Characterization of the Association of the Actin-binding Protein, IQGAP, and Activated Cdc42 with Golgi Membranes*

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IQGAP is a recently identified actin-binding protein, which is a putative target for the Cdc42 and Rac GTP-binding proteins. Cdc42 was localized to the Golgi (Erickson, J. W., Zhang, C., Kahn, R. A., Evans, T., and Cerione, R. A. (1996) J. Biol. Chem. 271, 26850–26854), and here we show by immunofluorescence that IQGAP has a perinuclear localization, that it can be co-immunoprecipitated with Cdc42 from Golgi-enriched fractions, and that purified Golgi membranes are recognized by specific antibodies raised against IQGAP and Cdc42 in negative-stain immunogold electron microscopy experiments. Addition of activated, recombinant Cdc42 or solubilization of endogenous Cdc42 from Golgi membranes by the Rho-GDP dissociation inhibitor protein fails to solubilize IQGAP, suggesting that it associates with these membranes in a Cdc42-independent manner. Detergent solubilization of Golgi membranes leaves IQGAP and actin in an insoluble pellet but releases Cdc42 to the supernatant, whereas treatments that release actin from this detergent-insoluble pellet also release IQGAP. Addition of the COOH-terminal half of the IQGAP protein, which contains the Cdc42-binding domain, removes Cdc42 from Golgi membranes in a dose-dependent manner. These data suggest that IQGAP and Cdc42 are part of a cytoskeletal complex in Golgi membranes that may mediate Cdc42-regulated effects on the actin cytoskeleton in these membranes.

Subcellular localization of signaling proteins is an important aspect of their function. Cdc42, a member of the Ras superfamily of low molecular weight GTP-binding proteins, has been shown to have a cytosolic form bound to the Rho GDP-dissociation inhibitor (RhoGDI)¹ (1), a Golgi membrane-bound form (2), and a plasma membrane-bound form (3, 4). These different cellular locations are likely to account for the involvement of Cdc42 in a variety of processes in mammalian cells including the formation of actin-containing filopodia, cell-cycle progression, and stress-activated pathways which impact on gene transcription (5–11). Identification of a number of proteins that interact specifically with the GTP-bound state of Cdc42 has provided evidence that the activation of Cdc42 and its ability to subsequently bind multiple cellular targets may be responsible for its pleiotropic effects on cells. Association of Cdc42-GTP with the p21-activated kinases is most likely responsible for Cdc42-mediated effects on stress-activated pathways (5, 6, 8). It is not yet clear how the association of Cdc42 with putative target/effectors such as WASP (12, 13), phospholipase D (14), phosphatidylinositol 3-kinase (PI 3-K) (15), ACK (16, 17), p70 S6 kinase (18), and IQGAP (19–21) are related to its various functions. However, by learning more about the subcellular localization of these different targets, we hope to identify the specific cellular effects that each target mediates.

IQGAP proteins have been identified by our laboratory (21) and others (19, 20, 22) as potential targets for Cdc42. There are two isoforms that have been identified, IQGAP1 and IQGAP2, which share 62% sequence identity with each other and have the same domain structures. IQGAP2 is an 180-kDa, liver-specific protein (22), whereas the 189-kDa IQGAP1 has a wider distribution and is enriched in placenta, lung and kidney (23). Both of these proteins bind to Cdc42 in its GTP-bound state and inhibit its rate of GTP hydrolysis (19, 21, 23). IQGAPs interact with Cdc42 through their COOH-terminal domains, which contain a region homologous to the RasGAP protein (19, 22); however, IQGAPs do not bind Ras. The IQGAPs also contain domains homologous to the actin-binding protein, calponin, as well as IQ domains similar to those found in myosin-like proteins that bind calmodulin, suggesting that they may mediate the effects of Cdc42 on the actin cytoskeleton and may be responsive to changes in intracellular calcium (19, 20, 24). In addition, the IQGAPs contain a WW domain and an NH₂-terminal repeat region, which is predicted to form a coiled-coil, possibly involved in IQGAP dimerization (25).

