Calmodulin regulates diverse Ca\(^{2+}\)-dependent cellular processes, including cell cycle progression and cytoskeletal rearrangement. A recently identified calmodulin-binding protein, IQGAP1, interacts with both actin and Cdc42. In this study, evidence is presented that, in the absence of Ca\(^{2+}\), IQGAP1 bound to Cdc42, which maintained Cdc42 in the active GTP-bound state. Addition of Ca\(^{2+}\) both directly abrogated the effect of IQGAP1 on the intrinsic GTPase activity of Cdc42 and, in the presence of calmodulin, dissociated Cdc42 from IQGAP1. In addition, in vitro binding assays revealed that calmodulin associated with both the calponin homology domain and the IQ motifs of IQGAP1. Moreover, F-actin competed with Ca\(^{2+}\)/calmodulin for binding to the calponin homology domain, but not the IQ motifs, of IQGAP1. Analysis of cell lysates revealed that calmodulin bound to IQGAP1 in a ternary complex with Cdc42. Increasing the Ca\(^{2+}\) concentration enhanced the interaction between calmodulin and IQGAP1, with a concomitant decrease in the association of IQGAP1 with Cdc42. Our data suggest that IQGAP1 functions as a scaffolding protein, providing a molecular link between Ca\(^{2+}\)/calmodulin and Cdc42 signaling.

The Ras superfamily comprises a group of small GTPases that function in intracellular signaling cascades, affecting cell growth and differentiation as well as cytoskeletal organization, vesicle trafficking, and nuclear transport (1). These proteins act as molecular switches, alternating between an active GTP bound form and an inactive GDP bound form. Regulators include GTPase-activating proteins (GAPs)\(^1\) that facilitate conversion from active to inactive states, guanine nucleotide exchange factors that catalyze release of GDP from the GTPase, and GDP-dissociation inhibitory factors that inhibit both GAP-mediated and intrinsic GTP hydrolysis. Deregulation of both GTPases and their regulators may be involved in cell cycle derangement and oncogenesis (2).

The Rho family GTPase Cdc42 is involved in cytoskeletal rearrangement and cell cycle progression (3, 4). Specifically, active Cdc42 stimulates filopodium and microspike formation in fibroblasts (5, 6) and polarization of actin and microtubules in T cells (7). In addition, microinjection of Cdc42 into Swiss 3T3 fibroblasts stimulates DNA synthesis (4). It is therefore not surprising that Cdc42 has been implicated in carcinogenesis. Transforming potential has been confirmed by overexpression studies in which dominant negative mutants of Cdc42 inhibit Ras-mediated transformation, whereas constitutively active mutants promote anchorage-independent growth in Rat1 fibroblasts (8).

Downstream effectors of Cdc42 are being elucidated and may include phosphatidylinositol 3-kinase (9) and pp70S6K (10), which are involved in cell cycle progression, and the Wiskott-Aldrich syndrome protein (11) and n-chimaerin (12), which may regulate actin polymerization. Cdc42 also stimulates p21-activated Ser/Thr kinases, which in turn regulate the activation of the nuclear mitogenic protein kinases, c-Jun kinase and p38 (13, 14). Interestingly, another potential downstream effector of Cdc42, IQGAP1, displays significant sequence similarity to Sary1 and the tumor suppressor neurofibromin (15), and is the major calmodulin-binding protein in Ca\(^{2+}\)-free breast cell lysates (16).

Calmodulin is a highly conserved, ubiquitous protein involved in diverse Ca\(^{2+}\)-dependent cellular processes, including cell cycle progression and proliferation, cyclic nucleotide metabolism, glycolgen metabolism, cytoskeletal arrangement, and smooth muscle contraction (17). It possesses four Ca\(^{2+}\)-binding sites, occupation of which effects a conformational change that facilitates association with multiple target proteins. Binding to calmodulin occurs via either basic amphiphilic a-helices or IQ motifs, 23 amino acid sequences with the consensus IQXXXRXXXXR (18). A “complete” IQ motif contains a C-terminal arginine in its consensus sequence, dictating no Ca\(^{2+}\) requirement for calmodulin binding. Alternatively, an “incomplete” IQ motif without arginine requires Ca\(^{2+}\) for binding (18).

