The Cdc42p GTPase Is Involved in a G2/M Morphogenetic Checkpoint Regulating the Apical-Isotropic Switch and Nuclear Division in Yeast*

(Received for publication, February 23, 1999, and in revised form, March 25, 1999)

Tamara J. Richman, Mathew M. Sawyer, and Douglas I. Johnson‡

From the Department of Microbiology and Molecular Genetics and the Markey Center for Molecular Genetics, University of Vermont, Burlington, Vermont 05405

The Cdc42p GTPase is involved in the signal transduction cascades controlling bud emergence and polarized cell growth in S. cerevisiae. Cells expressing the cdc42V44A effector domain mutant allele displayed morphological defects of highly elongated and multielongated budded cells indicative of a defect in the apical-isotropic switch in bud growth. In addition, these cells contained one, two, or multiple nuclei indicative of a defect in the apical plasma membrane; however, Cdc42V44AP localization was normal. Two-hybrid protein analyses showed that the V44A mutation interfered with Cdc42p’s interactions with Cla4p, a p21(Cdc42/Rac)-activated kinase (PAK)-like kinase, and the novel effectors Gic1p and Gic2p, but not with the Ste20p or Skm1p PAK-like kinases, the Bni1p formin, or the Iqg1p IQGAP homolog. Furthermore, the cdc42V44A morphological defects were suppressed by deletion of the Swe1p cyclin-dependent kinase inhibitory kinase and by overexpression of Cla4p, Ste20p, the Cdc12 septin protein, or the guanine nucleotide exchange factor Cdc24p. In sum, these results suggest that proper Cdc42p function is essential for timely progression through the apical-isotropic switch and G2/M transition and that Cdc42V44A p differentially interacts with a number of effectors and regulators.

Cdc42p is a member of the Rho/Rac family of GTPases, which play an essential role in the signal transduction pathways that lead to the establishment and maintenance of cell polarity and polarized cell growth in eukaryotic cells (1). In Saccharomyces cerevisiae, Cdc42p functions in selection of non-random bud sites, rearrangement of the actin cytoskeleton during bud emergence, and in directing actin-dependent secretion into an enlarging bud (2–5). The characteristic loss-of-function cdc42 phenotype, typified by the cdc42Δ, cdc42T17N, cdc42T35A, cdc42W97R, cdc42D118A, and cdc42C188S alleles (2, 4–8), is lethality with cells becoming large, round, and unbudded with multiple nuclei and delocalized actin and chitin. These alleles affect different aspects of Cdc42p function, but all lead to defects in bud emergence, suggesting that Cdc42p primarily functions in this process. However, Cdc42p localizes to the tips and sides of the enlarging bud following bud emergence (7), suggesting that Cdc42p regulates polarized cell growth after bud emergence. The mechanisms by which Cdc42p, in conjunction with various effectors and regulators, selects the bud site and maintains polarized growth throughout the cell cycle are still unclear.

There are a number of S. cerevisiae proteins that function as regulators of Cdc42p activity, including the guanine nucleotide exchange factor Cdc24p (9–13), and the Bem3p, Rga1p, and Rga2p potential GTPase-activating proteins (GAPs) (14–17). Cdc24p shows genetic as well as two-hybrid interactions with Cdc42p, exhibits in vitro guanine nucleotide exchange activity against Cdc42p, and is required for bud emergence. Bem3p, Rga1p, and Rga2p are potential Cdc42p GAPs, but only Bem3p has been shown to stimulate the intrinsic GTPase activity of Cdc42p in vitro. Characterization of bem3 rga1 mutants suggests that the GAPs do not have an essential role in bud emergence, but their elongated bud morphology (17) suggests that inactivation of Cdc42p to a GDP-bound state is necessary for cessation of apical polar bud growth. Analysis of these regulators suggests that the regulation of Cdc42p is critical for the establishment of bud emergence and may affect cellular processes later in the cell cycle.

GTP-bound Cdc42p displays physical and/or genetic interactions with a number of S. cerevisiae downstream effectors, including an IQGAP homolog Iqg1p/Cyk1p (18–20), a Wiskott-Aldrich syndrome protein (WASP) homolog Bee1p/Las17p (21), a formin-like protein Bni1p (22–25), novel Gic1p, Gic2p (26, 27), and the Ste20p, Cla4p, and Skm1p PAK-like kinases, which are members of the conserved p21(Cdc42/Rac)-activated kinase (PAK) family of serine/threonine protein kinases (6, 28–36). Characterization of the PAK kinases implicated both Ste20p and Cla4p in regulating the actin cytoskeleton (36) and Ste20p in mating and pheromone response (34) and Cla4p in mitosis and cytokinesis (28, 37), thereby suggesting that Cdc42p, in addition to its roles in bud emergence, regulates the pheromone response pathway, mitosis, and cytokinesis. In addition, Cla4p kinase activity, which is affected by binding to Cdc42p, peaks in G2/M (29) and cdc4 mutants display a mitotic delay (37), further implicating Cdc42p in a role later in the cell cycle beyond bud emergence. The functional consequences of Cdc42p interactions with its downstream effectors are not clear, but recent experiments using green fluorescent protein (GFP)-tagged Ste20p suggest that Cdc42p may function in localizing these proteins to the plasma membrane (31, 32).

* This work was supported by National Science Foundation Grants MCB-9405972, MCB-9723071, and MCB-9728218 and by the National Institutes of Health, Cancer Biology Training Grant T32-CA09286-19 (to T. J. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Microbiology and Molecular Genetics, 202 Stafford Hall, University of Vermont, Burlington, VT 05405. Tel.: 802-656-8203; Fax: 802-656-8749.

‡ To whom correspondence should be addressed: Dept. of Microbiology and Molecular Genetics, 202 Stafford Hall, University of Vermont, Burlington, Vermont 05405.
Alternatively, Cdc42p and Cla4p function in the localization of the S. cerevisiae septin proteins that are thought to be components of the 10-nm filament ring that forms at the bud site prior to budding and remains at the mother-bud neck region through cytokinesis (38).

To further elucidate Cdc42p cellular function during the cell cycle, the cdc42V44A allele was characterized. The V44A mutation lies within the Cdc42p effector domain and was originally isolated due to its ability to block two-hybrid protein interactions between Cdc2p and Cdc42p (6). cdc42V44A cells predominantly had elongated buds containing a single nucleus with a small percentage of cells conferring a multibudded, multinucleate phenotype indicative of a apical-isotropic switch defect, a small percentage of cells conferring a multibudded, multinucleate phenotype indicative of an apical-isotropic switch defect, and containing 2% glucose as a carbon source. To induce the GAL1 promoter, transformants were grown on solid or liquid media containing 2% raffinose + 2% galactose. Intragenic suppression of the cdc42V44A dominant-negative mutant and the cdc42G12V dominant-activated mutant by the V44A mutation in strain W303–1A was determined using plasmids pRS315(pGAL1/CDC42) (5), pRS315(pGAL1/cdc42V111A) (5), and pRS315(pGAL1/cdc42G12V) (5), and pRS315(pGAL1/cdc42G12V) (5). Transformants were selected on SC–Leu plates at 23 °C and individual transformants were streaked to SC–Leu containing 2% glucose + 2% galactose + 2% raffinose and incubated at 30 °C.

