We previously reported that Gβγ signaling regulates cell spreading or cell shape change through activation of a Rho family small GTPase, suggesting the existence of a Gβγ-regulated Rho guanine-nucleotide exchange factor (RhoGEF). In this study we examined various RhoGEF clones, found FLJ00018 to be a Gβγ-activated RhoGEF, and investigated the molecular mechanism of Gβγ-induced activation of Rho family GTPases. Co-expression of the genes for FLJ00018 and Gβγ enhanced serum response element-mediated gene transcription in HEK-293 cells. Combined expression of Gβγ and FLJ00018 significantly induced activation of Rac and Cdc42 but not RhoA. FLJ00018 also enhanced gene transcription induced by carbachol-stimulated m2 muscarinic acetylcholine receptor, and this enhancement was blocked by pertussis toxin. Furthermore, we demonstrated Gβγ to interact directly with the N-terminal region of FLJ00018 and the N-terminal fragment of this molecule to inhibit serum response element-dependent transcription induced by Gβγ/FLJ00018 and carbachol. In NIH3T3 cells, FLJ00018 enhanced lysophosphatidic acid-induced cell spreading, which was also blocked by the N-terminal fragment of FLJ00018. These results provide evidence for a signaling pathway by which G12-coupled receptor specifically induces Rac and Cdc42 activation through direct interaction of Gβγ with FLJ00018.

Rho family small GTPases belong to the Ras superfamily, comprise more than 20 distinct proteins, and control a wide variety of cellular processes. First identified as regulators of the actin cytoskeleton rearrangements, RhoA, Rac1, and Cdc42 induce stress fibers, lamellipodia, and filopodia formation, respectively, and it is now clear that Rho family proteins play pivotal roles in cell migration, outgrowth, extension and pathfinding of neuritis, and gene transcription. Like other small GTPases, Rho GTPases cycle between an inactive GDP-bound state and an active GTP-bound state (1, 2). This cycling of Rho GTPases is controlled by three distinct classes of regulatory proteins, namely (i) guanine-nucleotide dissociation inhibitors, which stabilize the inactive form (3), (ii) guanine-nucleotide exchange factors (GEFs), which catalyze GDP/GTP exchange (4, 5), and (iii) GTPases-activating proteins, which stimulate low, intrinsic GTPase activity of Rho GTPases (6). In particular, 60 different GEFs for Rho family members (RhoGEFs) have been described so far (4). A common feature of RhoGEFs is the Db1 homology (DH) domain responsible for exchange activity followed by a pleckstrin homology (PH) domain considered to be involved in subcellular localization. Besides this tandem motif, RhoGEFs often contain one or more additional signal transduction domains, such as PDZ, Src homology (SH) 2, SH3, and RGS (regulator of G protein signaling), which can function as molecular bridges between different signal transduction pathways.

It is well established that a large variety of G protein-coupled receptors (GPCRs), particularly those coupling to the G12/13 type of heterotrimeric G proteins, are upstream regulators of Rho proteins (7, 8). A family of RhoA-specific GEFs consisting of p115RhoGEF, PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG), which mediate this activation process, has been identified (7–10). All these proteins contain in addition to the DH/PH tandem motif an RGS (regulator of G protein signaling) homology domain for direct interaction with and activation by G12 type G proteins. More than a dozen Rac-specific RhoGEFs have been described so far, including for example Vav, Sos, Tiam, and P-Rex (4). Some of these GEFs are specific for Rac, and others have more broad targets, but all have activities that are tightly regulated, usually by protein kinases (11–13), phosphatidylinositol 3,4,5-trisphosphate, a lipid second messenger produced by class I phosphatidylinositol 3-kinase (14–16), and interaction with other proteins containing Gβγ subunits or adenomatous polyposis coli protein (16–18).