A substantial body of evidence implicates calmodulin in carcinogenesis. For example, the level of calmodulin is significantly increased in malignant tissue (19), including breast carcinoma (20), and overexpression of calmodulin alters cell morphology and shortens the cell cycle (21). Although a causal relationship between calmodulin concentration and malignancy has not been demonstrated, it is hypothesized that increased concentrations of calmodulin may contribute to neoplastic transformation.

The downstream effectors of such transformation are unknown. One candidate is the recently isolated 189-kDa protein, IQGAP1. IQGAP1 contains three complete IQ motifs, one incomplete IQ, and a N-terminal region homologous to the actin and calmodulin-binding domain of calponin (22, 23) (Fig. 1). In addition, IQGAP1 contains a region with significant sequence similarity to the catalytic domain of Ras-GAPs (15). IQGAP1 binds Rac and Cdc42 (22) and also cross-links microfilaments (24).

Recently, it was demonstrated that calmodulin binds the
IQGAP1 Integrates Ca\(^{2+}\)/Calmodulin and Cdc42 Signaling

N-terminal region of IQGAP1 (22), which contains both the calponin homology domain (CHD) and the IQ motifs. Ca\(^{2+}\)/calmodulin attenuates the association of IQGAP1 with Cdc42 (16) and F-actin (24). IQGAP1 thus appears to be an actin-associated protein that can transduce Ca\(^{2+}\)/calmodulin signals to Cdc42 at the cytoskeleton. To further explore the functional sequelae of the interaction between Ca\(^{2+}\)/calmodulin and IQGAP1, we isolated both full-length endogenous human IQGAP1 and glutathione S-transferase (GST) fusion constructs containing selected regions of IQGAP1. We show here that Ca\(^{2+}\) binds directly to IQGAP1 and modulates the IQGAP1-mediated inhibition of Cdc42-catalyzed GTP hydrolysis. We also present evidence that Ca\(^{2+}\)/calmodulin competes with F-actin for binding to the CHD of IQGAP1. Finally, we demonstrate that Ca\(^{2+}\) enhances the binding of calmodulin to IQGAP1 thereby inducing the release of Cdc42 from IQGAP1. We conclude that IQGAP1 may provide a molecular link between Ca\(^{2+}\)/calmodulin signaling pathways and Cdc42-mediated processes.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture reagents were obtained from Life Technologies Inc. Bovine serum was from BioWhittaker. Production of the GST-Cdc42 fusion protein has been described (22). Restriction enzymes and DNA Polymerase I (Klenow fragment) were purchased from New England Biolabs, Inc. Nucleotide primers for polymerase chain reaction were obtained from Genemed Biotechnologies. Radiocouliteptides were from DuPont. Calmodulin-Sepharose was purchased from Pharmacia Biotech Inc. G-actin was obtained from Sigma. All other reagents were of standard analytical grade.

Antibodies—Anti-GST and anti-Cdc42 polyclonal antibodies were purchased from Upstate Biotechnology Inc. and Santa Cruz Biotechnologies Inc. Fetal bovine serum was from Biowhittaker. Production of the anti-myoglobin monoclonal antibody was kindly provided by Dr. Jack Ladenson (Washington University School of Medicine, St. Louis, MO). Production of anti-calmodulin monoclonal antibody has been described previously (25). To produce anti-IQGAP1 antibody, GST-IQGAP1N (N-terminal region, amino acids 1–863) was affinity purified, digested with thrombin, and GST was removed by glutathione affinity chromatography. Anti-IQGAP1 antisera was raised by injecting rabbits with purified IQGAP1 fragment (22). Purification of IQGAP1—Full-length IQGAP1 was isolated from MCF-7 cells by calmodulin-Sepharose chromatography as described previously (16).