To determine if the Cdc42pV44A cytoplasmic domain function as a sole copy of Cdc42p within the cell, the integrating plasmid pRS306(cdc42V44A), which was linearized within the URA3 gene, was transformed into the CDC42::cdc2V111A::URA3::CDC2::cdc42V44A::URA3, under the control of the endogenous CDC42 promoter, integrated at the ura3 locus. Spores from 11 tetrad were grown at 23 °C and then streaked on selective media to determine marker distributions; segregants that contained the cdc42V44A mutant allele in a ∆cyc2 background were Ura-. All Ura- Trp- segregants grew at 16 °C, 23 °C, and 30 °C, but not at 37 °C, indicating that cdc42V44A-encoded a functional protein at low but not high temperatures. One of these temperature-sensitive segregants, designated TRY5–3A, was selected for further study. To characterize the cdc42V44A phenotype, strain TRY5–3A was grown at 20 °C in YEPD liquid media to early log phase, at which point the culture was shifted to the non-permissive temperature of 37 °C for 6 h. To characterize plasmid-mediated suppression of the cdc42V44A morphology, high copy plasmids YEp351 (43), YEp351(CDC42) (5), YEp13(BEM3) and YEp13(RGA1) (17), YEp351(CDC24), p425–75(STE20) (kindly provided by J. Kurjan), YEp351(CDC42), pKIN2 (kindly provided by D. Johnson), YEp51(CDC42), pKIN2 (kindly provided by D. Johnson), and YEp13(CDC24) (5) were transformed into the cdc42V44A strain TRY5–3A. Transformants were grown in SC–Leu liquid media at 23 °C to mid-log phase and sonicated briefly, and the morphology was quantified. The results presented represent the percent of abnormally budded cells in the total population of budded cells (n = 200 cells) and are representative of at least three independent transformation experiments.

**EXPERIMENTAL PROCEDURES**

**Reagents, Media, and Strains—**Enzymes, polymerase chain reaction (PCR) kits, and other reagents were obtained from standard commercial sources and used as specified by the suppliers. Oligonucleotide primers for sequencing and PCR were obtained from Genosys (The Woodlands, TX). Growth media and maintenance of bacterial and yeast strains were described previously (40, 41). The *S. cerevisiae* strains used are listed in Table I. The *cdc42::TRP1* marker was disrupted by HIS3 in strain TRY3-H by transforming the trp1::His3 marker swap fragment (42), released from pH4 by digestion with XhoI and EcoRI, into TRY1–6B (6). One of the stable His+ integrants was designated TRY3-H and used for further experiments. Yeast transformations were performed as described previously (41). Selection of transformants was performed on synthetic complete drop-out media lacking specified amino acids(s) and containing 2% glucose as a carbon source. To induce the GAL1 promoter, transformants were grown on solid or liquid media containing 2% raffinose + 2% galactose.

Intragenic suppression of the cdc42V111A dominant-negative mutant and the cdc42G12V dominant-activated mutant by the V44A mutation in strain W303–1A was determined using plasmids pRS315(pGAL1/CDC42), pRS315(pGAL1/cdc42V111A) (5), pRS315(pGAL1/cdc42G12V) (5), and pRS315(pGAL1/cdc42G12V) (5). Transformants were selected on SC–Leu plates at 23 °C and individual transformants were streaked to SC–Leu containing 2% glucose + 2% galactose + 2% raffinose and incubated at 30 °C.

To determine if the Cdc42pV44A cytoplasmic domain function as a sole copy of Cdc42p within the cell, the integrating plasmid pRS306(cdc42V44A), which was linearized within the URA3 gene, was transformed into the CDC42::cdc2V111A::URA3::CDC2::cdc42V44A::URA3, under the control of the endogenous CDC42 promoter, integrated at the ura3 locus. Spores from 11 tetrad were grown at 23 °C and then streaked on selective media to determine marker distributions; segregants that contained the cdc42V44A mutant allele in a ∆cyc2 background were Ura-. All Ura- Trp- segregants grew at 16 °C, 23 °C, and 30 °C, but not at 37 °C, indicating that cdc42V44A encoded a functional protein at low but not high temperatures. One of these temperature-sensitive segregants, designated TRY5–3A, was selected for further study. To characterize the cdc42V44A phenotype, strain TRY5–3A was grown at 20 °C in YEPD liquid media to early log phase, at which point the culture was shifted to the non-permissive temperature of 37 °C for 6 h. To characterize plasmid-mediated suppression of the cdc42V44A morphology, high copy plasmids YEp351 (43), YEp351(CDC42) (5), YEp13(BEM3) and YEp13(RGA1) (17), YEp351(CDC24), p425–75(STE20) (kindly provided by J. Kurjan), YEp351(CDC42), pKIN2 (kindly provided by D. Johnson), YEp51(CDC42), pKIN2 (kindly provided by D. Johnson), and YEp13(CDC24) (5) were transformed into the cdc42V44A strain TRY5–3A. Transformants were grown in SC–Leu liquid media at 23 °C to mid-log phase and sonicated briefly, and the morphology was quantified. The results presented represent the percent of abnormally budded cells in the total population of budded cells (n = 200 cells) and are representative of at least three independent transformation experiments.

**Plasmids and DNA Manipulations—**Recombinant DNA manipulations (40) and plasmid isolation from *E. coli* (44) were performed as

### TABLE I

**Strain**

| Strain | Genotype | Source |
|--------|----------|--------|
| C276–4A | MATa gal2 | (64) |
| DDJT2–16A | MATa cdc42–1 his4 leu2 trp1 ura3 | (3) |
| EGY48 | MATa ura3 his3 trp1 integrated lexAop-LEU2 integrated lexAop-lacZ | (46) |
| p1840 | MATa ade2 ste20–2 ADE2–3 his3–1,15 leu2–3,112 trp1–1 can1 | (65) |
| DJD6–11 | MATaMATα cdc42–1; TRP1+ + his3–200 + his4 + leu2 + can1 + | (4) |
| Y804 | MATa ura3–52 ade2–101 lys2–801 his3–200 trp1Δ | M. Snyder |
| W303–1A | MATα ade2–101 his3–11,5 leu2–3,112 trp1–1 D1 ura3–1 can1–100 | R. Rohde |
| DJMD3–23A | MATα cdc4–1 his3 leu2 trp1–11 trp1–1 | D. Johnson |
| DLY1028 | MATα ade2–101 lys2–801 trp1 Δ | D. Lew |
| JSO–1B | MATα cdc12–5 leu2 ura3 | B. Haarer |
| TRY1–9D | MATα ade2 leu2 his3 trp1 ura3 clc4::TRP1 | This study |
| TRY7–H | MATα ade2 leu2 his3 trp1 ura3 clc6::TRP1::HIS3 | This study |
| TRY3–H | MATα ade2 leu2 his3 trp1 ura3 clc6::TRP1::HIS3 | This study |
| TRY3–HV | MATα ade2 leu2 his3 trp1 ura3 clc6::TRP1::HIS3 | This study |
| TRY5 | MATαade2–101 lys2–801 his3–200 trp1–1 | This study |
| TRY5–3A | MATα ade2–101 lys2–801 his3–200 trp1–1 | This study |
| TRY5–8C | MATα ade2–101 lys2–801 his3–200 trp1–1 | This study |
| TRY1–117D | MATα ade2 leu2 ura3 trp1 his | This study |
| TRY15 | MATα ade2 leu2 ura3 trp1 his | This study |
| TRY15–12A | MATα ade2 leu2 ura3 trp1 his | This study |
| TRY15–23A | MATα ade2 leu2 ura3 trp1 his | This study |

