Conformational Activation of Radixin by G<sub>13</sub> Protein α Subunit*

Rita Vaiskunaite‡‡§, Vyacheslav Adarichev‡‡, Heinz Furthmayr¶, Tohru Kozasa‡, Andrei Gudkov¶, and Tatyana A. Vynn-Yasenetskaya***

From the ¶Department of Pharmacology, University of Illinois, Chicago, Illinois 60612, the §§Department of Pathology, Stanford University, California 94305, and the ***Department of Genetics, University of Illinois, Chicago, Illinois 60607

** G<sub>13</sub> protein, one of the heterotrimeric guanine nucleotide-binding proteins (G proteins), regulates diverse and complex cellular responses by transducing signals from the cell surface presumably involving more than one pathway. Yeast two-hybrid screening of a mouse brain cDNA library identified radixin, a member of the ERM family of three closely related proteins (ezrin, radixin, and moesin), as a protein that interacted with G<sub>13</sub>. Interaction between radixin and G<sub>13</sub> was confirmed by in vitro binding assay and by co-immunoprecipitation technique. Activated G<sub>13</sub> induced conformational activation of radixin, as determined by binding of radixin to polymerized F-actin and by immunofluorescence in intact cells. Finally, two dominant negative mutants of radixin inhibited G<sub>13</sub>-induced focus formation of Ras-1 fibroblasts but did not affect Ras-induced focus formation. Our results identifying a new signaling pathway for G<sub>13</sub> indicate that ERM proteins can be activated by and serve as effectors of heterotrimeric G proteins.

G<sub>13</sub> protein, one of the heterotrimeric guanine nucleotide-binding proteins (G proteins)§§ regulates diverse and complex cellular responses by transducing signals from the cell surface presumably involving more than one pathway. G<sub>13</sub> regulates Na<sup>+</sup>/H<sup>+</sup> exchanger activity (1), regulates the extracellular signal-regulated kinase (2, 3) and c-Jun NH<sub>2</sub>-terminal kinase pathways (3, 4), participates in embryonic development (5), and promotes assembly of actin stress fibers (6). It also induces mitogenesis and neoplastic transformation (2, 7) and apoptosis (8, 9). Changes in the organization of the actin cytoskeleton initiated by G<sub>13</sub> are RhoA-dependent (3, 6, 8), suggesting this small G protein is acting in downstream signaling. Recently, the exchange factor for RhoA, p115RhoGEF, was shown to act as a GTPase-activating protein for G<sub>13</sub> (10). However, only some G<sub>13</sub>-dependent cell responses can be explained by activation of the Rho-dependent pathway. For example, constitutively activated G<sub>13</sub> is a very potent oncogene (2, 11), whereas constitutively activated Rho is a weak oncogene (12), which indicates that G<sub>13</sub> may use Rho-independent signaling pathways to transduce mitogenic signal. Similarly, G<sub>13</sub> stimulates activity of the sodium/proton exchanger, NHE1, in both Rho-dependent and -independent manner (13).

Here we show that radixin, a member of the ERM family of proteins, interacts with G<sub>13</sub>. This was determined by yeast two-hybrid system, in vitro binding, and co-immunoprecipitation technique. Moreover, activated G<sub>13</sub> induced conformational activation of radixin. Finally, radixin mediated G<sub>13</sub>-induced neoplastic transformation. Our results identifying a new role for G<sub>13</sub> indicate that ERM proteins can be activated by and serve as effectors of heterotrimeric G proteins.

** Experimental Procedures

Yeast Two-hybrid Studies—The yeast two-hybrid MATCHMAKER LexA system (CLONTECH) was used for detecting specific protein-protein interactions (14). Plasmid pLexA-G<sub>13</sub>, containing the gene encoding G<sub>13</sub>, was constructed by cloning into poly linker of plasmid pLexA (in frame with the LexA coding region) of polymerase chain reaction product synthesized from pcdNA1-G<sub>13</sub>. Mouse brain cDNA library has been screened with pLexA-G<sub>13</sub>. Diploid yeast have been assayed for LEU2 and lacZ reporter gene activity, and G<sub>13</sub>-specific interactor clones have been picked. The cDNAs and deduced sequences of clones testing positive were determined and analyzed.

