Iqg1p, a Yeast Homologue of the Mammalian IQGAPs, Mediates Cdc42p Effects on the Actin Cytoskeleton

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Abstract. The Rho-type GTPase Cdc42p has been implicated in diverse cellular functions including cell shape, cell motility, and cytokinesis, all of which involve the reorganization of the actin cytoskeleton. Targets of Cdc42p that interface the actin cytoskeleton are likely candidates for mediating cellular activities. In this report, we identify and characterize a yeast homologue for the mammalian IQGAP, a cytoskeletal target for Cdc42p. The yeast IQGAP homologue, designated Iqg1p, displays a two-hybrid interaction with activated Cdc42p and coimmunoprecipitates with actin filaments. Deletion of IQG1 results in a temperature-sensitive lethality and causes aberrant morphologies including elongated and round multinucleated cells. This together with its localization at the mother–bud neck, suggest that Iqg1p promotes budding and cytokinesis. At restrictive temperatures, the vacuoles of the mutant cells enlarge and vesicles accumulate in the bud. Interestingly, Iqg1p shows two-hybrid interactions with the ankyrin repeat–containing protein, Akr1p (Kao, L.-R., J. Peterson, J. Ruiru, L. Bender, and A. Bender. 1996. Mol. Cell. Biol. 16:168–178), which inhibits pheromone signaling and appears to promote cytokinesis and/or trafficking. We also show two-hybrid interactions between Iqg1p and Afr1p, a septin-binding protein involved in projection formation (Konopka, J.B., C. DeMattei, and C. Davis. 1995. Mol. Cell. Biol. 15:723–730). We propose that Iqg1p acts as a scaffold to recruit and localize a protein complex involved in actin-based cellular functions and thus mediates the regulatory effects of Cdc42p on the actin cytoskeleton.

Key words: IQGAP • IQG1 • Cdc42 • morphogenesis • cytoskeleton

The Rho-type GTPase Cdc42p is structurally and functionally conserved from yeast to mammals. It has been implicated in a variety of fundamental cellular activities ranging from cytoskeletal organization (Ridley et al., 1995) to transcriptional activation (Bagrodia et al., 1995; Coso et al., 1995), cell proliferation (Olson et al., 1995; Qiu et al., 1995), intracellular trafficking (Singier et al., 1995; Erickson et al., 1996), and AIDS etiology (Sawai et al., 1996). Thus, Cdc42p may require a number of regulatory factors as well as target molecules to mediate its diverse cellular functions.

Like all members of the Ras superfamily, Cdc42p cycles between an inactive GDP-bound state and an active GTP-bound state at rates defined by specific regulatory proteins. These include members of the Dbl family of oncoproteins, which serve as guanine nucleotide exchange factors that stimulate GTP-GDP exchange (Cerione and Zheng, 1996), the Cdc42-GTPase–activating proteins (GAPs)1 and other members of the Rho-GAP family (Barfod et al., 1993; Zheng et al., 1993; Lamarche and Hall, 1994), and the Rho-GDP dissociation inhibitors (Rho-GDI) (Leonard et al., 1992; Koch et al., 1997).

The GTP-bound form of Cdc42p interacts with a number of different target/effectors and initiates downstream signaling cascades that result in biological responses. Among the best-known targets are members of the family of p21-activated serine/threonine kinases (Paks) that are stimulated upon the binding of activated Cdc42p or Rac, and are thought to initiate signaling pathways that lead to the nucleus and the activation of two stress-responsive nuclear Map kinases, the c-Jun kinase (JNK1) and p38 (Bagrodia et al., 1995; Coso et al., 1995; Minden et al., 1995; Nobes and Hall, 1995). More recently, various other putative targets for Cdc42p have been identified and proposed as possible interfaces between Cdc42p and the actin cytoskeleton including WASP (Symons et al., 1996) and the IQGAPs (Brill et al., 1996; Hart et al., 1996; McCallum et al., 1996; Erickson et al., 1997).

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1. Abbreviations used in this paper: CHD, calponin homology domain; GAP, Cdc42-GTPase–activating protein; HA, hemagglutinin.
The IQGAPs are especially interesting because they contain a number of different motifs, suggesting that these molecules may function as signaling scaffolds. These include a RasGAP homology domain that appears to contain the binding site for Cdc42p, a calponin homology domain (CHD) that most likely accounts for the binding of IQGAP to F-actin (Bashour et al., 1997; Erickson et al., 1997), and IQ motifs implicated in binding calmodulin. The binding of calmodulin to IQGAP has been suggested to weaken its affinity for F-actin (Bashour et al., 1997) and Cdc42p (Joyal et al., 1997).

Another interesting feature of the IQGAPs is that we have recently found that they are localized to the Golgi, as well as the plasma membrane and cytosol in mammalian cells (McCallum et al., 1998). The Golgi localization may reflect the presence of Cdc42p, which we earlier showed was predominantly present in the Golgi membranes of most cells, and whose cellular localization was influenced by the Arf GTPase (Erickson et al., 1996). This, taken together with the fact that Cdc42p can act with Arf to synergistically activate the Golgi membrane-associated phospholipase D (Brown et al., 1993), raises the possibility that in addition to influencing the cytoskeletal architecture and events in the nucleus, Cdc42p may participate in some aspect of intracellular trafficking.

Cdc42p may also play a role in cytokinesis (Dutartre et al., 1996). Transfection of dominant-active Cdc42p produces giant multinucleated cells defective in cytokinesis after the reorganization of F-actin. Targets for Cdc42p that interface actin microfilaments may mediate this role in cytokinesis and related processes involving the reorganization of the actin cytoskeleton. IQGAP is especially well suited for this role because, aside from its multidomain feature, it also appears to be a predominant target/effector for Cdc42p in most cells (Erickson et al., 1997). An understanding of its function and regulation could provide important insights into the biological actions of Cdc42p. However, thus far it has been difficult to study IQGAP function in mammalian cells and so we have turned to yeast, where a number of genetic studies have already been applied to Cdc42p and its regulators and targets (Adams et al., 1990; Johnson and Pringle, 1990; Chant and Herskowitz, 1991; Ziman et al., 1993; Stevenson et al., 1995; Evangelista, 1997). In this report, we characterize the yeast homologue of IQGAP, designated lqg1p, and present evidence suggesting that it provides a link between Cdc42p and pathways involved in yeast cell polarity, morphogenesis, and cytokinesis.