Additionally, Cdc42p and Cla4p function in the localization of the *S. cerevisiae* septin proteins that are thought to be components of the 10-nm filament ring that forms at the bud site prior to budding and remains at the mother-bud neck region through cytokinesis (38).
described previously (6). Automated DNA sequencing at the Vermont Cancer Center DNA Sequencing Facility was used to sequence all gene constructs. Site-directed mutagenesis was performed with the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The plasmid pRS15(CLA4) was created by inserting the HindIII plus SalI (3′) containing CLA4 fragment from pRS15(CL4A) into Yeplcl451 cut with HindIII plus SalI. pRS15(GFP-CDC3), which was digested by cutting TD150(GFP-CDC3) (kindly provided by B. Haarer) with BamHI plus NotI to release the GFP-CDC3 fragment, which was ligated to pRS15, was created by cutting TD150(GFP-CDC3) and ligated with BamHI plus NotI. To create plasmid pAS1-CYH2(cdc42V44A), pAS1-CYH2(cdc42V44A,D118A) (6) was cut with restriction enzymes EcoRI plus SalI, releasing the C-terminal portion of CDC42 containing the D118A mutation, which was replaced with the wild type sequence from pRS15(CDC42). To create pRS15(cdc42V44A,D118A), the C-terminal portion of CDC42 containing the D118A mutation was released from pRS15(cdc42V44A,D118A) (5) with EcoRI plus SalI and ligated into pRS15 to create pRS15(cdc42V44A,D118A). pRS15(cdc42V44A,D118A) was digested with EcoRI to release the N-terminal portion of CDC42 containing the C-terminal primer, which was ligated to pRS15, was created by digesting with EcoRI, thereby creating plasmid pRS15(cdc42V44A,D118A). The plasmid p145MET(GFP-H107V-CDC42) was constructed by cutting pREP3X(GFP-A2) with SpeI and SmalI to create a 730-base pair fragment (containing a GFP-H107V C-terminal in-frame fusion with eight alanine residues), which was ligated to pMT45 (kindly provided by M. Funk) cut with SpeI and SmalI. The CDC42 fragment was obtained from pGAL1-GFP(CDC42) (kindly provided by J. O’Dell and A. Adams) by cutting with HindIII, blunt ending the site with Klenow fragment of DNA polymerase I, and then cutting with SalI. The fragment was then ligated to p145MET23 (GFP-A2) cut with SmalI and SalI. To create p146MET(GFP-H107V,C188S-CDC42), a SpeI plus Xhol GFP-H107V,C188S-CDC42 fragment released from p145MET(GFP-H107V,C188S-CDC42) was subcloned into the SpeI/Xhol-digested pMT45 (kindly provided by M. Funk). PCR and Site-directed Mutagenesis—To create pEG202(cdc42V44A,D118A), cdc42V44A was amplified from pAS1-CYH2(cdc42V44A) by PCR using the Expand Long Template PCR system (Roche Molecular Biochemicals). The 5′-primer sequence used in the PCR reaction was GGATATCCATTATGCAC-AAGCTTAATTGTTG (underlined sequence is an EcoRI site; double underlined sequence is an NdeI site that contains the CDC42 start codon), and the 3′-primer sequence was CCGGGACAAGCTTTTGTAGATTG (underlined sequence contains a BamHI site followed by the CDC42 stop codon and the reverse complement of CDC42; Cys166 to Ser; underlined G is C in the wild type sequence). The PCR cycling parameters were 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The resulting 576-base pair PCR fragment was digested with EcoRI plus BamHI and inserted into EcoRI/BamHI-digested pEG202. The resulting construct was sequenced to verify the fusion and cdc42V44A,D118A sequence.

pEG202(cdc42V44AD118A) was created using the QuikChange™ site-directed mutagenesis kit from Stratagene. pEG202(cdc42V44A,D118A) was the DNA template for the mutagenesis. The nucleotide sequence of the forward mutagenic primer was GTCCGAGTATGCTGCGTGGAGAAACGC (Gly12 to Val; underlined T is G in the wild type sequence), and the reverse mutagenic primer was CCTTCTTTGAGCTTCTATCAAGG (Gly12 to Val; underlined A is C in the wild type sequence). The cycling parameters for the mutagenesis were 12 cycles of 95 °C for 30 s, 55 °C for 30 s, and 68 °C for 1 min. The resulting 576-base pair PCR fragment was digested with EcoRI plus NdeI to release the cdc42V44A,D118A fragment, which was inserted into pRS15 (pGAL1-CDC42) cut with NdeI. Sequencing of both plasmids resulting from the mutagenesis confirmed the sequence of the cdc42V44A,D118A and cdc42V44A,A320D118A alleles.

p146MET(GFP-H107V,C188S-CDC42) was also amplified using the QuikChange™ kit with p146MET(GFP-H107V,C188S-CDC42) as the DNA template. The nucleotide sequence of the forward mutagenic primer was CGAT-TAACATCTG-TGCTGATTTGTTGAG(AACG (Val14 to Ala; underlined C is T in the wild type sequence) and the reverse mutagenic primer was GTTTCATACATCAGCAGCAGGATGTTAC (Val14 to Ala; underlined G is A in the wild type sequence). The cycling parameters for the mutagenesis were as above.

Two-hybrid Protein Interactions—The methods for performing two-hybrid analysis have been described previously (45, 46). Strain EGY48-p1840 (provided by B. Brent and R. Finley, Harvard University) containing pAS1-CYH2(cdc42V44A) (28) (kindly provided by D. Lew), pEG202(cdc42V44A,D118A) (5), the various CDC42 constructs pEG202(CDC42), pEG202(cdc42V44A,D118A), pEG202(cdc42V44A,D118A,G12V), pEG202(cdc42V44A,D118A,C188S), pEG202(cdc42V44A,D118A,G12V,C188S) (kindly provided by D. Lew), pEG202(cdc42V44A,D118A,G12V,C188S) and pEG202(cdc42V44A,D118A,G12V,C188S) (kindly provided by B. White) were used to select on SC–His–Trp media containing ca. 2% lactose + 2% raffinose at 23 °C. Strain EGY48-p1840 containing pRF222(STE20) (kindly provided by M. Whiteway) or pGADC2(IQG1) (20) and the various pEG202(CDC42) constructs were selected on SC–His–Leu containing 2% glucose. LexA-DBD fusions in vector pEG202 are under the ADH constitutive promoter. GALA-AD fusions in vector pEG42–5 are under the pGAL1 inducible promoter. GALA-AD fusions in vector pEG222 or pGADC2 are under the ADH constitutive promoter. β-Galactosidase liquid assays were performed in triplicate, and β-galactosidase units were calculated as described previously (47).

Photomicroscopy—Cells were grown in the appropriate liquid media at 23 °C to mid-log phase. Cells were collected, sonicated, and examined morphologically. Methods for preparing and staining cells with 4′,6-diamidino-2-phenylindole (DAPI), Calcofluor, and rhodamine-phalloidin have been described previously (48). Cells containing GFP-Cdc12p, GFP-Cdc12p, and GFP-A2-Cdc42p constructs were grown to mid-log phase, sonicated and observed. Cells containing GFP-A2-Cdc42p constructs were grown in SC–Ura–Met media for expression from the methionine repressible promoter. Photomicroscopy using Hoffman modulation optics was performed on an Olympus BH-2 epifluorescence microscope. DAPI-stained cells were examined on a Nikon TE300 inverted microscope equipped with epifluorescence illumination and Nomarski optics. Video images were obtained using a VE1000SiT camera (Dage-MTI, Michigan City IN). The magnification of the image was adjusted with projection optics placed between the microscope and the video camera. Nikon filter cube UV-2A (excitation 330–380 nm, emission >420 nm) was used for visualizing DAPI fluorescence. Photomicroscopy using phase contrast optics (Fig. 2, B and C) was also performed on an E400 Nikon microscope (Omega Optical, Brattleboro, VT). GFP-A2-Cdc42p expressing cells were visualized on this microscope using Nikon optical filter cube XF100 and DAPI staining was visualized with Omega optical filter cube XF06 filter. All cell and plate images were digital images that were obtained using a Dage VE-VG-5 video frame grabber (Scion Corp., Frederick, MD) and analyzed in Adobe Photoshop 5.0 (Adobe Systems, Inc., San Jose, CA) on a PowerTower 180 Macos computer (PowerComputing, Round Rock, TX). Where indicated, cells from the same culture but different fields were manipulated into collages using Adobe Photoshop 5.0.

