Heterotrimeric G proteins are critical transducers of cellular signaling. In addition to their classic roles in relaying signals from G protein-coupled receptors (GPCRs), heterotrimeric G proteins also mediate physiological functions from non-GPCRs. Previously, we have shown that Go13, a member of the heterotrimeric G proteins, is essential for growth factor receptor-induced actin cytoskeletal reorganization such as dynamic dorsal ruffle turnover and cell migration. These Go13-mediated dorsal ruffle turnover and cell migration by growth factors acting on their receptor tyrosine kinases (RTKs) are independent of GPCRs. However, the mechanism by which RTKs signal to Go13 is not known. Here, we show that cholinesterase-8A (Ric-8A), a nonreceptor guanine nucleotide exchange factor for some heterotrimeric G proteins, is critical for coupling RTKs to Go13. Down-regulation of Ric-8A protein levels in cells by RNA interference slowed down platelet-derived growth factor (PDGF)-induced dorsal ruffle turnover and inhibited PDGF-initiated cell migration. PDGF was able to increase the activity of Ric-8A in cells. Furthermore, purified Ric-8A proteins interact directly with purified Go13 protein in a nucleotide-dependent manner. Deficiency of Ric-8A prevented the translocation of Go13 to the cell cortex. Hence, Ric-8A is critical for growth factor receptor-induced actin cytoskeletal reorganization.

Resistance to Inhibitors of Cholinesterase-8A (Ric-8A) Is Critical for Growth Factor Receptor-induced Actin Cytoskeletal Reorganization*

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Heterotrimeric G proteins are essential for the transmembrane signaling by G protein-coupled receptors (GPCRs).2 A structurally diverse repertoire of ligands activates GPCRs to elicit their physiological functions (1). Ligand-bound GPCRs function as guanine nucleotide exchange factors (GEFs) catalyzing the exchange of GDP bound on the Gα subunit with GTP in the presence of Gβγ. This leads to the dissociation of the Gα subunit from the Gβγ dimer to form two functional units (Gα and Gβγ) (2). Both Gα and Gβγ subunits signal to various cellular pathways. Based on the sequence and functional homologies, G proteins are grouped into four families: Gα, Gβ, Gγ, and G12 (3). Among these four subfamilies of G proteins, the physiological function of the G12 subfamily is less well understood. In this family, there are two members, G12 and G13. Gα12 knockout mice appeared normal (4). Gα13 knock-out mice displayed embryonic lethality (−/−) (5). The molecular basis that underlies the vascular defect observed in Gα13−/− mouse embryos has not been defined.

In addition to their classic roles in GPCR signaling, heterotrimeric G proteins have been genetically demonstrated to play important roles in GPCR-independent signaling (6). The best examples are in the mitotic spindle positioning and orientation (in the establishment of cell polarity) during asymmetric division in Caenorhabditis elegans embryos and in Drosophila neuroblasts (7–11). In these processes, Gα14-GDP binds to a protein with the tetratricopeptide-GoLoco domain (such as GPR-1/2 in C. elegans and Pins in Drosophila) and disrupts intramolecular tetratricopeptide-GoLoco interactions. Then, the tetratricopeptide-GoLoco protein binds a coiled-coil protein (LIN-5 in C. elegans and Mud in Drosophila). The formation of the complex of these three proteins, Gα14, GPR-1/2, and Pins-Mud in Drosophila, is required for spindle orientation (12). A nonreceptor GEF, Ric-8, has been implicated in these GPCR-independent processes (13–19).

Ric-8 (synembryn) was originally identified in C. elegans through genetic analysis (20). Ric-8 functions upstream of Gαi in regulating neurotransmitter secretion (20). Ric-8 also acts upstream of Gαq, and GPA16 (another Ga subunit in C. elegans) during asymmetric cell division of one-cell stage C. elegans embryos (13, 21, 22). In Drosophila, Ric-8 is also required for Gα-mediated spindle orientation and cell polarity during asymmetric cell division (16–18). In Ric-8 mutants, Gα failed to localize at the cell cortex (16–18). There are two distinct mammalian Ric-8-like genes, Ric-8A and Ric-8B. Ric-8A was identified in yeast two-hybrid screens of a rat brain embryonic cDNA library as a protein that interacted with a Gαq bait (23). The Ric-8A prey clone interacted with Gαi1, Gαi2, Gαq, and Gα, but not Gα, baits in pairwise two-hybrid interaction studies. In vitro biochemical studies have shown that Ric-8A is a GEF for Gαq, Gαi1, Gαi2, Gαq, and Gα, but not Gα (23, 24). On the other hand, Ric-8B interacts with Gα and Gα (23, 24). Mechanistically, Ric-8A binds to GDP-bound Ga proteins, promotes rapid GDP release, and forms a stable nucleotide-free transition state complex with the Ga that is disrupted upon GTP binding, thus leading to the formation of Gα-GTP. Furthermore, although...
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Ric-8A mRNA is expressed in a variety of tissues, Ric-8B mRNA is mainly expressed in the olfactory epithelium (25). Moreover, Ric-8A−/− mouse embryos died in the early stages of embryonic development (26).