Materials and Methods

Strains, Media, and Genetic Manipulations

The Escherichia coli strain used in this study for routine cloning was DH5α (GIBCO BRL, Gaithersburg, MD). Yeast strains used in this study are listed in Table I; plasmids used are listed in Table II. Yeast growth, media, and genetic techniques were performed as described in Guthrie and Fink (1991). Yeast transformation was done using a modified lithium acetate method (Elbe, 1992).

Cloning and Strain Construction

To construct the IQG1 deletion strain, a method described by Baudin et al. (1993) was followed. Briefly, two primers were designed (DEL5: 5'-GCTACG-...-3' and DEL3: 5'-GCTACG-...-3'). The confirmed deletion in the stable His2 haploid strain was amplified by PCR using primers for the flanking sequences of the IQG1 gene and the product (IQG1 ORF) was purified from agarose gel, transformed into the MO1 diploid strain, and then selected on plates lacking histidine. The resultant heterozygous diploid was prepared from both His2+ colonies and analyzed by PCR using primers for the flanking sequences to clone the IQG1 gene. Each contained sequences for deleting most of the IQG1 gene by homologous recombination followed by sequences for amplification of the HIS3 gene as a selectable marker. The plasmid Ydp was used to amplify the HIS3 gene and the product (IQG1::HIS3) was purified from agarose gel, transformed into the MO1 diploid strain, and then selected on plates lacking histidine. The resultant heterozygous diploid was sporulated and the tetrads were dissected and analyzed. The tetrads showed 2:2 segregation of the His+ marker at room temperature. To confirm the IQG1 deletion in the stable His+ colonies, genomic DNA was prepared from both His+ and His− (including a [MO2] and an a [MO3] colony) and their colonies and analyzed by PCR using primers for the flanking sequences of IQG1. IQG1 was amplified by PCR using primers 5'-ATGACAGCATATTCAGGCTCTCCTTCG-3' and 5'-ATGACAGCATATTCAGGCTCTCCTTCGTAAGATGACAGCATATTCAGGCTCTCCTTCG-3'. As expected, genomic DNA from three independent His− colonies yielded a 1.6-kb fragment corresponding to the marker gene plus some sequences of the IQG1 ORF. The His− colonies produced a 4.5-kb fragment corresponding to the IQG1 ORF. The confirmed a and a IQG1−/− strains were mated and y-genes were selected to produce a homozygous diploid (MO4). The primers, YQ5 and YQ3, were used in combination with different restriction site sequences to clone the IQG1 gene into the plasmids listed in Table II. The IQG1 contained within these plasmids fully complements the phenotypes of the His− colonies. To construct double mutants of IQG1 and each of AKR1 and AKR1, the IQG1::HIS3-deleting fragment was transformed into the haploid strains YQ55 (akr1::Δ) and YQ26 (akr1::Δ) and double mutants were selected on plates lacking histidine and uracil. To construct aakr1Δ akr1::Δ double mutants, the aakr1::Δ fragment was isolated from the pJK89 plasmid by digesting with BamHI and SalI and transformed into both aakr1Δ and aakr1Δ haploid strains to delete the AKR1 gene and double mutants were selected on plates lacking uracil.

Table I. Yeast Strains Used in This Study

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<th>Strain</th>
<th>Genotype</th>
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<td>CUY29</td>
<td>MATa Gal ura3-52 leu2-3,112 his3-D200 lys2-801 ade2</td>
<td>Huffaker Lab</td>
</tr>
<tr>
<td>CUY30</td>
<td>MATa Gal ura3-52 leu2-3,112 his3-D200 lys2-801 ade2</td>
<td>Huffaker Lab</td>
</tr>
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<td>MO1</td>
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<td>This study</td>
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<td>MO2</td>
<td>MATa Gal ura3-52 leu2-3,112 his3-D200 lys2-801 ade2</td>
<td>This study</td>
</tr>
<tr>
<td>MO3</td>
<td>MATa Gal ura3-52 leu2-3,112 his3-D200 lys2-801 ade2</td>
<td>This study</td>
</tr>
<tr>
<td>MO4</td>
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<td>This study</td>
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<td>Y935</td>
<td>MATa akr1-1ura32 trpl1 ade2 eu2</td>
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<td>JK211-5-3</td>
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<td>CB001</td>
<td>MATa ura3-5 trpl1 his3 leu2</td>
<td>Y. Kawasaki</td>
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<td>CTY10-5D</td>
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<tr>
<td>L40</td>
<td>MATa his3-D200 lys2-801 ade2ura3-1 trpl1-901 lys2-3,112 HIS3:: (LexAop)</td>
<td>R. Sternglanz</td>
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AACAGTTCGACAGCAATTTGTCAACAAAAAGTAAAGATTCCGCCTGTGCCGCTCCTTAGC-3' and DEL3: 5'-GCTTGTGCTTCATT-TAAATCTCATCCCTGCAAATCAGCTTCTCCTCTCGTTCA-GAATGACAGCG-3'. Each contained sequences for deleting most of the IQG1 gene by homologous recombination followed by sequences for amplification of the HIS3 gene as a selectable marker. The plasmid Zdp was used to amplify the HIS3 gene and the product (IQG1Δ::HIS3) was purified from agarose gel, transformed into the MO1 diploid strain, and then selected on plates lacking histidine. The resultant heterozygous diploid was sporulated and the tetrads were dissected and analyzed. The tetrads showed 2:2 segregation of the His+ marker at room temperature. To confirm the IQG1 deletion in the stable His+ colonies, genomic DNA was prepared from both His+ (including an a [MO2] and an a [MO3] colony) and His− colonies and analyzed by PCR using primers for the flanking sequences of IQG1. IQG1 was amplified by PCR using primers 5'-ATGACAGCATATTCAGGCTCTCCTTCG-3' and 5'-ATGACAGCATATTCAGGCTCTCCTTCGTAAGATGACAGCATATTCAGGCTCTCCTTCG-3'. As expected, genomic DNA from three independent His− colonies yielded a 1.6-kb fragment corresponding to the marker gene plus some sequences of the IQG1 ORF. The His− colonies produced a 4.5-kb fragment corresponding to the IQG1 ORF. The confirmed a and a IQG1−/− strains were mated and y-genes were selected to produce a homozygous diploid (MO4). The primers, YQ5 and YQ3, were used in combination with different restriction site sequences to clone the IQG1 gene into the plasmids listed in Table II. The IQG1 contained within these plasmids fully complements the phenotypes of the His− colonies. To construct double mutants of IQG1 and each of AKR1 and AKR1, the IQG1Δ::HIS3-deleting fragment was transformed into the haploid strains YQ55 (akr1::Δ) and YQ26 (akr1::Δ), and double mutants were selected on plates lacking histidine and uracil. To construct aakr1Δ akr1::Δ double mutants, the aakr1::Δ fragment was isolated from the pJK89 plasmid by digesting with BamHI and SalI and transformed into both aakr1::Δ and aakr1::Δ haploid strains to delete the AKR1 gene and double mutants were selected on plates lacking uracil.

