Ga\textsubscript{13} Stimulates Cell Migration through Cortactin-interacting Protein Hax-1*

V. Radhika‡, Djamila Onesime§§, Ji Hee Ha‡, and N. Dhanasekaran¶

From the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Ga\textsubscript{13}, the \(\alpha\)-subunit of the heterotrimeric G protein G13, has been shown to stimulate cell migration in addition to inducing oncogenic transformation. Cta, a Drosophila ortholog of G13, has been shown to be critical for cell migration leading to the ventral furrow formation in Drosophila embryos. Loss of Ga\textsubscript{13} has been shown to disrupt cell migration associated with angiogenesis in developing mouse embryos. Whereas these observations point to the vital role of G13-orthologs in regulating cell migration, widely across the species barrier, the mechanism by which Ga\textsubscript{13} couples to cytoskeleton and cell migration is largely unknown. Here we show that Ga\textsubscript{13} physically interacts with Hax-1, a cytoskeleton-associated, cortactin-interacting intracellular protein, and this interaction is required for Ga\textsubscript{13}-stimulated cell migration. Hax-1 interaction is specific to Ga\textsubscript{13} and this interaction is more pronounced with the mutationally or functionally activated form of Ga\textsubscript{13} as compared with the wild-type Ga\textsubscript{13}. Expression of Hax-1 reduces the formation of actin stress fibers and focal adhesion complexes in Ga\textsubscript{13}-expressing NIH3T3 cells. Coexpression of Hax-1 also attenuates Ga\textsubscript{13}-stimulated activity of Rho while potentiating Ga\textsubscript{13}-stimulated activity of Rac. The presence of a quadnary complex consisting of Ga\textsubscript{13}, Hax-1, Rac, and cortactin indicates the role of Hax-1 in tethering Ga\textsubscript{13} to the cytoskeletal component(s) involved in cell movement. Whereas the expression of Hax-1 potentiates Ga\textsubscript{13}-mediated cell movement, silencing of endogenous Hax-1 with Hax-1-specific small interfering RNAs drastically reduces Ga\textsubscript{13}-mediated cell migration. These findings, along with the observation that Hax-1 is overexpressed in metastatic tumors and tumor cell lines, suggest a novel role for the association of oncogenic Ga\textsubscript{13} and Hax-1 in tumor metastasis.

Cell migration plays a vital role in different biological processes ranging from embryogenesis to immune response (1, 2). However, an aberrant activation of cell migration in neoplastic cells results in tumor metastasis. Cells migrate in response to different cues through the coordinated interactions of actin- and/or microtubule-associated cytoskeletal proteins (3, 4). G protein-coupled receptors and their cognate G proteins play a major role in regulating cell migration and chemokinesis (5). The G12 family of G proteins, defined by \(\alpha\)-subunits Ga\textsubscript{12} and Ga\textsubscript{13}, has been shown to activate novel signaling pathways involved in cell growth and neoplastic transformation (6). Two lines of evidence indicate that the \(\alpha\)-subunit of G13, Ga\textsubscript{13}, is primarily involved in the regulation of cell migration (7–9). The first is the observation that Cta, an ortholog of Ga\textsubscript{13}, is critically required for cell movement during Drosophila embryogenesis (7). The second is the finding that Ga\textsubscript{13}-null (Ga\textsubscript{13}−/−) fibroblasts show the loss of chemokinetic response to thrombin or lysophosphatidic acid receptor-mediated cell movement (8, 9).