We have previously reported that Gβγ induces actin stress fiber and focal adhesion formation in a Rho-dependent manner in HeLa cells (19) and that Gγ signals through both Go12 and

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Gβγ regulate Rac and Cdc42 during lysophosphatidic acid (LPA)-induced cell spreading in NIH3T3 fibroblasts (20). Others have described Gβγ-coupled receptor signaling to induce cell migration and neurite outgrowth (21, 22). These results suggest the presence of G protein-regulated RhoGEFs, but the details of the underlying molecular mechanisms have yet to be clarified.

In the present study we searched for Gβγ-regulated RhoGEFs in various RhoGEF clones in the Kazusa huge data base and the NEDO data base and found FLJ00018/PLEKHG2 (23) to link specifically the Gβγ-coupled receptor to Rac and Cdc42 by directly interacting with Gβγ subunits. This RhoGEF, therefore, represents a hitherto unknown Gβγ effector molecule.

**EXPERIMENTAL PROCEDURES**

**Materials**—pCMV5-FLAG-RhoAT19N, pCMV5-FLAG-Rac1T17N, and pCMV5-FLAG-Cdc42T17N were generous gifts from Dr. H. Itoh (Nara Institute of Science and Technology). pcDNA3.1-Gα12QL, pcDNA3.1-Gα13QL, pcDNA3.1-Gα13L, pcDNA3.1-Gα11L, pcDNA3.1-Gα12L, pcDNA3.1-Gα13L, pcDNA3.1-Gβγ1, pcDNA3.1-Gβγ2, and pcDNA3.1-m2 muscarinic acetylcholine receptor (m2 receptor) were from UMR cDNA Resource Center. Complementary DNA clones for RhoGEF genes were isolated during the Kazusa human cDNA project, which aimed to accumulate information on the coding genes. This RhoGEF, therefore, represents a hitherto unknown Gβγ effector molecule.

**In Vitro Binding Assays**—The cellular level of GTP-loaded Rac, Cdc42, and RhoA was determined using a GST fusion protein containing the Rac/Cdc42 binding domain of PAK (GST-CRIB) expressed in and purified from Escherichia coli DH5α (20, 26) and the RhoA binding domain of Rhotekin (GST-RBD), purchased from Upstate. In brief, subconfluent HEK-293 cells were transfected with the indicated amounts of plasmid DNA or the corresponding empty vectors (2 μg/6-cm dishes). Thereafter, the cells were lysed in buffer containing 1% Nonidet P-40, and the particulate fraction was removed by centrifugation. The supernatant was then incubated for 1 h at 4 °C with GST-CRIB bound to glutathione-Sepharose beads. After three washes of beads, bound proteins were eluted with sample buffer and separated by SDS-PAGE (27). Rac, Cdc42, and RhoA were then detected by immunoblotting with specific monoclonal antibodies.

**Immunoprecipitation**—HEK-293 cells were seeded in 6-cm dishes and transfected at 80% confluence with 2 μg of the indicated cDNA constructs. After transfection, cells were solubilized in 300 μl of immunoprecipitation buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM Na3VO4, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5% Nonidet P-40) and incubated on ice for 10 min. After centrifugation, 2 μg of anti-c-Myc antibody was added to the clear supernatant, and the mixture was incubated for 2 h at 4 °C, added to protein G-agarose, and gently shaken for 1 h at 4 °C. Beads were washed three times with washing buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM Na3VO4, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 0.1% Nonidet P-40), and bound proteins were eluted with sample buffer and loaded onto a 7.5% polyacrylamide gel. After SDS-PAGE, immunoprecipitated proteins were transferred to nitrocellulose membranes and detected with anti-Myc and anti-Gβγ antibodies.

**Cell Culture and Transfection**—HEK-293 and NIH3T3 cells were grown in Dulbecco’s modified essential medium (DMEM) supplemented with 10% fetal bovine serum (HEK-293) or 10% calf serum (NIH3T3) at 37 °C. Transient transfection was performed using Lipofectamine Plus according to the manufacturer’s instructions (Invitrogen). To examine the effect of m2 muscarinic stimulus on serum response element (SRE)-dependent gene transcription, cells were co-transfected with the m2 receptor and FLJ00018 or other plasmids and cultured for 16 h in DMEM supplemented with 1× insulin-transferrin-selenium-X supplement (Invitrogen). Then cells were washed twice with DMEM, incubated for 2 h in DMEM, and stimulated with 1 mM carbachol for 6 h.