GST-IQGAP1 Fusion Constructs—The production of GST-IQGAP1N and GST-IQGAP1C (C-terminal region, amino acids 864–1657) (Fig. 1) has been described (22). The DNA for GST-IQGAP1CHD (calponin homology domain, amino acids 1–232) was generated by digestion of pGEX-2T-IQGAP1N with EcoRI, gel purification of vector, and religation. To obtain the cDNA for GST-IQGAP1IQ (IQ motifs, amino acids 740–869), polymerase chain reaction on pDNA3 vector containing full-length cDNA of IQGAP1 was performed using primers flanking nucleotides 2220 and 2608 of IQGAP1, with the 5’ primer containing a BglII site. The resulting 388-base pair product was gel purified and cut with EcoRI. Blunt ends were generated with DNA Polymerase I (Klenow fragment). The fragment was then cut with BglII and subcloned into the BamHI site of pGEX-2T. GST-IQGAP1 fusion proteins were expressed in Escherichia coli. Bacteria were lysed by sonication in 2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, and 10 mM diithiothreitol. Triton X-100 was added to 1% (v/v) and debris removed by centrifugation. Samples were loaded on glutathione-Sepharose columns and washed with PBS containing 10 mM diithiothreitol. GST fusion proteins were eluted with reduced glutathione and dialyzed against PBS. All GST fusion proteins migrated to the expected position on SDS-PAGE (see Fig. 4A).

Cdc42-catalyzed GTPase Activity Assay—2 μg of purified GST-Cdc42 was preincubated with 1 mM EDTA in a buffer containing 25 mM Tris (pH 7.5), 1 mM diithiothreitol, and 125 mM NaCl for 10 min. Cdc42 was then loaded with radiolabeled GTP by incubation with 0.1 mM [γ-\(^{32}P\)]GTP (30 μM MgCl\(_2\)) for 15 min. Various combinations of purified full-length IQGAP1, calmodulin, and 1 mM CaCl\(_2\) were added to equal aliquots of GTP-loaded GST-Cdc42. GTP hydrolysis was initiated by adding 7.5 μM MgCl\(_2\) at 22 °C. Control samples without added Mg\(^{2+}\) were processed in parallel. The assay was terminated after 15 min by adding ice-cold PBS containing 5 mM MgCl\(_2\). Samples were spotted on Millipore HA 0.45-μm filter membranes and filtered under vacuum with a Millipore Sampling Manifold Apparatus. Membranes were washed twice with ice-cold PBS containing 5 mM MgCl\(_2\) and radiolabeled GTP retained by Cdc42 was quantified by liquid scintillation spectrometry. GTP hydrolysis was the difference between [\(^{32}P\)]GTP retained in the presence and absence of Mg\(^{2+}\).

4\(^{6}Ca\)^{2+} Overlay—GST, GST-IQGAP1N, GST-IQGAP1C, and GST-IQGAP1CHD, GST-IQGAP1IQ (20 pmol each), and purified IQGAP1 (2.5 pmol) were adsorbed onto PVDF membrane. The membrane was incubated with \(^{46}Ca\)Cl\(_2\) at 2 μCi/ml in buffer containing 60 mM KCl, 5 mM MgCl\(_2\), and 10 mM imidazole (pH 6.8) for 10 min at 22 °C, washed once in 0.1 M NaH\(_2\)PO\(_4\) for 5 min, air dried, and exposed to x-ray film. To confirm that protein bound to the PVDF membrane, the dot blot was stained with 0.1% (v/v) Amido Black in 45% (v/v) methanol and 10% (v/v) acetic acid for 5 min at 22 °C, followed by destaining in 90% methanol and 2% acetic acid for 5 min.