The homology shared between IQGAPs and other actin-binding proteins would suggest a role for the IQGAPs in Cdc42-mediated cytoskeletal changes. Recently, Bashour et al. (25) have shown that IQGAP binds to F-actin and that this interaction can be attenuated by calmodulin binding to IQGAP. Calmodulin has also been shown to abolish the association of Cdc42-GTP with IQGAP in a calcium-dependent manner (24). Moreover, we have found that GTP-bound Cdc42 can form a ternary complex with IQGAP and F-actin (26). These findings argue that IQGAP may serve as an important interface between Cdc42 and F-actin.

Given that a pool of Cdc42 exists in Golgi membranes, we set out to identify Golgi-associated proteins that served as binding partners for Cdc42. Here, we show that an approximately 180-kDa rabbit liver Golgi membrane protein that specifically interacts with GTP-S-bound Cdc42 is IQGAP2. We describe the characteristics of the interactions of both IQGAP isoforms and Cdc42 with Golgi membranes and propose some possible roles for these interactions in Cdc42 function.
IQGAP Associates with Cdc42 in Golgi Membranes

EXPERIMENTAL PROCEDURES

Materials—The chemicals used in this study were obtained from Fisher. Nucleotides, glutathione-agarose, protein A-Sepharose, protease inhibitors, detergents, and antibodies to actin were obtained from Sigma. Rabbit anti-IQGAP antibodies were a generous gift from Dr. William Brown, Cornell University. Western blots were visualized using the enhanced chemiluminescence (ECL) system from Amersham International (Little Chalfont, Buckinghamshire, United Kingdom).

Preparation of Rabbit Liver Golgi Membranes and CHO Cell Golgi Membranes—Rabbit liver Golgi membranes were prepared as described (2). Briefly, fresh livers were collected in ice-cold 100 mM Tris, pH 7.4, 250 mM sucrose, plus protease inhibitors and homogenized in a Teflon glass homogenizer (Type C; Thomas Scientific, Swedesboro, NJ). The post-nuclear supernatant was centrifuged at 100,000 g for 90 min to yield a microsomal pellet. This microsomal pellet was loaded onto the bottom of a discontinuous sucrose density gradient; after centrifugation, Golgi membrane fractions were collected from the 0.86/1.55 m sucrose layer. Membranes were washed once in 250 mM sucrose, 100 mM Tris, pH 7.4, and resuspended, and then resuspended in the same buffer, pooled, aliquoted, and stored at −80 °C until use.

For preparation of Golgi membranes from CHO cells, 20 × 150-mm plates of cells were collected by trypsinization and suspended in homogenization buffer containing 250 mM sucrose, 10 mM Tris, pH 7.4, 1 mM DTT, 10 μg/ml aprotinin, and 200 μM phenylmethylsulfonyl fluoride. Cells were swelled in this buffer for 20 min on ice and centrifuged at 30,000 g for 10 min. Cells were decanted and resuspended in 4 ml of homogenization buffer before being homogenized (i.e. 25 strokes with a stainless steel Dounce homogenizer (Thomas Scientific)). The cell homogenate was recentrifuged as above, and the resultant post-nuclear supernatant was diluted to about 10 ml with homogenization buffer and centrifuged 90 min at 45,000 rpm in a T70 (Beckman) rotor (149,000 g). The microsomal pellet was further fractionated by loading it onto the bottom of a discontinuous sucrose density gradient and centrifuging for 3 h at 25,000 rpm (58,450 × g) in an SW 50.1 (Beckman) swinging bucket rotor. Gradient steps were 0.25, 0.86, and 1.15 m sucrose. The pellet was loaded in 1.22 m sucrose. Golgi membranes were collected from the 0.86/1.15 m sucrose layer, pooled, diluted with 250 mM sucrose and 100 mM Tris, and re-centrifuged at 40,000 rpm for 1 h in a TI50 rotor. The pellets were resuspended to 1 mg/ml total protein in 250 mM sucrose, 100 mM Tris, pH 7.4, and aliquots were stored at −80 °C until use.