**Assay of SRE-dependent Gene Transcription**—HEK-293 cells seeded on 24-well plates were co-transfected with the indicated expression plasmids (400 ng of total DNA/well) together with the pSRE.L-luciferase reporter plasmid and the pRL-SV40 control reporter vector. After transfection, cells were washed once with phosphate-buffered saline and lysed with passive lysis buffer (Nippon Gene). Luciferase activities were determined with the dual-luciferase reporter assay system (PicaGene Dual SeaPansy Luminescence kit, Nippon Gene). The activity of the experimental reporter was normalized against the activity of control vector.

**In Vitro Binding Assays**—GST or GST-p7 fusion proteins were expressed and extracted from E. coli strain DH5α and bound to glutathione-Sepharose 4B. Three μg of purified GST fusion protein (with the glutathione-agarose beads) and 3 μg of Gβγ subunit (purified from bovine brain) (28) were incubated for 3 h at 4 °C in buffer A (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 150 mM NaCl, 0.2% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride). Beads were washed three times with buffer A, and bound proteins were separated by SDS-PAGE and detected by Western blotting with an anti-Gβγ antibody.
RESULTS

Identification of a RhoGEF Inducing Gβγ-dependent Gene Transcription—To search for Gβγ-dependent RhoGEFs, we screened various RhoGEF clones in the Kazusa HUGE database and the NEDO database by co-expression with Gβγ in HEK-293 cells. To monitor Rho family GTPase activations in intact cells, we measured transcription of an SRE-controlled reporter gene (SRE-L-luciferase), which is known to be induced by Rho family activation. Overexpression of several RhoGEF clones containing KIAA0380 (no. 4), KIAA0424 (no. 5), KIAA0521 (no. 6), and FLJ00018 (no. 10) caused an increase in luciferase expression, and co-expression with Gβγ greatly increased FLJ00018-induced gene transcriptional activity and slightly enhanced KIAA0424-induced activity (Fig. 1). Co-expression of Gβγ with other RhoGEF clones caused a small increase or did not enhance RhoGEF-induced gene transcriptional activity (Fig. 1).

Cell Spreading Assays—NIH3T3 cells, which were transfected with GFP and indicated plasmids, were detached with trypsin-EDTA and washed with DMEM containing 0.3 mg/ml trypsin inhibitor and 1 mg/ml fatty acid-free bovine serum albumin. Cells were then re-suspended in DMEM, re-plated on fibronectin-coated glass coverslips, and incubated for 15 min in the presence or absence of 10 μM LPA. Cells were fixed in 4% paraformaldehyde in phosphate-buffered saline for immunocytochemistry. To quantitate cell spreading, images of cells were obtained using a laser-scanning microscope (LSM-510, Carl Zeiss), and the areas of GFP-positive cells were measured using LSM-510 image analysis software. For each experiment, the areas of 50 cells were quantitated.

Statistical Analysis—All results shown are the means ± S.D. of values from at least three independent experiments. The significance of group differences was analyzed by one-way analysis of variance followed by the Bonferroni correction. A p value of < 0.05 was considered significant.

Gβγ-activated RhoGEF, FLJ00018

FIGURE 1. Identification of Gβγ-activated RhoGEF clones. HEK-293 cells were co-transfected with pSRE-L-luciferase, pRL-SV40 plasmid DNAs, expression vectors for various RhoGEF clones, and Gβγ, as indicated. Luciferase activities were determined with the dual-luciferase reporter assay system, normalized for transfection efficiency, and relative activities are shown when the value of mock without Gβγ was taken as 1.0. Values are the means ± S.D. from four experiments. 1, KIAA0006 (D25304); 2, KIAA0142 (D63476); 3, KIAA0294 (AB002292); 4, KIAA0380 (AB002378); 5, KIAA0424 (AB007884); 6, KIAA0521 (AB011093); 7, KIAA0793 (AB018336); 8, KIAA0915 (AB020722); 9, KIAA1112 (AB29035); 10, FLJ00018 (AK024429).