Recently, we have discovered that G13 is essential for growth factor-induced cell migration, independent of GPCRs (27). This represents a novel cellular signal aspect and physiological function of heterotrimeric G proteins (28). Our finding that G protein-induced signaling to G13 (29). Ric-8A plays a critical role in PDGFR-induced actin cytoskeletal reorganization.

EXPERIMENTAL PROCEDURES

RNA Interference—RNA interference of Ric-8A was performed in mouse embryonic fibroblast (MEF) cells as described previously (30). The shRNA target sequence of Ric-8A used in most of experiments here was 5′-CCATGAAGCTAGTGAA-GUA, CAGGAUGCCAUGUGCGAGA, and CAGAGGAGGAGAUGCUGCGGAACA, AGAACUUUCCAUACGAGU-3′. shRNA-treated MEF cells in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) containing 10% FBS were seeded into wells of 24-multwell plates (BD Biosciences) (31, 32). After they grew to confluence, wounds were made with sterile pipette tips. Cells were washed with phosphate-buffered saline (PBS) and refreshed with medium containing 20 ng/ml platelet-derived growth factor (PDGF-BB). After ~16-h incubation at 37 °C, cells were fixed and photographed (with 100 × magnification).

Wound Healing Assay—Wild-type, LacZ shRNA, and Ric-8A shRNA-treated MEF cells in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) containing 10% FBS were seeded into wells of 24-multwell plates (BD Biosciences) (31, 32). After they grew to confluence, wounds were made with sterile pipette tips. Cells were washed with phosphate-buffered saline (PBS) and refreshed with medium containing 20 ng/ml platelet-derived growth factor (PDGF-BB). After ~16-h incubation at 37 °C, cells were fixed and photographed (with 100 × magnification).

Fluorescence Microscopy—Staining and observation of actin filaments were performed as described previously (35). Cells were plated onto coverslips coated with gelatin. Cells were then fixed with 3.7% formaldehyde. The fixed cells were then permeabilized in 0.1% Triton X-100 for 5 min. After washing in PBS, phalloidin conjugated to rhodamine (Molecular Probes) in a solution containing PBS and 1% BSA was added to stain actin. After incubation for 30 min at room temperature, the cells were washed extensively to reduce nonspecific interactions. The coverslips were then fixed onto slides and imaged using a Zeiss fluorescence microscope. For Gα13 staining, polyclonal anti-Gα13 antibody was from NewEast Biosciences. The immunostaining was done as described previously (35).

Protein Purification—GST-tagged Ric-8A, His6-tagged Gα13/i-5, and His6-tagged Gα13/i-5 proteins were purified from Escherichia coli as described previously (27). BL21 (DE3) E. coli cells harboring pET28a-Gα13/i-5 plasmids were grown to A600 = 0.5–0.6 in LB medium at 37 °C and induced with 30 μM isopropyl β-D-galactopyranoside at 30 °C overnight (36). Cells were harvested and lysed in 20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol, 1% Triton X-100, proteinase inhibitors, and purified with Ni-nitrilotriacetic acid beads (Qiagen). Gα13 proteins were induced with 1 mM isopropyl β-D-galactopyranoside at A600 = 0.5 for 18 h at 16 °C, then purified with Ni-nitrilotriacetic acid beads. GST-tagged Ric-8A protein was purified as described (37).