Cell Culture, Transfections, and cDNA Constructs—Stable cell lines of NIH3T3 cells expressing wild type constitutively active G<sub>13</sub> were prepared as described (2). Transient transfection of NIH3T3 cells was performed using LipofectAMINE reagent (Life Technologies, Inc.) according to manufacturer’s instruction. COS-7 cells were transiently transfected using DEAR-dextran as described (3). Hemagglutinin (HA)-tagged full-length, NH<sub>2</sub>- and COOH-terminal domains of radixin (obtained from F. Solomon (Ref. 15)) were subcloned into pcDNA 3.1 expression vector (Invitrogen). M. Symons provided RhoN19, RasV12, and RasN17.

Protein Purification, in Vitro Binding Assay, Immunoprecipitation, and Western Blotting—G-protein subunits were purified from recombinant baculovirus-infected Sf9 cells as described (10). To construct GST fusion proteins, DNA sequences corresponding to the indicated sequences of radixin complementary DNA were amplified by polymerase chain reaction and subcloned into vector pGEX-2T (Amersham Pharmacia Biotech). Each construct was confirmed by DNA sequencing. GST fusion proteins were expressed in DH5α cells and purified on glutathione-Sepharose. In vitro binding assay was performed as described (16). HA-tagged radixin and wild type or constitutively active G<sub>13</sub>, were transiently expressed in COS-7 cells. Cells were lysed in 20 mM Tris (pH 7.5), 1 mM dithiothreitol, 100 mM NaCl, 1 mM EDTA, 5 mM MgCl<sub>2</sub>, and 1% Triton X-100. Where indicated, AMP (30 μM AlCl<sub>3</sub>, 10 mM MgCl<sub>2</sub>, and 5 mM NaF) was included. Lysates were normalized for protein concentration, and proteins were immunoprecipitated with anti-HA 12CA5 antibody and protein A-agarose for 16 h at 4°C. Immunoprecipitates were washed, separated by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted with 12CA5 antibody (BabCo) or G<sub>13</sub> antibody (Santa Cruz Biotechnology, Inc.).

In Vivo and In Vivo F-actin Co-sedimentation Assay—Actin-binding protein kit (Cytoskeleton, Inc.) was used according to manufacturer’s instruction to determine the interaction of recombinant radixin with F-actin. To isolate total membrane and cytosol fractions, NIH3T3 cell lines stably expressing vector only or G<sub>13</sub>Q226L were scraped and...
and G Protein concentrations of cytosol fractions obtained from vector only.pended in homogenization buffer at 2 mg/ml protein concentration. Beckman Instruments ultracentrifuge. Membrane fraction was resus-

were obtained by centrifugation for 1 h at 4 °C at 150,000 g

interactions were all of about similar intensities. Rad-FL

transcription activation domain; Rad-N, NH2-terminal domain of radixin, amino acids 1–318; Rad-C, COOH-terminal domain of radixin, amino acids 319–583. A plus sign represents two-hybrid interaction; a minus sign represents no detectable signal. The positive interactions were all of about similar intensities. B, direct interaction of purified Gα13 with GST-radixin fusion protein in vitro. 0.4 μM recombinant Gα13 was incubated with 4 μg GST-radixin proteins in the absence or presence of AMF (50 μM AlCl3, 10 mM MgCl2, and 5 mM NaF) as indicated. GST fusion proteins were pull-down by centrifugation with GST-agarose beads, and bound Gα13 was analyzed by SDS-PAGE and Gα13 antibodies. C, expression of HA-radixin and wild type Gα13 (Gα13WT) or constitutively activated Gα13QL (Gα13QU) in COS-7 cells. Cell lysates were prepared in the absence or presence of AMF; equal amounts of protein from each sample were separated by SDS-PAGE and immunoblotted with anti-HA (12CA5) or Gα13 antibodies as indicated. D, co-immunoprecipitation of radixin and Gα13 with anti-HA epitope antibody. Cell lysates were prepared as described under “Experimental Procedures,” and proteins were immunoprecipitated with anti-HA epitope and immunoblotted with anti-HA or Gα13 antibodies.