RESULTS

cdc42V44A Mutant Cells Have an Apical-Isotropic Switch Defect and a Partial Cytokinesis Defect—The V44A mutation lies within the Cdc42p effector domain and was identified in a screen for point mutations that interfered with two-hybrid interactions between the Cdc42p (1), pJG4–5 (1), and pGADC2 (19) mutant protein and Cdc42p (6). The Cdc42p V44A mutant allele at 23 °C and the dominant lethal phenotype associated with the cdc42V44A allele at 23 °C (25) suggested that Cdc42p was partially functional at 23 °C. However, the cdc42V44A mutant allele could complement a Δcdc42 mutation in strain TRY5–3A, and these cells grew at 16 °C, 23 °C, and 30 °C, but not at 37 °C, indicating that cdc42V44A encoded a functional protein at low but not high temperatures. Western blotting of the cdc42V44A cells showed abnormally elongated buds and the cells appeared considerably larger than wild type cells (Fig. 1A, upper panel), suggesting that Cdc42pV44A was partially functional at 23 °C. Additionally, ~15% of the cells were multibudded (Fig. 1B). Staining of these cells with the fluorescent DNA stain DAPI (Fig. 1A, lower panel) indicated that 56% of the abnormally budded cells had 1 nucleus (Fig. 1A, arrowheads), 35% had two nuclei and 9% of the cells had more than two nuclei (Fig. 1A, lower panel)
Analysis of the \textit{cdc42}\textsuperscript{V44A} Effector Domain Mutant

\textbf{Figure 1. Morphological characterization of the \textit{cdc42}\textsuperscript{V44A} mutant.} A, the morphological phenotype and DNA content of the \textit{cdc42}\textsuperscript{V44A} strain TRY5–3A was compared with wild type C276–4A cells at 23 °C using Nomarski optics (upper panels) and DAPI staining (lower panels). Cells were grown in YEPD liquid media to mid-log phase and sonicated briefly before observation. Scale bar, 10 μm. B, multibudded \textit{cdc42}\textsuperscript{V44A} cells at 23 °C (strain TRY5–3A).

\textit{arrows}). The elongated-budded morphology along with the multinucleated cells indicated that Cdc42\textsuperscript{V44A}p conferred a delay in the apical-isotropic switch in bud growth and in the G2/M transition as well as a partial cytokinesis defect to cells at 23 °C.

To determine whether \textit{cdc42}\textsuperscript{V44A} had a cytokinesis or cell separation defect, \textit{cdc42}\textsuperscript{V44A} cells were fixed, treated with the cell wall-digesting enzyme gluclusalase, and the morphological phenotype quantified pre- and post-gluclusalase. The percentage of budded and multibudded cells in the gluclusalase-treated cells decreased by 30%, correlating with a 30% increase in the unabudded cell population. These results suggested that \textit{cdc42}\textsuperscript{V44A} cells have a cell separation defect. However, ~8% of the budded population post-gluclusalase were still multibudded, suggesting that cytokinesis may be defective or at least delayed in these cells.

The \textit{cdc42}\textsuperscript{V44A} phenotype at 37 °C was also characterized to determine the basis for the lethality. After 6 h at 37 °C, the \textit{cdc42}\textsuperscript{V44A} strain had a mixed population of cells with 62% being abnormally budded and 38% being large, round, and unbudded as compared with prototypic \textit{cdc42}\textsuperscript{–11a} cells, 69% of which were large, round, and unbudded (n = 100 cells). This 37 °C phenotype differed not only from the \textit{cdc42}\textsuperscript{–11a} phenotype, but also from the abnormal 20 °C morphology of \textit{cdc42}\textsuperscript{V44A} cells, in which no large, round, unbudded cells were observed.

The \textit{cdc42}\textsuperscript{V44A} Mutant Triggers the Swe1p-dependent Morphogenetic Checkpoint to Delay the Apical-Isotropic Switch and Nuclear Division—The \textit{cdc42}\textsuperscript{V44A} elongated-budded phenotype suggested that these cells have a delay in the apical-isotropic growth switch, resulting in hyperpolarized cortical actin. Indeed, \textit{cdc42}\textsuperscript{V44A} cells showed an abnormal cortical actin localization pattern as compared with wild type cells (Fig. 2A). Cortical actin was polarized to the tips of elongated buds, suggesting that the apical-isotropic growth switch that occurs during the G2/M transition was delayed in \textit{cdc42}\textsuperscript{V44A} cells. This actin localization pattern was similar to the patterns seen in abnormal \textit{Δala4} cells (Fig. 2A) and was less severe but similar to the actin localization seen in \textit{cdc12}\textsuperscript{ts} cells at non-permissive temperatures (data not shown; Ref. 49). These results also suggested that \textit{cdc42}\textsuperscript{V44A} defects may trigger the Swe1p-dependent G2/M morphogenetic checkpoint, which monitors the apical-isotropic switch through the Clb2p/Cdc28p regulatory kinase (50–53).

To examine whether \textit{cdc42}\textsuperscript{V44A} defects triggered the Swe1p-dependent morphogenetic checkpoint, the \textit{cdc42}\textsuperscript{V44A} mutant phenotype was examined in a \textit{swe1}\textsuperscript{Δ} background. A \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} double mutant no longer exhibited the elongated-budded phenotype characteristic of \textit{cdc42}\textsuperscript{V44A} cells at 23 °C (Fig. 2B), which supported an apical-isotropic switch delay that was Swe1p-dependent. However, the morphology of these cells was not completely normal, with 55% of the \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} budded cells displaying a multibudded or irregular morphology (Fig. 2B). DAPI staining revealed that 29% of all \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells contained greater than two nuclei (Fig. 2C), indicating that the \textit{swe1}\textsuperscript{Δ} mutation did not suppress and may exacerbate the \textit{cdc42}\textsuperscript{V44A} multinucleate phenotype. The multiple nuclei were also unevenly distributed in mother and daughter cells with some daughter cells having two nuclei (Fig. 2C, leftmost panel). The increased number of nuclei per cell and the abnormal multinucleated morphology of \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells indicated that loss of Swe1p exacerbated \textit{cdc42}\textsuperscript{V44A}p effects, highlighting the cytokinesis and/or cell separation defect. The nuclear staining of \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells (Fig. 1A) as compared with \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells also suggested that \textit{cdc42}\textsuperscript{V44A} cells had a nuclear division delay, which is characteristic of activation of the G2/M morphogenetic checkpoint.

To confirm whether these mutant cells truly had a cell cycle delay, cell synchrony experiments to follow the nuclear cycle were attempted. However, due to the mutant morphology, standard cell synchrony experiments, such as α-factor arrest, were unsuccessful. However, it was found that growth in 0.5 M NaCl remediated the abnormally budded morphological and multinucleate phenotypes at 23 °C with 82% of the budded \textit{cdc42}\textsuperscript{V44A} cells (strain TRY5–8C) having a normal budded morphology and none of the normally budded cells having more than 2 nuclei. Furthermore, \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells grown in NaCl and then shifted to restrictive temperatures in fresh media without NaCl were found to be a nearly uniform population of large unbudded cells. These results suggested that NaCl remediated the 23 °C mutant morphology but did not remediate the \textit{cdc42}\textsuperscript{V44A} temperature sensitivity.

