The IQGAP1 Protein Is a Calmodulin-regulated Barbed End Capper of Actin Filaments

POSSIBLE IMPLICATIONS IN ITS FUNCTION IN CELL MIGRATION

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IQGAP1 is a large modular protein that displays multiple partnership and is thought to act as a scaffold in coupling cell signaling to the actin and microtubule cytoskeletons in cell migration, adhesion, and cytokinesis. However, the molecular mechanisms underlying the activities of IQGAP1 are poorly understood in part because of its large size, poor solubility and lack of functional assays to challenge biochemical properties in various contexts. We have purified bacterially expressed recombinant human IQGAP1. The protein binds Cdc42, Rac1, and the CRIB domain of N-WASP in a calmodulin-sensitive fashion. We further show that in addition to bundling of filaments via a single N-terminal calponin-homology domain, IQGAP1 actually regulates actin assembly. It caps barbed ends, with a higher affinity for ADP-bound terminal subunits ($K_B = 4 \text{ nM}$). The barbed end capping activity is inhibited by calmodulin, consistent with calmodulin binding to IQGAP1 with a $K_C$ of 40 nM, both in the absence and presence of Ca$^{2+}$ ions. The barbed end capping activity resides in the C-terminal half of IQGAP1. It is possible that the capping activity of IQGAP1 accounts for its stimulation of cell migration. We further find that bacterially expressed recombinant IQGAP1 fragments easily co-purify with nucleic acids that turn out to activate N-WASP protein to branch filaments with Arp2/3 complex. The present results open perspectives for tackling the function of IQGAP1 in more complex reconstituted systems.

IQGAP1 is the major and best characterized member of the family of IQGAP proteins, which are present in all eukaryotes and comprise three members in humans, called IQGAP1, IQGAP2, and IQGAP3, which share similar structural organization and primary sequence (1, 2). IQGAP1 is a large (189 kDa, 1657 residues) scaffold protein characterized by its multiple partnership, which underlies its role of signal integrator in cell migration, proliferation, and tumorigenesis (3). Ligands of IQGAP1 include F-actin (4) and regulators of the actin cytoskeleton Cdc42 and Rac (5), adhesion proteins E-cadherin and $\beta$-catenin (6), calmodulin (CaM) (5), components of the MAP kinase pathway like ERK, MEK, B-Raf (Roy et al., Refs. 7, 8; Ren et al., Ref. 9), and components of the exocyst (Sakurai-Yageta et al., Ref. 10, Rittmeyer et al., Ref. 11). IQGAP1 has also been reported to activate N-WASP in vitro and to enhance formation of Arp2/3-branched filament arrays in lamellipodia (Bensenor et al., Ref. 12; Le Clainche et al., Ref. 13). Finally, IQGAP1 binds to APC and CLIP170 (Watanabe et al., Ref. 14; Fukata et al., Ref. 15), which are targeted to microtubule tips by EB1 (Bieling et al., Ref. 16), and to the formin mDia1 (Brandt et al., Ref. 17), which catalyzes processive barbed end assembly of actin filaments. Recent works thus suggest that the complex and pleiotropic effects of IQGAP1 in cell polarization, cell invasion, and adhesion might be mediated by its role in bridging the microtubule and actin cytoskeletons at the cell cortex in signal-controlled trafficking processes (18).

Elucidating the structure/function relationship in IQGAP1 is a challenging task due to its modular organization and multiple partnership, and to the lack of relevant basic functional assays. The binding sites of the known major ligands have been mapped on IQGAP1. The N terminus of IQGAP1 binds and bundles actin filaments via a single calponin homology domain (CHD) sufficient for F-actin binding, followed by 6 coiled-coil repeats which dimerize the protein. A moderate affinity of the actin-binding CHD (residues 1–210) has been measured in sedimentation assays ($K_D = 47 \mu\text{m}$, 19). The affinity of full-length IQGAP1 for F-actin is higher, and can be estimated to the micromolar range, based on one data point (20). The structure of the CHD (residues 26–210) has been solved by NMR (21). The CHD is followed by a WW region involved in ERK1 and ERK2 binding, and 4 IQ domains that bind CaM and also appear involved in association with MEK1, MEK2, S100B, and myosin ELC. Then follows a Ras-GAP-related domain (GRD, residues 962–1345) that has no GAP activity despite a crystal structure similar to other GAPS (22), but binds the GTP-bound forms of Cdc42 and Rac (23). The GRD also appeared as the minimal fragment of the C-terminal domain of IQGAP1 able to activate N-WASP in actin polymerization assays with Arp2/3 complex (13). The GRD is followed by a C-terminal region that binds E-cadherin and $\beta$-catenin. Finally the extreme C-terminal region (1503–1657) binds CLIP170, APC, and the formin domain; GRD, GAP-related domain; CP, capping protein; GTP $\gamma$S, guanosine 5’-3-O-(thio)triphosphate.
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The binding of CaM has been reported to inhibit the interaction of IQGAP1 with F-actin (20) as well as with Cdc42 (24, 25) and B-Raf (26). Recent results further indicate that IQGAP1 co-immunoprecipitates with EGFR in a calmodulin-sensitive fashion (27), and that the S100 protein also regulates IQGAP1 by binding to its IQ domains (28).

