Title: The effect of IQGAP1 on Xenopus embryonic ectoderm requires Cdc42

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Running Title: IQGAP1 activity in *Xenopus* embryos requires Cdc42
IQGAP1 contains a number of protein recognition motifs through which it binds to targets. Several *in vitro* studies have documented that IQGAP1 interacts directly with calmodulin, actin, E-cadherin, β-catenin, and the small GTPases Cdc42 and Rac. Nevertheless, direct demonstration of *in vivo* function of mammalian IQGAP1 is limited. Using a novel assay to evaluate *in vivo* function of IQGAP1, we document here that microinjection of IQGAP1 into early *Xenopus* embryos generates superficial ectoderm lesions at late blastula stages. This activity was retained by the mutated variants of IQGAP1 in which the calponin homology domain or the WW domain was deleted. By contrast, deletion of the IQ (IQGAP1-ΔIQ), Ras-GAP-related (IQGAP1-ΔGRD) or C-terminal (IQGAP1-ΔC) domains abrogated the effect of IQGAP1 on the embryos. None of the latter mutants bound Cdc42, suggesting that the binding of Cdc42 by IQGAP1 is critical for its function. Moreover, overexpression of IQGAP1, but not IQGAP1-ΔGRD, significantly increased the amount of active Cdc42 in embryonic cells. Coinjection of wild type IQGAP1 with dominant Cdc42, but not the dominant negative forms of Rac or Rho, blocked the effect of IQGAP1 on embryonic ectoderm. Together these data indicate that the activity of IQGAP1 in embryonic ectoderm requires Cdc42 function.
INTRODUCTION

The Ras superfamily of GTPases, which has over 80 members, can be divided into several subfamilies. One of these, the Rho family, is comprised of fourteen GTPases, the most extensively characterized of which are Rho, Rac and Cdc42 (1-3). The GTPases cycle between an inactive GDP-bound and an active GTP-bound form. In addition to controlling organization of the cytoskeleton, Cdc42 participates in cell movement, cell cycle progression, gene transcription and cell-cell attachment (1,2,4). These functions are elicited via target proteins which are regulated by direct interaction with GTP-Cdc42. Effectors include p21-activated kinases (5), the Wiskott-Aldrich syndrome protein (WASP) (6), the ACK tyrosine kinase (7), and IQGAP1 (8-11). IQGAP1, originally isolated from metastatic human osteosarcoma tissue (12), contains an IQ domain and a C-terminal region with sequence similarity to the catalytic domain of Ras GTPase activating proteins (GAPs). IQGAP1 contains several protein recognition motifs through which it binds to a number of targets. These motifs include a calponin homology domain (CHD), which binds actin (13); a WW polyproline-binding domain; four IQ motifs, which bind calmodulin (9,13); and a Ras-GAP related domain (GRD), which is necessary for Cdc42 and Rac1 binding (10). In addition, E-cadherin and β-catenin bind directly to IQGAP1 (14-16). IQGAP1 homologues, termed Iqg1/Cyk1p, Rng2p and DGAP1, have been identified in S. cerevisiae, S. pombe and D. discoideum, respectively (17-20).

Functional analysis reveals that yeast IQGAP1-related protein is an important regulator of cellular morphogenesis and induces actin-ring formation during cytokinesis (17,18). The function(s) of mammalian IQGAP1 are less clear. In vitro assays have demonstrated that mammalian IQGAP1 inhibits the intrinsic GTPase activity of Cdc42, keeping it in the active
GTP-bound state (13). Similar inhibition was obtained \textit{in vitro} with the C-terminal half of IQGAP1 (21). Moreover, mammalian IQGAP1 cross-links actin filaments \textit{in vitro} (22,23) and may have a role in cell-cell adhesion (14,16). However, direct demonstration of \textit{in vivo} function of mammalian IQGAP1 is limited. In the present study we demonstrate that IQGAP1 interferes with \textit{Xenopus} embryogenesis \textit{in vivo}, by causing development of ectodermal lesions around the site of RNA microinjection. This effect was not observed with mutant forms of IQGAP1 that do not bind Cdc42 and was abrogated by N17Cdc42, a dominant negative Cdc42 construct, indicating the important role of the interaction between IQGAP1 and Cdc42 \textit{in vivo}. 
EXPERIMENTAL PROCEDURES