Using this NaCl remediation as a means to increase cell synchrony, cell cycle reciprocal shift experiments were performed to determine if there was a delay in the nuclear cycle of \textit{cdc42}\textsuperscript{V44A} cells relative to \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells, \textit{cdc42}\textsuperscript{V44A} cells (strain TRY5–8C) and \textit{cdc42}\textsuperscript{V44A} \textit{swe1}\textsuperscript{Δ} cells (strain TRY15–21A) were grown in 0.5 M NaCl media to late-log phase,
Fig. 2. Actin localization and suppression of the cdc42V44A morphology by Δswe1. A, C276–4A (wild type), TRY5–3A (cdc42V44A), and TRY1–9D (Δcla4) cells were grown in YEPD at 23 °C to mid-log phase, fixed and stained with the actin fluorescent stain rhodamine-phalloidin using standard fixation and staining procedures. All cells were sonicated briefly before observation. B, TRY15–23B (Δswe1), TRY15–23A (cdc42V44A), and TRY15–21A (cdc42V44A Δswe1) were grown in YEPD liquid media at 23 °C to mid-log phase, sonicated briefly, and observed. C, TRY15–23B (Δswe1) and TRY15–21A (cdc42V44A Δswe1) were grown in YEPD liquid media at 23 °C to mid-log phase, stained with DAPI, and sonicated briefly before observation. Scale bars, 10 μm.

Analysis of the cdc42V44A Effector Domain Mutant

Washed, and then shifted to 37 °C in fresh media without NaCl for 1 h to obtain a population in which ~80% of the cells were unbudded and ~80% of the cells were uninuclear. These cells were then shifted to permissive temperature (23 °C) and observed over time with DAPI to determine when nuclear division occurred. Both cdc42V44A and cdc42V44A Δswe1 mutant cells began budding ~1 h after the shift to 23 °C and the abnormally elongated budded phenotype in cdc42V44A mutant cells became apparent ~30 min later with buds increasing in length over time. At 4 h after the shift to permissive temperatures, ~50% of the abnormally budded cells had two nuclei, suggesting that these cells eventually complete nuclear division. In contrast to cdc42V44A cells, ~50% of the cdc42V44A Δswe1 cells had two or more nuclei within 2 h after the temperature shift. These results indicated that cdc42V44A cells had an ~2-h nuclear division delay as compared with cdc42V44A Δswe1 cells and indicated further that the cdc42V44A cell cycle delay is Swe1p-dependent.

cdc42V44A Cells Have Abnormal Chitin Localization and Aberrant and Delocalized Septin Ring Structures—To determine if chitin was delocalized as seen in other cdc42 mutants, cdc42V44A cells were stained with the chitin stain Calcofluor. The haploid cdc42V44A cells had a normal axial budding pattern, but ~50% of the budded cells did not form a defined chitin ring at the mother-bud neck region and had diffuse chitin localized around the entire periphery of the cell (Fig. 3). The cdc42V44A mutant cells staining pattern was comparable to that seen in Δcla4 (Fig. 3) and cdc12 mutants (data not shown; Ref. 54). The mother-bud neck region of cdc42V44A cells also appeared wider than wild type cells and was comparable to the enlarged neck region of Δcla4 cells. These results suggested that one of the mechanistic defects in cdc42V44A cells was delocalized chitin deposition. Since chitin localization is dependent on the septins, these observations suggested that cdc42V44A cells had defects in septin ring localization.

To further explore a possible link between Cdc42V44A and septins, we compared the localizaton of Cdc3p and Cdc12p tagged with GFP, which were expressed from their endogenous promoters in plasmid pRS315, in wild type and cdc42V44A cells (Fig. 3; only GFP-Cdc12p is shown; GFP-Cdc3p gave identical results). In wild type cells, both GFP-Cdc3p and GFP-Cdc12p localized to the mother-bud neck region as a bright ring structure and many of the cells showed a double ring structure at the neck region, as was seen in immunofluorescence localization studies with anti-Cdc3p and anti-Cdc12p antibodies (Fig. 3; Refs. 55 and 56). It was also noted that the overexpression of either GFP-Cdc3p or GFP-Cdc12p did not alter the morphology of wild type cells. In contrast to wild type cells, expression of GFP-Cdc3p and GFP-Cdc12p did alter the morphology of cdc42V44A cells with ~50% of cells appearing normal. The elongated budded cells typically seen in the cdc42V44A cell population made up only a small percentage of the cdc42V44A cell population overexpressing either septin protein, suggesting that Cdc3p or Cdc12p suppressed the morphological defects seen in cdc42V44A cells alone. Overexpression of Cdc12p alone also showed the same quantitative level of suppression, but it did not suppress the cdc42V44A temperature sensitive defect. However, the morphology of the elongated budded cells overexpressing either GFP-Cdc3p, GFP-Cdc12p, or Cdc12p was altered from the typical cdc42V44A cell morphology as evidenced by the presence of cells with normal buds but a slightly elongated neck region (Fig. 3, arrows; normally budded cells with the slightly elongated neck regions were considered abnormally budded cells in the quantitative analysis of GFP-Cdc3p, GFP-Cdc12p, or Cdc12p suppression).

The cdc42V44A cells containing GFP-Cdc3p or GFP-Cdc12p...
that appeared to have normal buds had intact, properly localized septin rings. In normally budded cells with a slightly extended neck region, both GFP-Cdc3p and GFP-Cdc12p containing septin rings appeared to be intact. However, in these abnormal cells, the septin ring was localized at the base of the rounded bud at the point of constriction as opposed to at the mother-bud neck region (arrows in Fig. 3 show GFP-Cdc12p-containing cells which are representative of GFP-Cdc3p cells). Furthermore, of the elongated budded cells seen, many appeared to have a diffuse septin ring and GFP fluorescence could be seen at the tip and around the sides of the bud (Fig. 3, arrowheads). This aberrant septin ring localization was seen in 82% of the abnormally elongated budded cells. These results suggested that the Cdc42V44A had an adverse effect on the localization of Cdc3p and Cdc12p and the structural formation of the septin ring.

Abnormal localization of GFP-Cdc3p and GFP-Cdc12p was also observed in Δcla4 cells, with the abnormally elongated budded cells having a diffuse staining pattern and bud tip staining pattern that was similar to the patterns seen in cdc42V44A cells (Fig. 3, arrowheads). The Δcla4 cells also had intact septin rings in normal cells and intact rings that were wider or delocalized in elongated budded cells (Fig. 3, arrows). Interestingly, the percentage of abnormally budded cells in the Δcla4 cell population did not change upon overexpression of GFP-Cdc3p or GFP-Cdc12p, suggesting that overexpression of Cdc3p or Cdc12p could not suppress the abnormal morphology associated with Δcla4 cells (see “Discussion”).

**Fig. 3.** Chitin and GFP-Cdc12p localization in cdc42V44A mutant cells. C276–4A, TRY5–3A, and TRY1–9D (see Fig. 2 legend) were grown in YEPD at 23 °C to mid-log phase, fixed and stained with the chitin-specific fluorescent stain Calcofluor. To observe GFP-Cdc12p localization in cdc42V44A cells, pBS151/GFP-CDC12 was transformed into EGY48 (wild type), TRY5–8C (cdc42V44A) and TRY1–9D (Δcla4). Large arrows point to cells with slightly elongated neck. Arrowheads point to abnormal GFP-Cdc12p localization in a morphologically mutant cdc42V44A cells. GFP-Cdc12p images are collages from the same cell culture manipulated in Adobe Photoshop 5.0. Scale bar, 10 μm.