The above data suggest that several regions of the IQGAP1 molecule might regulate the actin cytoskeleton in a variety of signal-responsive functions. To get further insight into the possible multiple regulatory activities of IQGAP1 in actin dynamics, we purified bacterially expressed human recombinant IQGAP1 in amounts sufficient to carry out an in vitro biochemical functional analysis. We verified that the recombinant IQGAP1 protein displays the known CaM-regulated effector binding properties, thus behaves as native IQGAP1 isolated from adrenal glands (20). We then examined its activities in actin assembly and disassembly assays at the barbed and pointed ends of the filaments. Our results establish that IQGAP1 caps the barbed ends of actin filaments with a $K_0$ in the $10^{-8}$ M range. Barbed end capping is abolished by CaM. The C-terminal half moiety of IQGAP1, but not the N-terminal region, displays the capping activity with a conserved high affinity. Full-length IQGAP1 does not activate N-WASP. Activation of N-WASP by the GRD is observed on partially purified fragments containing the GRD region, but fails to be observed upon extensive purification of the GRD ensuring the complete removal of contaminating nucleic acids, which by themselves are found to activate N-WASP. These results provide insight into the possible role of IQGAP1 as an actual effector and not only as a scaffold in processes driven by actin and open avenues to further address its function in vivo and its regulation by signaling pathways.

EXPERIMENTAL PROCEDURES

Recombinant cDNA Constructs and Mutations—The cDNA of full-length IQGAP1 and the fragments 1–522 and 744–1657 were amplified by PCR and cloned into pET100/D-TOP0 vector (Invitrogen). The cDNA of the other IQGAP1 fragments were amplified by PCR and cloned into pET100/D-TOPO vector (Invitrogen). The cDNA of the other IQGAP1 fragments containing the GRD region, but fails to be observed upon extensive purification of the GRD ensuring the complete removal of contaminating nucleic acids, which by themselves are found to activate N-WASP. These results provide insight into the possible role of IQGAP1 as an actual effector and not only as a scaffold in processes driven by actin and open avenues to further address its function in vivo and its regulation by signaling pathways.

To generate the N-WASP-CRIB H211D point mutant, the cDNA of N-WASP-(142–276) was amplified from the N-WASP cDNA containing the point mutation H211D. The resulting cDNA was cloned into plasmid pGEX-6P-1 (GE Healthcare). All constructs were verified by sequencing.

Protein Purification—Actin was purified from rabbit muscle, isolated in CaATP-G-actin-form by gel filtration in G-buffer (5 mM Tris-Cl pH 7.8, 1 mM DTT, 0.1 mM CaCl2, 0.2 mM ATP) and was pyrene-labeled (29). Arp2/3 complex from bovine brain, human His-tagged N-WASP, human His-tagged VCA, and human gelsolin were purified as described (30). Spectrin-actin seeds were prepared from human erythrocytes (31).

His$_6$-tagged IQGAP1 was expressed in E. coli BL21. Bacteria were lysed with lysozyme in the presence of benzamidine, in lysis buffer (50 mM phosphate pH 8.0, 0.5 mM KCl, 50 mM imidazole, 1% Triton X-100, 5 mM DTT, 0.1 mM EDTA, 0.2 mM PMSF, protease inhibitors (Roche), and 10 µg/ml benzamidine. His$_6$-IQGAP1 was purified with HisTrap FF Crude (GE Healthcare) affinity chromatography. The centrifuged bacterial extract was loaded in lysis buffer without PMSF nor benzamidine, and eluted with 500 mM imidazole in the same buffer. The eluted protein was dialyzed overnight against buffer C (50 mM Tris-Cl, pH 7.5, 1 mM DTT, 0.2 mM sucrose, 0.5 mM KCl, 2 mM CaCl$_2$), added to the dialyzed material prior to loading on a CaM-Sepharose 4B (GE Healthcare) affinity column equilibrated in the same buffer. His$_6$-tagged IQGAP1 was eluted from CaM-Sepharose 4B with 10 mM EGTA and 0.5 mM KSCN, dialyzed in 20 mM Tris-Cl pH 7.5, 0.5 mM KCl, 1 mM DTT, and 200 mM sucrose, and centrifuged 30 min at 90,000 rpm. The protein was flash-frozen on liquid nitrogen and stored at −80°C. The barbed end capping activity was fully recovered after thawing, up to at least 6 months storage at −80°C. The yield was typically 0.4 mg IQGAP1 per liter of culture.