Existing models of cell movement suggest that the initial movements in cell migration involve polymerization of filamentous actin at the leading edge forming membrane extensions (3, 4). Subsequent adhesion at the leading edge is followed by the translocation of the cell body by the forward flow of the cytosol. Finally, whereas the leading edge of the cell is still attached to the substratum, the rear-end is detached and retracted into the cell body thereby effecting cell movement. All of these distinct phases of cell movement, with the exception of cytosolic translocation, involve temporal and spatial regulation of actin polymerization and depolymerization. Although, many different proteins control actin polymerization, several lines of evidence indicate that the interaction between cortactin and actin-related proteins 2/3 plays a critical role in actin polymerization leading to cell movement (10–12). Cortactin is a major substrate for tyrosine kinases such as Src, Fer, and Syk, and is usually present in the cytosol. Upon growth factor stimulation, it is translocated by activated Rac-1 to cell periphery where it interacts with F-actin and stimulates actin-related protein 2/3-mediated actin polymerization (13). As a result, tyrosine kinases as well as small GTPases converge on cortactin to stimulate actin polymerization and consequent cell movement. Although Ga\textsubscript{13} has been known to be involved in the regulation of thrombin and lysophosphatidic acid-receptor-stimulated cell migration of fibroblasts (8, 9), the mechanism by which Ga\textsubscript{13} stimulates cell movement or the identity of the signaling components involved in Ga\textsubscript{13}-mediated cytokinesis is largely unknown. Therefore, we sought to identify the signaling components involved in Ga\textsubscript{13}-mediated cell movement. Here we demonstrate that Ga\textsubscript{13} physically associates with intracellular protein Hax-1, which has been previously identified as a cortactin-interacting protein (14) and that Hax-1 promotes Ga\textsubscript{13}-mediated cell migration. Furthermore, we show that Ga\textsubscript{13} and Hax-1 exists in a complex consisting of Rac and cortactin. We also demonstrate that the coexpression of Hax-1 enhances Ga\textsubscript{13}-mediated Rac activity while inhibiting Rho activity, both of which can promote cell movement. These results describe for the first time a critical role for Hax-1 in cell movement mediated by Ga\textsubscript{13}.

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‡ These authors made equal contributions to this study.

§ Present address: Laboratoire de Génétique Moléculaire et Cellulaire Institut National de la Recherche Agronomique-Centre National de la Recherche Scientifique, Institut National Agronomique Paris-Grignon, F-78850 Thiverval-Grignon, France.

¶ To whom correspondence should be addressed. Tel.: 215-707-1941; Fax: 215-707-5963; E-mail: danny001@temple.edu.

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Experimental Procedures

Plasmids, Strains, and Cells—The yeast expression vector pLexA-Gα13ED was constructed by ligating the PCR-derived cDNA insert encoding amino acids 221–347 of Gα13 into pBTM116 vector. Expression vector pcDNA3-Hax-1 was constructed by ligating the EcoRI-SpeI-excised Hax-1 insert from pME18S-Hax-1 into the EcoRI and Xbal site of pcDNA3 vector. C-terminal S-protein-tagged Hax-1 was constructed by replacing the stop codon of Hax-1 with the coding sequence for the S-tag (KETAAAKFERQHMDS) followed by a stop codon. The resultant Hax-1-S-tag was cloned into the pcDNA3 vector. Cells lines Gα13-QL- and Gα13WT-NH3T3 have been previously described (31). All the constructs were verified by sequencing. Transformation of NIH3T3 cells was carried out using the calcium phosphate method as previously described (32). COS-7 cells were transfected using FuGENE 6 reagent (Roche Applied Science) according to the manufacturer’s protocol.

Yeast Two-hybrid Screen—The yeast two-hybrid screen was performed in yeast strain L40 transformed with pLexA-Gα13ED and plasmid pGAD-GH containing an oligo(dT)-primed HeLa cell cDNA library (Clontech, Palo Alto, CA). Of the 4 × 10^9 transformants screened, 550 clones were found to grow in the absence of His. The His+ colonies were restreaked on SD-His−/Leu−/Trp− plates and subjected to β-galactosidase activity using a filter assay. The resultant 454 His+ LacZ+ colonies were grown in SD-Leu medium to enable the segregation of pLexA-Gα13ED. The Trp-Leu+ segregates were mated to yeast strain AMR70 that had been pre-transformed with the plasmid pLex-Lamin or pLexA-Gα13ED. The leu− trp+ diploids were then assayed for transcription of the lacZ reporter by a filter assay. The positive clones totaling 115 that showed β-galactosidase activity only in the presence of pLexA-Gα13ED were isolated and the library plasmids were recovered. The cDNA inserts of the library plasmids were amplified by PCR and 10 representative clones were further sequenced. The sequence analysis of the cDNA inserts revealed that they are five independent fusions of human Hax-1.