**Analysis of the cdc42V44A Effector Domain Mutant**

**Calcofluor**

**GFP-Cdc12p**

**Wild Type**

**cdc42V44A**

**Δcla4**

Three-hybrid and Genetic Evidence Indicated Cdc42V44Ap Had Altered Interactions with Downstream Effectors Cla4p, Gic1p, and Gic2 but Not Ste20p, Bni1p, Bni1p, or Ig1p—Since the V44A mutation is located in the effector domain, it seemed likely that the primary defect of Cdc42V44A was due to altered interactions with its regulators and effectors. Previous two-hybrid experiments had indicated that the V44A mutation affected interactions between Cdc42p and Cdc24p (6) supporting this hypothesis. To further explore Cdc42V44Ap interac-

---

3 M. M. Sawyer and D. I. Johnson, manuscript in preparation.
Fig. 4. GFP-A1-Cdc42p localization. p146MET(GFP-H67T-A1-
CDC42) and p146MET(GFP-H67T-A1-cdc42V44A) were transformed into
TRY1–13D (wild type) and TRY1–9D (Δcl4A). Transformants were
selected on SC−Ura plates, and then selected transformants were grown
at 23 °C to mid-log phase in SC−Ura−Met liquid media for expression
from the methionine-repressible promoter.

tions, two-hybrid analysis was performed with known Cdc42p
effectors. Two-hybrid interactions between Cla4p and the
Cdc42V44A,C188Sp mutant protein were significantly decreased,
but not abolished, in comparison to interactions between Cla4p
and the Cdc42C188Sp (Fig. 5; the C188S mutation was intro-
derminated GDP-bound Cdc42Ap as was previously reported
for synthetic lethality. The cdc42–1 mutant allele (28). These results indicate that
Cdc42p in the cell without a functional Cla4p. Similar
synthetic lethality was observed with
cdc42–1 cla4::trp1::HIS3 diploid strain TRY3-HV was sporulated and, of 95 spores (from
50 tetrads), none were Ura+ Trp+ His+ , suggesting that the
cdc42V44A,C188Ap Δcl4A double mutant is inviable at 23 °C. To confirm
this apparent synthetic lethality, a LEU2-based Cdc42p plas-
mid, pRS315(CDC42), was transformed into TRY3-HV, and
upon sporulation and tetrad dissection, three Ura+ Trp+ His+ Leu+ spores were isolated, indicating that a cdc42V44A,C188Ap Δcl4A double mutant could be rescued by Cdc42p on a plasmid. All
three segregants were stably Leu+, indicating that these
spores required plasmid-borne Cdc42p for survival and con-
fiming that cdc42V44A,C188Ap Δcl4A double mutant displayed syn-
thetistic lethality. Similar synthetic lethality was observed with
the cdc42–1 Δcl4A double mutant allele (28). These results indicate that
these mutant Cdc42 proteins cannot function as the sole copy of
Cdc42p in the cell without a functional Cla4p. Similar
cdc42V44A,C188Ap constructs were made with ste20, bem3, arg1, cdc5–1, and cdc12–5 mutants, but none of these double
mutants displayed synthetic lethality.

DISCUSSION

S. cerevisiae Cdc42p has been implicated in a number of
different processes from bud emergence to pheromone
response, but mostly through indirect information obtained from
characterization of known Cdc42p interacting regulators and
effectors. Insight into Cdc42p direct functions and interactions
has been limited to characterization of mutants that primarily
have defects that disrupt the polarity of the actin cytoskeleton
and prevent bud emergence or affect the nucleotide-binding
state of Cdc42p. The cdc42V44A allele gave rise to an elongated
multibudded, multinucleate phenotype, a phenotype that dif-
fered from other previously characterized cdc42 mutant alleles.
Analysis of this novel effector domain mutant cdc42V44A
showed that Cdc42p was required for triggering the apical
isotropic growth switch and for timely progression through the
G/M transition.

Based on the altered two-hybrid interactions with Cla4p, the
dosage-dependent suppression by CLA4 and the synthetic leth-
ality of the cdc42V44A Δcl4A double mutant, Cdc42pV44Ap effects
on morphology and the cell cycle were likely manifested
through Cla4p. Data implicating Cdc42p in the regulation of

at 23 °C and 37 °C. Plasmids containing CDC42 completely
complemented the cdc42V44A morphological defect at 23 °C and
could also complement the temperature sensitive lethality.
However, it could only partially complement the morphological
phenotype at 37 °C (data not shown), suggesting that the V44A
mutation was semi-dominant at 37 °C. Overexpression of the
other seven proteins could not suppress the cdc42V44A temper-

Cla4p kinase activity during the cell cycle have been reported previously. Cla4p kinase activity was found to be most active during mitosis, and when the Cla4p CRIB domain was deleted, kinase activity peaked closer to G1/START (29). Cla4p also has been shown to biochemically and genetically interact with the Gin4p and Nap1p kinases, which are thought to play a role in regulating the Clb2p/Cdc28p kinase-dependent G2/M transition (37, 57, 58). Therefore, Cdc42 V44Ap altered interactions with Cla4p may be affecting the regulatory pathway that controls passage through the G2/M transition to cause a nuclear division delay that at least partially contributes to the mutant phenotype.

Recently, the septin proteins, among their many roles during the cell cycle, have also been implicated in regulating entry into mitosis (58, 59), which suggests that the septin structural defects seen in cdc42V44A may trigger the G2/M checkpoint. Septin ring formation occurs in the G1 phase of the cell cycle ~15 min prior to bud emergence (56), and is required for proper chitin ring formation prior to bud emergence at the presumptive bud site, but is not required for bud emergence itself (38, 54). Previously, cdc42 loss of function mutants were found to have delocalized chitin and were unable to localize or form a septin ring (38). The delocalized chitin deposition and lack of a chitin ring in 50% of cdc42V44A cells suggested that Cdc42V44A may affect the function or structure of the septin ring and the delocalization of GFP-Cdc3p and GFP-Cdc12p corroborated this hypothesis. The diffuse and delocalized GFP-Cdc3p and GFP-Cdc12p staining patterns in cdc42V44A elongated-budded cells (Fig. 3) suggested that Cdc42V44A affected septin ring formation or maintenance but not localization of the septins to the bud. Therefore, along with its role in localizing the septins to the presumptive bud site, Cdc42p may play a role in the structural assembly and maintenance of the septin ring.

The correlation between the cdc42V44A morphological defects and Cdc42p interactions with Cla4p and the aberrant ring structure seen in these mutant cells suggests that together Cdc42p and Cla4p regulate the septins. There are several lines of evidence that support a functional relationship between Cdc42p, Cla4p, and the septins. First, a Δcla4 mutant had defects in the maintenance of the septin ring to the mother-bud neck region as shown in Fig. 3 that were similar to the septin ring defects seen in cdc42V44A cells. cla4Δ ste20 double mutant cells have also been shown to have similar defects in septin ring maintenance (28). Second, several genetic interactions between Cdc42p, Cla4p, and the septins have been established including synthetic lethality observed with Δcla4 cdc42V44A and Δcla4 cdc42–1 double mutants as well as cla4 cdc12 double mutants (28). Further evidence to support this hypothesis is that Gin4p mitosis-specific phosphorylation is dependent on Cla4p whose mitosis-specific phosphorylation is dependent on Nap1p and Cdc42p (37). Gin4p kinase activity promotes septin organization and the septins interact with, and are dependent on Gin4p for proper ring structure (60). Therefore, Cdc42V44A reduced interaction with Cla4p may be affecting Cla4p functional association with Gin4p thereby interfering with septin ring structure. The ability of Cla4p and Cdc12p to suppress cdc42V44A could be due to the overexpressed proteins localizing and functioning properly, allowing cells to progress through G2/M.