N-WASP-(142–276) (CRIB) wt and N-WASP-(142–276) (CRIB) H211D glutathione S-transferase (GST) fusion proteins were expressed in Escherichia coli BL21 and purified according to standard purification procedures.

The GST-tagged IQGAP1 fragment 1–216 was purified, after lysis by sonication, on glutathione-Sepharose 4B (Invitrogen), cleaved with PreScission Protease (GE Healthcare), dialyzed in 20 mM Tris-HCl (pH 7.8), 0.1 mM KCl, 1 mM DTT, and flash frozen.

The GST-tagged IQGAP1 fragments 1–1345, 675–1345, 657–1675, 744–1345, 962–1583 were purified, after enzymatic lysis, on glutathione-Sepharose 4B (Invitrogen), cleaved with PreScission Protease (GE Healthcare) or eluted with 50 mM reduced l-glutathione, dialyzed in 20 mM Tris-HCl (pH 7.5), 0.5 mM KCl, 1 mM DTT, 0.2 mM sucrose, centrifuged 30 min at 90k rpm, 4°C and flash frozen. Directly after cleavage 962–1345 was centrifuged 20 min at 90k rpm, 4°C and gel filtered on a Superdex 75, HiLoad 16/60, 120 ml (GE Healthcare) in 20 mM Tris-HCl (pH 7.5), 0.5 mM KCl, 10 mM MgCl$_2$, 1 mM DTT, and 0.2 mM sucrose.

The His$_6$-tagged IQGAP1 fragment 1–522 was purified, after lysis by sonication, on Ni-NTA-agarose (Qiagen), eluted with 0.5 mM imidazole, dialyzed in 20 mM Tris-HCl (pH 7.5), 0.5 mM KCl, 1 mM DTT, and 10% glycerol, centrifuged 30 min at 90,000 rpm, 4°C, and flash frozen.

The His$_6$-tagged IQGAP1 fragments 744–1657 and 744–1502 were purified, after enzymatic lysis, exactly as described for the full-length IQGAP1 protein, by His-trap, and CaM affinity chromatography and stored identically.

GST-Rac1 and GST-Cdc42 proteins in vector pGEX-2T were expressed in E. coli and purified using glutathione-Sepharose 4B (GE Healthcare). The proteins were eluted with 50 mM l-glutathione and dialyzed in 10 mM Tris-Cl pH 7.6, 0.1 mM KCl, 5 mM MgCl$_2$, 1 mM DTT, and 0.1 mM GTP. Cdc42 Q61L was cleaved with thrombin in 20 mM Hepes pH 7.7, 0.1 mM KCl, 5 mM MgCl$_2$, and 10% glycerol. The thrombin was then removed with p-aminobenzamidine resin.

Loading of Rac1 and Cdc42 with GTPyS—GST-Rac1 and GST-Cdc42 were loaded with GTPyS under the following conditions. Purified GDP-bound protein (14–17 µM) in buffer L (20 mM Tris-Cl buffer pH 7.8, 0.1 mM KCl, 1 mM MgCl$_2$, 0.1% Tween 20), was incubated in 1 mM GTPyS and 5 mM EDTA for...
30 min at room temperature. The GDP/GTPγS exchange reaction was stopped by addition of 10 mM MgCl2.

Coprecipitation Assays—His6-tagged IQGAP1 (120 or 180 nM) in buffer L was mixed with 1 μM of GST, GST-Rac1-GTPγS, GST-Cdc42-GTPγS, or GST-CRIB in the presence or absence of CaM (17 μM), or with GST-CRIB or GST-CRIB-H211D in the presence or absence of the Cdc42 Q61L (1 μM) in 100 μl of buffer L and incubated for 1 h at 4 °C on a rotating wheel. The protein solutions were added to 20 μl of glutathione-Sepharose 4B (GE Healthcare) and incubated for 1 h at 4 °C on a rotating wheel. The beads were washed three times with buffer L, boiled in SDS-loading buffer, and the bound proteins were separated by SDS-PAGE, followed by Coomassie Blue staining or Western blotting. For Western blotting, IQGAP1 was detected by using a mouse monoclonal antibody (BD Transduction Laboratories). For experiments using CaM, the reaction was supplemented with 0.1 mM CaCl2.