α Factor Arrest and Halo Assays

Haploid cells were grown to early log phase, α factor was added to 4 μg/ml, and growth was continued for 3 h. Cells were fixed directly in the growth medium by adding 37% formaldehyde to 3.7% and incubated for another
Table II. Plasmids Used in This Study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Source</th>
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<td>pBTM116</td>
<td>2 μmTRP1LeXa (1–202)</td>
<td>S. Fields</td>
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<tr>
<td>pACT2</td>
<td>2 μmLEU2 Gal4 (768–881)</td>
<td>S. Elledge</td>
</tr>
<tr>
<td>pGAD-C2</td>
<td>2 μmLEU2 Gal4 (768–881)</td>
<td>James et al., 1996</td>
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<td>YEpD</td>
<td>pUC9-HIS3 cassette</td>
<td>Beren et al., 1991</td>
</tr>
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<td>pEG202CDC42</td>
<td>pEG202 with LEXA-CDC42 gene fusion</td>
<td>Stevenson et al., 1995</td>
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<td>pEG202 with LEXA-CDC42 gene fusion with isoprenylation site mutation</td>
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<td>pEG202-CDC42G12V</td>
<td>pEG202 with LEXA-CDC42 constitutively activated mutant gene fusion</td>
<td>Stevenson et al., 1995</td>
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<tr>
<td>pEG202-CDC42G12V+</td>
<td>pEG202 with LEXA-CDC42 constitutively activated mutant gene fusion</td>
<td>Stevenson et al., 1995</td>
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<td>pJG4-5 CDC42G12V</td>
<td>pEG202 with LEXA-CDC42 constitutively inactive mutant gene fusion</td>
<td>Stevenson et al., 1995</td>
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<td>pLAF</td>
<td>pBTM116-LEXA-AFR1 gene fusion</td>
<td>Konopka et al., 1995</td>
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<td>pJK38</td>
<td>Bluescript-af1::URA3</td>
<td>Konopka et al., 1995</td>
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<td>pBP659</td>
<td>pBTM116-LEXA-AKR1 gene fusion</td>
<td>Kao et al., 1996</td>
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<td>pBT17</td>
<td>pBTM116-LEXA-IQG1 gene fusion</td>
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<td>pA14</td>
<td>pGAD-C2-GAL4-IQG1 gene fusion</td>
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<td>pBT6</td>
<td>pBTM116-LEXA-IQG1 (GRD-domain) gene fusion</td>
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<td>pC11</td>
<td>pGAD-C2-GAL4-IQG1 (GRD-domain) gene fusion</td>
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<tr>
<td>pA1</td>
<td>pACT2-GAL4-HA-IQG1 gene fusion</td>
<td>This study</td>
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</table>

3 h; the cells were then briefly sonicated and processed for immunofluorescence or visualized by light microscopy. For halo assays, 15 μl of 40 μg/ml α factor were spotted on sterile filler disks placed on lawns of plates, plates were incubated for 3 d at room temperature, and then photographed.

Indirect Immunofluorescence

Formaldehyde-fixed diploid (MOI and MO4) cells were processed by procedures previously described (Kilmartin and Adams, 1984; Ziman et al., 1993). For Cdc42p immunofluorescence, an antibody sandwich technique was used as described in Ziman et al. (1993); affinity-purified anti-Cdc42p (1:50) was followed by AffiniPure goat (1:1,000), and rhodamine-labeled goat anti–rabbit (1:80) antibodies. AffiniPure secondary antibodies were obtained from Jackson Immuno Research Laboratories (West Grove, PA). Affinity-purified anti-calmodulin antibody (1:200) was a gift from Dr. T. Davis (Washington University, Ithaca, NY) and FITC goat anti–rat IgG antibodies were used.

Comunoprecipitation of Iqg1p and Actin

A modification of the procedure described in Erickson et al. (1997) was used to detect immune complexes containing Iqg1p and F-actin. Cells transformed with HA-tagged Iqg1 (plasmid pA1) were grown on selective media to an OD600 of 0.8, pelleted, washed in cold lysis buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% Triton X-100, 5% glycerol), and then resuspended in the same buffer containing 20 μl of Fungal Protease Inhibitor Cocktail (Sigma Chemical Co., St. Louis, MO). Cold acid-washed glass beads were added and the suspension was vortexed (four times) at 4°C (1 min each with 2-min intervals of cooling). The supernatant was collected by centrifugation and 0.5 ml was added to 1 μg of anti-HA polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), together with 25 μM phalloidin, and then incubated at 4°C overnight with rocking. 20 μl of protein A–Sepharose beads equilibrated in the same buffer were added to the immune complexes and incubated at 4°C for 1 h with rocking. The beads were collected by centrifugation at 14,000 g for 20 s and extensively washed (four times) with 1 ml lysis buffer and resuspended in 2× SDS sample buffer, boiled for 5 min, and then loaded onto a 10% SDS–polyacrylamide gel. Western blotting was carried out as described (Ausubel et al., 1992), and the filters were stained with either anti-yeast actin antibodies (1:2,000; a gift from Dr. T. Bretscher, Cornell University) or with a polyclonal anti-HA probe (Santa Cruz Biotechnology, Inc.).

Fluorescence Microscopy

Nuclear DNA staining was performed with ~2 × 105 cells, which were grown at low phase, harvested, fixed in 70% ethanol, and then stained with DAPI (4',6'-diamidino-2-phenylindole) according to Futter (1993). When staining actin with phalloidin, cells were fixed in the growth media by the addition of 37% formaldehyde solution to 3.7% final concentration for 3 h. Staining with rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR) was carried out as described by Adams and Pringle (1991). To visualize chitin deposition on the cell wall, cells were collected and stained with Calcofluor (Fluorescent Brightener; Sigma Chemical Co.) as described by Pringle (1991).