Interestingly, overexpression of GFP-Cdc12p did not suppress the Δcla4 elongated-bud phenotype, but was able to partially suppress the cdc42V44A phenotype, suggesting that the cdc42V44A mutant had a milder septin localization defect than the Δcla4 mutant. It should also be noted that the penetrance of the elongated-bud morphological defect was higher in cdc42V44A cells (80% of budded cells) than in Δcla4 mutant cells (50% of budded cells). This difference in penetrance suggested that the cdc42V44A phenotype was not solely the result of altered interactions with Cla4p. Two-hybrid analysis revealed that the V44A mutation, in addition to reducing interactions with Cla4p, affected interactions with Cdc24p (6) and effectors Gic1p and Gic2p (Fig. 5). The correlation between the reduced
interactions between Cdc42V44A and Gic1p or Gic2p is difficult to assess since the functions of Gic1p and Gic2p are not well defined and Gic2p, unlike Cla4p, was unable to suppress the cdc42V44A morphological defect. However, gic1 gic2 double mutants appear to have defects in nuclear migration (27), suggesting that the reduced two-hybrid interactions seen between Cdc42V44A and Gic1p and Gic2p were contributing to the nuclear division delay seen in cdc42V44A cells. Furthermore, genetic interactions between Gic1p, Gic2p, and Cla4p have been observed (27), suggesting that these proteins may have overlapping roles as Cdc42p effectors. Therefore, the reduced interactions between Cdc42V44A and Cla4p, Gic1p, and Gic2p are all likely contributing to the cdc42V44A phenotype and may explain why the cdc42V44A phenotype is more severe than the cla4 mutant phenotype. Taken together, these results suggested that the severity of the cdc42V44A G2/M delay was not due to a specific alteration of Cdc42p function and/or protein interactions, but was the result of a combination of altered functions and interactions with several regulators and effectors including Cdc24p, Cla4p, Gic1p, and Gic2p.

The proper localization of the GFP-Cdc42V44A fusion protein suggested further that the effects of the V44A mutation are most likely the result of altered protein interactions as opposed to a localization defect (Fig. 4). It is interesting to note that wild type GFP-A4p-Cdc42p localized not only to the pre-bud site and the tips and sides of elongating buds but also to the mother-bud neck region in some larger budded cells, suggesting a function in cytokinesis and/or cell separation. The partial cytokinesis and cell separation defect in cdc42V44A cells also supports a role for Cdc42p at later stages in the cell cycle although what that function is remains unclear. GFP-A4p-Cdc42p also localized properly regardless of the presence of Cla4p, suggesting that Cdc42p localization is not dependent on Cla4p.

The cdc42V44A Δswe1 phenotype (Fig. 2) is consistent with Cdc42V44A activating the G2/M morphogenetic checkpoint that controls the apical-isotropic growth switch and entry into mitosis. Swe1p, the S. cerevisiae homolog of the Schizosaccharomyces pombe CDC11 CDC2-dependent kinase Wee1p, phosphorylates the Cdc28p cyclin-dependent kinase on residue Y19 (61, 62), thereby inhibiting Cdc28p kinase activity and resulting in a G2 phase cell-cycle delay. Swe1p is required for the morphogenetic checkpoint, and monitors the actin cytoskeleton and possibly other structural components required during the budding process (51, 53, 63), and Δswe1 mutants no longer delay at G2 phase. The aberrant phenotype associated with the cdc42V44A Δswe1 double mutant suggested that Δswe1 suppressed the elongated-budded phenotype characteristic of a G2/M delay but did not alleviate the aberrant multibudded and multinucleate defects associated with Cdc42V44A, suggesting that the G2/M delay is necessary to repair or compensate for the effects of Cdc42V44A in order to allow the cell to proceed through the checkpoint. However, since some cdc42V44A cells are multibudded and multinucleate even post-glusulase treatment, which indicated that a percentage of the cells do have a cytokinesis defect, the G2/M delay may not always be sufficient for alleviating Cdc42V44A effects.

Actin depolarization prior to relocation to the mother-bud neck region for cytokinesis is characteristic of the apical-isotropic growth switch that occurs during the G2/M transition and requires activation of the Cln2p/Cdc28p kinase complex (51, 52). However, the cdc42V44A G2/M delay did not seem to be the result of a depolarized cytoskeleton or a loss of bud emergence. Rather, proper actin polarization and bud emergence in cdc42V44A cells suggested that Cdc42V44A may exert different effects than in the G2/M transition that is initiating the G2/M morphogenetic checkpoint. Since one of the mechanistic defects observed in cdc42V44A cells is in septin ring structure, passage through the G2/M checkpoint in these cells may be regulated through the septins. If this is the case, then cdc42V44A cells that were able to form a stable septin ring during the mitotic delay may have progressed through the checkpoint allowing for viability, while those cells that never formed a stable ring did not recover from the checkpoint resulting in the cytokinesis defect seen in some cells. The septin proteins have recently been implicated in regulating the Swe1p morphogenetic checkpoint pathway through their interactions with Has1p, a Nip1p-like protein that negatively regulates Swe1p, and Has1p homologs Kec1p and Gin4p (59). Δswe1 also suppressed the cdc12 elongated-budded phenotype and the Δswe1 cdc12 double mutant had similar morphological and nuclear phenotypes to those seen in cdc42V44A Δswe1 cells (59). Therefore, Cdc42p-dependent regulation of septin organization may be required for progressing through the G2/M morphogenetic checkpoint.

The data presented here strongly suggest that Cdc42p is required for timely cell cycle progression and is dependent on differential interactions with its regulators and downstream effectors for function. The differential interactions seen between Cdc42V44A and various regulators and effectors most likely contributed to the severe morphological defects associated with cdc42V44A. Surprisingly, this mutant is still viable, suggesting that Cdc42p functioning with other proteins in the cell can compensate for these altered interactions. The characteristics of cdc42V44A also suggested that Cdc42p has a direct role in septin ring structural maintenance that is mediated by Cla4p. What still remains unclear is how Cdc42p signals are passed through its various interactions with regulators and downstream effectors to structural cellular components like actin and the septins. Future characterization of cdc42 mutants that mechanistically disrupt interactions with various regulators, effectors and structural components of the cell polarity pathway should be useful in discerning the Cdc42p-dependent signals required for bud emergence, the G2/M transition and cytokinesis.

Acknowledgments—We thank Ben Benton, Fred Cross, Janet Kurjan, Maria Molina Martin, George Sprague, Brian Haarer, Malcolm Whiteway, Daniel Lew, Johanna O'Dell, Clarence Chan, Charles Boone, Alison Adams, and Alia Merla for sharing valuable reagents. We also thank David Pederson and members of the Johnson laboratory for valuable discussions and critical comments on the manuscript.