IQGAP1 Plasmid Construction – A Myc-tagged wild type human IQGAP1 in pcDNA3 vector (9) was used. IQGAP1 lacking the IQ region (IQGAP1-ΔIQ; amino acids 699-905 deleted) or the C-terminal region (IQGAP1-ΔC; amino acids 1502-1657 deleted) were described previously (16). Deletion of the CHD (IQGAP1-ΔCHD; amino acids 35-265 deleted) was performed by digesting full length IQGAP1 with BamH1 and inserting the resultant 2.6 kilobase (kb) BamH1-BamH1 fragment into pBluescript-KS at the BamH1 site. This construct, named pBluescript-IQN, was cut with Blp1 and AatII and a 4.85 kb fragment was purified from low melting agarose, blunted with T4 polymerase and self-ligated. The construct was named pBluescript-IQN-ΔCHD. Deletion of the WW region (IQGAP1-ΔWW; amino acids 643-744 deleted) was performed by cutting pBluescript-IQN with MscI and partially digesting with DraI. Blunt ends were generated with T4 polymerase. A 5.24 kb fragment was purified from low melting agarose and self-ligated. The construct was named pBluescript-IQN-ΔWW. The BamH1-BamH1 fragments of pBluescript-IQN-ΔCHD and pBluescript-IQN-ΔWW were re-inserted into pcDNA3-IQGAP1 and clones with the right orientation were selected. IQGAP1 lacking the GRD (IQGAP1-ΔGRD; amino acids 1122-1324 deleted) was prepared by cutting the IQGAP1 pcDNA3 with BspE1, generating blunt ends with S1 nuclease and cutting with NheI. Blunt ends were generated with Klenow, a 10.35 kb fragment was isolated from low melting agarose gel and self-ligated. The sequence of all constructs was confirmed by both restriction mapping and DNA sequencing. All mutants migrated to the predicted region on SDS-PAGE (Fig. 3B). Plasmids were purified with a QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer’s instructions.
Cell Culture and Transient Transfection – Chinese hamster ovary (CHO) cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum in a 37 °C humidified incubator. Transient transfections of wild type or mutant IQGAP1 were performed with Fugene 6 (Roche Molecular Biochemicals) following the instructions provided by the manufacturer. Briefly, CHO cells were grown to 70-80% confluence in a 100 mm dish. Fugene 6 (6 µl) was mixed with 2 µg of plasmid DNA and added to the cells. After 48 h, cells were harvested, lysed and processed as described below.

Immunoprecipitation and Western Blotting – CHO cells were washed three times in phosphate-buffered saline (PBS) (145 mM NaCl, 12 mM Na₂HPO₄, and 4 mM Na₂HPO₄, pH 7.2), lysed in 50 mM Tris, pH 7.4, 150 mM NaCl, and 1% (v/v) Triton X-100 (buffer A) with 1 mM CaCl₂ or 1 mM EGTA and quick frozen in methanol/solid CO₂. Samples were clarified by centrifugation at 15,000 x g for 5 min at 4 °C and precleared with protein A-Sepharose. Protein concentrations were measured, equal amounts of protein were immunoprecipitated with anti-IQGAP1 antiserum (16) or nonimmune rabbit serum for 2 h at 4 °C and immune complexes were collected with protein A-Sepharose. After sedimentation by centrifugation, samples were washed five times with buffer A, resolved by SDS-PAGE, and transferred to PVDF. Blots were probed with anti-IQGAP1 (16), anti-Cdc42 (24), anti-Myc (16) or anti-actin (Santa Cruz Biotechnology) primary antibodies, followed by the appropriate horseradish peroxidase-conjugated secondary antibody, and developed by enhanced chemiluminescence (ECL, Amersham). Embryonic lysates were prepared by homogenization of microinjected embryos cultured until stages 10-10+ (ten embryos per 200 µl of buffer A). Lysates were cleared by centrifugation and processed for immunoprecipitation and Western blotting as described above.
**Preparation of mRNA and embryo microinjections** – Capped synthetic mRNAs were transcribed in vitro from linearized plasmid DNA with SP6 or T7 RNA polymerase (25) using mMESSAGE mMACHINE kits (Ambion, Austin, TX) following manufacturer’s instructions. The wild type and mutant IQGAP1 mRNAs were linearized with *Sma*I and synthesized with T7 RNA polymerase. Myc-tagged dominant negative forms of Rho, Rac and Cdc42 – N19RhoA, N17Rac1 and N17Cdc42, respectively – and constitutively active Cdc42 (L61Cdc42) (26) were kindly provided by Alan Hall (University College of London). The Rho family plasmids were linearized with *Dra*III and transcribed with SP6 polymerase. All RNAs were evaluated and quantified by formaldehyde-agarose gel electrophoresis (27) prior to microinjection.