Co-precipitation and Immunoblot Analysis—Co-precipitation studies were carried out with COS-7 cells using S-protein-agarose (Novagen, EMD Biosciences, Inc., Madison, WI) or antibodies specific to the protein of interest. At 24 h following transfection, cells were lysed and cell lysate protein (1 µg each) was incubated with 35 µl of S-protein-agarose for 4 h at 4 °C. After repeated washes with lysis buffer, the S-protein-agarose-bound proteins were separated by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes. Co-immunoprecipitation analyses were carried out by incubating cell lysate protein (1 µg each) with 1–5 µg of the respective antibodies for 4 h at 4 °C followed by the addition of 30 µl of 50% slurry of protein A-Sepharose (Amersham Biosciences). Antibodies to cortactin (05-180) and Rac (05-389) were from Upstate Signaling Solutions (Charlottesville, VA), whereas antibodies to the hemagglutinin epitope (2362) and Hax-1 (05-389) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibodies to Gαi (1521), 89-2) raised against the C terminus of Gαi3, were a kind gift from Dr. David Manning, University of Pennsylvania, Philadelphia, PA. For immunoblot and immunoprecipitation studies for Gα12 WT and Gα12QL, rabbit polyclonal antibodies (AS1-89-2) raised against the C terminus of Gα12 were used. After washing the immunoprecipitates twice with lysis buffer, the immunoprecipitated proteins were separated by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes. Immunoblot analyses with specific antibodies were carried out following the previously published procedures (31). Co-localization of Hax-1 and Gα13—Cells were grown on coverslips for 48 h, fixed with 3% paraformaldehyde in PBS1 for 10 min, per-
FIG. 2. Association of Gα13 with Hax-1. A, interaction of Gα13 and Hax-1 in COS-7 cells. COS-7 cells were transfected with pCDNA3 constructs encoding activated mutant (Gα13QL) or wild-type (Gα13WT) along with S-tagged Hax-1 (Hax-1-S) for 24 h. Gα13 was co-precipitated with S-protein-agarose (upper panel), whereas Hax-1 was co-immunoprecipitated (IP) with anti-Gα13 antibodies (lower panel). Expression of the transfected Gα13WT, Gα13QL, and Hax-1 were monitored by immunoblot (IB) analyses of the cell lysates (panel under Lysates) with the respective antibodies. B, effect of aluminium fluoride-stimulated activation of Gα13 on Hax-1 interaction. COS-7 cells were co-transfected with a vector encoding epitope-tagged Hax-1 along with Gα13 (Hax-1 + Gα13WT) or vector control (pcDNA3 + Hax-1) for 24 h. The transfected were preincubated with 10 mM AlCl3 plus 10 mM NaF for 15 min (+AlF) prior to lysis along with the non-treated control group (−AlF). Gα13 subunits co-precipitated along with Hax-1 by S-protein-agarose (P: Hax-1-S) were identified by immunoblot analysis using Gα13 antibodies (upper panel). Expression levels of Hax-1 and Gα13 in these transfectants were monitored by immunoblot analyses with respective antibodies (lower panel). C, specificity of Gα13-Hax-1 interaction. COS-7 cells were transfected with pCDNA3 constructs encoding activated mutants of Gα13 (Gα13QL), Gα12 (Gα12QL), Gα12 (Gα12Q11L), and Gα12 (Gα12QL) along with S-tagged Hax-1 (Hax-1-S) for 24 h. Gα subunits that were co-precipitated along with Hax-1-S by S-protein-agarose were identified by immunoblotting with the antibodies to the respective Gα-subunits as indicated. The expression levels of these subunits in the lysates were monitored for comparison (under panel labeled lysate). D, colocalization of Gα13 and Hax-1. NIH3T3 cells stably expressing Gα13QL were transiently transfected with vectors encoding Hax-1 for 48 h. The cells were immunostained with antibodies to Gα13 followed by Texas Red-labeled anti-rabbit IgG (red) for the expression of Gα13, and antibodies to Hax-1 followed by Alexa 488-labeled anti-mouse IgG (green) for the expression of Hax-1. Colocalization of Gα13 and Hax-1 is shown by dual channel imaging of red and green channels (yellow). These results are representative of triplicate experiments. The scale bar (20 μm) is common to all.
RESULTS

