peptides strongly suggest that the carboxyl-terminal tail of FPRs is required for G protein-coupled activation of MAPKs and phosphatidylinositol C-dependent calcium mobilization. Our data suggest that the mechanism of inhibition of MAPK activation and calcium mobilization in FPRdel HL-60 cells is uncoupling of the receptor-G protein interaction. This conclusion is based on several findings. First, FMLP failed to stimulate guanine nucleotide exchange by G proteins in FPRdel membranes, as shown by the absence of a concentration-dependent increase in GTPγS binding. Second, basal GTPγS binding was significantly reduced in FPRdel membranes, compared with FPRwt membranes. We have previously shown that uncoupling of formyl peptide receptors from G proteins by pretreatment with pertussis toxin results in a reduction of basal GTPγS binding (6). Third, GTPγS failed to inhibit FMLP binding in FPRdel membranes. Guanine nucleotides reduce G protein-coupled receptor affinity for ligand, resulting in a reduced level of ligand binding (36–38). Fourth, photoaffinity labeling of G proteins in FPRwt and FPRdel membranes showed that FMLP failed to stimulate increased labeling of G proteins in FPRdel membranes, and basal labeling was reduced compared with FPRwt membranes. Fifth, the concentration of fluoresceinated formyl peptide required to saturate binding sites was greater in FPRdel HL-60 cells. Because the number of receptors was similar in the two groups of cells, this finding suggests a reduced affinity for ligand of FPRdel. Finally, pertussis toxin pretreatment did not alter either stimulated or basal ERK and p38 activities in FPRdel HL-60 cells.

Basal ERK and p38 kinase activities were significantly higher in FPRdel HL-60 cells, than in FPRwt HL-60 cells. This finding suggests that removal of the carboxyl-terminal tail of FPR may result in a constitutively active receptor. The differences in basal MAPK activity was not due to selection of a specific clone of FPRdel cells with high MAPK activity, because
transfected HL-60 cells were selected by antibiotic resistance. Immunoprecipitation of specific Gα subunits following AA-GTP photoaffinity labeling in the presence of FMLP confirmed that FPRwt couple to G proteins containing αq but not to those containing Goαi2. FPRdel are not constitutively active for Gi proteins, because there was reduced basal photoaffinity labeling of immunoprecipitated αq in membranes from FPRdel HL-60 cells, and pertussis toxin did not alter this labeling. The increased basal MAPK activity in FPRdel cells failed to be the result of uncoupling of FPR from Gi2 proteins, because pertussis toxin pretreatment of FPRwt cells failed to reproduce the increase in basal ERK or p38 kinase activity. Okamoto et al. (43) showed that removal of a portion of the carboxy-terminal tail or a conformational hindrance of the carboxyl-terminal tail acts to inhibit activation of an alternative signal transduction pathway when FPRs are uncoupled from Gi2 in membranes or following FMLP stimulation of FPRwt cells, because there was reduced basal photoaffinity labeling. We considered the possibility that FPRdel constitutively activate other G proteins. Immunoprecipitation of αq11 subunits failed to demonstrate increased photoaffinity labeling in FPRdel membranes or following FMLP stimulation of FPRwt and FPRdel membranes. No basal or stimulated labeling of αq12 or αq13 was seen, and immunoblotting did not detect these subunits in HL-60 cells. We were unable to directly examine photoaffinity labeling of αq16, because an immunoprecipitating antibody was unavailable. However, the absence of increased basal or FMLP-stimulated photo labeling in solubilized membranes from FPRdel HL-60 cells suggests that this pathway was not activated. Our results suggest that the carboxy-terminal tail acts to inhibit activation of an alternative signal transduction pathway when FPRs are uncoupled from αq2-containing G proteins. Elimination of the carboxy-terminal tail not only uncouples FPRs from Gi proteins but alters the FPR by either unmasking of an active site upon removal of stearic hindrance of the carboxy-terminal tail or a conformational change in the remaining receptor. The mutant receptor is then constitutively active for an alternative pathway resulting in ERK and p38 MAPK activation. The failure of FPRdel membranes to show increased basal photo labeling with AA-GTP suggests that the alternative pathway is G protein-independent; however, the components of this pathway remain to be determined.

Acknowledgment—We acknowledge the excellent technical assistance of Suzanne Eades.