Ric-8A Activity Assay—Ten 10-cm plates of MEF cells were starved in DMEM without serum overnight and then treated with or without 20 ng/ml PDGF-BB for 10 min. Cells were harvested and lysed in 50 mM Na-HEPES, pH 7.4, 100 mM NaCl, 1% Triton X-100, 1 mM PMSF, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, and 1 mM Na3VO4. Ric-8A proteins were precipitated with rabbit polyclonal anti-Ric-8A antibodies.
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**FIGURE 1. Ric-8A is required for PDGF-BB-induced dorsal ruffle turnover.** A, upper panel, Western blot with anti-Ric-8A antibody showing the knock-down of Ric-8A protein levels in Ric-8A shRNA-treated MEF cells, but not in the control LacZ shRNA-treated MEF cells. Lower panel, Western blot with anti-tubulin antibody showing the whole cell lysate loading. B, cell proliferation assay. LacZ shRNA-treated and Ric-8A shRNA-treated cells were cultured for days, and the number of the cells in the plate was counted every day. Results are mean ± S.D. (error bars) of three plates. C, actin filament staining. LacZ shRNA-treated and Ric-8A shRNA-treated MEF cells were stained overnight or treated with 20 ng/ml PDGF-BB. Cells were fixed and stained with phalloidin-rhodamine. Arrowheads indicate either peripheral membrane ruffles (upper) or dorsal ruffles (lower). D, distribution of peripheral membrane ruffles and dorsal ruffles in control LacZ shRNA-treated cells (n = 179) or in Ric-8A shRNA-treated cells (n = 215). Data are representative of three to five experiments.

(Millipore). 50 μl of precipitated proteins was mixed with 50 μl of 2 μM His₆-G₁₁ or GST₁₃/1.5 proteins contained in GTPγS loading buffer (50 mM HEPES, pH 8.0, 100 mM NaCl, 100 mM MgCl₂, 1 mM dithiothreitol, 20 μM GTPγS, and 1 μM [³⁵S]GTPγS). Aliquots were removed after 2 min, and binding of GTPγS was stopped by the addition of ice-cold buffer containing 20 mM Tris-HCl, pH 7.7, 100 mM NaCl, 2 mM MgSO₄, 1 mM GTP, and 0.02% C₁₂E₁₀. The quenched reactions were passed through BA-85 nitrocellulose filters and washed with 20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 1% Triton X-100, 1 mM PMSF, 0.25 mM GST-Ric-8A or GST protein and 20 μl of glutathione beads. The mixtures were rotated at 4 °C for 30 min. 10 μg of the nucleotide loaded Gα₁₃/i-5 proteins were added into 1 ml of binding buffer containing 20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 1% Triton X-100, 1 mM PMSF, 0.25 μM GST-Ric-8A or GST protein and 20 μl of glutathione beads. The mixtures were rotated at 4 °C for 2 h and washed three times with 1 ml of binding buffer. The beads were boiled with SDS buffer, resolved by PAGE, and blotted with anti-His₆ antibody.

**Confocal Microscopy**—Cells were seeded on acid-washed glass coverslips and allowed to grow overnight. After appropriate treatments, the cells were fixed for 10 min at room temperature with 3.7% formaldehyde in PBS, permeabilized with PBS containing 0.1% Triton X-100 for 5 min, and then washed three times with PBS. The fixed cells were incubated for 30 min with PBS containing 1% BSA to block nonspecific binding and then incubated overnight at 4 °C with rabbit polyclonal antibody against Gα₁₃ from NewEast Biosciences at 1:100 dilution. The cells were washed three times with PBS and incubated with fluorescence-conjugated secondary antibody (Molecular Probes) for 1 h. Actin polymer staining was performed using fluorescent-labeled phalloidin (Molecular Probes). The coverslips were mounted onto glass slides and imaged with a Zeiss LSM 510 laser scanning confocal microscope.

**Statistical Analysis**—Data are expressed as mean ± S.D. and analyzed by one-way ANOVA followed by Dunnett’s Multiple Comparison test with significance defined as p < 0.05.