FIGURE 2. Involvement of Rac and Cdc42 in Gβγ/FLJ00018-induced SRE-dependent gene transcription. A, HEK-293 cells were co-transfected with pSRE-L-luciferase, pRL-SV40 plasmid DNAs, expression vectors for FLJ00018 (GEF), Gβγ, Gγ2, and dominant negative (DN) mutants of RhoA, Rac1, and Cdc42, as indicated. Luciferase activity obtained with Mock was taken as 1.0, and relative activities are shown. Values are the means ± S.D. from four experiments. B, cells were co-transfected with expression vectors for FLJ00018, Gβγ, and Gγ2, as indicated. Cell extracts were incubated with GST-Myc-CRIB or GST-RBD-glutathione beads, and bound proteins were analyzed by immunoblotting using anti-Rac1, anti-Cdc42, and anti-RhoA antibodies. Rac, Cdc42, and RhoA activities are indicated by the amounts of Myc-CRIB-bound Rac and Cdc42 and RBD-bound RhoA, and relative activities are shown when the value of Mock was taken as 1.0. Total cell lysates were also directly immunoblotted for the levels of total Rac, Cdc42, or Rho for normalization. Results are the means ± S.D. from four experiments.
FIGURE 3. Gβγ subunits, but not Ga subunits, enhance FLJ00018-induced SRE-dependent gene transcription. A, HEK-293 cells were co-transfected with pSRE-Luciferase, pRL-SV40 plasmid DNAs, and expression vectors for FLJ00018 (GEF), Gα1, Gγ2, Gα12Q13L (QL), Gα13, Gα12Q20L, Gα12Q209L, Gα12Q213L, and Gα13Q226L as indicated. B, cells were co-transfected with pSRE-Luciferase, pRL-SV40 plasmid DNAs, and expression vectors for FLJ00018, Gβ1, plus Gγ2, Gγ5, Gγ7, or Gγ12, as indicated. Luciferase activity obtained with Mock was taken as 1.0, and relative activities are shown. Values are the means ± S.D. from four experiments.

The α subunits of Gi family G proteins were specifically ADP-ribosylated by pertussis toxin, then became unable to couple with GPCRs. Pretreatment with pertussis toxin eliminated carbachol-stimulated luciferase production in cells co-transfected with m2 receptor and FLJ00018 (Fig. 4B).

Wortmannin Does Not Influence Gβγ/FLJ00018-induced SRE-dependent Gene Transcription and Rac Activation—The RacGEF, P-Rex1, is directly regulated by Gβγ-like FLJ00018, but full activation of P-Rex1 requires the binding of phosphatidylinositol 3,4,5-trisphosphate (16). To examine the requirement of phosphatidylinositol 3,4,5-trisphosphate for FLJ00018 activation, we used an inhibitor for phosphatidylinositol 3-kinase, wortmannin. The treatment of wortmannin did not influence Gβγ/FLJ00018-induced SRE-dependent gene transcription (Fig. 5A). Wortmannin seemed to be functional, because 100 nm wortmannin inhibited 10% fetal bovine serum-stimulated SRE-dependent gene transcription by 40% as compared with control. Gβγ/FLJ00018-induced Rac activation was also unaffected by wortmannin (Fig. 5B). The activation mechanism of FLJ00018 seems to be different from the one of P-Rex1. In the future, however, it is necessary to compare the phosphoinositide dependence of two molecules in detail.

