Selective Uncoupling of $\alpha_{12}$ from Rho-mediated Signaling*

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The heterotrimeric G protein $G_{12}$ has been implicated in such cellular regulatory processes as cytoskeletal rearrangement, cell-cell adhesion, and oncogenic transformation. Although the activated $\alpha$-subunit of $G_{12}$ has been shown to interact directly with a number of protein effectors, the roles of many of these protein-protein interactions in $G_{12}$-mediated cell physiology are poorly understood. To begin dissecting the specific cellular pathways engaged upon $G_{12}$ activation, we produced a series of substitution mutants in the regions of $G_{12}$ predicted to play a role in effector binding. Here we report the identification and characterization of an altered form of $G_{12}$ that is functionally uncoupled from signaling through the monomeric G protein Rho, a protein known to propagate several $G_{12}$-mediated signals. This mutant of $G_{12}$ fails to bind the Rho-specific guanine nucleotide exchange factors p115RhoGEF and LARG (leukemia-associated RhoGEF), fails to stimulate Rho-dependent transcriptional activation, and fails to trigger activation of RhoA and the Rho-mediated cellular responses of cell rounding and c-jun N-terminal kinase activation. Importantly, this mutant of $G_{12}$ retains coupling to the effector protein E-cadherin, as evidenced by its ability both to bind E-cadherin in vitro and to disrupt E-cadherin-mediated cell-cell adhesion. Furthermore, this mutant retains the ability to trigger $\beta$-catenin release from the cytoplasmic domain of cadherin. This identification of a variant of $G_{12}$ that is selectively uncoupled from one signaling pathway while retaining signaling capacity through a separate pathway will facilitate investigations into the mechanisms through which $G_{12}$ proteins mediate diverse biological responses.

Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins)* mediate cellular signaling from heptahelial cell surface receptors to a wide variety of downstream effector proteins that in turn propagate signals to elicit certain cellular responses and changes. The $\alpha$-subunits of the $G_{12}$ subfamily of G proteins, $G_{12a}$ and $G_{12b}$, have been linked to cellular events such as cytoskeletal rearrangements (1, 2), cell proliferation (3), Na+/H+ exchange (4, 5), activation of phospholipase C (6), activation of phospholipase D (7), down-regulation of cell-cell adhesion (8), activation of radixin (9), and modulation of tight junction-mediated paracellular permeability (10). Furthermore, mutationally activated $G_{12a}$ and $G_{12b}$ have been demonstrated to elicit oncogenic transformation of several cell lines (11, 12). Although a number of proteins involved in these processes have been shown to bind directly to activated (GTP-ligated) $G_{12}$ and/or $G_{13}$ (13), the roles of these effector proteins in mediating particular $G_{12}$-dependent cellular events are still largely undefined.

Two subsets of G$12$ effectors that are the best characterized are a class of Rho-specific guanine nucleotide exchange factors that includes p115RhoGEF, leukemia-associated RhoGEF (LARG), and PDZ-RhoGEF, as well as members of the cadherin superfamily of cell surface adhesion proteins (14–19). Cell-free studies using purified proteins have demonstrated that $G_{13}$ directly stimulates the ability of p115RhoGEF to enhance guanine nucleotide exchange on the monomeric G protein RhoA and, reciprocally, that p115RhoGEF binding accelerates the rate of GTP hydrolysis by $G_{12a}$ and $G_{12b}$ (16, 18). Also, activated $G_{12a}$ and $G_{13}$ have been demonstrated to bind the cytoplasmic domain of several cadherins in vitro, and expression of mutationaly activated $G_{12a}$ and $G_{12b}$ in cells disrupts the extracellular adhesive function of epithelial cadherin (E-cadherin) in a manner that requires direct $G_{12}/\beta$-catenin interaction (8, 19). Furthermore, binding of activated $G_{12}$ to cadherin results in release of the cytoplasmic protein $\beta$-catenin from cadherin, allowing $\beta$-catenin to act as a transcriptional activator of genes involved in cell proliferation, differentiation, and oncogenesis (17, 19).