Flow Cytometry

Yeast cells were grown to early log phase (OD600 = 0.3) at 23°C and incubated at 23°, 30°, and 37°C for 4 h. Cells were stained with propidium iodide as described by Futter (1993) and analyzed with the FACScan® in the Cornell Biotechnology Flow Cytometry and Imaging Facility.

Two-Hybrid Analysis

PCR-amplified IQG1 gene was cloned, in frame, into pBTM116 and pACT2 to produce LexA- and Gal4-Iqg1 fusion proteins respectively for two-hybrid analysis. These plasmids complement the phenotypes of the IQG1 deletion strain. Cdc42 fusion proteins were previously described (Stevenson et al., 1995). Plasmids were cotransformed into the CTY10-5D deletion strain. Cdc42 fusion proteins were previously described (Stevenson et al., 1995). Plasmids were cotransformed into the CTV10-5D strain and transformants were selected on medium lacking tryptophan, histidine, and uracil. To detect interactions with Af1p and Akr1p, plasmids pLAF and pPB659, respectively, were used. For cotransformation of L40 strain with pA1 (pACT2-IQG1) and transformants were selected on medium lacking histidine, tryptophan, and leucine. Colonies thus isolated were cultured into liquid medium lacking the same amino acids to OD600 of 0.3, then processed and sectioned in the Cornell Integrated Microscopy Facility.
Results

The Existence of a Saccharomyces cerevisiae Homologue, IQG1, for the Mammalian IQGAPs

A search of the yeast *Saccharomyces cerevisiae* genome database revealed a gene (these sequence data are available from GenBank/EMBL/DDBJ under accession number Z67751) predicted to encode a protein homologous to the mammalian IQGAPs. We have cloned this homologue and designated it Iqg1p. Alignment (Fig. 1) of the predicted protein sequence for Iqg1p with the mammalian IQGAP1 protein revealed significant homology over the entire coding region, with most of the sequence motifs from the mammalian IQGAPs being conserved within Iqg1p (Fig. 1 A). These include the region of homology with RasGAP (Fig. 1 C), which appears to be responsible for high affinity binding to the human Cdc42p (Cdc42Hs) but not Ras, the CHD (Fig. 1 A), a putative actin-binding site, and IQ motifs that represent potential calmodulin-binding sites. The WW motif, which is present in the mammalian IQGAPs and represents a potential binding site for proline-rich regions (Sudol et al., 1995), appears to be absent or less conserved in Iqg1p. The *IQG1* sequence was assigned GenBank/EMBL/DDBJ accession number AF019644.

<table>
<thead>
<tr>
<th>Table III. Two-Hybrid Interaction between Iqg1p and Cdc42 Protein</th>
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<tr>
<td>Protein</td>
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<td>pEG202-CDC42</td>
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β-Galactosidase activity (Miller units) was calculated from five independent transfectants.

Two-Hybrid Interactions between Iqg1p and the Saccharomyces cerevisiae Cdc42p

Given that Iqg1p contains the RasGAP homology domain, which appears to be responsible for the binding of mammalian IQGAP to activated Cdc42Hs, we used the two-hybrid analysis (Fields and Song, 1989; Gyuris et al., 1993) to detect interactions between Iqg1p and Cdc42p. The re-
Results in Table III show that a significant interaction was detected between the Iqg1 molecule and the GTPase-defective yeast Cdc42p (Cdc42pG12V,C188S), which was also mutated at position 188 to prevent membrane localization and thus facilitate the nuclear localization of the GTP-binding protein. A similar interaction was observed between another dominant-active, GTPase-defective Cdc42p mutant (Cdc42pQ61L,C188S) and Iqg1p. In contrast, neither the dominant-negative Cdc42 mutant (Cdc42p D118A, C188S) nor the Cdc42p C188S showed significant interactions with Iqg1p compared with the activated forms.

Iqg1p Concentrates at Sites of Yeast Cell Growth and at the Septum

As an initial characterization of Iqg1p, we used indirect immunofluorescence to determine its localization in yeast cells. The distribution of Iqg1p in an asynchronous cell culture appeared to vary with the cell cycle. In cells that have not yet undergone a complete budding event, the Iqg1p localized as a patch at the region of the cell from which the bud was emerging (Fig. 2A). In cells with smaller buds, Iqg1p appears more diffuse and is located throughout the bud. Cells with larger buds, which have presumably completed mitosis, show that Iqg1p is localized at the neck between the mother and daughter cells at cytokinesis (Fig. 2B). In the majority of the round unbudded cells, the distribution of Iqg1p appeared as punctate and/or diffuse (Fig. 2C). This pattern was not observed in cells transformed with vector alone. Overall, the localization of Iqg1p to the bud site and to the septum is similar to what has been reported for Cdc42p (also, see below) and proteins involved in cytokinesis (Kim et al., 1991; Brockerhoff and Davis, 1992; Ziman et al., 1993; Konopka et al., 1995).

Deletion of IQG1 Affects Yeast Cell Growth and Polarity

To begin to examine the in vivo function of Iqg1p, homologous recombination was used to replace one copy of IQG1 amino acids 38–1,382 in a wild-type diploid strain as described in Materials and Methods. Tetrad analysis revealed four viable spores per tetrad at room temperature. The His+::His– marker segregated 2:2 indicating that Iqg1p is not essential at least in this strain background. Growth of the diploid (MO1) and the homozygous diploid iqg1Δ::HIS3 (MO4) were examined at different temperatures ranging from 18°C to 37°C. At 18°C, the deletion strain grew on plates similar to wild-type strains (data not shown). At 30°C, the deletion strain culture grew more slowly than the corresponding wild-type strain but was un-
able to grow at 37°C indicating that the deletion of *IQG1* is temperature sensitive.

*iqg1Δ* cells grown at 23°, 30°, and 37°C were examined by light microscopy. At 23° and 30°C, the cells did not exhibit a uniform phenotype in liquid culture. Approximately 5% of the cells appeared normal, such that their size was comparable to those of the isogenic wild-type strain. However, the majority of the *iqg1Δ* cells were typically larger in size, rounder in shape, or elongated (Fig. 3 A, b–g; also see Figs. 4 E, 5 D, 8 B, and 9 C, below) and defective in budding. In some cases at 30°C, an elongated bud, tubular in shape, was attached to a large mother cell (Fig. 3 A, b). A fraction of the cells appeared amorphous.