resuspended in homogenization buffer containing 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol, and protease inhibitors. Cells were homogenized using a 27-gauge needle, and nuclei were removed by centrifugation at 800 x g for 10 min. Membrane and cytosol fractions were obtained by centrifugation for 1 h at 4 °C at 150,000 x g in Beckman Instruments ultracentrifuge. Membrane fraction was resus-

inization buffer at 2 mg/ml protein concentration. Protein concentrations of cytosol fractions obtained from vector only and Gα13Q226L-expressing cells were equilibrated and formation of F-actin was initiated by a 40-fold polymerization buffer containing 200 mM MgCl2, 4 mM KCl, 100 mM ATP. The samples were incubated for 1 h at 37 °C, and F-actin was pelleted by ultracentrifugation for 1 h at 4 °C at 150,000 x g. The F-actin-containing pellets were rinsed with homogen-

ization buffer and resuspended in 200 μl of the same buffer. Membrane, cytosol, and F-actin fractions were separated by 8% SDS-PAGE and immunoblotted with radixin and Gα13 antibodies (Santa Cruz Biotechnology, Inc.) and actin antibody (gift of Mark Rasenick).

Immunofluorescent and Confocal Microscopy—Cells were grown on gelatin-coated coverslips, serum-starved for 24 h, washed with phospho-

buffered saline, fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 2% bovine serum albumin for 30 min. Thereafter, cells were incubated for 1 h at room temperature with 15 μg/ml Gα13, or radixin (Santa Cruz Biotechnology, Inc.) polyclonal rabbit or goat antibodies, correspondingly. Following three washes in phosphate-buffered saline, fluorescein isothiocyanate-conju-
gated or tetramethyl rhodamine isothiocyanate-conjugated species-appropriate secondary antibodies (10 μg/ml, Pierce) were added for an additional 30 min. Co-localization studies of Gα13 and radixin in NIH3T3 cells were performed using dual-wavelength laser scanning confocal microscopy with a Zeiss LSM 510 equipped with 40x water-immersion objective.

Focus Formation Assay—Focus formation assay was performed as described (2). Rat-1 cells (60-mm dishes in triplicate) were transfected with indicated cDNA constructs and cultured for 18 days in the presence of 5% calf serum. Foci were stained with Giemsa and counted in three independent experiments.

RESULTS AND DISCUSSION

Gα13 Interacts with Radixin in the Yeast Two-hybrid Screen-
ing—To further delineate components of signaling pathways that are important for apparently diverse cellular functions, we used yeast two-hybrid screening of a mouse brain cDNA library (total 4.3 x 108 cDNAs) to search for proteins interacting with Gα13. Using full-length mouse Gα13 as “bait,” the screening yielded five positive clones, one of which was a fragment (171–583 amino acids) of radixin. To characterize this interaction of Gα13 with radixin further, pairs of hybrid plasmids containing full-length and domain fragments of radixin were transformed together with Gα13 into the yeast reporter strain EGY48 and co-transformants were selected on synthetic drop-out media lacking leucine at 30 °C for 4 days. Growth on selective media and β-galactosidase activity indicated that Gα13 interacted only with the full-length protein and the amino-terminal (amino acid residues 1–318), but not carboxyl-terminal domain fragment of radixin (Fig. 1A). This places the site for interaction to the membrane-binding region of radixin.

Gα13 Interacts with Radixin in an Activation-dependent Manner—To confirm that amino-terminal domain of radixin interacted directly with Gα13, we examine their binding using purified components in vitro. Purified Gα13 bound to purified GST-radixin-FL (full-length) and to GST-radixin-N (amino-terminal domain), whereas Gα13 binding to GST-radixin-C (carboxy-terminal domain) was very limited (Fig. 1B). This was no detectable binding of Gα13 to control GST. Importantly, Gα13 bound to GST-radixin-FL and GST-radixin-N only in the presence of AlF4−, an activator of the Gα subunit that promotes a conformation similar to that of the transition state for GTP hydrolysis (17), suggesting a potential role of radixin as a Gα subunit effector protein. AlF4−-dependent interaction was also detected between GST-radixin-FL and Gα13, but not Gαi or Gαq (data not shown).

We next demonstrated binding of Gα13 with radixin by co-
immunoprecipitation technique (Fig. 1, C and D). Wild type, GDP-bound Gα13 or a constitutively activated GTP-bound muta-
tant form of Gα13 (Gα13Q226L) (1) were co-expressed with HA-tagged radixin in COS-7 cells (Fig. 1C). Antibodies against
HA epitope co-immunoprecipitated appreciable amounts of Ga13Q226L, and only trace amounts of wild type GDP-bound Ga13 (Fig. 1D). However, when AlF4 was added, a 2–3-fold increase in the amount of immunoprecipitated wild type Ga13 was detected (Fig. 1D). We did not detect interaction of endogenous Ga13 with HA-radixin (Fig. 1D), probably due to low abundance of Ga13 in most cell types. Taken together, these data indicate that Ga13 binds radixin in an activation-dependent manner.