Eggs and embryos were obtained from *Xenopus* females and cultured in 0.1x MMR as described (28). Embryonic stages were determined according to Nieuwkoop and Faber (29). For microinjection, embryos were transferred to 3% Ficoll 400 (Pharmacia) in 0.5x MMR and injected at the 4-8-cell stage with 10 nl of a solution containing 2-4 ng of IQGAP1 RNAs, or Rho family RNAs, unless specified otherwise. Injections were carried out in two opposite animal pole blastomeres for morphological and biochemical analysis. Data represent the results obtained from at least three independent experiments.

**Detection of Activated Cdc42** – Active Cdc42 was measured with a glutathione S-transferase (GST) fusion construct of the GTPase binding domain (GBD) of WASP as previously described (24). Briefly, each blastomere of four-cell embryos was injected with 4 ng of either wild type IQGAP1 or IQGAP1-ΔGRD mRNAs and embryos were cultured until st. 10+. Lysates prepared from equal size experimental groups (ten embryos/200 µl of buffer A), were incubated with 5 µg of GST-WASP-GBD for 2 h at 4 °C. Complexes were collected with glutathione-Sepharose,
washed and resolved by SDS-PAGE. The resultant Western blots were probed with anti-Cdc42 antibody (24).

$Miscellaneous$ – ECL signals were analysed by densitometry and quantified with NIH Image. Statistical significance was evaluated by Student’s $t$ test with InStat software (GraphPad Software, Inc). Protein concentrations were measured with the DC Protein Assay (Bio-Rad).
RESULTS

The Effect of IQGAP1 on Xenopus Ectoderm - To gain insight into IQGAP1 functions, we synthesized mRNA encoding full length IQGAP1 protein and injected it into Xenopus early embryos, a rapid in vivo model for gene expression. Injected embryos developed normally until midblastula stages, when compared to uninjected controls (Fig. 1). At mid-late blastula stages, depigmentation of embryonic blastomeres was observed around the injection site, suggesting that pigment granules lost their cortical localization due to rearrangement of cytoskeletal structure (30). By gastrulation, the animal hemisphere of injected embryos developed ectodermal lesions, in which cells appeared to have lost adhesion (Fig. 1). These lesions were observed in the majority of injected embryos (77%, n=92). By contrast, control uninjected embryos and embryos injected with LacZ RNA developed normally (Fig. 1, n=150, and data not shown). The observed morphological abnormalities of embryos overexpressing IQGAP1 reflect an in vivo activity of IQGAP1 and provide a novel functional assay.