After 2 h at 37°C, the majority of the *iqg1Δ* cells were large and round with a large vacule that occupied nearly the entire volume of the cell (Fig. 3 C). Some of the *iqg1Δ* cells at 37°C still exhibited an elongated cell phenotype similar to that caused by mutations in proteins involved in cytokinesis (Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim et al., 1991; Field et al., 1996; Longtine et al., 1996). As the incubation continued over 3 h at 37°C, the majority of the cells appeared amorphous or lysed.

To further examine the issue of polarity in *iqg1Δ* cells, haploid *iqg1Δ* cells (MO2) and their isogenic wild-type cells were treated with α factor. Under conditions where wild-type cells were arrested at G1 (Fig. 3 D), a small population of *iqg1Δ* cells formed at least two projections, whereas the majority of the mutant cells were extensively elongated or misformed (Fig. 3, E–G; also see Figs. 4 B and 7 B). Halo assays (Fig. 3 H) confirmed that *iqg1* cells are more sensitive to pheromone than their respective wild-type cells. Thus, the machinery for polarized growth appears to be hyperactive or otherwise deregulated in the absence of Iqg1p. This phenotype was rescued by the pA1 plasmid encoding an HA-Iqg1 fusion protein.

**Localization of Cdc42p in *iqg1Δ* Cells**

Because Iqg1p is a putative cytoskeletal target for Cdc42p, we examined the cellular localization of Cdc42p in *iqg1Δ* cells. In wild-type cells treated with α factor, Cdc42p is localized to the tip of the shmoo (Fig. 4 A), whereas in budding cells, Cdc42p is found in the bud neck, along the sides of the bud, and in the plasma membrane (Fig. 4 C) as previously reported (Ziman et al., 1993). In the isogenic *iqg1Δ* cells, Cdc42p was located throughout the α factor–treated cells (Fig. 4 B), and is found primarily in the plasma membrane but not in the necks of budded cells (Fig. 4 D). However, in *iqg1Δ* cells that exhibited the elongated bud (Fig. 4 E), Cdc42p localized to ridges along the bud as well as at the neck between the mother–daughter cells. The Cdc42p localization in these long buds resembles that of actin and chitin in *iqg1Δ* cells (see below).

**Iqg1p Coprecipitates with F-Actin and Is Essential for Proper Actin Filament Localization**

Because the predicted protein sequence of Iqg1 harbors a potential binding site for F-actin in its NH2 terminus similar to that found in α-actinin and filamin (Lebart et al., 1994; Castresana and Saraste, 1995), we examined both the ability of Iqg1p to interact with actin and the organization of actin filaments in *iqg1Δ* cells. Under non-permissive conditions, actin mutants arrest as unbudded cells and enlarge uniformly without directing material to the bud (Drubin, 1990). Similarly, *cdc42* mutants grow isotropically and delocalize actin filaments (Adams et al., 1990; Ziman et al., 1991). We examined the organization of the actin filaments in *iqg1Δ* cells using rhodamine-phalloidin

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**Figure 4.** Immunofluorescence localization of Cdc42p in α factor–arrested and budding *iqg1Δ* cells. (A) *MATa* wild-type cells treated with α factor. (B) *MATa iqg1Δ* cells treated with α factor. (C) Wild-type (MO1) cells. (D) *iqg1Δ* (MO4). (E) Nomarski image of an elongated bud attached to the mother cell in *iqg1Δ* (MO4). (F) Cdc42p localization at presumptive septa (arrows).

**Figure 5.** Actin filament organization defects caused by *iqg1Δ* mutation. The images show rhodamine-phalloidin staining of MO1 (wild-type) and MO4 (*iqg1Δ*) budding cells grown at 30°C. (A) Budding wild-type cells. (B and C) Budding *iqg1Δ* cells. (D) Large *iqg1Δ* cell with an elongated bud; arrows pointing at ridges of the presumptive septa locations.
staining. As expected, actin patches in wild-type cells are concentrated in the small bud and at the tip of the cells during polarized growth (Fig. 5 A), and at the septum during cytokinesis. By contrast, actin filaments in iqg1Δ cells appear to be randomly distributed throughout the mother cell and the bud (Fig. 5, B and C). However, in iqg1Δ cells that exhibit extremely elongated buds, actin patches, while still randomly scattered in the large mother cell, appear to be concentrated at ridges along the elongated bud (Fig. 5 D). We propose that these ridges represent presumptive sites for the septa in aborted separation of the mother and daughter cells. Despite the apparent correct localization of actin and other cell materials such as chitin (see below) and Cdc42p (above) at these presumptive septa locations, the buds grew as tubular projections and showed no obvious constrictions. This phenotype is similar to that observed for mutations in proteins implicated in cytokinesis (Holtzman et al., 1993; Konopka, 1993; Bi and Pringle, 1996; Kao et al., 1996), raising the possibility that Igglp is involved in some of the early steps of cytokinesis and is required for the polarized distribution of actin filaments during cell growth.

To examine whether Igglp interacts with actin in vitro, an HA-tagged Igglp was immunoprecipitated from total yeast cell lysates and then the resuspended precipitate was Western blotted to detect F-actin. As shown in Fig. 6, F-actin coprecipitates with Igglp (compare lanes 1 and 2) in the presence of phalloidin, which induces a net increase in polymerized actin filaments (Estes et al., 1981). Beads incubated with HA-tagged Igglp in total cell lysates and phalloidin did not retain an F-actin band (Fig. 6, lane 3). Similarly, expression of the HA tag without Igglp did not precipitate an F-actin band. Fig. 6, lane 4 shows actin in total cell lysates.

Thus, as reported for the mammalian IQGAP (Bashour et al., 1997; Erickson et al., 1997), Igglp also appears to interact with F-actin.

Calmodulin Is Delocalized in iqg1Δ Cells

Calmodulin is involved in bud growth, cytokinesis, and chromosome segregation (Davis, 1992) and localizes to sites of cell growth similar to the polarity establishment proteins and overlaps actin (Brockerhoff and Davis, 1992). The mammalian IQGAP binds calmodulin (Brill et al., 1996; Bashour et al., 1997; Joyal et al., 1997), which modulates the interactions of IQGAP with both F-actin and Cdc42Hs (Joyal et al., 1997); because Igglp contains at least four IQ motifs and thus is likely to bind calmodulin, we examined whether the localization of calmodulin was affected in iqg1Δ cells using indirect immunofluorescence in α-factor–arrested cells. In wild-type cells, calmodulin concentrated at the tip of the forming shmoo (Fig. 7 A) as previously described (Brockerhoff and Davis, 1992). In isogenic iqg1Δ haploid cells, calmodulin was located throughout the mis-shapen cell (Fig. 7 B). These results suggest that Igglp is involved in mediating the correct localization of calmodulin at growth sites.