Protein concentrations were determined by the method of Bradford (27) by using bovine serum albumin in 250 mM sucrose, 100 mM Tris, pH 7.4, as a standard. In some cases, such as with proteins on beads, protein concentrations were estimated from Coomassie Blue-stained gels using bovine serum albumin as a standard.

Cell Cultures—CHO cells stably transfected with triple hemagglutinin-tagged (HA) wild-type Cdc42Hs were maintained in Dulbecco’s minimal essential medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and anti-mycotic/antibiotic solution. Immunofluorescence experiments were carried out as described previously (2) using a 1:50 dilution of the rabbit anti-IQGAP1 affinity-purified antibody that was raised against the amino-terminal half of insect cell-expressed IQGAP1 (4, 19). This antibody only detects IQGAP1 and IQGAP2 in Western blots of Golgi membrane fractions from rabbit liver or CHO cell lysates.

GST-Protein Affinity Precipitations and Immunoprecipitations—GST fusion proteins were expressed in Escherichia coli purified on glutathione-agarose beads as described previously (28). For GST-protein affinity precipitation experiments, 10% of insect cell-expressed IQGAP1 (4, 19). This antibody only detects IQGAP1 and IQGAP2 in Western blots of Golgi membrane fractions from rabbit liver or CHO cell lysates.

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Detergent Insolubility and Actin Depolymerizing Conditions—10–25 μg of washed CHO cell Golgi membranes were incubated with either 5.25 μM GST-RhoGDI or with an equal volume of GST elution buffer (10 mM glutathione, 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 5 mM MgCl2, 25% glycerol). In experiments using the carboxyl-terminal half of IQGAP1 (which contains the RasGAP-related domain, designated GRD), the GRD was added in quantities shown in the figures, or Glu-Glu elution buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 200 μM phenylmethylsulfonyl fluoride) was added as a control. These additions were made to a reaction mix containing 50 mM Tris, 6 mM MgCl2, 100 mM NaCl, and 125 mM sucrose in a total volume of 60 μl. Samples were incubated 30 min at room temperature, and then membranes were pelleted by Airfuge centrifugation and total supernatants and pellets analyzed by Western blotting for IQGAP1 and (HA)-tagged Cdc42. For experiments with different guanine nucleotides, membranes were first incubated for 15 min in a sucrose/Tris buffer containing 1 mM EDTA to deplete nucleotides from the GTP-binding proteins and then the reaction mix, containing 1 mM GTPγS, 1 mM GDP, or 40 mM EDTA, or 0.6 M KI as indicated. Actin depolymerizing conditions were adapted from Cano et al. and Robertson et al. (31, 32). After incubation, membranes were centrifuged for 30 min
GST-Cdc42 was loaded with either GTP using GST-Cdc42 fusion proteins as affinity reagents. The Golgi membranes which interacted specifically with Cdc42 by Cdc42 is localized to the Golgi apparatus (2). Thus, we set out a large pool of the low molecular weight GTP-binding protein A, rabbit liver fractions.

itated with the beads were separated by 8% SDS-PAGE and visualized with GTP, GTP S, GDP or nucleotide depleted with EDTA, or incubated with GST on glutathione-agarose beads, alone, as a control. Proteins precipitated with the beads were separated by 8% SDS-PAGE and visualized by silver staining. B, rabbit liver Golgi membranes were solubilized as in A and incubated with GST, GST-Cdc42, GST-Rac1, GST-RhoA, or GST-Ha-Ras in the nucleotide states indicated and precipitating proteins were analyzed as in A. C, GST-Cdc42-coated beads were loaded with GTP, GTP S, or GDP as indicated, and then incubated with Golgi membranes as above and samples were analyzed for associating proteins by silver stain.

in an Eppendorf centrifuge at 14,000 rpm, and total supernatants and pellets were analyzed by 5–15% SDS-PAGE followed by Western blotting.