In Vitro Binding Assays—Approximately 20 pmol of GST, GST-IQGAP1N, GST-IQGAP1C, and GST-IQGAP1CHD were incubated with calmodulin-Sepharose in buffer A containing 1 mM
CaCl₂ or 1 mM EGTA for 1 h at 22 °C on a rotator. Where indicated, 200 pmol of F-actin or bovine serum albumin were added. Samples were washed four times in buffer A. Complexes were resolved by SDS-PAGE and transferred to PVDF membrane. Blots were probed with anti-GST antibody. Antigen-antibody complexes were visualized with horseradish peroxidase-conjugated secondary antibody and developed by ECL.

Actin competition analysis was also performed by incubating 0.16 μM GST fusion protein and different concentrations of F-actin in 50 mM Tris (pH 7.4), 75 mM NaCl, 75 mM KCl, 1 mM CaCl₂, and 0.1% Triton X-100 for 15 min at 22 °C. Where indicated, 1.2 μM calmodulin, samples were incubated for 60 min at 22 °C. Fusion proteins were isolated with glutathione-Sepharose and washed 4 times with PBS. Samples were resolved by SDS-PAGE, transferred to PVDF membrane, and blots were probed with anti-calmodulin antibody. Antigen-antibody complexes were visualized with horseradish peroxidase-conjugated secondary antibody and developed by ECL. Full-length IQGAP1 was isolated from MCF-7 cells lysates with calmodulin-Sepharose. After washing the beads four times with buffer A containing 1 mM CaCl₂ or 1 mM EGTA for 2 min at 4 °C, supernatants were equalized for protein concentration. Equal aliquots of lysate were precleared with blocked Affi-Gel in buffer A with 1 mM CaCl₂ or 1 mM EGTA for 1 h at 22 °C on a rotator. Complexes were washed 4 times with buffer A, resolved by SDS-PAGE, transferred to PVDF membrane, and blots were probed with anti-IQGAP1 antibody.

In Vivo Binding Assays—MCF-7 cells were lysed in buffer A containing 1 mM CaCl₂ or 1 mM EGTA and subjected to centrifugation at 15,000 × g for 5 min at 4 °C. Supernatants were divided into two equal aliquots, one of which was brought to 5 mM CaCl₂. Samples were precleared with protein A-Sepharose for 30 min at 4 °C. Anti-IQGAP1 antiserum or non-immune rabbit serum was added to the supernatant for 2 h at 4 °C. Immune complexes were collected by incubating samples for 2 h at 4 °C with protein A-Sepharose. Complexes were sedimented by centrifugation, washed five times with buffer A, and heated for 2 min at 100 °C in solubilization buffer. Samples were resolved by SDS-PAGE, transferred to PVDF, and blots were probed with anti-IQGAP1, anti-Cdc42, or anti-calmodulin antibodies. Antigen-antibody complexes were visualized with the appropriate (rabbit or mouse) horseradish peroxidase-conjugated secondary antibody and developed by ECL.

Immunoprecipitation—MCF-7 cells were lysed in buffer A containing 1 mM EGTA and subjected to centrifugation at 15,000 × g for 5 min at 4 °C. Supernatants were divided into two equal aliquots, one of which was brought to 5 mM CaCl₂. Samples were precleared with protein A-Sepharose for 30 min at 4 °C. Anti-IQGAP1 antiserum or non-immune rabbit serum was added to the supernatant for 2 h at 4 °C. Immune complexes were collected by incubating samples for 2 h at 4 °C with protein A-Sepharose. Complexes were sedimented by centrifugation, washed five times with buffer A, and heated for 2 min at 100 °C in solubilization buffer. Samples were resolved by SDS-PAGE, transferred to PVDF, and blots were probed with anti-IQGAP1, anti-Cdc42, or anti-calmodulin antibodies. Antigen-antibody complexes were visualized with the appropriate (rabbit or mouse) horseradish peroxidase-conjugated secondary antibody and developed by ECL.