Chitin Is Delocalized in iqg1Δ Cells

In S. cerevisiae, chitin is essential for cell growth and is localized at the incipient bud site, bud neck, and bud scars (Bulawa, 1993). During cytokinesis, chitin is localized to the primary septum between the mother and daughter cells. Polarity establishment proteins participate in the organization of chitin in the cell wall. Mutations in cdc42 (Adams et al., 1990) cause actin delocalization as well as...
affect chitin deposition. However, how these effects are mediated by Cdc42p is not well understood. Because Iqg1p is a putative target for Cdc42p, we examined whether the absence of Iqg1p affects chitin deposition. Calcofluor staining of an asynchronous cell culture showed that in wild-type cells, chitin is correctly deposited at the opposite poles, and at the incipient bud site, the septum, and the lateral wall (Fig. 8A). In some iqg1Δ cells (5%), chitin appeared to be correctly deposited. However, more typically, chitin was found over the entire surface of the iqg1Δ cells (Fig. 8B and C), yielding a similar phenotype to what has been observed for mutants defective in actin (Novick and Botstein, 1985) and in mutants that affect actin function (Liu and Bretscher, 1992). In large iqg1Δ cells bearing elongated buds, chitin was localized throughout the cell wall, but was concentrated at sites such as the presumptive septa and some bud scars (Fig. 8C). These results suggest that Iqg1p is required for directed deposition of chitin in the cell wall.

**iqg1Δ Cells Accumulate Nuclei**

Mutations in cdc42 (Adams et al., 1990; Hart et al., 1996) and in other proteins that influence the actin cytoskeleton accumulate nuclei to varying degrees (Holtzman et al., 1993; Bi and Pringle, 1996; Li, 1997). When stained with DAPI, as described in Materials and Methods, iqg1Δ cells also accumulate nuclei. At 23°C, 20% of the cells appeared to be bi- or multinucleated. This was especially clear in the elongated cells (compare Fig. 9B and D). However, the majority of the cells appeared to have masses of RNA, which was difficult to score as bi-nucleate or multinucleate. Many of the rounded cells had a mass of RNA either at one or both sides of the cell (Fig. 9E and F). Therefore, we suspected that 20% (n = 200) may represent an underestimation of the number of multinucleated iqg1Δ cells. To more clearly delineate the amount of RNA contained in these cells, we used flow cytometry to measure the DNA content of individual iqg1Δ cells at various temperatures. As shown in Fig. 10, wild-type cells contained both 1C and 2C DNA peaks, whereas iqg1Δ cells contained only 2C and 4C DNA at all temperatures tested. We obtained identical results comparing haploid or diploid strains. One possible explanation for these results is that the iqg1Δ cells have all diploidized. However, because we can detect cells with multiple nuclei (Fig. 9), we suspect that the results shown in Fig. 10 may reflect a situation where RNA replication and nuclear division continue in these cells, but both budding and cytokinesis are blocked in the absence of Iqg1p, thereby causing the cells to appear polyploid. To examine whether tubulin orientation was affected in iqg1Δ cells, we performed immunofluorescence studies with anti-tubulin antibodies. The spindles appeared to be fully extended but curved along or across the cell axis and were typically observed along one periphery of the cell, thus demonstrating that they were misoriented in the absence of Iqg1p (compare Fig. 9G [wild-type] and H [mutant]). This finding resembles that of actin mutations and points to a possible role for Iqg1p in organizing the actin cytoskeleton.
Genetic and Physical Interactions between IQGAP and Proteins Involved in Cytokinesis

Because the phenotypes of \textit{iqg1Δ} cells, namely the elongated cells and the accumulation of nuclei, appeared to resemble those of many mutants involved in cytokinesis, we reasoned that IQGAP may be part of a complex involved in organizing the actin cytoskeleton during cell cycle progression. One such candidate, AKR1, contains six ankyrin repeats suggesting that it is a cytoskeletal protein. The deletion of AKR1 is conditionally lethal and at restrictive temperatures produces an elongated cell phenotype (Kao et al., 1996) reminiscent of the IQG1 deletion. By contrast, the deletion of AFR1 has no discernible phenotypic effect but the overexpression of Afr1p produces a phenotype (Konopka, 1993) similar to that caused by \textit{iqg1Δ::HIS3}, \textit{akr1Δ-1}, and septin mutations. Thus, Afr1p may also act to regulate the functions of IQGAP and AKR1, similar to its proposed actions on the septins (Konopka et al., 1995). In addition, Afr1p localizes to the neck similar to IQGAP and interacts with Cdc12p (Konopka et al., 1995), a septin involved in neck filament formation (Haarer and Pringle, 1987), thus providing an intimate link to cytokinesis. We examined the possible interactions between these two proteins and IQGAP using double mutant and two-hybrid analyses. We transformed the \textit{iqg1Δ::HIS3} deleting fragment into both JK26 (\textit{afriΔ}) and JK211-5-3 (AFR1) strains. No transformants were recovered from the \textit{afriΔ} strain, whereas a high number of transformants was recovered from its parental wild-type strain. Thus, the double deletion of IQG1 and AFR1 appears to result in synthetic lethality at room temperature, suggesting a physical interaction between these two proteins that we then confirmed by the two-hybrid system (Table IV). The double mutation of \textit{iqg1Δ} and \textit{akr1Δ-1} resulted in slower growth and an enhanced cytokinetic defect with extremely elongated and large cells at room temperature, similar to the \textit{akr1Δ-1} phenotype at 37°C (Kao et al., 1996; and our unpublished results) suggesting functional synergy between IQGAP and AKR1. Indeed, an in vivo interaction was detected in the two-hybrid system between IQGAP and AKR1 (Table IV).