Gβγ Interact Directly with the N-terminal Region of FLJ00018—To investigate whether FLJ00018 directly interacts with Gβγ, Gβ1, Gγ2, and N-terminal Myc-tagged FLJ00018 were co-expressed in HEK-293 cells, and cell lysates were immunoprecipitated with anti-Myc antibody and analyzed for co-precipitated Gβ protein. As shown in Fig. 6A, the Gβ subunit co-precipitated with the wild-type FLJ00018 (WT). To identify the part of FLJ00018 on which this interaction takes place, we performed similar experiments with seven truncated mutants; WT (amino acids (aa) 1–1386), p1 (aa 1–964), p2 (aa 1–464), p3 (aa 1–309), p4 (aa 108–309), p5 (aa 1–108), p6 (aa 1–149), and p7 (aa 1–134). The mutant p4 was immunoprecipitated to a similar extent as WT and other mutants with the anti-Myc antibody but did not form a complex with the Gβ subunit (Fig. 6A). In contrast, the three mutants containing the N-terminal region (amino acids 1–108), p5, p6, and p7, co-precipitated with the Gβ subunit. To examine whether the N-terminal region of FLJ00018 binds directly Gβγ, Gβγ proteins purified from bovine brain were mixed with GST and GST-p7 fusion protein in vitro. As shown in Fig. 6B, the Gβ subunit bound p7-GST fusion protein but not GST (Fig. 6B).

To further study the properties of these mutants, we investigated the ability of each to activate SRE-dependent transcrip-
tion (Fig. 6C). The mutants lacking the PH domain such as p3, p4, and p5 were inactive, suggesting this domain is strictly required. These results resemble those with PDZ-RhoGEF deletion mutants (9), and the binding of RhoGEF to lipids or other molecules through the PH domain seems to be necessary...
for the activation of several RhoGEF molecules. In contrast, p1 and p2 enhanced luciferase production more than WT, but the levels of luciferase production induced by co-expression of G\(\gamma1\)/H9253 were similar to that induced by the combination of WT and G\(\gamma1\)/H9253 (Fig. 6C). These results suggest that the C-terminal region of FLJ00018 is inhibitory for the GEF activity, and binding of G\(\gamma1\)/H9253 may release this inhibition.

The P7 Deletion Mutant of FLJ00018 Inhibits G\(\beta\)γ/FLJ00018-Dependent Gene Transcription—Because the deletion mutant p7, like p5 and p6, binds G\(\beta\)γ dimer but has no GEF activity, expression of this mutant would be expected to block G\(\beta\)γ-induced FLJ00018 activation. Indeed, co-expression of p7 with FLJ00018, G\(\beta1\)/H9253, or G\(\beta1\)/H9253 plus FLJ00018 dose-dependently reduced the luciferase production (Fig. 7A). In addition, p7 also suppressed induction of luciferase production by the m2 receptor stimulated by carbachol (Fig. 7B).

The P7 Deletion Mutant of FLJ00018 Inhibits LPA/FLJ00018-Induced Cell Spreading—We have previously shown Rac and Cdc42 to be activated by G\(\gamma\) during LPA-induced cell spreading in NIH3T3 fibroblasts (20). To examine whether the expression of FLJ00018 and p7 influences LPA-induced cell spreading, we co-transfected these plasmids and GFP into NIH3T3 cells and re-plated in the presence or absence of LPA. FLJ00018 expression significantly enhanced cell spreading in the presence of LPA after 15 min, whereas LPA did not (Fig. 8A). Co-expression of p7 markedly diminished LPA/FLJ00018-induced cell spreading. To examine the requirement of functional domains (PH domain) in cell spreading, we transfected the deletion mutants...
of PH domain and re-plated on fibronectin-coated coverslips in the presence of LPA. The cell spreading of the mutant-expressed cells was similar to mock cells (mock, 166.2 ± 21.2; wild type, 478.0 ± 59.6; mutant, 135.6 ± 21.5). These observations suggest that FLJ00018 functions as a Rac/Cdc42 RhoGEF downstream of Gβγ during LPA-induced cell spreading.