As the number of known $G_{12}$ binding partners has increased, so has the apparent complexity of the signaling networks that originate with the receptor-driven activation of $G_{12}$ proteins. To elucidate the biological significance of the interactions between $G_{12}$ proteins and their various effectors, reagents that selectively manipulate the interaction between $G_{12a/b}$ and individual target proteins would be of great value, as these tools could reveal the role of particular $G_{12}$-effector interactions in specific signaling events. To this end, we have introduced a series of mutations into the primary sequence of $G_{12a}$ and from these have identified a mutant that is impaired both in binding

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1 The abbreviations used are: G protein, guanine nucleotide-binding protein; $G_{\alpha}$, $\alpha$-subunit of the heterotrimeric G protein; QL, mutationally activated Gln-to-Leu variant of $G_{\alpha}$ protein; E-cadherin or E-cad, epithelial cadherin; p115RhoGEF and harboring its RGS domain; LARG, leukemia-associated RhoGEF; $\Delta p115-G_{12a}^{\Delta K}$, variant of $G_{12a}^{\Delta K}$ that lacks the ability to interact with p115RGS; GFP, green fluorescent protein; PBS, phosphate-buffered saline; GST, glutathione S-transferase; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; TLCK, N'-p-tosyl-L-lysine chloromethyl ketone; JNK, c-jun N-terminal kinase; PIPES, 1,4-piperazinediethanesulfonic acid; SRF, serum response factor.
Rho-specific guanine nucleotide exchange factors and in activating Rho-mediated signaling pathways. This mutant retains normal binding to E-cadherin as well as the ability to disrupt cadherin function when expressed in cells. This variant of Go12 provides a novel reagent for dissecting the roles of distinct downstream effector pathways that are triggered following Go12 activation and also provides important new structure-function information regarding the nature of the interaction between G12 proteins and Rho-specific guanine nucleotide exchange factors.

**EXPERIMENTAL PROCEDURES**

**Materials**—The Myc-p115RGS plasmid and the plasmid containing a GST fusion to the Rhoetkin RhoA-binding domain (GST-RBD) was kindly provided by Robert Lefkowitz (Duke University, Durham, NC). The reporter plasmid SRE-L was a gift from Channing Der (University of North Carolina, Chapel Hill), and the internal control reporter plasmid pRL-TK and dual-luciferase system were purchased from Promega. Anti-Go12 and anti-RhoA antibodies were purchased from Santa Cruz Biotechnology. Mouse monoclonal antibody specific for phospho-SAPK (stress-activated protein kinase)/JNK (Thr183/Thr185) was purchased from Cell Signaling Technology (Beverly, MA). Protease inhibitors were purchased from Sigma.

**Construction of Plasmids**—The Myc epitope tag (EQKLISEEDL) was introduced into mutational activated Go12 (Go12mt) by first creating a silent AgeI restriction site within the sequence encoding the amino acid sequence 1–252 of the Myc-p115RGS plasmid with restriction sites incorporated into the pcDNA3.1 plasmid, or various NAAIRS mutants of Myc-tagged Go12mt using Lipofectamine reagent (Invitrogen) according to the manufacturer’s instructions. Approximately 48 h post-transfection, cells were washed twice with phosphate-buffered saline (PBS) and harvested by scraping in PBS, pelleted by centrifugation at 800 g for 5 min, and then resuspended in lysis buffer (containing 0.1% polyoxyethylene-10-lauryl ether). Material was resuspended and subjected to SDS-PAGE and immunoblot analysis as described (17) in order to detect Go12mt or its NAAIRS variants.