Immunodepletion of IQGAP1 with Anti-calmodulin Antibody—MCF-7 cells were lysed in buffer A containing 1 mM CaCl₂ or 1 mM EGTA and subjected to centrifugation at 15,000 × g for 5 min at 4 °C. Supernatants were equalized for protein concentration. Equal aliquots of lysate were precleared with blocked Affi-Gel in buffer A with 1 mM CaCl₂ or 1 mM EGTA for 1 h at 4 °C on a rotator. Where indicated, the addition of either anti-calmodulin or anti-myoglobin monoclonal antibody linked to Affi-Gel for an additional 3 h at 4 °C. Complexes were sedimented by centrifugation and removed. Proteins remaining in the supernatants were precipitated by trichloroacetic acid, heated at 100 °C for 2 min in solubilization buffer, resolved by SDS-PAGE, and transferred to PVDF. An equal aliquot of untreated MCF-7 lysate was processed in parallel. Blots were probed with anti-IQGAP1 antibody. Antigen-antibody complexes were visualized with horseradish peroxidase-conjugated secondary antibody and developed by ECL.

RESULTS

Ca²⁺ Inhibits the Effect of IQGAP1 on Cdc42-catalyzed GTPase Activity—We previously demonstrated that Ca²⁺/calmodulin modulates the association of IQGAP1 with Cdc42 (16), but no analysis of function was performed. To examine the role of both Ca²⁺/calmodulin and IQGAP1 on Cdc42 function, GTPase activity was assessed by [γ-32P]GTP-Cdc42 filter binding assays (Fig. 2). Ca²⁺ alone did not significantly alter GTP hydrolysis in the control samples. Similarly, neither Ca²⁺/calmodulin nor apocalmodulin produced a significant alteration of the intrinsic GTPase activity of Cdc42 (Fig. 2). In contrast, IQGAP1 inhibited GTPase activity of Cdc42 by 38% (± 13% (mean ± S.E., n = 4) (p < 0.05)). This inhibition was not affected by the addition of calmodulin (Fig. 2). However, addition of Ca²⁺ abrogated the effect of IQGAP1 on Cdc42-catalyzed GTP hydrolysis. Incubation of Ca²⁺/calmodulin, which prevents the association of Cdc42 with IQGAP1 (16), concurrently with IQGAP1 similarly eliminated regulation of the GTPase activity of Cdc42 by IQGAP1 (Fig. 2).

IQGAP1 Binds Ca²⁺ —The direct effect of Ca²⁺ on the modulation of Cdc42 activity by IQGAP1 suggested that IQGAP1 may bind Ca²⁺. To test this hypothesis, GST, GST fusion proteins containing the N- or C-terminal regions of IQGAP1, and purified IQGAP1 were immobilized on PVDF (Fig. 3A). Amidro Black staining confirmed that all proteins bound to the membrane with similar affinity (data not shown). 45Ca²⁺ bound directly to full-length IQGAP1 and to the N-, but not the C-terminal region of IQGAP1 (Fig. 3A). To further localize the Ca²⁺-binding site, GST fusion proteins of the IQ and CHD regions of IQGAP1 were evaluated. This strategy demonstrated Ca²⁺ binding to the CHD of IQGAP1, but not to the IQ region (Fig. 3B). GST alone did not bind Ca²⁺.
Calmodulin Binds to Both the CHD and IQ Motifs of IQGAP1—Calmodulin binds to the 95-kDa N-terminal region of IQGAP1 (22), but the exact location of the calmodulin-binding site on IQGAP1 had not been determined. Candidate regions include the four IQ motifs and the CHD, which displays sequence similarity to the calmodulin-binding region of calponin (22). Therefore, we examined the binding of GST fusion proteins containing the N-terminal, C-terminal, CHD, or IQ motifs of IQGAP1 to calmodulin-Sepharose. As anticipated, calmodulin bound to both the N-terminal domain and IQ motifs of IQGAP1 (Fig. 4B). As was observed with full-length IQGAP1 (16), binding affinity of calmodulin to both of these fusion proteins was higher in the presence than in the absence of Ca\(^{2+}\). Ca\(^{2+}\)/calmodulin also bound to the CHD of IQGAP1, but apocalmodulin did not bind (Fig. 4B). Based on densitometry, it is estimated that the binding affinity of Ca\(^{2+}\)/calmodulin for the CHD was approximately 20-fold lower than for the IQ motifs. Consistent with previous data (22), neither GST-IQGAP1 \(^{N}\) nor GST alone bound to calmodulin (Fig. 4B). The lower molecular weight bands present in the GST-IQGAP1 \(^{N}\) and GST-IQGAP1 \(^{IQ}\) lanes likely represent degradation fragments of the fusion proteins (see Fig. 4A).