Pyrene-actin Polymerization Assay—Actin polymerization was monitored by the increase in fluorescence of 10% pyrenyl-labeled actin. Polymerization was induced by the addition of 0.1 M KCl, 1 mM MgCl2 and 0.2 mM EGTA to a solution of 2 or 2.5 μM CaATP-G-actin in G-buffer (5 mM Tris-HCl pH 7.8, 0.1 mM CaCl2, 0.2 mM ATP, 1 mM DTT) containing the proteins of interest. Fluorescence measurements were carried out in a Safas Xenius model FLX (Safas, Monaco) spectrophotometer.

Spectrin-actin seeds (0.1 nM) were added to the reaction for barbed end elongation measurements, and gelsolin-actin (1:2) complexes (5–10 nM) were added for pointed end elongation measurements. For experiments using Ca-CaM, the reaction was supplemented with 0.1 mM CaCl2. The affinity of IQGAP1 for barbed ends (Kb) was derived as follows. The initial elongation rate was taken as a measure of the free barbed ends, with all barbed ends being free when [IQGAP1] = 0. The fraction of free barbed ends is defined by \( V_0 \).

\[
\frac{V_o}{V_F} = \frac{K_b}{K_b + I}
\] (Eq. 1)

\( V_o \) and \( V_F \) represent the barbed end growth rate in the absence and presence of IQGAP1 respectively, \( K_b \) represents the equilibrium dissociation constant for binding of IQGAP1 to barbed ends, and \( I \) is the concentration of free IQGAP1, which in this case may be considered equal to the total concentration \( I_o \).

The affinity of IQGAP1 for CaM (\( K_c \)) was derived in the same way. Data were fitted using Equation 1 in which the value of \( K_b \) measured in the absence of CaM was introduced (\( K_b = 34 \) nM), and the value of \( I \) was defined as follows in Equation 2.

\[
I = \frac{I_o - C_0 - K_c \pm \sqrt{(I_o + C_0 + K_c)^2 - 4I_oC_0}}{2}
\] (Eq. 2)

\( I_o \) is total [IQGAP1] and \( C_0 \) is total [CaM].

Pyrene-actin Depolymerization Assay—Actin depolymerization was monitored by the decrease in fluorescence of 50% pyrenyl-labeled F-actin (2.5 μM) upon 40-fold dilution in F-buffer (G-buffer with 0.1 mM KCl, 1 mM MgCl2, and 0.2 mM EGTA). Equation 1 was used to fit the data.

RESULTS

Bacterially Expressed Recombinant IQGAP1 Binds Cdc42, Rac, and the BR-CRIB Domain of N-WASP in a Calmodulin-sensitive Fashion—The bacterial recombinant full-length His-tagged IQGAP1 protein was purified by Ni affinity and CaM affinity (Fig. 1a). Pull-down assays using GST-tagged, GTPγS-bound Cdc42 or Rac and the basic region-CRIB domain (BR-CRIB) of N-WASP (residues 142–276) were performed in the absence and presence of CaM. The results (Fig. 1b) show that the IQGAP1 protein binds its regular ligands Cdc42 and Rac1 and that both interactions are strongly inhibited by CaM. We

![FIGURE 1. Purification of bacterially expressed human IQGAP1 and characterization of its interactions with various ligands. a, recombinant His6-tagged IQGAP1 was produced in E. coli and purified by affinity chromatography on Ni-Sepharose and CaM-Sepharose. IQGAP1 was eluted from CaM Sepharose with 10 mM EGTA and 0.5 M KSCN. The yield was typically 0.4 mg per liter of culture. Samples at the different steps of the purification were analyzed on SDS-PAGE and Coomassie Blue staining. b, IQGAP1 binds Rac1 and Cdc42 in a CaM-sensitive fashion. His6-tagged IQGAP1 (180 nM) coprecipitation assays with GST (1 μM), GST-Rac1-GTPγS (1 μM) or GST-Cdc42-GTPγS (1 μM) in the absence or presence of CaM (5 μM). Bound fractions were analyzed by Western blot using an anti-IQGAP1 antibody. c, IQGAP1 binds BR-CRIB (wt) and to a lesser extent BR-CRIB (H211D) of N-WASP in a CaM-sensitive fashion. His-tagged IQGAP1 (120 nM) coprecipitation assays with GST (1 μM) or GST-BR-CRIB wt (1 μM) or GST-BR-CRIB-H211D in the absence or presence of CaM (5 μM). Bound fractions were analyzed by Western blot as in panel b. d, map of full-length N-WASP and the BR-CRIB fragment with the position of the mutation H211D.](image-url)
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(a) Pyrene-fluorescence over time for different conditions:
- actin + SP
- actin + GA
- actin + GA + IQGAP1
- actin + 32 nM IQGAP1
- actin + 160 nM IQGAP1