RESULTS

Identification of an 180-kDa Rabbit Liver Golgi Protein That Binds to Cdc42-GTP\textsubscript{S} as IQGAP2—We have reported that a large pool of the low molecular weight GTP-binding protein Cdc42 is localized to the Golgi apparatus (2). Thus, we set out to identify proteins from Triton X-100 solubilized rabbit liver Golgi membranes which interacted specifically with Cdc42 by using GST-Cdc42 fusion proteins as affinity reagents. The GST-Cdc42 was loaded with either GTP\textsubscript{S} or GDP, or was nucleotide-depleted by addition of EDTA, in order to probe for proteins which bind to a specific nucleotide state of Cdc42. GST alone on beads was used as a control for nonspecific binding. Fig. 1A shows that an approximately 180-kDa protein co-precipitated with Cdc42 in its GTP\textsubscript{S}-bound state but not with Cdc42-GDP, nucleotide-depleted Cdc42 (EDTA-treated), or with GST alone.

Our previous work had identified similarly sized proteins in the cytosol as IQGAP1 and IQGAP2 (21), and thus we thought it likely that the 180-kDa Golgi membrane protein was also an IQGAP. In support of this idea, the 180-kDa Golgi protein (designated Golgi p180) showed many of the same characteristics as the cytosolic IQGAPs. First, like the cytosolic forms of IQGAP, Golgi p180 also bound to Rac1 but not to RhoA nor Ha-Ras (Fig. 1B). In this particular experiment, we used an excess of the Rac protein, which gave rise to a large band of p180. However, we routinely observed that Golgi p180 bound Rac1 more weakly than Cdc42. Second, like the cytosolic IQGAPs (21), Golgi p180 was able to form a stable complex with GTP-bound GST-Cdc42 (Fig. 1C). This is due to the ability of IQGAPs to inhibit the GTPase activity of Cdc42 as indicated in real-time fluorescence assays of Cdc42 labeled with a fluorescence reporter group (6-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexanoyl)2. Finally, similar to the cytosolic IQGAPs (21), binding of Golgi p180 to Cdc42 was competed by the p21-activated kinase, a putative target for Cdc42, but not by the Cdc42-GAP (data not shown).

Fig. 2 shows that the Golgi p180 protein reacts with an antibody raised against the amino-terminal half of human IQGAP1, which recognizes both IQGAP1 and IQGAP2 from rabbit liver cytosol. The Golgi protein, precipitated with GST-Cdc42-GTP\textsubscript{S} and recognized by the IQGAP antibody, migrated with the same mobility as IQGAP2, which appears to be the predominant isoform in the liver (22). IQGAP2 from Golgi membranes appears to bind specifically to the GTP\textsubscript{S}-bound form of Cdc42.

Studies of IQGAP Localization to Golgi Membranes in Other Cell Types—Given that IQGAP2 is liver-specific (22) and that IQGAP1 is more widely distributed (23), we investigated whether IQGAP1 is Golgi-localized in cells that do not express IQGAP2. As shown in Fig. 3, we found that in CHO cells that stably over-expressed epitope-tagged (HA) wild-type Cdc42 or from non-transfected (parental) CHO cells. Solubilized membranes were incubated with the 12CA5 antibody against the HA-epitope tag and precipitated with protein A-Sepharose. Immunoprecipitated proteins were separated by 8% SDS-PAGE and immunoblotted with antibodies raised against the NH\textsubscript{2}-terminal half of IQGAP1.

Fig. 3. Immunoprecipitation of Cdc42 and IQGAP1 from CHO cell Golgi membranes. Golgi membranes were purified from CHO cell stably transfected with epitope-tagged (HA) wild-type Cdc42 or from non-transfected (parental) CHO cells. Solubilized membranes were incubated with the 12CA5 antibody against the HA-epitope tag and precipitated with protein A-Sepharose. Immunoprecipitated proteins were separated by 8% SDS-PAGE and immunoblotted with antibodies raised against the NH\textsubscript{2}-terminal half of IQGAP1.