Interaction of Gα13 with Hax-1—To identify novel signaling proteins that interact with Gα13, a yeast two-hybrid screen was carried out in which the effector interacting domain of Hax-1 spanning amino acids 221–347, deduced from the crystal structures of the Gα13 and Gα13, was used as bait in a human HeLa cell cDNA library. Analyses of a set of transformants that were positive for Gα13 interaction identified Hax-1 (HS-1 associated protein X-1) as a Gα13-interacting protein. Previous studies have identified Hax-1 as an intracellular, 35-kDa HS-1 or cortactin-interacting protein (14, 16). Sequence analyses of Hax-1 inserts rescued from these transformants revealed that Hax-1 coding sequences of varying lengths interact with Gα13 (Fig. 1A). The interactions between these Hax-1 inserts and Gα13 were also verified using β-galactosidase activity of the LacZ reporter gene (Fig. 1B). Sequence alignment of the different Gα13-interacting Hax-1 fragments indicated that amino acids 178–247 of Hax-1 was sufficient for its interaction with Gα13 (Fig. 1C).

To examine the in vivo interaction between Gα13 and Hax-1, co-immunoprecipitation studies were carried out in COS-7 cells that were cotransfected with an expression vector containing a cDNA insert encoding S-epitope-tagged Hax-1 and a vector containing an insert encoding wild-type Gα13 (Gα13 WT) or its activated mutant (Gα13, QL). Examination of Gα13 immunoprecipitates for the presence of Hax-1 indicated that Hax-1 was co-immunoprecipitated with Gα13 (Fig. 2A). Similarly, examination of Hax-1 immunoprecipitates by immunoblot analysis showed that Gα13 was coimmunoprecipitated with Hax-1, thereby confirming the physical interaction between these two proteins. Although both Gα13, WT and its activated mutant Gα13, QL could be seen to interact with Hax-1, the interaction between Gα13, QL and Hax-1 is more pronounced than that of the wild-type (Fig. 2A). Furthermore, pretreatment of Gα13, WT transfecants with aluminum fluoride for 15 min, which is known to convert the unstimulated wild-type α-subunit to an active conformation (17, 18), drastically enhanced the interaction of Hax-1 with Gα13 WT, demonstrating that the active configuration of Gα13 more avidly interacts with Hax-1 (Fig. 2B). Co-immunoprecipitation analyses to investigate whether Hax-1 interacts with the α-subunits of other G proteins sub-families represented by Gα5, Gα12, and Gα13, indicated that Hax-1 failed to interact with any of these α-subunits (Fig. 2C). Interestingly, a similar analysis to determine the interaction between Hax-1 and Gα12, the α-subunit closely related to Gα13, indicated that Hax-1 does not interact with Gα12 thereby indicating the specificity of Gα13-Hax-1 interaction (Fig. 2C). These results are significant in light of the observations that only Gα13, but not Gα12, is involved in the cell migratory response (9). The interaction between Gα13 and Hax-1 can also be observed in NIH3T3 cells stably expressing Gα13, QL in which Hax-1 is transiently expressed. Double immunofluorescent labeling of Gα13 and Hax-1 followed by image-merging analysis indicated the colocalization of Gα13 and Hax-1 in NIH3T3 cells, further confirming their interaction in a cell-type independent manner (Fig. 2D). These findings, taken together with the previous observations, that Gα13 regulates cell migratory response (8, 9) and Hax-1 interacts with cortactin (16), which promotes cell migration (10–13), suggested to us the interesting possibility that Hax-1 is involved in linking Gα13 to cell migration-associated actin cytoskeletal motor.

Cytoskeletal Changes in Gα13-Hax-1 Co-transfectants—Previous studies from others, as well as us, have also shown that

![Fig. 3. Reduction of stress fibers by Hax-1. NIH3T3 cells stably expressing the empty vector (NIH3T3-pcDNA3) or Gα13, QL (NIH3T3-Gα13, QL) were transiently transfected with pcDNA3-Hax-1. At 48 h following transfection, the transfecants were stained for actin using fluorescein isothiocyanate-phalloidin and fluorescent imaging was carried out under a fluorescent microscope (Scale bar, 20 μm). Photomicrographs compare the effect of Hax-1 on expression on actin stress fibers (right panel) in NIH3T3 cells expressing NIH3T3-pcDNA3 or NIH3T3-Gα13, QL cells (left panel). Three independent experiments yielded similar results and the results of a representative experiment are shown.