**RESULTS**

**Ric-8A Is Involved in PDGF-BB-induced Dorsal Ruffle Turnover**—First, we investigated whether Ric-8A is involved in PDGF receptor-induced dorsal ruffle formation and cell migration. The earliest ultrastructural changes of cells treated with growth factors are the intensive bursts of ruffling of the dorsal surface plasma membranes as seen under the phase-contrast microscope (38 – 40). The physiologic functions of dorsal ruffles, including macropinocytosis, cell migration, and invasion, are continually expanding (41 – 44). It has been suggested that one major function of dorsal ruffles is to reorganize the actin cytoskeleton to prepare a static cell for motility (45). We used RNA interference to down-regulate the protein levels of Ric-8A in MEF cells (Fig. 1A). We transfected plasmid DNAs carrying shRNAs either against Ric-8A or against LacZ (as control) into MEF cells and selected stable cell lines expressing these shRNAs. Although Ric-8A shRNA decreased the protein level of Ric-8A in MEF cells, LacZ shRNA did not (Fig. 1A). Decrease of Ric-8A protein levels had no effects on MEF cell proliferation within the first 48 h of cell culture after splitting the cells (Fig. 1B), although it increased cell proliferation after 72 h. Because
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our experiments (described below) were done within 1 h (dorsal ruffle turnover), 6 h (Boyden chamber cell migration assays), or 16 h (wound-healing assays), cell proliferation was not a contributing factor here.

In serum-starved fibroblasts, PDGF-BB induces at least two types of membrane ruffles: peripheral membrane ruffles (or lamellipodia) and dorsal ruffles (46). Dorsal ruffles are dynamic structures. They form and disassemble rapidly (47, 48). Previously, we have reported that, in MEF cells, dorsal ruffles formed within ~5 min after PDGF-BB (20 ng/ml) treatment (33). These dorsal ruffles were disassembled ~10 min after PDGF-BB treatment. Dorsal ruffles formed only one time after PDGF-BB stimulation. After the disassembly of these dorsal ruffles, protrusion of large peripheral membrane ruffles was observed (33). Without PDGF-BB treatment, actin filaments were uniformly distributed in MEF cells (Fig. 1C). After PDGF-BB treatment for 10 min, control cells (treated with LacZ shRNA) showed dorsal ruffles in <10% of all cells and peripheral membrane ruffles in ~60% of cells (Fig. 1, C and D). On the other hand, in Ric-8A shRNA-treated cells, dorsal ruffles could still be observed in ~80% of cells and peripheral membrane ruffles in <5% of the cells (Fig. 1, C and D). These data imply that deficiency of Ric-8A delayed the dorsal ruffle turnover, similar to our previous observation with Go13 deficiency (33). These data demonstrate that Ric-8A is involved in PDGF-BB-initiated dynamic dorsal ruffle turnover.

Ric-8A Is Required for RTK-initiated Cell Migration—Next, we used cell migration as the second model to investigate the function of Ric-8A in actin cytoskeletal reorganization. Although some believe that dorsal ruffle turnover is part of the cell migration process and indeed required for cell migration, this notion is still under debate. Therefore, here, we treated these as two events of actin cytoskeletal reorganization. To investigate a possible role of Ric-8A in PDGF-BB-initiated cell migration, we have used two approaches to compare the migration of Ric-8A-down-regulated cells and control cells. One approach is the qualitative in vitro wound-healing assay, the other the quantitative Boyden chamber assay (31, 32). For the wound-healing assay, control LacZ shRNA-treated cells and Ric-8A shRNA-treated cells were grown to confluence. A wound (small scratch) was made in the middle of the tissue culture plate with a pipette tip. After ~24 h in the presence of PDGF-BB, control cells migrated and covered the wound, yet Ric-8A shRNA-treated cells did not (Fig. 2A). Therefore, PDGF-BB-induced migration of Ric-8A-down-regulated cells was markedly reduced compared with the migration of control cells. These results were confirmed with Boyden chamber assays (Fig. 2B). Different cell proliferation rates of control MEF cells and Ric-8A shRNA-treated cells might affect their migratory rates in wound-healing assays. However, we have shown that proliferation of Ric-8A shRNA-treated cells was faster than control cells after 72 h of culture (Fig. 1B), the slower rate of migration exhibited by Ric-8A shRNA-treated cells is not due to a slower proliferation rate. Furthermore, to investigate the dynamic turnover of dorsal ruffles and peripheral ruffles in cells at the wound edge, we examined the actin cytoskeletal reorganization of LacZ-shRNA-treated and Ric-8A shRNA-treated cells at the wound edge (Fig. 2C). After a 10-min treatment with PDGF-BB, cells treated with control (LacZ) shRNA displayed lamellipodia at the edges facing the wound (65% of the cells), indicating that the dorsal ruffles had turned over, similar to the nonmigrating cells in Fig. 1C. On the other hand, cells treated with Ric-8A shRNA exhibited dorsal ruffles (70% of the cells) (Fig. 2C), confirming a delayed dorsal ruffle turnover in Ric-8A down-regulated cells. After PDGF-BB treatment for 2 h, LacZ shRNA-treated cells showed weaker lamellipodial staining than Ric-8A shRNA-treated cells (~80% of the cells) (Fig. 2C). It is interesting to note that, even though Ric-8A shRNA-treated cells did not migrate, they still formed lamellipodia, albeit delayed. Together, these data demonstrate that Ric-8A is required for PDGF-BB-induced cell migration.