**DISCUSSION**

It has been well documented that Gβγ subunits induce activation of Rho family GTPases in a variety of cells and tissues (19, 20, 29, 30). Because there is increasing evidence that such activation occurs independently of the phospholipase C/protein kinase C pathway, it is most likely that hitherto unidentified RhoGEFs are involved. In this report we present several lines of evidence that FLJ00018, a novel member of the Dbl family of GEFs, might represent the specific GEF or at least one of the GEFs, mediating the response. First, FLJ00018 greatly enhanced the transcriptional activity with Gβγ subunits but not with GTPase-deficient mutants of various Gα subunits. This increase in transcriptional activity was completely sensitive to the dominant negative mutant of Rac1. Second, FLJ00018 enhanced the transcriptional activity when the primarily G1αi-coupled m2 receptor was stimulated by carbachol. This enhancement was blocked by pertussis toxin. Similarly, FLJ00018 enhanced LPA-induced cell spreading. Third, co-expression of FLJ00018 and Gβγ subunits synergistically increased the cellular amount of activated Rac and Cdc42 proteins, although Rac was more intensely activated. Finally, FLJ00018 directly interacted with Gβγ subunits, as detected by means of direct binding in vitro and co-immunoprecipitation as well as by functional inhibition of Gβγ-induced gene transcription and cell spreading by the N-terminal fragment of FLJ00018 including the Gβγ binding domain (p7). Therefore, our data indicate that FLJ00018 is a novel RhoGEF for Rac and Cdc42 that is regulated by Gβγ subunits.

So far it has been shown that Gβγ could activate several RhoGEFs: Ras-GRF1 (31), P-Rex1 (16), P-Rex2 (32, 33), and p114RhoGEF (34). Ras-GRF1, P-Rex1, and P-Rex2 are Rac-specific RhoGEFs, and p114RhoGEF is a RhoGEF for RhoA and Rac. Ras-GRF1 has to be tyrosine-phosphorylated to display RacGEF activity (31). p114RhoGEF binds Gβγ subunits both via its DH/PH tandem and its C terminus, but it is not known whether they regulate the catalytic activity of this GEF (34), identical to KIAA0521 (no. 6 in Fig. 1), which increased SRE-dependent gene expression but did not further increase it with co-expression of Gβγ in our experiments. Dimers of the Gβ1 subunit with different Gγ subunits varied in their ability to stimulate P-Rex1 in vitro. The β1γ12, β1γγ1, β1γ10, and β1γ13HEA dimers all activated P-Rex1 with similar EC50 values, whereas dimers composed of β1γ12 had lower EC50 values (35). In the present study, co-expression of Gβ1 and various Gγ subunits including Gγ12 showed similar effects on SRE-dependent gene transcription through FLJ00018 activation. These results indicate that Gβγ-induced activation of FLJ00018 is clearly different from that of P-Rex family, Ras-GRF, or p114RhoGEF.

Structural analyses of these RhoGEFs further showed FLJ00018 to be a novel type of GEF. FLJ00018 is 71% identical to the mouse orthologue, Clg, overall, with 94% identity in the DH domain, 96% identity in the PH domain, and differing most in C-terminal region (36). Although FLJ00018 contains DH and PH domains in the N-terminal region like P-Rex1, the sequence in other regions is completely different (16). For example, P-Rex1 contains two DEP domains, two PDZ domains, and a C-terminal InsP4-phosphatase domain, but these are not present in FLJ00018. In PC12 cells, both PDZ and InsP4-phosphatase domain, but not PH domain, are required for optimal activity of P-Rex1 (37). In contrast, PH domain is required for optimal activity of FLJ00018 (Fig. 6C). The C-terminal region of FLJ00018 is predicted to have a Formin homology 1 domain.
FIGURE 9. Proposed mechanism whereby GPCRs stimulate Rac/Cdc42. The stimulation of Gαs-coupled receptors, including the m2 receptor, releases free Gβγ subunits, which in turn interact with FLJ00018 through the N terminus region, and membrane polyphosphoinositides (PIPs) interact with FLJ00018 through PH domain, thereby causing the activation of FLJ00018 by a still unclear mechanism. Subsequently, the activated FLJ00018 catalyzes the exchange of GDP for GTP on Rac and Cdc42 through its DH domain, leading to activation of the Rac/Cdc42-dependent pathways and causing gene expression and cell spreading.