![Fig. 4. Hax-1 modulation of Gα13-mediated activities of Rho GTPases. A, activation of Rho by Gα13 is attenuated by Hax-1. NIH3T3 cells stably expressing empty vector or Gα13, QL were co-transfected with vector encoding Hax-1. At 48 h after transfection, the cells were lysed and the lysates were incubated with Rhotekin-RBD-(7–89)-agarose beads (20 μg) at 4 °C for 45 min. The beads were washed, and bound Rho-GTP was detected by immunoblot analysis using polyclonal antibodies that recognize Rho-A, Rho-B, and -C (upper panel). Immunoblot analysis of Rho in the cell lysate is presented for comparison (lower panel). B, activation of Rac by Gα13 is attenuated by Hax-1. NIH3T3 cells stably expressing empty vector or Gα13, QL were co-transfected with pcDNA3 vector encoding Hax-1. At 48 h after transfection, the cells were lysed and cell lysates were incubated with PAK-PBD-(67–150)-agarose beads (10 μg) at 4 °C for 45 min. The beads were washed, and bound Rac-GTP was detected by immunoblot analysis using polyclonal antibodies to Rac (upper panel). Immunoblot analysis of Rac in the cell lysate is presented for comparison (lower panel).]
Gα13 and Hax-1 in Cell Migration

Gα13 stimulates the formation of focal adhesion complexes through the small GTPase Rho (19, 20). It has also been demonstrated that Gα13 stimulates signaling pathways regulated by the small GTPase Rac (17). Whereas Rho-stimulated stress fiber and focal adhesion complex formations are often associated with cell adhesion, Rac-stimulated membrane ruffling and lamellipodia formation are associated with cell protrusion during cell movement (21, 22). Although these two responses seem to contradict each other, cell migration often involves a sequential as well as spatio-temporal regulation of adhesion, de-adhesion, and protrusional mechanisms involving both of these GTPases (3, 4). Therefore, it is possible that Gα13 can transmit adhesion and protrusion signals to the actin cytoskeleton by recruiting different signaling components in a context-specific manner. If such a differential signaling for cell movement is promoted by Gα13-Hax-1 interaction, a decrease in stress fiber formation with a concomitant increase in actin-rich structures at the leading edges of the cell, a characteristic feature of cell migration, should be observed. This was investigated by analyzing the actin organization in cells expressing Gα13-QL and Hax-1 (Fig. 3). Consistent with previous findings (19, 20), actin staining showed that NIH3T3 cells expressing Gα13-QL as well as the control group showed extensive stress fiber formations. However, upon expression of Hax-1, cells expressing Gα13-QL showed a drastic reduction in the number of stress fibers along with the formation of actin-rich structures within the periphery of the membrane ruffles. It should be noted here that the observed actin reorganization is analogous to the one stimulated by Ras during cell migration, wherein Ras promotes Rac-mediated lamellipodia formation with a reduction in actin stress fibers so that the cells can repetitively adhere, de-adhere, and move (23).

**Differential Effects of Hax-1 on Gα13-mediated Activation of Rho-GTPases**—Because stress fiber formation has been shown to be directly correlated with Rho activity (19, 20) and the co-expression of Gα13-QL and Hax1 showed a reduction in stress fiber formation (Fig. 3), we sought to investigate whether the co-expression of Hax-1 would suppress Gα13-QL-mediated Rac activity. Whereas the expression of Gα13-QL stimulated Rho activity confirming previous results (19, 20, 24, 25), co-expression of Hax-1 drastically attenuated the Rho activity stimulated by Gα13-QL (Fig. 4A). Although, the mechanism(s) through which Hax-1 inhibits Rho activity remains to be resolved, it is possible that Hax-1 sequesters Gα13 thereby preventing it from interacting with the Rho-guanine-nucleotide exchange factor in stimulating Rho. Based on the formation of actin-rich structures in these cells, often associated with Rac (26), it can be deduced that the co-expression of Hax-1 would potentiate Gα13-QL-mediated Rac activation. To test this postulate, we investigated whether the coexpression of Hax-1 modulated any changes in Gα13-QL-stimulated Rac activity.