Furthermore, Ric-8A is also required for EGF-induced migration of MEF cells and human breast tumor MDA-MB-231 cells (Fig. 3). Although EGF induced the migration of MEF cells treated with control (LacZ) shRNA, EGF-induced migration was impaired in MEF cells treated with Ric-8A shRNA (Fig. 3A). In addition, we have investigated the effect of Ric-8A in human breast tumor MDA-MB-231 cells (Fig. 3, B–D). Both PDGF-BB and EGF induced the migration of MDA-MB-231 cells treated...
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FIGURE 3. Ric-8A is needed for EGF-initiated cell migration. A, wound-healing assay of cell migration. LacZ shRNA-treated control cells and Ric-8A shRNA-treated MEF cells were assayed in the absence (starvation) or presence of EGF for 16 h. Representative images are shown. B, upper, Western blot with anti-Ric-8A antibody showing the knock-down of Ric-8A protein levels in Ric-8A siRNA-treated MDA-MB-231 cells, but not in the control siRNA-treated MDA-MB-231 cells. Lower, Western blotting with anti-tubulin antibody to show the whole cell lysate loading. C and D, Boyden chamber assay of cell migration of control siRNA-treated and Ric-8A siRNA-treated MDA-MB-231 cells in the presence of 20 ng/ml PDGF-BB (C) or EGF (D). The number of migrated cells in each microscopic field in the absence of PDGF-BB or EGF was subtracted from the number in the presence of PDGF-BB or EGF. Results are mean ± S.D. (error bars; n = 3, p < 0.05). Data are representative of three to five experiments.

with control siRNAs (Fig. 3, B–D). However, PDGF-BB– or EGF-induced migration of MDA-MB-231 cells treated with Ric-8A siRNAs was impaired (Fig. 3, C and D). Pretreatment with a PDGFR inhibitor AG1296 prevented the effect of PDGF-BB on cell migration (Fig. 3C). Together, these data indicate that Ric-8A plays roles in RTK-induced cell migration.

PDGF Increases Ric-8A Activity in Cells—To investigate the mechanism by which PDGF receptors signal through Ric-8A to induce actin cytoskeletal reorganization, we examined the effect of PDGF-BB on the activity of Ric-8A. First, we showed that purified Ric-8A protein could increase the rates of [35S]GTPγS loading onto purified Go13 (Fig. 4A) and purified Go113 (Fig. 4B), demonstrating the working of the assay conditions in our hands and confirming the GEF activity of Ric-8A for Go13 and Go113. Next, we treated wild-type MEF cells with PDGF-BB. Whole cell lysates were prepared. Ric-8A protein was immunoprecipitated. The GEF activity of Ric-8A was measured by following [35S]GTPγS loading assays with purified Go13 proteins. The initial rate of [35S]GTPγS loading was compared without or with PDGF-BB treatment. Representatives of three similar experiments are shown. D, direct interaction of Ric-8A and Go113. Purified GST-Ric-8A proteins (lanes 2 and 3) or control GST proteins (lane 1) were used to pull down purified His-tagged Go113 proteins, in the presence of GDP (lane 2) or GTPγS (lane 3). The pull-downed Go113 was shown by anti-His6 antibody (upper panel). Lower panels are Ponceau S staining showing the used GST-Ric-8A proteins or GST proteins. Data are representative of three to five experiments.