\textbf{iqg1Δ Cells Accumulate Post-Golgi Vesicles}

Yeast cells initiate growth at a specific site on the cell surface and undergo polar growth because of the localized fusion of vesicles with the plasma membrane (Sloat et al., 1981). There is mounting evidence that an aberrant actin cytoskeleton results in delocalized growth and accumulation of secretory vesicles (Novick and Botstein, 1985; Liu and Bretscher, 1992, Mulholland et al., 1997). Cdc42p controls cellular polarity and actin cytoskeleton organization (Adams et al., 1990; Johnson and Pringle, 1990; Johnson, 1993), and its localization to the plasma membrane is essential for its function (Ziman et al., 1991; Stevenson et al., 1995). To examine whether the \textit{iqg1Δ} phenotypes are due to effects at the plasma membrane, we visualized cells with electron microscopy as described in Materials and Methods. After shifting to the restrictive temperature (37°C), many of the \textit{iqg1Δ} cells displayed a scalloped shape membrane structure (not shown), with large vacuoles as previously revealed by Nomarski optics (Fig. 3 C) and CD-CFDA staining (not shown). As shown in Fig. 11, a population of \textit{iqg1Δ} cells (10 out of 21 cells with small buds), that appeared to be less severely affected or lysed, accumulated a large number of vesicles in the bud. Presumably, these represented post-Golgi secretory vesicles at the polarized cell surface. By contrast, isogenic wild-type cells did not accumulate these vesicles (0 out of 75 small-budded cells). These results may explain the slow growth phenotype and cytokinesis defect of \textit{iqg1Δ} cells.

\textbf{Discussion}

The Rho-type GTP-binding protein, Cdc42p, has been implicated in various functions both in yeast and mammalian cells, such as cell polarity, cell motility, and cytokinesis, by reorganizing the actin cytoskeleton, presumably as an outcome of its interactions with cytoplasmic targets. Despite numerous microinjection, genetic, and biochemical studies, little is known about the downstream signals that mediate the roles of Cdc42p in cell cycle-dependent morphological changes. In this study, we provide evidence that IQGAP, a homologue of the mammalian IQGAPs, mediates Cdc42p function in cytokinesis and other actin-based cellular processes in yeast.

It should be noted that recently, two other studies have appeared which describe the same homologue (Epp and Chant, 1997; Lippincott and Li, 1998). In both of these

\begin{table}
\centering
\begin{tabular}{lcc}
\hline
\textbf{pACTII-IQG1 (Miller units)} & \textbf{pACTII (Miller units)} \\
\hline
pBTM116-AFRI & 115.3 ± 0.7 & 0.2 ± 0.1 \\
pBTM116-AKRI & 89.7 ± 2.0 & 1.3 ± 0.6 \\
pBTM116 & 0.2 ± 0.1 & 0.1 ± 0.0 \\
\hline
\end{tabular}
\caption{Two-Hybrid Interaction between IQGAP and Each of AKR1 and AFR1}
\end{table}

\begin{figure}
\includegraphics[width=\textwidth]{figure11.png}
\caption{Electron micrographs showing \textit{iqg1Δ} cells accumulate vesicles in the bud. Cells were grown to early log phase at 23°C and shifted to 37°C for 3 h. Thin-sectioning electron microscopy was done as described in Materials and Methods. (A) \textit{iqg1Δ} cell. (B) Wild-type cell. (C) The bud in A at higher magnification.}
\end{figure}
In Vivo and In Vitro Evidence That Iqg1p Is Involved in Cytokinesis

The deletion of IQG1 (also called CYK1 by Lippincott and Li, 1998) was reported to be lethal. At present, we do not know the underlying genetic basis for the differences in these studies versus our own, which indicate IQG1 deletion to be conditionally lethal. However, this conditional lethality has allowed us to examine how the loss of Iqg1p expression impacts on its suspected binding partners including Cdc42p and actin.

Iqg1p May Mediate Cdc42p Functions by Reorganizing the Actin Cytoskeleton and Localizing Cdc42p

By homology with the mammalian IQGAP protein, we have identified and cloned the yeast homologue, Iqg1p protein. Our results suggest that Iqg1p may mediate the functions of Cdc42p in reorganizing the actin cytoskeleton, as well as help localize Cdc42p to sites of cell growth (Table III; Figs. 4-6). Further support for this idea comes from earlier biochemical studies with mammalian cells showing that Cdc42Hs, IQGAP, and actin form a ternary complex (Erickson et al., 1997; Fukata et al., 1997). Moreover, Iqg1p and its mammalian homologue, IQGAP, each contain a CHD. These motifs have been previously implicated in cross-linking actin filaments into bundles (Brown et al., 1995a,b; Bashour et al., 1997) and in binding and recruiting signaling proteins to the interface between actin and the plasma membrane (Brown et al., 1995a,b).

In addition, as is the case for the mammalian IQGAPs, Iqg1p is likely to bind calmodulin (Brill et al., 1996; Joyal et al., 1997). Iqg1p contains conserved IQ motifs at positions corresponding to the mammalian IQGAP motifs (Brill et al., 1996; Hart et al., 1996; Joyal et al., 1997) and we have shown that calmodulin distribution is diffuse in cells lacking Iqg1p (Fig. 7), implying that Iqg1p mediates the proper localization of calmodulin. In yeast, calmodulin colocalizes with actin and its function is affected in actin mutants (Davis, 1992). Furthermore, results from mammalian cells have shown that calmodulin modulates the interactions between Cdc42p and actin (Joyal et al., 1997).

Iqg1p Promotes Cytokinesis

Cytokinesis is the final stage of the cell cycle that produces two cells. Before cytokinesis, cells cease polarized growth and assume an isotropic expansion. Numerous proteins are thought to be involved in this process by virtue of their localization and the phenotypes of their mutations. However, the signals that regulate this process are thus far unknown. Our results suggest that Iqg1p is required for the completion of cytokinesis in yeast cells perhaps by transducing a signal from Cdc42p. The elongated cell phenotype (Figs. 3 A, 4 E, 6 D, 8 C, and 9 C), the accumulation of nuclei in iqlΔ cells (Figs. 9 and 10), and the localization of Iqg1p at the mother–bud neck (Fig. 2 B) at cytokinesis support this view. The localization of Iqg1p to sites of cell growth and the septum appears to overlap that of actin, Cdc42p, and calmodulin. These putative Iqg1p-binding partners are also implicated in morphogenesis and cytokinesis (Drubin, 1990; Davis, 1992; Dutartre et al., 1996). Immunofluorescence experiments performed on iqlΔ cells showed elongated buds containing ridges of localized proteins at positions we proposed to be septa locations. Namely, Cdc42p (Fig. 4 F), and actin (Fig. 5 D) localized to ridges across the tubular buds that displayed no constrictive formation that precedes cell separation. Whereas Cdc42p, actin, and chitin all appeared to be correctly localized to the presumptive septum, the absence of Iqg1p alone apparently accounts for the defect in the completion of the cell cycle and the separation of the mother and daughter cells. Whether the mislocalization of other proteins involved in cytokinesis, such as the septins, also contribute to this defect in cytokinesis needs to be further investigated.