In contrast to its effect on Rho activity, Hax-1 potentiated Gα13-QL-stimulated Rac activity in these cells. An analysis of Rac activity indicated that while Rac is stimulated by the expression of Gα13-QL in NIH3T3 cells (Fig. 4B), coexpression of Hax further enhanced such Gα13-QL-stimulated Rac activity. Thus, the co-expression of Hax-1 appears to differentially modulate the ability of Gα13 to stimulate Rho and Rac. Although
Role of Hax-1 in G\(_{\alpha_13}\)-mediated Cell Movement—To investigate the role of Hax-1 in G\(_{\alpha_13}\)-mediated cell migration, Hax-1 was transiently expressed in NIH3T3 cells stably expressing G\(_{\alpha_13}\)-QL. When these cells were analyzed for migration, the results indicated that Hax-1 increased the migration of G\(_{\alpha_13}\)-QL-NIH3T3 cells by 3-fold (Fig. 5, A and B). Because lysophosphatidic acid and lysophosphatidic acid-like agonists in the serum can activate wild-type G\(_{\alpha_13}\) (G\(_{\alpha_13}\)-WT) through their cognate receptors (26), we tested whether Hax-1 promotes receptor-stimulated migration of NIH3T3-G\(_{\alpha_13}\)-WT toward 5% serum. The results clearly demonstrated that Hax-1 increased the migration of these cells over the vector controls by 2.5-fold (Fig. 5, A and B). Expression of Hax-1 in migrated cells was verified by monitoring the fluorescence of the co-transfected pCEFL-GFP in NIH3T3-G\(_{\alpha_13}\)-WT and NIH3T3-G\(_{\alpha_13}\)-QL cells (Fig. 5C).

Further analysis was carried out to test the effects of inhibiting Hax-1 on G\(_{\alpha_13}\)-mediated cell migration. siRNAs specific to Hax-1 or nonspecific control siRNAs were transfected into NIH3T3-G\(_{\alpha_13}\)-WT cells and the migrations of these transfec-
tants toward 5% serum were analyzed. Silencing of the endogen-
ous Hax-1 by Hax-1-siRNA reduced the migration of NIH3T3-G\(_{\alpha_13}\)-WT as well as NIH3T3-G\(_{\alpha_13}\)-QL cells by 60% (Fig. 6B). The extent of reduction in cell migration upon silencing Hax-1 can be correlated with the siRNA-mediated reduction in the Hax-1 expression levels (Fig. 6B, lower panel). Quantification of the immunoblots showed that Hax-1 protein expression was reduced to 50–65% by 96 h after transfection. These results strongly indicate that Hax-1 plays a critical role in cell migration upon silencing Hax-1 can be correlated with the siRNA-mediated reduction in the Hax-1 expression levels (Fig. 6B, lower panel). Quantification of the immunoblots showed that Hax-1 protein expression was reduced to 50–65% by 96 h after transfection. These results strongly indicate that Hax-1 plays a critical role in cell migration.

**Fig. 6. Effect of silencing Hax-1 on G\(_{\alpha_13}\)-mediated cell migration.** A, inhibition of G\(_{\alpha_13}\)-mediated cell motility by siRNA to Hax-1. Micrographs showing the inhibition of cell migration by silencing endogenous Hax-1 in NIH3T3 cells stably expressing pcDNA3, G\(_{\alpha_13}\)-WT, and G\(_{\alpha_13}\)-QL were transfected with control nonspecific siRNA duplexes or siRNA (Control siRNA) targeted at Hax-1 (Hax-1 siRNA). The migration assay was carried out against 5% serum with appropriate controls. Migration of NIH3T3 cells expressing empty vector (NIH3T3-pcDNA3, top panel), G\(_{\alpha_13}\)-WT (NIH3T3-G\(_{\alpha_13}\)-WT, middle panel), and G\(_{\alpha_13}\)-QL (NIH3T3-G\(_{\alpha_13}\)-QL, bottom panel) in which the expression of Hax-1 was silenced (+Hax-1 siRNA) were compared with those that expressed control siRNA (+ control siRNA). B, quantification of Hax-1-siRNA-mediated inhibition. Cell migration profiles were quantified by enumerating the migrated cells in a minimum of three fields. Results are expressed as number of cells migrated per field and bars represent the mean from four independent experiments (mean ± S.D.). Silencing of endogenous Hax-1 by Hax-1-siRNA was monitored by Hax-1 immunoblot analysis (lower panel). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
The analyses of G13/QL (Hax-1 and cortactin) indicated that Hax-1 and cortactin stimulating cell migration by counteracting G13-Hax-1-mediated cell adhesion (Fig. 8). The studies presented here demonstrate for the first time a novel interaction between G13 and the cytoskeleton-associated protein Hax-1, thereby identifying a cytoskeletal signaling locus for G13 in cell movement.