(b) Fraction of growing ends as a function of IQGAP1 concentration:
- $K_d = 34$ nM

(c) Pyrene-fluorescence over time for different IQGAP1 concentrations:
- actin + 19 nM IQGAP1
- actin + 9 nM IQGAP1
- actin + 5 nM IQGAP1
- actin

(d) Fraction of depolymerizing ends as a function of IQGAP1 concentration:
- $K_d = 4$ nM

(e) Pyrene-fluorescence over time for different Holo-CaM concentrations:
- actin + SP
- actin + 144 nM IQGAP1
- actin + 144 nM IQGAP1 + 1.46 μM Holo-CaM
- actin + 144 nM IQGAP1 + 439 nM Holo-CaM
- actin + 144 nM IQGAP1 + 256 nM Holo-CaM
- actin + 144 nM IQGAP1 + 73.1 nM Holo-CaM
- actin + 144 nM IQGAP1

(f) Pyrene-fluorescence over time for different Apo-CaM concentrations:
- actin + SP
- actin + 79 nM IQGAP1
- actin + 79 nM IQGAP1 + 1.5 μM Apo-CaM
- actin + 79 nM IQGAP1 + 500 nM Apo-CaM
- actin + 79 nM IQGAP1 + 150 nM Apo-CaM
- actin + 79 nM IQGAP1 + 100 nM Apo-CaM
- actin + 79 nM IQGAP1

(g) Fraction of growing ends as a function of CaM concentration:
- $K_d (Holo-CaM) = 30$ nM
- $K_d (Apo-CaM) = 30$ nM
further show that full-length IQGAP1 binds the CRIB domain of N-WASP in a CaM-sensitive fashion (Fig. 1c). In pull-down assays, IQGAP1 bound much more weakly to GST-BR-CRIB(H211D), which does not interact with Cdc42 (32) than to GST-BR-CRIB (Fig. 1d). All the shown experiments were reproduced three times with quantitatively identical results using different recombinant IQGAP1 preparations.

In conclusion, the bacterially expressed IQGAP1 appears as a fully active, CaM-responsive protein that shares the main known properties and probably the fold of native eukaryotic IQGAP1. In addition we have shown that CaM abolishes the binding of Rac1 to IQGAP1.

**IQGAP1 Caps Filament Barbed Ends in a CaM-sensitive Fashion**—Preliminary actin polymerization assays showed that IQGAP1 potently inhibited spontaneous assembly of actin at substoichiometric concentrations. Measurements of filament elongation at either barbed end or pointed end showed specific barbed end growth inhibition by IQGAP1 with a $K_\text{d}$ of 34 nM (Fig. 2, a and b). This result was corroborated by the inhibition of barbed end depolymerization by IQGAP1, in which IQGAP1 displayed a 10-fold higher affinity ($K_\text{d} = 4 \text{ nM}$), which suggests that IQGAP1, like twinfilin (33), binds with higher affinity to ADP-terminal subunits exposed at barbed ends in a depolymerization regime, than to ATP/ADP-P$_\text{~}$terminal subunits in a growth regime (Fig. 2, c and d). Again, these results were quantitatively reproducible with several IQGAP1 preparations. Both in filament growth and filament depolymerization assays, the initial rate was established after the mixing dead time (5 to 10 s) and was independent of the order of addition of reactants to the solution, which testifies that IQGAP1 binds in rapid equilibrium to the barbed ends.

IQGAP1 thus appears to be a novel barbed end capping protein. The barbed end capping activity of IQGAP1 was inhibited by CaM in a concentration-dependent fashion. The data were quantitatively analyzed to derive a $K_\text{d}$ value of 30 nM for the binding of CaM to IQGAP1. Intriguingly, in the absence of Ca$^{2+}$ ions, apo-CaM inhibited the capping activity of IQGAP1 with the same affinity as Ca-CaM (Fig. 2, e–g).