For binding assays utilizing untagged or “S-labeled variants of Go12mt, the proteins were produced using a TnT in vitro coupled transcription/translation system (Promega) according to the manufacturer’s instructions. Reactions were diluted into reduced detergent lysis buffer (see above) and incubated with GST fusion proteins as described above, and then proteins were separated by SDS-PAGE and gels fixed in 10% acetic acid, 1% glycerol, dried under vacuum, and analyzed by autoradiography.

**Luciferase Reporter Assays**—HEK293 cells were transfected with the SRE-L plasmid (containing the cDNA for firefly luciferase positioned downstream of the RGS domain of LARG) and the plasmid containing the cDNA for Renilla luciferase positioned downstream of a thymidine kinase promoter) plus a plasmid encoding Go12mt or a mutant variant. Approximately 36 h post-transfection, cells were washed with serum-free Dulbecco’s modified Eagle’s medium and then incubated in the same medium for an additional 16 h. Cells were washed with PBS and then incubated in Passive lysis buffer (Promega) for 20 min. Lysates were cleared by centrifugation and then assayed by luminescence for firefly and Renilla luciferase activities using a dual-luciferase assay system (Promega). Firefly luciferase activity measurements were normalized for the corresponding Renilla luciferase values. Measurements were performed using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA).

**Rho Activation Assays**—MDA-MB-231 cells were seeded at a density of 200,000 cells/dish on 35-mm glass-bottom Petri dishes (MatTek, Ashland, MA) and allowed to grow for 24 h. Cells were infected with adenovirus harboring a cDNA encoding GFP plus either Go12mt or a variant of Go12mt, or no cDNA. Infections were allowed to proceed for 4 h, and then cells were serum-starved for 15–16 h. Cell rounding phenotype was visualized using an Eclipse TE300 inverted microscope (Nikon).

**Rho Activation Assays**—MDA-MB-231 cells were seeded at a density of ~250,000 cells/well in 6-well plates and allowed to grow for ~24 h. Cells were infected with adenovirus harboring a cDNA encoding GFP plus either Go12mt, a variant thereof, or no cDNA. Infected cells were incubated for 4 h and then serum-starved for an additional 17–20 h. Rho activation assays were performed as described previously (21). Briefly, cells were washed quickly with PBS while on ice, and cold lysis buffer (25 mM HEPES, pH 8.0, 150 mM NaCl, 10 mM MgCl2, 1 mM EDTA, 1% Nonidet P-40, 2% glycerol, 10 µg/ml leupeptin, 10 µg/ml aprotinin) was added to the wells. Cells were lysed for 10 min and then centrifuged at 16,000 × g for 5 min at 4°C. Supernatants were assayed for protein concentration. An aliquot was saved from each lysate to determine total endogenous RhoA levels, and equal amounts of protein incubated with the GST fusion of the RhoA-binding domain of rhokinetin immobilized on glutathione-Sepharose. Samples were mixed for 1 h at 4°C, and then the glutathione-Sepharose was pelleted by centrifugation at 700 × g and washed three times with cold lysis buffer. Pelleted material was resuspended in SDS-PAGE sample buffer, separated by gel electrophoresis, and subjected to immunoblot analysis to detect RhoA.
Mutational Uncoupling of Gα12 from Rho

**c-jun N-terminal Kinase Activation Assays—**MB-MB-231 cells were seeded at ~250,000 cells/well in 6-well plates and allowed to grow for 24 h. Cells were infected with adenovirus harboring a cDNA encoding GFP plus either Gα12QL, a variant thereof, or no cDNA. Infections were allowed to proceed for 4 h, and then cells were serum-starved for 24 h. Cells were then washed quickly twice with cold PBS while on ice, and cold c-jun N-terminal kinase (JNK) assay buffer (50 mM Tris, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 50 mM NaF, 150 mM NaCl, 2 mM DTT, 0.2 mM sodium vanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 3 μg/ml leupeptin, 4 μg/ml aprotinin, 30 μM TPCK, 29 μM TLCK, 133 mM phenylmethylsulfonyl fluoride) was added to the wells. Cells were lysed for 10 min and then centrifuged at 16,000 × g for 5 min at 4 °C. Supernatants were assayed for protein concentration, and then equal amounts of total protein from lysates were separated by SDS-PAGE and subjected to immunoblot analysis to detect levels of phospho-JNK.