Based on the results, it can be envisioned that Hax-1 plays a central role in G13-mediated cell motility by affecting three distinct, but closely related, signaling loci. First, by associating with G13, Hax-1 sequesters G13 from activating Rho and associated cell adhesion pathways. Second, through the potentiation of G13-stimulated Rac activity, Hax-1 promotes Rac-mediated translocation of cortactin to the periphery where cortactin along with actin-related proteins induces cell protrusion and migration. Finally, by tethering G13 to cortactin in a complex containing Rac, Hax-1 provides a signaling nexus that facilitates the dynamic and uninterrupted transmission of signals from G13 to cortactin through Rac to promote cell protrusion and migration.

Our present study does not address the mechanism(s) through which Hax-1 potentiates G13-mediated Rac activation. However, it is interesting to note that Hax-1 contains the characteristic PXXP motif (amino acids 198–202, PXXP motif flanked by PXXP motifs on either side), which is known to be the binding motif for the PIX family of Rac/CDC42 guanine-nucleotide exchange factors (27). Therefore, it is possible that Hax-1 potentiates Rac activation by bringing G13 closer to a specific Rac-guanine-nucleotide exchange factor. Although our initial studies did not identify such interaction between PX1-β and Hax-1,2 it is possible that a closely related guanine-nucleotide exchange factor may interact with Hax-1 through this site or other PXXP sites that are present in Hax-1. Further studies should define the mechanisms through which Hax-1 potentiates G13-stimulated activation of Rac.

Previous studies have shown that G13 is critically required for the development of mouse embryos as G13 is resorbed by day 10.5 (8). The lethality of the G13−/− genotype is ascribed to the loss of G13-mediated cell motility associated with embryonic angiogenesis. It is intriguing to note that G13 is required for the development of mouse embryos as G13 is resorbed by day 10.5 (8). The lethality of the G13−/− genotype is ascribed to the loss of G13-mediated cell motility associated with embryonic angiogenesis. It is intriguing to note that G13−/− /H9251 mutant mice die by embryonic day 10.5, and G13−/− /H9251 mutant mice exhibit severe defects in cell motility and angiogenesis.

and Rac (Fig. 7), it is reasonable to conclude that both G13 and Hax-1 are part of a signaling complex involved in cell motility. It is likely that such proximal positioning of G13, Rac, Hax-1, and cortactin stimulates cell migration by counteracting G13-Rho-mediated cell adhesion (Fig. 8). The studies presented here demonstrate for the first time a novel interaction between G13 and the cytoskeleton-associated protein Hax-1, thereby identifying a cytoskeletal signaling locus for G13 in cell movement.
although it shares 67% amino acid identity with Go_{13} (6), failed to compensate for the loss of Go_{13} in these embryos. Because both Go_{13} and Go_{13} can be stimulated by the same receptors to activate similar cellular responses, the molecular basis for the differential effect on cell motility remained elusive. In this context, the results presented here demonstrate that Hax-1 specifically interacts with Go_{13} but not Go_{12} and that such interaction is critical for Go_{13}-mediated cell motility that provides a molecular basis for such unique and differential signaling by Go_{13}. Further studies should define the role of Go_{13}-Hax-1 complexes in physiological responses that require cell movement or cytoskeletal changes mediated by Go_{13}. In light of the recent findings that Hax-1 is differentially expressed in different metastatic tumors as indicated by SAGE analysis (28) and overexpressed in many different tumor cell lines (29), it is more likely that Go_{13}-Hax-1 interaction plays a critical role in the metastatic phenotype of these tumors.

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V. Radhika, Djamila Onesime, Ji Hee Ha and N. Dhanasekaran

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