REFERENCES

1. Johnson, D. I. (1999) Microbiol. Mol. Biol. Rev. 63, 54–105
2. Adams, A. F., Johnson, D. I., Longnecker, R. M., Sloat, B. F., and Pringle, J. R. (1990) J. Cell Biol. 111, 131–142
3. Johnson, D. I., and Pringle, J. R. (1990) J. Cell Biol. 111, 143–152
4. Miller, P. J., and Johnson, D. I. (1997) Yeast 13, 561–572
5. Ziman, M., O'Brien, J. M., Ouellette, L. A., Church, W. R., and Johnson, D. I. (1995) Mol. Cell. Biol. 12, 3457–3544
6. Davis, C. R., Richman, T. R., Deliduka, S. B., Blaisell, J. O., Collins, C. C., and Johnson, J. L. (1996) J. Cell. Biol. 132, 849–861
7. Ziman, M., Preuss, D., Mulholland, J., O'Brien, J. M., Botstein, D., and Johnson, D. I. (1995) Mol. Cell. Biol. 4, 1307–1316
8. Ziman, M., and Johnson, J. L. (1991) Yeast 7, 463–474
9. Hart, M. J., Eva, A., Evans, T., Aaronson, S. A., and Cerierne, R. A. (1991) Nature 341, 311–314
10. Hartwell, L. H., Mortimer, R. K., Cuolotti, J., and Cuolotti, M. (1973) Genetics 74, 267–286
11. Sloat, B. F., Adams, A., and Pringle, J. R. (1983) J. Cell Biol. 89, 395–405
12. Sloat, B. F., and Pringle, J. R. (1987) Science 236, 1171–1173
13. Zheng, Y., Cerione, R., and Bender, A. (1994) J. Biol. Chem. 269, 2366–2372
14. Zheng, Y., Hart, M. J., Shinjo, K., Evans, T., Bender, A., and Cerierne, R. A. (1998) J. Cell. Biol. 150, 2469–2484
15. Bender, A., and Pringle, J. R. (1991) Mol. Cell. Biol. 11, 1295–1305
16. Stevenson, B. J., Rhodes, N., Errede, B., and Sprague, G. F. (1992) Genes Dev. 6, 1293–1304
17. Stevenson, B. J., Ferguson, B., De Vargillo, C., Bie, E., Pringle, J. R., Ammerer, G., and Sprague, G. F. (1995) Genes Dev. 9, 2949–2963
18. Epp, J. A., and Chant, J. (1997) Curr. Biol. 7, 921–926
19. Lippincott, J., and Li, R. (1998) J. Cell Biol. 140, 355–366
20. Osman, M. A., and Cerierne, R. A. (1998) J. Cell Biol. 142, 443–455
Analysis of the cdc42\textsuperscript{V44A} Effector Domain Mutant

21. Li, R. (1997) J. Cell Biol. 136, 649–658
22. Kohno, H., Tanaka, K., Mino, A., Umikawa, M., Imamura, H., Fujitani, T., Fujita, Y., Hotta, K., Qadota, H., Watanabe, T., Ohya, Y., and Takai, Y. (1996) EMBO J. 15, 6060–6068
23. Evangelista, M., Blundell, K., Longtine, M. S., Chow, C. J., Adams, N., Pringle, J. R., Peter, M., and Boone, C. (1997) Science 276, 118–122
24. Imamura, H., Tanaka, K., Hihara, T., Umikawa, M., Kamei, T., Takahashi, K., Sasaki, T., and Takai, Y. (1997) EMBO J. 16, 2745–2755
25. Lee, L., Klee, S. K., Evangelista, M., Boone, C., and Pellman, D. (1999) J. Cell Biol. 144, 947–961
26. Brown, J. L., Jaquenoud, M., Gulli, M.-P., Chant, J., and Peter, M. (1997) EMBO J. 16, 2972–2982
27. Chen, G.-C., Kim, Y.-J., and Chan, C. S. M. (1997) J. Biol. Chem. 272, 29071–29074
28. Cvrčková, F., De Virgilio, C., Manser, E., Pringle, J. R., and Nasmyth, K. (1997) EMBO J. 16, 83–97
29. Peter, M., Neiman, A. M., Park, H.-O., van Lohuizen, M., and Herskowitz, I. (1996) EMBO J. 15, 7046–7059
30. Leberer, E., Dignard, D., Harcus, D., Thomas, D. Y., and Whiteway, M. (1992) J. Cell Biol. 119, 939–953
31. Leberer, E., Wu, C., Leevu, T., Furest-Lieuvin, A., Segall, J. E., and Thomas, D. Y. (1997) EMBO J. 16, 83–97
32. Peter, M., Neiman, A. M., Park, H.-O., van Lohuizen, M., and Herskowitz, I. (1996) EMBO J. 15, 7046–7059
33. Martin, H., Menduza, A., Rodriguez-Pachón, J. M., Molina, M., and Nombela, C. (1997) Mol. Microbiol. 23, 431–444
34. Wu, C., Whiteway, M., Thomas, D. Y., and Leberer, E. (1995) J. Biol. Chem. 270, 15984–15992
35. Wu, C., Leevu, T., Leberer, E., Thomas, D. Y., and Whiteway, M. (1998) J. Biol. Chem. 273, 28107–28115
36. Eby, J. J., Holly, S. P., van Drogen, F., Grishin, A. V., Peter, M., Drubin, D. G., and Blumer, K. J. (1998) Curr. Biol. 8, 967–970
37. Tjandra, H., Compton, J., and Kellogg, D. (1998) Curr. Biol. 8, 991–1000
38. Longtine, M. S., DeMarini, D. J., Valenčíková, F., and Pringle, J. R. (1996) Curr. Opin. Cell Biol. 8, 106–119
39. Burbele, P. D., Drechsel, D., and Hall, A. (1995) J. Biol. Chem. 270, 29071–29074
40. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
41. Sherman, F., Fink, G. R., and Hicks, J. B. (1986) Methods in Yeast Genetics: Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
42. Cross, F. R. (1997) Yeast 13, 647–653
43. Hill, J. E., Myers, A. M., Koerner, T. J., and Tsangoloff, A. (1986) Yeast 2, 163–167
44. Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523
45. Chien, C.-T., Bartel, P. L., Stern-glanz, R., and Fields, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9578–9582
46. Gyužys, J., Golemis, E., Chertkov, H., and Brent, R. (1993) Cell 75, 791–803
47. Reynolds, A., and Lundblad, V. (1989) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) Vol. 2, pp. 13.6.2–13.6.4, John Wiley & Sons, Inc., New York
48. Pringle, J. R., Preston, R. A., Adams, A. E. M., Stearns, T., Drubin, D. G., Haarer, B. K., and Jones, E. W. (1989) Methods Cell Biol. 31, 357–435
49. Adams, A. E. M., and Pringle, J. R. (1994) J. Cell Biol. 100, 934–945
50. Sia, R. A. L., Bardes, E. S. G., and Lew, D. J. (1998) EMBO J. 17, 6678–6688
51. McMillan, J. N., Sia, R. A. L., and Lew, D. J. (1998) J. Cell Biol. 142, 1487–1499
52. Lew, D. J., and Reed, S. I. (1993) J. Cell Biol. 120, 1305–1320
53. Lew, D. J., and Reed, S. I. (1995) J. Cell Biol. 129, 739–749
54. DeMarini, D. J., Adams, A. E. M., Fares, H., DeVirgilio, C., Valle, G., Chuang, J. S., and Pringle, J. R. (1997) J. Cell Biol. 139, 75–93
55. Haarer, B. K., and Pringle, J. R. (1987) Mol. Cell. Biol. 7, 3678–3687
56. Kim, H. B., Haarer, B. K., and Pringle, J. R. (1991) J. Cell Biol. 112, 535–544
57. Altman, R., and Kellogg, D. (1997) J. Cell Biol. 138, 119–130
58. Carroll, C. W., Altman, R., Schröder, J. J., and Kellogg, D. (1998) J. Cell Biol. 143, 709–717
59. Barral, Y., Parra, M., Bildingmaier, S., and Snyder, M. (1999) Genes Dev. 13, 176–187
60. Longtine, M. S., Fares, H., and Pringle, J. R. (1998) J. Cell Biol. 143, 719–736
61. Russell, P., Moreno, S., and Reed, S. I. (1989) Cell 57, 295–303
62. Bhorser, R. N., Deshaies, R. J., and Kirschner, M. W. (1993) EMBO J. 12, 3417–3426
63. Sia, R. A. L., Herald, H. A., and Lew, D. J. (1996) Mol. Biol. Cell 7, 1657–1666
64. Wilkinson, L. E., and Pringle, J. R. (1997) Exp. Cell Res. 295, 175–187
65. Akada, R., Kallal, L., Johnson, D. I., and Kurjan, J. (1996) Genetics 143, 103–117