Binding of Cdc42 to IQGAP1 – In order to identify the target which mediated the IQGAP1 effect on embryonic ectoderm, we generated a series of mutant forms of IQGAP1 (Fig. 2A). All of the major identified protein interaction motifs and the C-terminal tail of IQGAP1 were individually deleted. The ability of these mutant constructs to bind endogenous Cdc42 was examined. CHO cells were transiently transfected with IQGAP1 and equal amounts of cell lysate were immunoprecipitated. As previously demonstrated (13), Cdc42 co-immunoprecipitated with wild-type IQGAP1 (Fig. 2B). Deletion of the CHD or WW domains did not alter binding of Cdc42 to IQGAP1. By contrast, Cdc42 binding was essentially abolished by deleting the IQ domain, the
GRD or the C-terminal region of IQGAP1 (Fig. 2B). Because endogenous IQGAP1 concentrations in CHO cells are low, the amount of recombinant IQGAP1 is several fold higher than the endogenous protein (Fig. 2C, compare vector and IQGAP1-transfected samples).

Effect of Mutant IQGAP1 Constructs on Xenopus Ectoderm – To define which domain of IQGAP1 is critical for its functional activity in embryonic ectoderm, we tested the ability of different mutated forms of IQGAP1 to induce ectodermal lesions. RNA microinjections revealed that deletion of the CHD or WW domains did not alter the activity of IQGAP1 ($n=70$ and $n=62$, respectively), whereas deletion of the IQ domain, the GRD or the C-terminus of IQGAP1 completely abrogated its activity in early embryos (Fig. 3A, $n=61$, 76 and 49, respectively).

To ensure that the injected mRNA was translated into protein, after microinjection embryos were lysed and proteins were resolved by SDS-PAGE. Immunoblotting revealed that all of the IQGAP1 constructs were expressed (Fig. 3B). Probing the blots for actin verified that equal amounts of protein lysate were loaded on the gel (Fig. 3B). These findings confirm that the indicated IQGAP1 mutants, although present, did not have a functional effect. Importantly, the constructs that lost functional activity are the same constructs that no longer bind Cdc42 (Fig. 2B). This result indicates that the binding of Cdc42 is essential for the functional activity of IQGAP1 in the embryos.

The Effect of IQGAP1 on Animal Pole Cells Requires Cdc42 – Comparison of mutant constructs suggested that the property of IQGAP1 to produce ectodermal lesions may be due to the modulation of the activities of the Rho family GTPases in early embryos. To address this
hypothesis, we compared the amount of active Cdc42 that binds GST-WASP-GBD (24) in embryos injected with the wild-type or mutant IQGAP1. Since GST-WASP-GBD binds only GTP-bound Cdc42, the amount of bound Cdc42 reflects the relative amount of active Cdc42 in the lysate (24). Injection of mRNA for wild type IQGAP1 augmented the amount of active Cdc42 in embryo lysates by 41% (p<0.05) (Fig. 4A). By contrast, IQGAP1-ΔGRD did not increase the amount of active Cdc42. This result indicates that overexpression of IQGAP1 increases the amount of active Cdc42 in the embryo, and is consistent with our earlier observation that wild type IQGAP1 downregulates the intrinsic GTPase activity of Cdc42 in vitro (13).

We next tested the hypothesis that the effect of IQGAP1 on embryonic ectoderm is due to activation of endogenous Cdc42. This was evaluated by co-injecting dominant negative forms of Rho family proteins, namely N19Rho, N17Rac1 or N17Cdc42 (26), with wild type IQGAP1 (Fig. 5A). Consistent with the failure of IQGAP1 to bind RhoA (10), N19Rho did not have the effect on the morphology or frequency of ectodermal lesions triggered by IQGAP1. Lesions were observed in 80-95% of embryos coinjected with N19Rho and IQGAP1 RNAs (n=65).

Although Rac1 binds to IQGAP1 (10), N17Rac1 did not attenuate the effect of IQGAP1 (n=82). By contrast, co-injection of N17Cdc42 almost completely abrogated the change in Xenopus animal pole cells elicited by IQGAP1. Small lesions formed in less than one quarter of injected embryos, whereas others developed normally (n=78, Fig. 5A). Injection of the same doses of N19Rho, N17Rac1 or N17Cdc42 without IQGAP1 did not have a significant effect on normal embryonic development (data not shown).