Actin Inhibits the Binding of Calmodulin to the CHD of IQGAP1—The CHD displays sequence similarity to the actin-binding region of calponin (22, 23). Since actin associates with IQGAP1 (24), the ability of actin to compete with calmodulin for binding to the CHD of IQGAP1 was examined. Addition of F-actin prevented the binding of the GST-IQGAP1 \(^{CHD}\) to calmodulin (Fig. 5). An equimolar amount of bovine serum albumin had a negligible effect, impeding binding by only 15% (Fig. 5A).

F-actin competition analysis was also performed with calmodulin in solution and complexes were isolated with glutathione-Sepharose (Fig. 5B). Probing the resultant blots with anti-calmodulin antibody confirmed that F-actin substantially decreased calmodulin binding to the CHD of IQGAP1. Bovine serum albumin did not decrease calmodulin binding to the CHD (data not shown). Separate analysis performed by actin immunoblotting revealed that F-actin bound to the CHD of IQGAP1 (data not shown). By contrast, calmodulin binding to the IQ region (Fig. 5B) and to full-length IQGAP1 (Fig. 5C) were not altered by F-actin; increasing the F-actin concentration by 8-fold (up to 6.4 μM) had no effect (data not shown). Hence, there is substantially more calmodulin in the GST-IQGAP1 \(^{IQ}\) sample than in the GST-IQGAP1 \(^{CHD}\) sample in Fig. 5B because of the significantly higher affinity of calmodulin for the IQ region (see Fig. 4B).

Ca\(^{2+}\)/Calmodulin Prevents the in Vivo Association of IQGAP1 with Cdc42—Ca\(^{2+}\)/calmodulin inhibits the in vitro binding of IQGAP1 to Cdc42 (16). To characterize this interaction in a normal cellular milieu, we examined the effect of calmodulin on the binding of GST-Cdc42 to endogenous IQGAP1 in MCF-7 human breast epithelial cell lysates. Endogenous IQGAP1 bound to activated GST-Cdc42 (Fig. 6A). Binding affinity was greater in the absence than in the presence of Ca\(^{2+}\). In addition, calmodulin was isolated from cell lysates by GST-Cdc42 affinity chromatography (Fig. 6A). Because calmodulin does not associate directly with Cdc42 (16, 22), the calmodulin was presumably bound to IQGAP1 in a ternary complex with Cdc42. The reduced amount of calmodulin detected upon addition of Ca\(^{2+}\) (Fig. 6A) can be accounted for by two possible mechanisms. First, Ca\(^{2+}\)/calmodulin may mediate a decrease in the affinity of IQGAP1 for Cdc42 (16). An alternative mechanism that has to be excluded is a decrease in the affinity of IQGAP1 for Ca\(^{2+}\)/calmodulin. To distinguish between these two possibilities, calmodulin-Sepharose affinity chromatography was performed. Ca\(^{2+}\) increased the binding of endogenous IQGAP1 to calmodulin by 2.39 ± 0.25-fold (mean ± S.E., n = 4), with a concomitant decrease in the amount of endogenous Cdc42 that was retained by the calmodulin-Sepharose.
Modulates the Binding of Cdc42 and Calmodulin to IQGAP1—The specificity of the anti-IQGAP1 antibody was evaluated before using it in immunoprecipitation analysis. IQGAP1 was the only protein recognized in MCF-7 cell lysates by the anti-IQGAP1 antibody (Fig. 7A). The antibody, which was raised against the N-terminal fragment of IQGAP1, bound to GST-IQGAP1<sup>CHD</sup> but not GST-IQGAP1<sup>IQ</sup> (data not shown). The effect of Ca<sup>2+</sup> on the interaction among calmodulin, Cdc42, and IQGAP1 was analyzed further by co-immunoprecipitation. Ca<sup>2+</sup> did not alter the binding of IQGAP1 to its antibody (Fig. 7B). By contrast, Ca<sup>2+</sup> increased by 1.5-fold the amount of Cdc42 that co-immunoprecipitated with IQGAP1 (Fig. 7B). Consistent with the calmodulin-Sepharose data in Fig. 6, substantially more calmodulin co-immunoprecipitated with IQGAP1 in the presence of Ca<sup>2+</sup> than in the absence of Ca<sup>2+</sup> (Fig. 7B). Overexposure of the ECL image revealed that a small amount of calmodulin co-immunoprecipitated with IQGAP1 in the absence of Ca<sup>2+</sup> (data not shown). The specificity of the interactions is revealed by the absence of immunoreactive proteins in the samples immunoprecipitated with non-immune serum (Fig. 7B, lanes 3 and 4).