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branes, which have been treated with primary antibodies raised against mannosidase II, IQGAP, and HA-Cdc42. In panel A, mannosidase II is labeled with 20 nM gold particles and HA-Cdc42 is labeled with 15 nM gold particles (see arrows). In panel B, HA-Cdc42 is labeled with 15 nM gold and IQGAP is labeled with 20 nM gold (see arrows). In addition, we found that in immunofluorescence experiments in Swiss-3T3 cells, we could detect IQGAP1 staining to a perinuclear region which overlaps with a Golgi resident marker, 10E6 (Fig. 5). These experiments show that IQGAP1 does have a Golgi membrane-bound pool, as well as the previously observed cytosolic and plasma membrane-bound pools (19–21).

Biochemical Characterization of Cdc42 and IQGAP Association with Golgi Membranes—As IQGAPs lack predictable transmembrane domains, we investigated whether they were solely dependent on Cdc42 for membrane-binding or were associated with other proteins in Golgi membranes. Fig. 6A shows experiments in which we attempted to perturb the association of IQGAP with CHO cell Golgi membranes. Specifically, we incubated Golgi membranes with GTPγS (i.e. to promote activation of Cdc42), EDTA (to promote nucleotide depletion of Cdc42) or excess non-isoprenylated Cdc42 (which is not able to bind membranes) and then pelleted the membranes in an Airfuge at 100,000 × g. We analyzed the supernatants and resuspended pellets by Western blotting for HA-Cdc42 and IQGAP. The expectation was that if IQGAP was only binding to Cdc42 in these membranes, it would be unaffected by GTPγS treatment but dissociated from the membrane by either excess Cdc42 or by reducing the amount of GTP-bound Cdc42 (i.e. by nucleotide depletion upon the addition of EDTA). However, we found that neither IQGAP nor Cdc42 binding to Golgi membranes was affected by treatment with GTPγS, EDTA, or excess Cdc42 and both proteins still fractionated with the membrane pellets. In Fig. 6B, similar experiments were performed to examine the affects of various treatments on the binding of Cdc42 and IQGAP to CHO cell Golgi membranes. Treatment with 2 M NaCl, or exposure to acidic or basic pH, had no effect on Cdc42 or IQGAP binding to Golgi membranes.

These experiments suggested that IQGAP and Cdc42 might be part of a larger complex in Golgi membranes, which is resistant to high salt and extreme pH treatments and is not available to competition by excess (non-isoprenylated) Cdc42. We next examined the ability of detergents to solubilize these proteins with the aim of finding the lowest effective concentration of detergent that would solubilize a Cdc42-IQGAP complex without disrupting it. As shown in Fig. 7A (see lower panel under Pellets), treatment of Golgi membranes with 2% Triton X-100 and subsequent centrifugation for 30 min (in an Eppendorf centrifuge at 15,000 × g), effectively removed Cdc42 from the pellet fractions. Similar results were obtained when octyl glucoside and CHAPS were used at concentrations above their critical micelle concentrations (data not shown). However, only a small amount of IQGAP was removed from the pellet fractions by detergent treatment (Fig. 7A, upper panel). As detergent insolubility is a characteristic of the actin cytoskeleton, we Western blotted for actin and found that the detergent-insoluble pellets also contained actin (Fig. 7A, middle panel). These

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Fig. 4. Immunogold labeling of purified CHO cell Golgi membranes. Purified Golgi membranes from stably transfected (HA-Cdc42) CHO cells were fixed onto grids for negative-stain electron microscopy and then double-labeled with primary antibodies raised against mannosidase II and HA-Cdc42 (A) or HA-Cdc42 and IQGAP1 (B). HA-Cdc42 was labeled with 15 nM gold particles (small arrows), whereas IQGAP1 and mannosidase II were labeled with 20 nM gold particles (large arrowheads). Grids were stained for EM with 0.2% phosphotungstic acid. Scale bar = 0.25 μm. Gold labeling was not detected in the absence of primary antibodies (negative control).
results indicate that Cdc42 can be solubilized from Golgi membranes under conditions where IQGAP and actin are retained in a low speed, detergent-insoluble pellet.