Biochemical analysis was performed to evaluate the effects of the dominant negative Rho constructs on the amount of active Cdc42. In agreement with the morphological data, N17Cdc42
abrogated the increase in GTP-Cdc42 formation produced by microinjection of IQGAP1 (Fig. 4B). By contrast, N19Rho and N17Rac1 did not attenuate the increase in active Cdc42 observed in cells microinjected with IQGAP1. The dominant band in the Western blot of total Cdc42 (Fig. 4B, lane 2) is the injected N17Cdc42 construct. Longer exposure of the blot revealed that equal amounts of total Cdc42 were present in all the samples (data not shown).

To verify that the Rho family mRNAs were translated into protein, aliquots of embryo lysates were subjected to Western blotting. Probing the blots with anti-Myc antibody revealed the expression of the dominant negative Rac, Cdc42 and Rho proteins (Fig. 5B). Inspection of the blots also verified that injected IQGAP1 protein was expressed in the embryos and that equal amounts of protein lysate were loaded on the gel (Fig. 5B, top and middle panels, respectively). Note that IQGAP1 is not detectable in the Western blots from uninjected embryos (Fig. 4A). The anti-IQGAP1 antibody, which was generated using the N-terminal region (amino acids 1-863) of human IQGAP1 (13), may not recognize Xenopus IQGAP1.
DISCUSSION

In this report, we describe the *in vivo* activity of IQGAP1 overexpressed in early frog embryos. Injection of wild type IQGAP1 into *Xenopus* early embryos induced changes at the mid-blastula stages, with more dramatic effects evident by gastrulation. Based on the morphological appearance of induced lesions in presumptive ectoderm, IQGAP1 is likely to affect the cytoskeletal architecture and cell adhesion. IQGAP1 interacts with several proteins that could modulate the cytoskeleton, including actin, E-cadherin, β-catenin, Cdc42 and Rac (10,13,14). To identify the target, we created a series of mutant IQGAP1 constructs. All of the IQGAP1 regions that are known to interact with targets or contain domains likely to be involved in protein-protein interactions, e.g., the WW domain, were deleted. The known binding domains are the CHD, actin binding domain (13); IQ region, calmodulin-binding domain (13); GRD, necessary for Cdc42 and Rac binding (10) and the C-terminal tail, which is required for binding of E-cadherin and β-catenin (14). Analysis of these mutant IQGAP1 proteins revealed that Cdc42 binding was necessary to produce the phenotype.

The specific region of IQGAP1 to which Cdc42 binds has not been identified. *In vitro* analysis with purified proteins demonstrated that the C-terminal half of IQGAP1 (amino acid residues 864-1657) bound Cdc42, but deletion of the GRD prevented Cdc42 binding (10). Interestingly, residues in addition to the GRD, particularly near the C-terminus, were required for binding of Cdc42 to the recombinant C-terminal half of IQGAP1 (10). Our data with IQGAP1-ΔGRD and IQGAP1-ΔC extend these observations to reveal that both the GRD and C-terminal region are necessary for binding of intact IQGAP1 to endogenous Cdc42 in a normal cell milieu. Less anticipated was the finding that IQGAP1-ΔIQ was unable to bind Cdc42. Because
Ca\textsuperscript{2+}/calmodulin, which binds predominantly to the IQ region of IQGAP1 (13), prevents binding of Cdc42 to IQGAP1 (9), it seems reasonable to infer that the N-terminal half of IQGAP1 imparts structural information to the intact protein. Furthermore, the conformation of IQGAP1-ΔIQ is likely to resemble that of wild type IQGAP1 bound to Ca\textsuperscript{2+}/calmodulin. Detailed structural analysis of the IQGAP1 constructs is necessary to validate this hypothesis.