Attempts were made to measure the shift in critical concentration from 0.1 to 0.6 $\mu$m (the critical concentration for actin assembly at pointed ends) due to IQGAP1 capping of barbed ends. However the IQGAP1 protein was not stable during the overnight incubation period required to reach steady state and derive critical concentration plots and its aggregation interfered with the assay.

To map the barbed end capping activity, several fragments of IQGAP1 were expressed, purified and assayed (Fig. 3). The N-terminal fragment (1–216), which contains the calponin homology domain and the actin-binding flanking regions, known to be responsible for filament bundling, did not inhibit barbed end growth. The larger N-terminal fragment (1–522) inhibited barbed end growth with an affinity too low to be considered as relevant (Fig. 3a). In contrast, the C-terminal fragment (744–1657) capped barbed ends with a $K_\text{d}$ of 68 nM (Fig. 3, b and c). CaM again inhibited the capping activity, with a somewhat lower affinity ($K_\text{d} = 120 \text{ nM}$) than for the full-length protein (Fig. 3, d and e).

The C-terminally truncated fragment (744–1502) capped barbed ends as efficiently as (744–1657) and in a calmodulin-sensitive fashion. Consistently, the isolated short C-terminal fragment (1503–1657), which contains the binding sites of $\beta$-catenin, E-cadherin, APC, CLIP 170, and more recently DBR, standing for Diaphanous-Binding-Region (17), had no capping activity. In conclusion the extreme C-terminal region of IQGAP1 plays no role in barbed end capping.

To determine whether the IQ motifs are dispensable for a calmodulin-insensitive, constitutive capping activity, IQ-truncated fragments (962–1345) and (962–1583) derived from the active C-terminal region were expressed, but purification of high amounts of soluble protein was hampered by technical difficulties. It is possible that the presence of the IQ motifs assists the folding of IQGAP1 peptides.

Altogether these results suggest that the integral capping activity resides in the C-terminal half of IQGAP1, excluding the extreme C-terminal RGC1 region. Thus, this activity is distinct from and does not require the N-terminal F-actin binding calponin-homology region.

**Neither IQGAP1 Full-length nor Its C-terminal GRD Domain Are Activators of N-WASP**—The fact that full-length IQGAP1 binds the CRIB domain of N-WASP prompted us to examine whether the full-length protein was an activator of N-WASP, as was previously reported for the isolated GRD and other larger C-terminal domains (13). We first assayed the effect of IQGAP1 on the VCA-induced assembly of actin in branched filaments with Arp2/3 complex, to obtain a reference in which only the barbed end capping activity of IQGAP1 would be involved (Fig. 4a). As expected, IQGAP1 caused an increased delay in filament assembly and lowered the maximal rate at mid-polymerization. Both effects result from the barbed end capping activity of IQGAP1. Consistently, at concentrations of IQGAP1 high enough to exceed 90% capping of barbed ends and establish the high critical concentration for assembly of G-actin at pointed ends, the extent of change in fluorescence reached at steady state decreased, due to sequestration of actin by VCA, because the WH2 domain of VCA supports barbed end assembly when it is in complex with G-actin, like profilin-actin, but does not support pointed end assembly (30).