We examined the subcellular distribution of Ga13 and radixin in NIH3T3 cells stably expressing Ga13Q226L using laser scanning confocal microscopy. Optical sectioning (0.5-μm-thick confocal sections) of antibody-labeled cells showed that there was a striking overlap of Ga13 and radixin (Fig. 2). Both proteins were co-localized at the plasma membrane, at the nuclear membrane, and in the form of small dots. This co-distribution is consistent with our observation that Ga13 and radixin form a complex.

Ga13 Induces Conformational Activation of Radixin—Radixin and other members of ERM family of membrane-actin-linking proteins exist in structurally different forms and conformational activation is required to expose otherwise masked interaction sites (15, 18–20). The concept that ERM proteins are regulated by conformational changes has gained much support in last years (21). The conformational activation of ERM proteins results in unmasking of a high affinity binding site for F-actin in the COOH-terminal domain of ERM (20, 22, 23).

Therefore, to test whether interaction between Ga13 and radixin results in activation of radixin, we first examined the ability of radixin to bind F-actin, as a way of assessment of radixin’s conformational activation. Unmasking of radixin’s actin-binding site was determined using in vitro co-sedimentation assay (20, 24, 25) (Fig. 3A). Purified actin was polymerized in the presence of divalent cations and ATP, and after addition of purified radixin and AlF4-treated Ga13, F-actin, and any bound proteins were separated by ultracentrifugation. Western blotting showed that radixin alone did not co-sediment with F-actin. However, in the presence of activated Ga13, about 50% of radixin was associated with F-actin (Fig. 3A), whereas addition of GDP-bound Ga13 did not significantly increase the association of radixin with F-actin (Fig. 3A). Additionally, Ga13 was equally distributed between soluble and pellet fractions in the presence of radixin but did not co-sediment with F-actin in the absence of radixin (Fig. 3B), suggesting that interaction with radixin may have caused the co-sedimentation of Ga13 with F-actin.

To corroborate these results, we determined whether Ga13 promoted binding of radixin to endogenous F-actin. We hypothesized that if Ga13 promotes conformational activation of radixin, then in cells expressing Ga13Q226L larger fraction of endogenous radixin will be associated with F-actin. To test this hypothesis, the cytosol fractions of NIH3T3 cells expressing vector only or Ga13Q226L were prepared by ultracentrifugation in low ionic strength buffer in the absence of divalent cations (see “Experimental Procedures”); under these conditions F-actin spontaneously depolymerizes (24). Actin polymerization was then induced in the cytosol fraction (25), and F-actin was separated from non-sedimentable forms of actin by ultracentrifugation. Western blotting detected radixin in comparable amounts in both control and Ga13Q226L-expressing cells (Figs. 3C and 4D). After polymerization of actin and ultracentrifugation, the pellet obtained from control cells contained only trace amounts of radixin and Ga13 (Fig. 3C). Remarkably, the pellet from Ga13Q226L-expressing cells contained considerable amounts of both radixin and Ga13, suggest-
ing that the two proteins co-sedimented with F-actin (Fig. 3C). To test for specificity, we probed cytosol and membrane fractions with antibodies against primarily membrane-bound \( \text{Ga}_q \) and cytosolic mitogen-activated protein kinase/extracellular signal-regulated kinase kinase. Both proteins could not be detected in the F-actin pellet.\(^2\)

We finally examined whether activated \( \text{Ga}_{13} \)Q226L induced conformational activation of radixin in vivo. Another manifestation of the conformationally active state of radixin in cells is its redistribution to newly formed apical microvilli in the cells (26). Whether or not \( \text{Ga}_{13} \) is associated with a conformationally active state of radixin was tested using immunofluorescence microscopy of NIH3T3 fibroblast cell lines stably expressing either vector alone or the constitutively active \( \text{Ga}_{13} \)Q226L sub-unit. In these cells, \( \text{Ga}_{13} \)Q226L induced assembly of actin stress fibers (data not shown) and caused the appearance of numerous short apical membrane microvilli (Fig. 4, A and B); however, radixin did not localize along stress fibers but instead showed diffused staining (Fig. 4, A and B). Importantly, the