(38) from Pfam 21.0 analysis, although the sequence homology is low. Several reports demonstrated that the Formin homology 1 domain is a binding site for profilin (38–41), apart from having potential for binding Src homology 3 or WW domain-containing signaling proteins (42). Because profilin is a G-actin-binding protein that promotes the addition of monomers to the barbed-end of actin filaments, its binding to Formin homology 1-like domains of FLJ00018 would be expected to contribute to efficient formation of actin filaments around the areas where FLJ00018 is recruited and Rac/Cdc42 is activated in cells.

Recently, a series of interactions between Rho-GEFs and scaffolding proteins containing PDZ domains have been described (23). PDZ domains are small protein interaction domains that mediate protein targeting and the assembly of multiprotein complexes. FLJ00018 also contains a putative PDZ binding motif at the C terminus. The function of the region is remains uncertain in the regulatory mechanism of FLJ00018 activity.

In summary, the data presented herein define a new signaling pathway for GPCRs, with FLJ00018 serving as a direct Gβγ effector molecule as shown in Fig. 9. Its postulated linkage of GPCRs to Rac/Cdc42 and Rac/Cdc42-dependent pathways ultimately would be predicted to affect the cytoskeletal structure, nuclear gene expression, and cellular growth.

REFERENCES
1. Hall, A. (1998) Science 279, 509–514
2. Etienne-Manneville, S., and Hall, A. (2002) Nature 420, 629–635
3. DerMardirosian, C., and Bokoch, G. M. (2005) Trends Cell Biol. 15, 356–363
4. Schmidt, A., and Hall, A. (2002) Genes Dev. 16, 1587–1609
5. Rossman, K. L., Der, C. J., and Sondek, J. (2005) Nat. Rev. Mol. Cell Biol. 6, 167–180
6. Moon, S. Y., and Zheng, Y. (2003) Trends Cell Biol. 13, 13–22
7. Kozasa, T., Jiang, X., Hart, M. J., Sternweiss, P. M., Singer, W. D., Gilman, A. G., Bollag, G., and Sternweiss, P. C. (1998) Science 280, 2109–2111
8. Hart, M. J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweiss, P. C., and Bollag, G. (1998) Science 280, 2112–2114
9. Fukuhara, S., Murga, C., Zohar, M., Igishi, T., and Gutkind, J. S. (1999) J. Biol. Chem. 274, 5868–5879
10. Fukuhara, S., Chikumi, H., and Gutkind, J. S. (2000) FEBS Lett. 485, 183–188
11. Fleming, I. N., Elliott, C. M., and Exton, J. H. (1998) FEBS Lett. 429, 229–233
12. Bustelo, X. R. (2000) Mol. Cell. Biol. 20, 1461–1477
13. Kato, J., Kazziro, Y., and Satoh, T. (2000) Biochem. Biophys. Res. Commun. 268, 141–147
14. Das, B., Shu, X., Day, G. J., Han, J., Krishna, U. M., Falcé, I. R., and Broek, D. (2000) J. Biol. Chem. 275, 15074–15081
15. Fleming, I. N., Gray, A., and Downes, C. P. (2000) Biochem. J. 351, 173–182
16. Welch, H. C., Coadwell, W. J., Elson, C. D., Ferguson, G. J., Andrews, S. R., Erdjument-Bromage, H., Tempst, P., Hawkins, P. T., and Stephens, L. R. (2002) Cell 108, 809–821