Notwithstanding the documentation that IQGAP1 interacts with several proteins that regulate the cytoskeleton and cell-cell adhesion, our data identify Cdc42 as an essential component of the effect of IQGAP1 on \textit{Xenopus} ectoderm. This interpretation is supported by several findings. All deletion mutants of IQGAP1 that bound Cdc42 produced the phenotype, while none of the IQGAP1 deletion mutants that failed to bind Cdc42 elicited a detectable effect. In addition, injection of wild-type IQGAP1, but not IQGAP1-ΔGRD that does not bind Cdc42, significantly increased the amount of active Cdc42 in embryo lysates. Although exhibiting substantial sequence similarity to the catalytic domain of RasGAPs (12), \textit{in vitro} analysis revealed that IQGAP1 inhibits (rather than increases, as would be anticipated for a GAP) the intrinsic GTPase activity of Cdc42 (13,21). These observations led Ho et al. to hypothesize that, by maintaining Cdc42 in the active GTP-bound form, IQGAP1 could potentially enhance Cdc42 function in cells (13). The findings in this work validate that hypothesis.

Additional evidence indicating that Cdc42 is required for IQGAP1 function is the demonstration that co-injection of the dominant negative Cdc42 construct, N17Cdc42, abrogated the effect of IQGAP1 on \textit{Xenopus} ectoderm \textit{in vivo}. Rho, which has pronounced effects on the cytoskeleton (3), does not bind to IQGAP1 (10). Consistent with this observation, N19Rho did not attenuate the effect of IQGAP1 on the embryo. Moreover, although Rac organizes the cytoskeleton and binds to IQGAP1 (10), N17Rac did not modulate the phenotype produced by
IQGAP1. Overexpression of L61Cdc42, a constitutively active Cdc42, RNA resulted in a qualitatively different phenotype, suggesting that activation of Cdc42 is not sufficient for the observed IQGAP1 effect. At high doses (100-500 pg), L61Cdc42 RNA blocked cytokinesis, whereas at lower doses (10-40 pg), it had no visible effect on Xenopus ectoderm (data not shown and (31). Thus, while our data do not rule out the involvement of other targets of IQGAP1, they clearly indicate that Cdc42 is necessary, but not sufficient, for the IQGAP1-induced phenotype in early Xenopus embryos.

The mechanism by which the interaction between IQGAP1 and Cdc42 generates the phenotype in the Xenopus embryos is not completely understood. At the present time, we have not identified the downstream target(s) that mediate the morphological changes produced by the IQGAP1-Cdc42 complex. IQGAP1, which forms a link between Cdc42 and the actin cytoskeleton (32), exhibits increased F-actin-cross-linking activity in vitro in response to active Cdc42 (22). Moreover, multiple effector proteins for Cdc42 have been identified (for review, see Ref (1), several of which are known to alter cell morphology directly or indirectly. These range from protein kinases to lipid kinases to scaffolding proteins. While many of these targets are effectors for both Rac and Cdc42, some appear to be selective for Cdc42. For example, activation of PAK4, an effector for Cdc42, induces morphological changes and alters cell adhesion (33). Cdc42 is also implicated in the regulation of cadherin function (14,34). Keratinocytes overexpressing CEPs (Cdc42 effector proteins) had reduced F-actin and E-cadherin at adherens junctions (34). However, many questions remain unanswered and the specific role of Cdc42 in the regulation of cell-cell adhesion is not fully understood (35). Regardless of the mechanism, our data establish that IQGAP1 maintains Cdc42 in the active GTP-bound state, culminating in functional sequelae in intact cells.
REFERENCES


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The abbreviations used are: GAP, GTPase activating protein; PVDF, polyvinylidene difluoride; PAGE, polyacrylamide gel electrophoresis; CHD, calponin homology domain; GRD, GTPase regulatory domain; WASP, Wiskott Aldrich Syndrome protein; GBD, GTPase binding domain; GST, glutathione S-transferase; CHO, Chinese hamster ovary.
FIGURE LEGENDS

Fig. 1. **Effect of IQGAP1 on Xenopus ectoderm.** Two animal blastomeres of 4-8 cell embryos were each injected with 2 ng of IQGAP1 mRNA and allowed to develop. Control uninjected embryos are shown in the upper panels (animal pole view). Embryos injected with IQGAP1 mRNA are shown at st. 8.5 and 10 (lower panels). Large ectoderm lesions are visible in the animal pole of injected embryos at st. 10.