\(^{2}\) R. Vaiskunaite, V. Adarichev, H. Furthmayr, T. Kozasa, A. Gudkov, and T. A. Voyno-Yasenetskaya, unpublished data.
apparent intensity of radixin staining in cells expressing Ga13Q226L was dramatically increased (Fig. 4, A versus B), although the protein concentration of radixin remained unchanged in both cell lines as determined by Western blotting (Figs. 3C and 4D). This indicates that the increase in intensity of the immunofluorescence staining was not due to up-regulation of radixin expression. Similarly, radixin distribution between cytosol and membrane fractions did not differ in Ga13Q226L-expressing cells when compared with vector-only-expressing cells (Fig. 4D). Therefore, the change in immunofluorescence staining was not due to recruitment of radixin from the cytosol to the membrane, but was the result of an increase in epitope availability for antibody due to a change in the conformational state of radixin (21). The Ga13Q226L-induced unmasking of radixin epitope for antibodies was not dependent on RhoA, because dominant negative RhoA mutant did not affect immunofluorescence of radixin (Fig. 4, A–C), while inhibited Ga13-induced actin polymerization, presumably mediated by RhoA (data not shown).

Because Ga13 functionally interacts with thrombin receptors (5), we next examined the intracellular distribution of radixin in serum-starved human microvascular endothelial cells in response to thrombin. The apparent distribution of endogenous radixin changed, most notably due to the increase in staining intensity and the appearance of intensely stained membrane protrusions (Fig. 5, A and B). Western blotting analysis did not detect changes in cytosol and membrane contents of radixin before and after the stimulation of the endothelial cells with thrombin (Fig. 5C), supporting the notion that Ga13-dependent increase of apparent intensity and distribution of radixin staining resulted from a change in the conformational state of radixin. However, as thrombin receptors are coupled to multiple G proteins, we are currently investigating the possible involvement of individual G proteins in a thrombin-induced redistribution of radixin. Taken together, these data indicate that Ga13 directly induces conformational activation of radixin.

As ERM proteins are the substrates of serine/threonine and tyrosine kinases, phosphorylation may positively regulate their activities by stabilizing ERMs in conformationally "open" state (20, 21). In Swiss 3T3 cells, mutationally activated Rho induces phosphorylation of ERM proteins (26). However, Rho kinase
does not phosphorylate full-length radixin in a conformationally “inactive” state (20). Radixin can serve as a Rho kinase substrate mostly in conformationally “active” state in vitro; full-length radixin is a poor substrate for Rho kinase, whereas COOH-terminal domain of radixin can be readily phosphorylated by Rho kinase (20). In addition, recently it was shown that phosphorylated inositol bisphosphate is involved in the Rho-dependent activation of ERM proteins (27). Our data suggest that Gα13-induced conformational changes of radixin may provide its availability as a substrate for Rho kinase, which also can be indirectly activated by Gα13 (30). This potentially suggests that Gα13 may recruit two independent signaling pathways resulting in “stabilized” activation radixin. Although this hypothesis is currently under investigation, here we demonstrate a novel mechanism of activation of radixin via direct interaction with Gα13.

**Radixin Inhibited Gα13, but Not Ras-dependent Cell Transformation**—To further investigate the physiological relevance of the interaction between Gα13 and radixin, we studied the modulation of Gα13-dependent cellular responses by radixin. The recognized cellular effect of Gα13 is neoplastic transformation, which occurs by yet unknown mechanism (2, 11). Therefore, we next examined the effect of radixin on cellular transformation. Activated Gα13 induced focus formation when transfected into Rat-1 cells (Fig. 6, Table I), which is consistent with previously published data (2, 11). This cellular transformation was not Ras- or Rho-dependent, because it could not be inhibited by dominant negative Ras (RasN17) or dominant negative Rho (RhoN19) (Fig. 6, Table I). Under the same conditions, RasN17 was functionally active since it was able to inhibit Ras-induced focus formation (Fig. 6, Table I). Similarly, the functional activity of RhoN19 was also confirmed by its ability to inhibit Ras-induced focus formation (Fig. 6, Table I).