17. Nishida, K., Kazziro, Y., and Satoh, T. (1999) FEBS Lett. 459, 186–190
18. Kawasaki, Y., Senda, T., Ishidate, T., Koyama, R., Morishita, T., Iwayama, Y., Higuchi, O., and Akiyama, T. (2000) Science 289, 1194–1197
19. Ueda, H., Itoh, H., Yamauchi, J., Morishita, R., Kazziro, Y., Kato, K., and Asano, T. (2000) J. Biol. Chem. 275, 2098–2102
20. Ueda, H., Morishita, R., Yamauchi, J., Itoh, H., Kato, K., and Asano, T. (2001) J. Biol. Chem. 276, 6846–6852
21. Srinivasan, S., Wang, F., Glavas, S., Ott, A., Hofmann, F., Aktories, K., Kalman, D., and Bourne, H. R. (2003) J. Cell Biol. 160, 375–385
22. Yokomizo, T., Isumi, T., Chang, K., Takuwa, Y., and Shizumi, T. (1997) Nature 387, 620–624
23. Garcia-Mata, R., and Burridge, K. (2007) Trends Cell Biol. 17, 36–43
24. Nakajima, D., Okazaki, N., Yamakawa, H., Kikuno, R., Ohara, O., and Nagase, T. (2002) DNA Res. 9, 99–106
25. Morishita, R., Kato, K., and Asano, T. (1988) Eur. J. Biochem. 174, 87–94
26. Sander, E. E., van Delft, S., ten Klooster, J. P., Reid, T., van der Kammen, M., Erdjument-Bromage, H., Tempst, P., Hawkins, P. T., and Stephens, L. R. (1998) J. Cell Biol. 143, 1385–1398
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Asano, T., Morishita, R., Matsuda T., Fukuda, Y., Yoshizawa, T., and Kato, K. (1993) J. Biol. Chem. 268, 20512–20519
29. Ma, A., D., Metjian, A., Bagrodia, S., Taylor, S., and Abrams, C. S. (1998) Mol. Cell. Biol. 18, 4744–4751
30. Belisle, B., and Abo, A. (2000) J. Biol. Chem. 275, 26225–26232
31. Kiyono, M., Satoh, T., and Kazziro, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4826–4831
32. Donald, S., Hill, K., Lecureuil, C., Barnoun, R., Krugmann, S., John Coadwell, W., Andrews, S. R., Walker, S. A., Hawkins, P. T., Stephens, L. R., and Welch, H. C. (2004) FEBS Lett. 572, 172–176
33. Rosenfeldt, H., Vázquez-Prado, J., and Gutkin, J. S. (2004) FEBS Lett. 572, 167–171
34. Niu, J., Profirovic, J., Pan, H., Vaiksnaita, R., and Vovoyn-Yasenetskaya, T. (2003) Circ. Res. 93, 848–856
35. Mayeunuddin, L. H., McIntire, W. E., and Garrison, J. C. (2006) J. Biol. Chem. 281, 1913–1920
36. Himmel, K. L., Bi, F., Shen, H., Jenkins, N. A., Copeland, N. G., Zheng, Y., and Largaespada, D. A. (2002) J. Biol. Chem. 277, 13463–13472
37. Yoshizawa, M., Kawauchi, T., Sone, M., Nishimura, Y. V., Terao, M., Chihamo, K., Noshima, Y., and Hoshino, M. (2005) J. Neuroscience 25, 4406–4419
38. Higgins, H. N. (2005) Trends Biochem. Sci. 30, 342–353
39. Sagot, I., Rodal, A. A., Moseley, J., Goode, B. L., and Pelman, D. (2002) Nat. Cell Biol. 4, 626–631
40. Pring, M., Evangelista, M., Boone, C., Yang, C., and Zigmond, S. H. (2003) Biochemistry 42, 486–496
41. Kovar, D. R., Kuhn, J. R., Tichy, A. L., and Pollard, T. D. (2003) J. Cell Biol. 161, 875–887
42. Wallar, B. J., and Alberts, A. S. (2003) Trends Cell Biol. 13, 435–446