**FIGURE 2. IQGAP1 is a barbed end capper.** a, IQGAP1 blocks barbed end growth. Barbed end growth of 2 $\mu$m MgATP-G-actin (10% pyrenyl-labeled) was measured in the presence of 105 pm spectrin-actin seeds (SP) and the indicated concentrations of IQGAP1. Inset, IQGAP1 does not affect pointed end growth. 2.5 $\mu$m MgATP-G-actin (10% pyrenyl-labeled) was polymerized with 10 nM gelsolin-actin (GA2) complexes in the presence or absence of 300 nM IQGAP1. b, initial rates of barbed end growth corresponding to data in a (+ additional data points) were plotted versus the concentration of IQGAP1. The best fit of the data gave a $K_\text{d}$ of 34 nM. c, IQGAP1 caps ADP-bound barbed ends with a higher affinity than ADP-Pi-bound barbed ends. Dilution-induced depolymerization of 60 nM F-actin (10% pyrenyl-labeled) was measured in the presence of the indicated concentrations of IQGAP1. d, initial rates of depolymerization corresponding to the data in c were plotted versus the concentration of IQGAP1. The best fit curve was calculated with a $K_\text{d}$ of 4 nM. e and f, CaM inhibits barbed end capping by IQGAP1. Barbed end growth was measured in the presence of 105 pm spectrin-actin seeds (SP), 2 $\mu$m MgATP-G-actin (10% pyrenyl-labeled), and the indicated concentrations of IQGAP1 and Ca-CaM (red) or apo-CaM (blue). The best fit of the data gave the same $K_\text{d}$ value of 30 nM for binding of apo- and holoc-Alo-CaM to IQGAP1. g, initial rates of barbed end growth corresponding to the data in e, f were plotted versus the concentration of Ca-CaM (red) and apo-CaM (blue). The curve is calculated with a $K_\text{d}$ of 40 nM for the IQGAP1-CaM complex.
FIGURE 3. Localization of the barbed end capping activity of IQGAP1. a, N-terminal fragment (residues 1–216) that does not contain the dimerizing coiled coil domain does not inhibit barbed end growth. The N-terminal fragment comprising the CH domain (1–522) inhibits barbed end polymerization with low affinity. A C-terminal fragment of IQGAP1 (744–1657) inhibits barbed end polymerization with an affinity close to the full-length protein. 2.5 μM MgATP-G-actin (10% pyrenyl-labeled) was polymerized in the presence of 110 pM SP seeds and the indicated concentrations of the various IQGAP1 fragments. b and c, IQGAP1 fragment 744–1657 inhibits actin assembly at barbed ends with a value of $K_d$ of 68 nM. 2.5 μM MgATP-G-actin (10% pyrenyl-labeled) was polymerized in the presence of 105 pM SP seeds and the indicated concentrations of the IQGAP1 fragment 744–1657. d and e, CaM relieves barbed end capping by the IQGAP1 fragment 744–1657. Barbed end growth was measured in the presence of 110 pm spectrin-actin seeds (SP), 2 μM MgATP-G-actin (10% pyrenyl-labeled), and the indicated concentrations of IQGAP1-(744–1657) and Holo-CaM. For clarity, only some data points are shown. f, SDS-PAGE of the various recombinant IQGAP1 fragments purified and used in this work. g, diagram showing the structural organization of IQGAP1, its identified binding partners, and the fragments used in a and b.
The effect of IQGAP1 on N-WASP-stimulated polymerization of actin with Arp2/3 presented the same inhibitory features as on VCA with Arp2/3, over the whole range of concentrations assayed (Fig. 4b). No activation of N-WASP by IQGAP1 was detected in any preparation of pure IQGAP1. Capping protein (CP) was used as a standard barbed end capper to compare its effects on the kinetics of filament branching to those recorded in the presence of IQGAP1. Data (supplemental Fig. S1) indicate...
cate that CP slows down the polymerization process with N-WASP and Arp2/3 in a manner qualitatively similar to IQGAP1, with the difference that CP interacts with high affinity and slow kinetics with barbed ends, while IQGAP1 binds barbed ends in rapid equilibrium with lower affinity.

The activation of N-WASP by several C-terminal fragments of IQGAP1 was not observed either when the fragments were pure. However it was found that partially purified preparations of several fragments (1–1345, 675–1345, 744–1345, 962–1345, 962–1583), all containing the GRD domain previously shown to activate N-WASP (13) strongly activated N-WASP following PreScission cleavage on the glutathione-affinity resin (Fig. 4).

**DISCUSSION**

**IQGAP1 Caps the Barbed Ends of Actin Filaments**

IQGAP1 Caps the Barbed Ends of Actin Filaments —The main point of this work is that IQGAP1 interacts directly with actin not only as a filament bundling factor using its N-terminal calponin homology domain, but also as an actual regulator of assembly dynamics at barbed ends, which IQGAP1 caps through its C-terminal domain. Both filament bundling and capping activities are inhibited by CaM (Ref. 20 and the present work). In our *in vitro* assays we find identical inhibition of capping by calmodulin in the absence and presence of Ca²⁺ ions, while only Ca-calmodulin is reported to inhibit other interactions of IQGAP1 with G-proteins, F-actin, β-catenin (1), E-cadherin, or the BR-CRIB domain of N-WASP. It is possible that the binding of other ligands to IQGAP1 modulates the specificity of Ca-calmodulin to inhibit barbed end capping *in vivo*. In a simple comprehensive view of the data, the protein is tentatively proposed to exist in two main conformations, a CaM-bound "closed" inactive conformation and an "open" active conformation in which a large number of ligand binding sites are exposed (Fig. 5). In this open conformation, we have not detected significant effect of Cdc42, Rac1, and BR-CRIB domain of N-WASP on the barbed end capping activity.