Overexpression of full-length radixin or its deletion mutants had no effect on basal rate of Rat1 transformation (Fig. 6, Table I). However, overexpression of both COOH- and NH2-terminal domains of radixin dramatically inhibited Gα13-induced focus formation by 62% and 76%, respectively (Fig. 6, Table I). The COOH- and NH2-terminal domains were shown to exert dominant negative effect on radixin in different cellular systems (18), presumably by competing with radixin’s F-actin binding site and membrane binding site, respectively. Thus, our data suggest that endogenous radixin mediates Gα13-induced transformation, and its binding to both F-actin and to the membrane are necessary for this function. Importantly, the deletion mutants of radixin did not affect Ras-induced focus formation (Fig. 6, Table I). This is consistent with our data showing that Gα13-induced transformation is not Ras-dependent (Fig. 4, Table I), and this also indicates that Ras-induced transformation does not require radixin, but recruits a distinct signaling pathway. Overexpression of full-length radixin did not additionally enhance Gα13-induced transformation, but rather slightly reduced the effects of both Gα13Q226L and Ras, the significance of which is questionable. This suggests that the amounts of endogenous radixin in Rat1 cells are sufficient for mediating the effect of Gα13Q226L.

The mechanism by which radixin mediates Gα13-dependent focus formation is not yet understood. Recently ezrin, a protein closely related to radixin, has been shown to interact with p85 subunit of phosphatidylinositol (PI) 3-kinase and promote cell survival (28). Our preliminary data show that PI 3-kinase is also involved in Gα13 function, although the connection between PI 3-kinase and radixin is yet to be examined. Furthermore, the role of cortical cytoskeleton, which is obviously regulated by radixin at focal adhesions and other cell surface structures (21), could be important in Gα13-induced transformation. Thus, the relative importance of one or the other pathways and the precise sequence of molecular events under different conditions of cell activation remain to be established.

In conclusion, we have shown that Gα13 directly interacts with radixin, a member of a distinct family of ERM proteins (Fig. 7). Interaction of Gα13 with radixin occurs in a Gα13-activation-dependent manner and results in conformational activation of radixin. This represents a novel signaling pathway induced by Gα13. Functionally, radixin mediates the Gα13-dependent neoplastic transformation. To our knowledge, this is the first evidence that an ERM proteins can be activated by and serve as direct effectors of a heterotrimeric G protein. Finally, because ERM proteins and proteins containing homology to membrane-binding domain of ERM proteins (29), such as the brain tumor suppressor neurofibromatosis 2 gene product mer-

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**TABLE I**

| Transfection | Focus formation |
|--------------|-----------------|
|              | # foci/dish     |
| PcDNA3, 4 µg | 10 ± 2          |
| Radixin, full-length, 1 µg | 15 ± 9         |
| Radixin, N-domain, 1 µg | 16 ± 6         |
| Radixin, C-domain, 1 µg | 16 ± 7         |
| Gα13, Q226L, 1 µg | 320 ± 35       |
| Gα13, Q226L + radixin, full-length | 250 ± 22       |
| Gα13, Q226L + radixin, N-domain | 120 ± 19      |
| Gα13, Q226L + radixin, C-domain | 75 ± 10        |
| RasV12, 0.1 µg | 605 ± 59      |
| RasV12 + radixin, full-length | 490 ± 53       |
| RasV12 + radixin, N-domain | 520 ± 58       |
| RasV12 + radixin, C-domain | 550 ± 49       |
| RasN17, 1 µg | 0               |
| RasV12 + RasN17 | 120 ± 26       |
| RhoAN19, 1 µg | 350 ± 44        |
| Gα13, Q226L + RhoAN19 | 350 ± 40       |
| RasV12, 0.1 µg + RhoAN19, 1 µg | 350 ± 40       |

Fig. 7. Proposed signaling pathways for Gα13. Activated Gα13 binds to radixin and induces conformational activation of radixin. Radixin mediates Gα13-dependent neoplastic transformation. Gα13 contributes to Rho activation via interaction with p115RhoGEF (10). Direct interactions are shown in solid lines. Indirect effects are in dashed lines. Potential cross-talk between radixin and RhoA pathways, which are currently under investigation, are in dotted lines.
lin, cell-cell-contact protein talin, cytoplasmic tyrosine phosphatase, regulate a variety of signal transduction pathways, the $\alpha_{13}$-ERM protein link should provide a new means to extend G-protein signaling to a broad range of physiological processes.

Acknowledgments—We thank S. Vogel for the help with quantification of the fluorescent images; M. Panchenko and E. Gerasimovskaya for help with F-actin co-sedimentation assay; F. Solomon and M. Symons for providing cDNA constructs; and D. Barber, O. Colamonici, N. Dulin, H. Hamm, A. Malik, R. Minshall, M. Symons, and R. Ye for critical reading of the manuscript.

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