**Cell Aggregation Assays—**Parental MDA-MB-435 cells and those stably expressing E-cadherin were infected with recombinant adenoviruses harboring Gα12QL, a variant of Gα12, or a control adenovirus. Three days post-infection, cells were subjected to fast-aggregation assays as described previously (8).

**Indirect Immunofluorescence—**DLD-1 and HEK293 cells grown on glass coverslips were washed twice in PHEM buffer (60 mM PIPES, pH 6.9, 25 mM HEPES pH 7.0, 10 mM EGTA, 4 mM MgSO4) and then incubated for 37 °C for 20 min in the same buffer containing 4% paraformaldehyde. Cells were then permeabilized by a 5-min incubation in PHEM buffer containing 0.5% Triton X-100 followed by three 5-min washes in PHEM containing 0.1% Triton X-100. A blocking solution of PHEM buffer containing 10% goat serum (InVitrogen) was added, cells were incubated for 30 min at 37 °C, and then primary antibody to β-catenin was applied at a 1:100 dilution in PHEM buffer plus 0.5% goat serum. Following an overnight incubation at 4 °C, cells were washed three times in PHEM plus 0.1% Triton X-100. A Cy3-conjugated secondary antibody was applied at a 1:500 dilution in PHEM buffer plus 5% goat serum. Following an overnight incubation at 4 °C, cells were washed three times in PHEM containing 0.1% Triton X-100 and then primary antibody to α-catenin was added, cells were incubated for 30 min at 37 °C, and then Cy3-conjugated secondary antibody was applied at a 1:500 dilution in PHEM buffer plus 0.5% goat serum for 1 h. Cells were then washed three times as described above and incubated for 5 min in Hoechst stain (Molecular Probes, Eugene, OR) at a 1:1000 dilution in PHEM buffer, washed with distilled water, and mounted onto slides using ProLong antifade solution (Molecular Probes). Cells were visualized using an LSM-410 laser scanning confocal microscope (Carl Zeiss).

**RESULTS**

To identify structural determinants of Gα12 necessary for its coupling to downstream effector proteins, we designed a series of substitution mutants within the mutationally activated (QL) form of Gα12. To allow for proper post-translational modification (e.g. acylation) of these variants of Gα12QL, the proteins were expressed in HEK293 cells. To distinguish the ectopically expressed Gα12QL variants from endogenous, wild-type Gα12, a Myc epitope tag was inserted into Gα12QL at the αBαC loop within the helical domain of the protein. Structural analyses of other Gα subunit proteins have revealed that this highly conserved region is spatially removed from the GTP-binding and effector-binding domains of the Switch I region to just downstream of the Switch III region (20). Myc-tagged Gα12QL expressed in HEK293 cells exhibited binding to the Gα12 effector E-cadherin and p115RhoGEF (Fig. 1A), which was essentially identical to that of untagged Gα12QL (data not shown).

We first attempted to uncouple Gα12 from the Gα12-specific RGS protein p115RhoGEF by converting a highly conserved glycine within the “Switch I” region of Gα12 to a serine, because other Gα subunits in mammalian cells and yeast have been uncoupled from RGS proteins by this alteration (22, 23). We generated this Gly-to-Ser mutation within Myc-tagged Gα12QL. This variant, denoted as G208S-Gα12QL, was produced in HEK293 cells and extracted from membrane preparations of the cells, and then binding to GST fusions of either the cytoplasmic C-terminal domain of E-cadherin or the N-terminal RGS domain of p115RhoGEF was assessed. As shown in Fig. 1A, Myc-tagged Gα12QL bound strongly to both of these effector proteins, and the apparent affinity of G208S-Gα12QL for both effector proteins was not significantly changed.