We next examined whether IQGAP could be removed from this detergent-insoluble complex under conditions that successfully removed actin. Fig. 7B (center panel) shows that treatments which caused the apparent depolymerization of actin and removed it from the pellet fractions also removed IQGAP (upper panel). The apparent depolymerization of actin was dependent on the presence of EDTA, presumably to chelate the Mg\(^{2+}\) required for nucleotide binding and stabilization of the polymer. Treatment with 0.6 M KI is another treatment that has been shown to remove actin from detergent-insoluble pellets (33). These experiments were performed in the presence of 2% Triton X-100, i.e. conditions that completely solubilized Cdc42 (Fig. 7A). However, in the presence of actin depolymerizing buffer, plus Mg\(^{2+}\), Cdc42 was also detected in the pellet (Fig. 7B, lower panel). Apparently, under these conditions some Cdc42 is retained in a complex containing IQGAP and actin. Taken together, these results support a model in which an actin-rich complex is tethering IQGAP to Golgi membranes. When we attempted to examine these solubilized fractions by sucrose density gradient sedimentation, the majority of the IQGAP and actin moved to a low sucrose concentration, which contained most of the soluble proteins (data not shown). This suggested that the actin depolymerizing treatments caused the dissociation of the putative complex into its monomeric components.

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IQGAP Effects on the Membrane Binding of Cdc42—Previous work from our laboratory characterizing the RhoGDI protein had shown that this regulator has the ability to solubilize Cdc42 from membranes (34). We used this observation to test the association of Cdc42 and IQGAP with Golgi membranes. Fig. 8A shows that, whereas we were able to remove a portion of the Cdc42 from Golgi membranes (this ranges from 10 to 40%) using the RhoGDI protein (compare the supernatant fractions in the presence and absence of RhoGDI), IQGAP was not removed from the membrane pellet upon RhoGDI treatment. These observations suggested that the pool of Cdc42 which was...
unavailable to RhoGDI might be tethered to Golgi membranes by IQGAP. We set out to test this possibility by using the Cdc42-binding domain of the IQGAP molecule (i.e. GRD) as a competitor versus the membrane-associated, full length IQGAP. The expectation was that if Cdc42 is held in Golgi membranes by IQGAP, then the addition of the soluble GRD should compete with IQGAP for Cdc42 and thus facilitate its release from membranes. As shown in Fig. 8B, we found that the GRD did stimulate the release of Cdc42 from Golgi membranes (compare supernatant fractions from buffer- and GRD-treated samples). However, the GRD was no more effective than the RhoGDI in releasing Cdc42 from membranes and their membrane releasing activities were not additive (under the supernatant lanes, compare GST-GDI to GRD + GST-GDI). Thus, these results argue against the idea that IQGAP makes Cdc42 insensitive to membrane release by RhoGDI, but rather suggests that the pool of Cdc42 available for membrane release by the GDI is the same pool available to membrane release by the GRD. As shown in Fig. 9A, as much as 40% of the total Cdc42 can be solubilized from Golgi membranes by the GRD with an apparent Kd value of 109 nM. We typically found that more Cdc42 was released by GRD when the membranes were pretreated with GTPγS or GTP compared with GDP or EDTA (i.e. to induce the nucleotide-depleted state) (Fig. 9B). This was in contrast to the RhoGDI, which exhibited no nucleotide dependence in this assay (34).