Fig. 2. **Effect of IQGAP1 mutations on Cdc42 binding.** A, schematic representation of IQGAP1 constructs. Wild type and deletion mutants of IQGAP1 are depicted. The identified protein interaction motifs and the specific amino acid residues deleted are indicated for each mutant. B, CHO cells were transiently transfected to equivalent concentrations with wild type (WT) IQGAP1, the indicated mutant forms of IQGAP1 or vector (V) as described under “Experimental Procedures”. After 48 h, cells were lysed and protein concentrations were measured. Equal amounts of protein lysate were immunoprecipitated with anti-IQGAP1 antibody (IP:αIQGAP1), and samples were resolved by SDS-PAGE and immunoblotting. The upper and lower halves of the blot were probed with anti-IQGAP1 and anti-Cdc42 antibodies, respectively. C, equal amounts of protein lysate were resolved by SDS-PAGE and transferred to PVDF, and blots were probed with anti-IQGAP1 antibody (upper panel) or anti-Cdc42 antibody (lower panel). Data are representative of three independent experiments.

Fig. 3. **Different domains of IQGAP1 are necessary for its activity in Xenopus animal pole cells.** A, *Xenopus* embryos were injected with mRNAs encoding the wild-type or different
mutated forms of IQGAP1 as described in Fig. 1 and morphological phenotypes were assessed at st. 10. Data are representative of 3 independent experiments. B, expression of different forms of IQGAP1 in injected embryos. Lysates of embryos shown in panel A were subjected to Western analysis. Blots were probed with anti-IQGAP1 (upper panel) or anti-actin (lower panel) antibodies. A representative experiment is shown.

Fig. 4. Embryos injected with IQGAP1 mRNA contain an increased amount of GTP-Cdc42. A, embryos were injected with mRNA of wild type IQGAP1 (WT) or IQGAP1-ΔGRD (ΔGRD), or left uninjected (C). Protein lysates were prepared at st. 10. Lysates were loaded directly onto the gel for analysis with anti-IQGAP1 (top panel) or anti-Cdc42 (second panel) antibodies, or incubated with GST-WASP-GBD, washed and probed with anti-Cdc42 antibodies (third panel, Active Cdc42). The relative amount of active Cdc42 in each sample was quantified by densitometry. Results, presented in the bottom panel, are expressed relative to uninjected samples (C) and represent the means ± S.E. of four independent experimental determinations with two different preparations of mRNA. *, significantly different from control (p <0.05). B, embryos at the 4 cell stage were left uninjected (C) or each blastomere was injected with 2 ng IQGAP1 mRNA and mRNAs encoding the dominant negative forms of Cdc42 (N17Cdc42), RhoA (N19RhoA) or Rac1 (N17Rac1). Samples were processed for total and active Cdc42 as described for panel A, above. Data are representative of three independent experimental determinations.

Fig. 5. The effect of IQGAP1 on animal pole cells requires Cdc42. A, Xenopus embryos at the 4-8 cell stage were co-injected with 2 ng of IQGAP1 mRNA and mRNAs encoding
dominant negative forms of RhoA, Rac1 and Cdc42 into two animal pole blastomeres, as indicated. The effect was scored morphologically at stage 10. Data show a representative set of four independent experiments. B, lysates of the injected embryos shown in panel A were subjected to Western analysis. Blots were probed with anti-IQGAP1 (top panel), anti-actin (middle panel) or anti-Myc (bottom panel) antibodies. The same pattern of migration of the dominant negative Rho family proteins was observed when the cDNAs were transiently transfected into mammalian cells (data not shown). Data are representative of two independent experimental determinations.
3B.

IQGAP1

Actin

WT ΔCHD ΔWW ΔIQ ΔGRD ΔC
4A.

IQGAP1

Total Cdc42

Active Cdc42

![Graph showing Active Cdc42 levels for C, ΔGRD, and WT conditions with a significant difference marked by an asterisk.](image)
5A.
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