Because the single point mutant of Gα12 did not yield the type of altered function we sought, we embarked on a more global strategy to identify mutant forms of Gα12 impaired in effector binding. To this end, we produced a series of substitution mutants within the region of Gα12 that encompasses all three of the “Switch regions” that are known to play a critical role in other Gα-effector interactions (24, 25). To produce each mutant, a sextet of consecutive amino acids in the primary sequence of Myc-tagged Gα12QL was replaced by the sequence Asn-Ala-Ala-Ile-Arg-Ser (NAAIERS), which is believed to be a well tolerated substitution in proteins because of its appearance in both β-sheet and α-helical secondary structures (26). This NAAIERS mutagenesis strategy has been successfully employed to dissect functional domains in the retinoblastoma protein (27) and in telomerase (28).

The panel of Gα12 NAAIERS mutants was expressed in HEK293 cells and then extracted from membranes. The amount of plasmid DNA used for transfecting cells was varied to achieve similar levels of ectopic Gα12 expression, as determined by immunoblot analysis (data not shown). These Gα12 variants were screened for binding to p115RhoGEF and E-cadherin as described above. In all, 13 variants were analyzed that covered the primary sequence of Gα12 from just upstream of the Switch I region to just downstream of the Switch III region (see Fig. 1B). None of these proteins bound to immobilized GST lacking a protein adduct (Fig. 1A, and data not shown). The majority of these variants exhibited binding to the GST-p115RGS and GST-E-cadherin proteins that was not markedly different from that of parental Gα12QL (for an example, see results for the Δ238–243 variant in Fig. 1A; others are not shown). A few additional variants showed a marked decrease in binding to both effector proteins; an example of this is
the Δ196–201 variant shown in Fig. 1A. This may reflect a nonspecific, global effect of the mutation on the structure of Ga12, and therefore these variants were not pursued further. However, one NAAIRS variant, designated Δ244–249 in Fig. 1A, showed a nearly complete loss of binding to p115RhoGEF while retaining normal binding to E-cadherin. This variant also bound to GST fusions of the cytoplasmic domains of neural cadherin and cadherin-14 (data not shown). The region of Ga12 altered in the Δ244–249 variant lies immediately downstream of the Switch II region of Ga12 (Fig. 1B). To ensure that the Myc epitope tag did not influence binding of Ga12QL or its variants to these effector proteins, several of the Ga proteins, including Ga12QL, G208S-Ga12QL, and the Δ244–249 variant, were produced without the Myc tag in a cell-free transcription/translation system (see “Experimental Procedures”). Pull-down experiments testing these proteins for binding to GST-E-cadherin and GST-p115RGS yielded essentially the same results observed in Fig. 1A (data not shown). Based on these initial findings, we designated this variant of Ga12QL as Δp115-Ga12QL to denote loss of its interaction with p115RhoGEF; this designation is used for the remainder of this report.

The impaired binding of the Δp115-Ga12QL variant to p115RhoGEF suggested that this protein would be functionally uncoupled from Rho signaling. To test this hypothesis, we first examined the ability of Δp115-Ga12QL to activate serum response factor (SRF)-mediated transcription in cultured cells. Ga12 signaling to SRF has been well characterized as a Rho-dependent pathway because of its sensitivity to specific inhibitors of Rho (29). HEK293 cells were transfected with a reporter plasmid harboring a luciferase cDNA downstream of an SRF-responsive element. Co-transfection with a plasmid encoding Ga12QL elicited an approximately 6-fold increase in SRF activity (Fig. 2A). This effect was blunted by the co-expression of p115RGS, which contains the G protein-interacting domain of p115RhoGEF and acts as a dominant negative by sequestering activated Ga12 (18, 30). In contrast to the results obtained with Ga12QL, expression of Δp115-Ga12QL failed to stimulate SRF-mediated transcriptional activation (Fig. 2A). Immunoblot analysis of cell lysates verified that Δp115-Ga12QL was expressed at levels comparable with Ga12QL in these experiments (Fig. 2B).