DISCUSSION

Previous studies have shown that Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus and that its localization is regulated by Arf (2), a GTP-binding protein that has been implicated in intracellular trafficking and membrane fusion (35, 36). In addition, work by Singer and colleagues (14) has shown that Cdc42 can act synergistically with Arf to activate a Golgi membrane-associated phospholipase D (PLD) activity, and it has been proposed that PLD activation may underlie the role of Arf in trafficking. Cdc42 also has been shown to activate PI 3-K (15), which has been implicated in Golgi-to-lysosome trafficking processes in mammalian systems (37), whereas its Saccharomyces cerevisiae homolog, Vps34, has been implicated in Golgi trafficking to the vacuole (38). Thus, the localization of Cdc42 to the Golgi may highlight an important role for this GTPase in cellular trafficking.

The association of IQGAP with activated Cdc42 in Golgi membranes highlights the possible involvement of actin in Golgi processes. That IQGAP is a cytoskeletal element has been inferred from its primary sequence and from the fact that...
it binds to actin. Domains homologous to myosin, and regions of putative coiled-coil structure suggest that IQGAP may have a rigid structure similar to cytoskeletal elements. Work from our laboratory and others has shown that IQGAP binds to actin (25), and we have further shown that a ternary complex of Cdc42-GTP, IQGAP, and actin can be immunoprecipitated using antibodies against Cdc42 (26). To our knowledge, the present study represents the first report of an actin-binding protein in the Golgi that is likely to be regulated by a GTP-binding protein with known effects on the actin cytoskeleton.

The recent identification of Golgi-specific isoforms of spectrin (39) and ankyrin (40) have led Nelson and colleagues (41) to propose a role for actin-binding proteins in sorting events in the Golgi analogous to those that are already known for the cortical actin cytoskeleton. The spectrin membrane skeleton has been hypothesized to anchor membrane proteins such as the Band 3 anion exchanger in erythrocytes and a Golgi-specific isoform of the Band 3 protein has been identified in non-erythroid cells (42). The finding that the \textit{Dictyostelium} actin-binding protein, p24 comitin, is localized to the Golgi further implicates actin-mediated processes in Golgi structure or function (33, 43). Finally, the purification of a detergent-insoluble “Golgi matrix” has yielded the identification of a cytoskeletal-like complex which contains the Golgin proteins (44–48). Golgin proteins are predicted to play a role in Golgi structure, perhaps by anchoring Golgi-localized enzymes (48) or by providing docking sites for specific vesicles (49). These detergent-insoluble proteins have coiled-coil domains, and some have proline-rich regions. The biochemical and putative structural similarities between Golgins and IQGAP proteins may implicate IQGAP in similar processes, and the proline-rich regions in the Golgin proteins could provide potential binding sites for the WW domain of IQGAP. Studies are under way in our laboratory to examine whether the Golgin proteins directly associate with IQGAP.

The association of IQGAP with Golgi membranes is not dependent on the nucleotide state of Cdc42 and is resistant to treatments with high salt, extremes of pH, and detergents. The presence of IQGAP and actin in a detergent-insoluble fraction suggests that the IQGAPs may be part of an actin-rich complex in Golgi membranes. The ability of the GRD of IQGAP to remove Cdc42 from Golgi membranes further suggests that Cdc42 and IQGAP interactions may provide a mechanism by which Cdc42 cycles between membranes and cytosol to interact with its various targets. Fig. 10 depicts a working model based on the data that we have obtained thus far. Two pools of activated Cdc42 in Golgi membranes are depicted. The first is an activated (GTP-bound) pool that is membrane-associated through its isoprenylation. This pool of Cdc42 is susceptible to

**Fig. 9.** Dose dependence and nucleotide specificity of GRD-mediated solubilization of HA-Cdc42 from transfected CHO cell Golgi membranes. A, Golgi membrane samples were treated with increasing doses of GRD as indicated and then centrifuged and analyzed by Western blotting as in Fig. 8B. Western blots were visualized by ECL, and x-ray film was scanned and densitometries quantitated by the Scan Analysis program (BIOSOFT, Ferguson, MO). Data from these scans was plotted using the Igor program. B, CHO cell Golgi membranes were loaded with GTP-\textgamma-S or GDP or were nucleotide-depleted by addition of EDTA and then the ability of 2.75 \( \mu M \) GRD to solubilize HA-Cdc42 was measured as in Fig. 8B. Buffer was used as a negative control and GST-RhoGDI as a positive control for this assay. Densitometries of samples were analyzed as in A.