Anti-calmodulin Antibody Depletes MCF-7 Lysates of IQGAP1—Previous in vitro assays have shown that Ca<sup>2+</sup> does not impair the binding of IQGAP1 to Cdc42 (16). However, less IQGAP1 from MCF-7 lysates bound to GST-Cdc42 in the presence than in the absence of Ca<sup>2+</sup> (Fig. 6A). Moreover, Ca<sup>2+</sup> decreased the amount of Cdc42 that co-immunoprecipitated with IQGAP1 (Fig. 7). An explanation for these findings may be that in the normal cellular milieu, Ca<sup>2+</sup>/calmodulin binds a substantial amount of endogenous IQGAP1, thereby preventing its association with Cdc42. To evaluate this hypothesis, MCF-7 cell lysates were subjected to immunodepletion with anti-calmodulin antibody. Notably, in the presence of Ca<sup>2+</sup>, but not EGTA, incubation with anti-calmodulin antibody reduced by 53 ± 1% (mean ± S.E., n = 3) the amount of IQGAP1 in MCF-7 lysates (Fig. 8). Incubation with an irrelevant isotype-identical monoclonal antibody (anti-myoglobin IgG<sub>2a</sub>) did not alter the amount of IQGAP1 remaining in the supernatant. These data confirm that a significant proportion of endogenous
IQGAP1 is a signaling protein composed of multiple domains, including a C-terminal region that binds actin. Experimental evidence suggests that the C-terminal region of IQGAP1 has significant sequence similarity to the muscle protein MP-20, which is involved in the regulation of Cdc42, a small GTPase involved in the regulation of the cytoskeleton. IQGAP1 interacts with Cdc42 and maintains it in its active GTP-bound state, thus stabilizing its activity and linking it to downstream effectors such as F-actin. The interaction of IQGAP1 with Cdc42 is modulated by Ca2+/calmodulin, which can dissociate IQGAP1 from Cdc42 and thus influence the cytoskeletal rearrangements mediated by Cdc42.

Our previous work revealed that calmodulin in the presence of Ca2+ plays a role in the dissociation of IQGAP1 from Cdc42. In vitro, Ca2+/calmodulin appears to be a negative regulator of Cdc42 activity. The presence of Ca2+/calmodulin inhibits the binding of IQGAP1 to Cdc42 and may dissociate IQGAP1 from F-actin, which is essential for cytoskeletal rearrangements. Additionally, the interaction of IQGAP1 with Cdc42 and F-actin is regulated by Ca2+/calmodulin, which may influence cell migration and invasion.

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