The Ga12 subfamily α-subunit Ga12 stimulates the ability of p115RhoGEF to trigger guanine nucleotide exchange on the small monomeric G protein Rho. Although Ga12 interacts strongly with the RGS domain of p115RhoGEF in vitro (16) (also see Fig. 1A), there are little data available on the ability of Ga12 to stimulate Rho activation specifically through p115RhoGEF. However, evidence has emerged that a closely related Rho-specific guanine nucleotide exchange factor, LARG, is functionally activated by Ga12 in cells (31). Therefore, we next tested Ga12QL and the Δp115-Ga12QL variant for interaction with LARG. As shown in Fig. 2C, Ga12QL was clearly bound by an immobilized GST fusion of the RGS domain of LARG, but Δp115-Ga12QL exhibited essentially no binding to this LARG fusion protein, even though analysis of cell extracts confirmed similar expression levels of these Ga proteins (Fig. 2C). Hence, the Δp115-Ga12QL variant is compromised in binding to both p115RhoGEF and the closely related LARG.

Two additional, well documented readouts of Ga12QL-dependent cellular signaling mediated through RhoA are cell rounding (8, 32, 33) and the activation of JNK (34–36). Therefore, as additional tests of whether the Δp115 variant is uncoupled from Rho-mediated signaling pathways, we examined the abilities of Ga12QL and Δp115-Ga12QL to stimulate these responses when ectopically expressed in cells. As shown in Fig. 3, A and B, essentially all MDA-MB-231 cells infected with a recombinant adenovirus encoding Ga12QL exhibited a marked change from a flattened, splayed morphology to a distinctly rounded morphology, whereas a control adenovirus caused no such effect. However, cells infected with an adenovirus encoding the Δp115-Ga12QL variant retained the normal, flattened appearance that most closely resembled the control cells (Fig. 3C), even though cells expressed similar levels of the variant as of Ga12QL (Fig. 3D).
Fig. 3. Ability of Go12QL and the Δp115-Go12QL variant to trigger cell rounding and stimulate RhoA activation and JNK activation. A–C, MDA-MB-231 cells were infected with a control adenovirus (A), an adenovirus harboring Go12QL (B), or Δp115-Go12QL (C), incubated for 4 h, serum-starved overnight, and then analyzed by bright field and fluorescence microscopy for cell rounding and successful adenoviral infection. D, cells were lysed after imaging, and lysates were subjected to immunoblot analysis using anti-Go12 antibody to assess expression levels of Go12QL and Δp115-Go12QL. E, MDA-MB-231 cells infected with the recombinant adenoviruses described above were incubated post-infection for 4 h and then serum-starved for 24 h before being lysed (see “Experimental Procedures”). Immunoblot analyses of cell lysates for levels of phosphorylated JNK (P-JNK) and expression of Go12 proteins are shown. F, MDA-MB-231 cells infected with the recombinant adenoviruses described above were incubated post-infection for 4 h and then serum-starved for 17 h before being lysed. Lysates were subjected to pull-down assays using a GST fusion of the activated RhoA-binding domain of rhotekin, and levels of precipitated RhoA were determined by immunoblot analysis using anti-RhoA antibody. Total levels of RhoA and Go12 in the lysates prior to pull-down experiments are also shown. All panels are representative of three or more separate experiments.

To assay for JNK activation, MDA-MB-231 cells were infected with the recombinant adenoviruses described above, and cells were lysed and then subjected to immunoblot analysis using an antibody that specifically recognizes the activated, phosphorylated form of JNK. Although Go12QL significantly increased the level of activated JNK in these cells, expression of the Δp115-Go12QL variant did not lead to an increase in the phospho-JNK epitope above basal levels (Fig. 3E). Finally, we also directly examined the ability of Δp115-Go12QL to activate RhoA in MDA-MB-231 cells through pull-down assays using a GST fusion of the RhoA-binding domain of rhotekin. Although expression of Go12QL led to an increase in the level of activated RhoA as expected, the level of activated RhoA in cells expressing Δp115-Go12QL was similar to that seen in control cells (Fig. 3F).