**Fig. 10.** Model of the putative Cdc42-IQGAP complex in Golgi membranes. IQGAP is depicted as a dimer as proposed by Bashour et al. (25) with its calponin-homology (CH) domain bound to actin (as predicted based on sequence analysis) and its COOH-terminal RasGAP homology (GRD) domain bound to Cdc42. The possible existence of proteins X and/or Y is considered under “Discussion.”
solubilization by either the RhoGDI or the GRD of cytosolic IQGAP. The other pool of activated (GTP-bound) Cdc42 is suggested to be complexed to a Golgi membrane-associated protein(s) (designated X in Fig. 10). These Cdc42 molecules are unable to be released from the membranes by the addition of non-prenylated Cdc42, which suggests that the isoprenoid modification may contribute to the association of Cdc42 with the Golgi membrane-protein(s). GTP-bound Cdc42, IQGAP, and actin are suggested to exist within a multi-protein complex. Neither the RhoGDI nor the GRD of cytosolic IQGAP is able to release Cdc42 from this complex. The inaccessibility of some Cdc42 to membrane removal by the GRD further argues that Cdc42 is associated with another protein in these membranes (i.e. in addition to IQGAP). However, addition of detergent does remove Cdc42 without disrupting the actin-IQGAP Golgi membrane complex. Depolymerization of actin then disrupts this complex. The IQGAP and/or actin may also be associated with Golgi membranes via the interactions with one or more Golgi membrane proteins (e.g. possibly spectrin or ankyrin isoforms (39, 40), depicted by Y in the figure). An important emphasis of future studies will be the identification of Golgi membrane-associated binding partners for IQGAP and Cdc42. Thus far, we have not been able to identify such proteins through standard biochemical and purification approaches. However, we and others (50, 51) have found an IQGAP homolog in S. cerevisiae, and the hope is that through yeast genetics, it will be possible to identify candidate binding partners that are homologous to mammalian proteins.

Perhaps the most important question concerns how an actin/IQGAP-rich complex might be operating in Golgi membranes. One possibility is that IQGAP binding to calmodulin could regulate its association with actin and thus its association with these membranes. Calmodulin antagonists have been shown to have effects on Golgi function (52). Bashour et al. (25) showed that the binding of calmodulin by IQGAP inhibited its interactions with actin in a calcium-independent manner. In addition, recently, calmodulin has been reported to inhibit the association of Cdc42 with IQGAP in a calcium-dependent manner (24). We have not found the interaction between Cdc42 and Golgi membrane-associated IQGAP to be affected by excess calcium. However, removal of IQGAP from the Golgi by disruption of its interaction with actin could also remove Cdc42 from the membranes and allow it to interact with its cytosolic targets. Alternatively, IQGAP could aid in the recruitment of Cdc42-targets to Golgi membranes and/or facilitate target interactions with Cdc42 by maintaining Cdc42 in its GTP-bound state and in close proximity to actin. A particularly interesting possibility is that Golgi-associated IQGAP, acting in this manner, could bring activated Cdc42 and actin into proximity with lipid kinases (PI 3-K) or PLD, which are then activated by Cdc42. The bring activated Cdc42 and actin into proximity with lipid kinases could result in the activation of a similar lipid product. More specifically, the lipid products generated could have affects on both the actin cytoskeleton and on protein sorting events (53, 54). The precise role of the Cdc42-IQGAP complex in Golgi membranes is unclear at this point, but elucidation of its potential role in activation of Golgi PLD and PI 3-K could provide important new insights into the physiological functions of this GTP-binding protein.

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