The capping activity of IQGAP1 belongs to the conserved C-terminal region including the IQ motifs and the GRD, which suggests that all members of the IQGAP family share the barbed end capping activity. IQGAP1 has no sequence similarity with any known capping protein. The same absence of sequence signature of the capping activity is displayed by Eps8, another protein that binds both to the sides and to the barbed ends of filaments, so as to bundle and cap them respectively (34). Secondary structure elements are common to several known barbed end cappers. Binding of an amphipathic α-helix to a hydrophobic pocket between subdomains 1 and 3 at the barbed face of actin supports the capping activity of Eps8 (35), gelsolin (36), CapZ (37), twinfilin (38), capping protein (39) as well as the multiple activities of β-thymosin/WH2 domains (40–43). The protein VASP also binds both to the sides of filaments via an F-actin binding region and to the barbed face of actin via a WH2 domain (44), which may assist its processive tracking of barbed ends (45, 46). More detailed studies of the minimum segment of IQGAP1 that displays barbed end capping should reveal whether a similar structural element accounts for barbed end capping activity of IQGAP1.
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Possible Implication of the Barbed End Capping Activity of IQGAP1 in Its in Vivo Function—IQGAP1 plays multiple roles and co-localizes with actin in motile processes such as lamellipodia and invadopodia formation, phagocytosis, exocytosis, cytokinesis, and pedestal formation by EPEC (49). So far all the functions of IQGAP1 and the effects of mutations and deletions have been discussed in relation with its binding to actin filaments via the N-terminal calponin homology domain. The present results question this sole actin binding element as being responsible for the role of IQGAP1 in cellular processes involving actin, and call for a re-examination of the possible functions of IQGAP1 in terms of a CaM-regulated barbed end capper in these processes. Two of these processes, cytokinetic ring regulation and lamellipodia extension, are attractive processes for a putative capping function of IQGAP1 because in both processes regulated filament turnover is playing a role. Lamellipodia and invadopodia are actin-based motile processes driven by the growth of a dendritic array of branched actin filaments induced by WASP/WAVE proteins with Arp2/3 complex, and are known to require capping proteins (50). Capping proteins are also essential for the reconstituted actin-based motility of N-WASP-coated beads (51, 52). The stimulat-
ing effect of IQGAP1 on these processes was first attributed to its activation of N-WASP (12, 13). This conclusion was difficult to reconcile with the facts that lamellipodia form in the total absence of N-WASP (53–55). The present work shows that bacterially expressed full-length IQGAP1 does not activate N-WASP. We cannot dismiss the possibility that full-length IQGAP1 expressed in sf9 cells may have activated N-WASP due to some posttranslational modification (12). However this possibility seems unlikely since the activation was also observed with bacterially expressed C-terminal (13) or N-terminal (12) fragments of IQGAP1. We propose that in previous works N-WASP may have been activated by nucleic acids contaminating the bacterially expressed fragments.

The localization and role of IQGAP1 in lamellipodia and invadopodia would appear consistent with a capping function. The barbed ends should be more than 95% saturated for the capping effect to be effective, which would be difficult to reach with IQGAP1 alone, since it binds barbed ends at least 10-fold less tightly than usual cappers. Consistently, using the IQGAP1 fragment 744–1657 at a concentration of 0.5 μM (the highest amount at which the protein remains soluble), only 88% of barbed end capping was reached, which was not sufficient to promote propulsion of N-WASP-coated beads in the reconsti-
tuted motility assay mimicking lamellipodium extension (supple-
mental Fig. S2). It is possible that binding of other ligands or coherent modifications of IQGAP1 like phosphorylation of Ser-1443 by PKCe (56) up-regulate its capping activity. The fact that a mutant of IQGAP1 that lacks the C-terminal region is unable to promote cytokinesis in yeast but does localize to the cytoki-
netic ring (57), suggests that the barbed end capping activity in the C-terminal region of IQGAP1 supports its function in cyto-
kinesis, whereas the CHD at the N-terminal is sufficient for its localization.

IQGAP1 is thought to link the actin and microtubule cytoskeleton via its direct interaction with APC and CLIP170, two proteins that are targeted to the plus end of growing microtu-

bules by EB1 (16, 58). APC and EB1, like IQGAP1, are involved in mitosis, cell polarity and cell invasion. The binding site of CLIP170 has been located in the C-terminal region (residues 1503–1657) of IQGAP1 (15), which also contains the mDia1-binding region. Future experiments should tell whether IQGAP1 is not merely a passive scaffold but also a coordinator of assembly of the two major polymers of the cytoskeleton.

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