we have also shown that Go113 is essential for PDGF-BB-initiated dorsal ruffle turnover and cell migration. Do Ric-8A and Go113 act in the same pathway or in parallel pathways? Because Ric-8A interacts directly with Go113 (Fig. 4D) and to catalyze guanine nucleotide exchange on Go113 (Fig. 4B), it is likely that Ric-8A and Go113 work in the same pathway and that Ric-8A functions upstream of Go113. It would be helpful to investigate the activation of Go113 by PDGF-BB in cells in the presence or absence of Ric-8A. However, for technical reasons, it is rather difficult to measure Go113 activation in cells. Because one common effect of Ric-8A on heterotrimeric G proteins in C. elegans and in Drosophila is to mediate the cell cortex translocation of heterotrimeric G proteins (even on Go proteins that Ric-8 has no GEF activity), we examined the cell cortex translocation of Go113 in response to PDGF-BB, in the presence or absence of Ric-8A. Without PDGF-BB treatment, Go113 was uniformly distributed in cells (Fig. 5A). Phalloidin staining showed actin
stress fibers, in both control shRNA-treated and Ric-8A shRNA-treated cells (Fig. 5A). PDGF-BB treatment led to lamellipodial formation with strong cell cortex actin filament staining in both control shRNA-treated and Ric-8A shRNA-treated cells (Fig. 5A). However, although Gα13 was translocated to the cell cortex in control shRNA-treated cells, Gα13 was absent in the cortex in Ric-8A shRNA-treated cells (Fig. 5A, dashed line circled area). As summarized in Fig. 5B, ~70% of LacZ shRNA-treated controls (n = 109) showed Gα13 staining on the cell cortex after PDGF-BB treatment. On the other hand, only ~15% of Ric-8A shRNA-treated cells (n = 111) displayed cell cortex staining of Gα13. These data show that Ric-8A is needed for Gα13 cell cortex translocation in response to PDGF-BB and imply that Ric-8A functions upstream of Gα13 in PDGFR signaling.

DISCUSSION

We have shown that a nonreceptor GEF, Ric-8A, plays a critical role in PDGFR-induced actin cytoskeletal reorganization.

Down-regulation of Ric-8A in cells decreased PDGF-BB-induced dorsal ruffle turnover and cell migration. PDGFR is able to activate Ric-8A in cells. Ric-8A is required for Gα13 cell cortical translocation in response to PDGF-BB stimulation. Thus, Ric-8A links PDGFR signal to Gα13 in this pathway (Fig. 6).

Our data could provide a molecular mechanism by which RTKs, and possibly other non-GPCR receptors, use heterotrimeric G proteins (in addition to Gα13) to signal and to regulate various physiological functions. Although Ric-8 has been implicated in receptor-independent activation of heterotrimeric G proteins in asymmetric division, our report is the first to reveal that Ric-8A functions downstream of a RTK. Thus, this might provide a molecular mechanism for linking RTKs to heterotrimeric G proteins. Indeed, previously, heterotrimeric G proteins have been shown to play roles in RTK signaling. Various approaches including toxins, inhibitors, and antisense constructs have been used to inhibit the function or to reduce the level of heterotrimeric G proteins (49–52). These treatments led to impairment of RTK cellular signaling (53). Although in some cases a direct interaction between the Gα subunit and a RTK had been proposed, in most instances the mechanism was not known. Future experiments will be directed to investigate the molecular mechanism by which RTKs regulate the activity of Ric-8A.

It remains unclear how Gα13 controls the dynamic turnover of dorsal ruffles. Previously, we have shown that, in wild-type fibroblast cells, dorsal ruffles form and disappear quickly (33). However, in the absence of Gα13, the dorsal ruffles stay much longer (33). Because dorsal ruffles take up much of the actin polymers, a slow disassembly of dorsal ruffles would slow down the formation of other actin polymer-based structures such as peripheral membrane ruffles that are required for cell migrat-
tion. Given that the formation of these dorsal ruffles is controlled by Ras, Rac, and Rab5 small GT Pases (43, 48, 54, 55), one way to slow down the disassembly of dorsal ruffles (in the absence of Gα13) would be to slow the conversion of Ras (or Rac, Rab5) from its active GTP-bound state to the inactive GDP-bound state, i.e., to slow the GTP hydrolysis. In other words, the presence of Gα13 could accelerate the conversion. It is interesting to note that we have previously shown that Gα13 could activate a specific Ras GTPase-activating protein, Gap1(13), to shorten the duration of activated Ras in cells (56). Furthermore, in a yeast two-hybrid assay, Ras-GAP III (Gap1IP4BP, related to Gap1(13)) was shown to interact with Gα13 (23). Moreover, Rap1GAP has also been shown to interact directly with Gαπ, Gαo, and Gαq (57–59). Thus, Gα13 could act through a GAP for Ras, Rac, or Rab5 to accelerate the actin cytoskeletal turnover and, hence, cell migration.

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