Additional support for the involvement of Iqg1p in cytokinesis came from genetic and two-hybrid interactions with Akr1p and Afr1p (Table IV). The deletion of the ankyrin repeat–containing Akr1p results in a similar elongated cell phenotype as the Iqg1p deletion, and the double mutants displayed significant growth and cytokinesis defects compared with each of the single mutant iqlΔ and akr1Δ cells, thus suggesting synergy of function between these proteins. Afr1p appears to antagonize the functions of Iqg1p and Akr1p, as suggested by the fact that the ectopic expression of Afr1p produces phenotypes (Konopka et al., 1995) similar to the deletions of AKR1 and IQG1. In addition, Afr1p localizes to the septum and interacts with a septin, Cdc12p, thus lending a further connection to cytokinesis.

Recently, three IQGAP homologues (Faix and Dittrich, 1996; Adachi et al., 1997; Lee et al., 1997) were identified in Dictyostelium. The three molecules also appear to be involved in cytokinesis at different levels. Both Cdc42Hs (Dutartre et al., 1996) and its Dictyostelium relative RacE (Larochelle et al., 1996) were previously implicated in cytokinesis. More recently, two reports (Epp and Chant, 1997; Lippincott and Li, 1998) have also described cytokinesis as a primary function for the yeast Iqg1p. Together, these findings suggest that Iqg1p function is well conserved among organisms.

Figure 12. Summary of Iqg1p interactions and proposed functional outcome. Interactions between Iqg1p Cdc42p, actin, Akr1p, and Afr1p (this study); Afr1p and Cdc12p (Konopka et al., 1995); Akr1p, Bem1p, and Gcs1p (Kao et al., 1996).
Similarly, calmodulin has been implicated in cytokinesis both in yeast and Dictyostelium (Davis, 1992; Liu et al., 1992). Our results suggest that Iqg1p participates in localizing calmodulin to sites of active growth (Fig. 7) supporting the view that Iqg1p recruits and maintains a larger protein complex to execute its functions.

A Possible Role for Iqg1p in the Regulation of Cell Polarity

The localization of Iqg1p at the site of the incipient bud (Fig. 2) points to a possible involvement in bud morphogenesis. This localization overlaps that of many proteins involved in bud formation such as actin, Cdc42p, calmodulin, and the septins (Drubin, 1990; Brockerhoff and Davis, 1992; Ziman et al., 1993). These proteins may use the same signal to localize at the site of bud formation. Based on our findings, it would appear that the role of Iqg1p is to promote isotropic growth of the bud and subsequently cytokinesis. Two pieces of evidence support this suggestion; the localization of Iqg1p throughout the small buds and the hyperpolarization and pheromone sensitivity of iqg1Δ cells (Fig. 3, E–H). The hyperpolarization displayed by iqg1Δ cells treated with α factor also suggests that Iqg1p may actually inhibit projection formation, perhaps through an interaction with Akr1p. It appears that Akr1p inhibits signaling in the pheromone response pathway in cooperation with Ste4p, the Gβ subunit of the pheromone receptor–coupled G protein (Kao et al., 1996). Thus, Iqg1p and Akr1p may work in synergy to inhibit pheromone signaling by Cdc42p. However, the expression of another Iqg1p-binding partner, Afr1p, is induced by mating pheromone and cells lacking AFRI are defective in α factor–induced projections (Konopka, 1993). The ectopic expression of Afr1p in vegetative cells, where Afr1p localizes to the neck, causes abnormal morphologies (Konopka et al., 1995) similar to those caused by mutations in Iqg1p and Akr1p. Taken together, these findings suggest that interactions between Iqg1p, Afr1p, and Akr1p (Fig. 12) may result in a complex regulation of projection formation.

Iqg1p May Influence Trafficking

The large vacuole phenotype of iqg1Δ cells (Fig. 3, C), the accumulation of vesicles at the growing bud (Fig. 11), and the interaction of Iqg1p in the two-hybrid system with Akr1p (Table IV) all suggest a possible involvement of Iqg1p in secretion or some aspect of protein trafficking. The accumulation of vesicles is analogous to that caused by mutations in Iqg1p and Akr1p. The kinetics of an Iqg1p-mediated recruitment of different proteins would likely be important. Our immunofluorescence studies show that the elongated buds contain ridges of localized proteins that we propose to represent septa locations. The fact that the various proteins that we have examined were not correctly localized at the outset (compare Figs. 4, D and F, and 5, C and D) suggest that there might be a delay in their localization. This delayed localization may explain the slow growth phenotype of iqg1Δ cells. The lethality of iqg1Δ cells at 37°C may reflect the instability of a protein complex involved in growth and cytokinesis.

Mechanism of Action of Iqg1p

We propose that the Iqg1p is involved in recruiting and maintaining the organization of a number of cytoskeletal proteins at sites of cell growth and therefore, may act as a scaffold. The kinetics of an Iqg1p-mediated recruitment of different proteins would likely be important. Our immunofluorescence studies show that the elongated buds contain ridges of localized proteins that we propose to represent septa locations. The fact that the various proteins that we have examined were not correctly localized at the outset (compare Figs. 4, D and F, and 5, C and D) suggest that there might be a delay in their localization. This delayed localization may explain the slow growth phenotype of iqg1Δ cells. The lethality of iqg1Δ cells at 37°C may reflect the instability of a protein complex involved in growth and cytokinesis.

The multidomain structure of Iqg1p suggests that it can interact with a variety of proteins to negatively regulate cell polarization and promote isotropic growth and subsequently cytokinesis. This is especially apparent by the hyperpolarized phenotypes exhibited by iqg1Δ cells and suggested by the observed two-hybrid interactions between Iqg1p, Akr1p, and Afr1p. Given that Akr1p appears to inhibit the pheromone response pathway (Kao et al., 1996) and promote cytokinesis, we suspect that it may serve to mediate the effects of Cdc42p and Iqg1p on these events. The two-hybrid interactions detected between Iqg1p and both Akr1p and Afr1p point to a complicated scheme by which Cdc42p and Iqg1p may regulate a number of fundamental processes in yeast (Fig. 12). Future studies will be directed at exploring the interplay between Iqg1p, Akr1p, and Afr1p in more detail and in particular examining the possibilities that Akr1p and Afr1p represent positive and negative effectors, respectively, for the actions of Cdc42p and Iqg1p.

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