The finding that the Δp115-Go12QL variant retained normal binding to the cytoplasmic domain of E-cadherin (see Fig. 1) suggested that even though signaling to Rho by this Go12QL mutant was abrogated, its ability to regulate cadherin function would be preserved. To test this hypothesis in a cellular context, we performed cell-cell adhesion assays using breast cancer cells stably expressing E-cadherin. These cells have been shown to form large aggregates, in a Ca2+-dependent manner, that can be reversed upon introduction of activated Go12QL via a process that involves the ability of the activated Go protein to trigger release of β-catenin from the cytoplasmic domain of E-cadherin (8). Consistent with previous results, breast cancer cells expressing E-cadherin (termed 435-E-cad cells) formed large, tightly clumped aggregates in a so-called “fast-aggregation” assay performed in the presence of Ca2+ (Fig. 4A). Formation of these large aggregates was not observed in cells lacking E-cadherin (termed 435-puro cells, Fig. 4C). Although infection of 435-E-cad cells with a control adenovirus prior to the assay did not affect aggregate formation (Fig. 4A), infection with an adenovirus expressing Go12QL caused a dramatic reduction in aggregate size (Fig. 4D), as reported previously (8). Importantly, very similar results were obtained when the Δp115-Go12QL variant was expressed in these cells, i.e. this protein caused disruption of aggregate formation to essentially the same degree as Go12QL (Fig. 4G) indicating that the Δp115-Go12QL variant retained the ability to modulate cadherin function in an intact cell. In all cases, inclusion of the Ca2+ chelator EGTA in the assay disrupted aggregate formation (Fig. 4, B, E, and H) confirming that the cell-cell interactions observed were mediated by cadherins.

As noted in the Introduction, activated Go12 disrupts the interaction between cadherin and its associated cytoplasmic
protein β-catenin in a manner that requires direct Go12-cadherin interaction (17, 19). This effect of Go12QL binding to cadherins can be observed directly in cells by following the shift in subcellular localization of β-catenin from a peripheral, plasma membrane-associated pattern to a more diffuse staining throughout the cell when activated Go12 is introduced (19). In DLD-1 cells (Fig. 5A) and HEK293 cells (data not shown) infected with a control adenovirus, β-catenin was observed to localize most prominently at the cell periphery, whereas in cells expressing Go12QL this perimeter staining was much more prominent relative to the staining in the cytoplasm and nucleus (Fig. 5B). In cells expressing Δp115-Go12QL, the staining pattern of β-catenin most closely resembled that of cells expressing Go12QL (Fig. 5C), providing additional evidence that the Δp115-Go12QL variant retains the ability to interact with the cadherin cytoplasmic domain in cells and thereby trigger a redistribution of β-catenin.

**DISCUSSION**

The purpose of this study was to identify determinants within the heterotrimeric G protein Go12 that are necessary for its interaction with certain downstream effector proteins. To this end, a series of variants of constitutively active Go12 (Go12QL) were constructed by replacing sextets of consecutive amino acids with the well tolerated amino acid motif NAAIRS. This approach has proven successful in identifying domains of intracellular signaling proteins. A full understanding of the signaling networks emanating from the activation of Go12 will require reagents that allow selective uncoupling of this G protein from particular effector proteins. Hence, mutants of Go12 that have lost the ability to interact with one or more effectors while retaining normal affinity for other effectors will provide valuable tools for such studies. The work reported here represents a significant advance toward this goal, in the form of a mutant of Go12 that is uncoupled from a major, well characterized signaling arm, namely Rho-mediated pathways, while retaining the ability to signal through the cadherin/β-catenin pathway.

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Mutational Uncoupling of Go12 from Rho

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