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# Mutational Analysis of *CDC42Sc*, a *Saccharomyces cerevisiae* Gene That Encodes a Putative GTP-Binding Protein Involved in the Control of Cell Polarity

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**The *Saccharomyces cerevisiae CDC42* gene product, a member of the *ras* superfamily of low-molecular-weight GTP-binding proteins, is involved in the control of cell polarity. We have analyzed the effects of three *CDC42* mutations (Gly to Val-12, Gln to Leu-61, and Asp to Ala-118) in the putative GTP-binding and hydrolysis domains and one mutation (Cys to Ser-188) in the putative isoprenylation site. The first three mutations resulted in either a dominant-lethal or dose-dependent dominant-lethal phenotype when present on plasmids in haploid *cdc42-1$^{ts}$* or wild-type strains. Both wild-type and *cdc42-1$^{ts}$* cells carrying plasmids (p*GAL*) with either the *CDC42*$^{Val-12}$ or *CDC42*$^{Leu-61}$ alleles under the control of a *GAL* promoter were arrested with a novel phenotype of large cells with elongated or multiple buds. Cells carrying p*GAL-CDC42*$^{Ala-118}$ were arrested as large, round, unbudded cells reminiscent of *cdc42-1$^{ts}$* arrested cells. The different phenotype of the *CDC42*$^{Ala-118}$ mutant versus the *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ mutants was unexpected since the phenotypes of all three analogous *ras* mutants were similar to each other. This suggests that aspects of the biochemical properties of the Cdc42 protein differ from those of the Ras protein. The *cdc42*$^{Ser-188}$ mutant gene was incapable of complementing the *cdc42-1$^{ts}$* mutation and was recessive to both wild-type and *cdc42-1$^{ts}$*. In double-mutant alleles, the *cdc42*$^{Ser-188}$ mutation was capable of suppressing the dominant lethality associated with the three putative GTP-binding and hydrolysis mutations, suggesting that isoprenylation is necessary for the activity of the wild-type and mutant proteins.**

The *Saccharomyces cerevisiae CDC42* gene product is involved in the control of several morphogenetic events during the cell cycle, including the generation of cell polarity, development of normal cell shape, localization of secretion, and deposition of cell-surface material (1, 2, 20). Its predicted amino acid sequence (20) is similar to that of members of the *ras* superfamily of low-molecular-weight GTP-binding proteins from *S. cerevisiae* and higher eukaryotes (16), especially in those domains that have been implicated in the binding and hydrolysis of GTP and in carboxyl-terminal modifications. *CDC42* shows the greatest degree of sequence similarity to the products of two related human cDNAs, *CDC42Hs* (39) and *G25K* (27), which encode isoforms of the low-molecular-weight GTP-binding protein previously referred to as $G_p$ or G25K (11, 33). Both of these human gene products are 80% identical (88% related amino acids) in predicted amino acid sequence to the *S. cerevisiae CDC42* gene product, which we have redesignated *CDC42Sc*, and they are 95% identical to each other. These human genes, when expressed in *S. cerevisiae* under the control of a yeast promoter on a multicopy plasmid, can functionally complement the *cdc42-1$^{ts}$* mutation (27, 39). The $G_p$/G25K protein is an excellent in vitro phosphosubstrate for the epidermal growth factor receptor tyrosine kinase (17), but its in vivo function is unknown.

In order to further elucidate the role of the *CDC42Sc* gene product in the control of cell polarity, we have generated new mutations in *CDC42Sc* by using site-directed mutagenesis (see Fig. 1). A carboxyl-terminal mutation predicted to eliminate isoprenylation results in a nonfunctional product. Mutations in the putative guanine-nucleotide-binding domain lead to a lethal phenotype that can be suppressed by combination with the carboxyl-terminal mutation. This lethality is manifested as several different and unique morphological abnormalities, which have provided clues to the function of the *CDC42Sc* gene product in controlling cell polarity.

## MATERIALS AND METHODS

**Reagents.** Enzymes, M13 dideoxy sequencing kit, mutagenesis kit, and other reagents were obtained from standard commercial sources and used according to the suppliers' specifications. $^{35}$S-dATP was obtained from Amersham Corp. (Arlington Heights, Ill.). Anti-yeast actin and anti-yeast tubulin antibodies were a gift from Sue Lillie and Brian Haarer (The University of Michigan, Ann Arbor, Mich.). Calcofluor (fluorescent brightener) and 4',6-diamidino-2-phenylindole (DAPI) were obtained from Sigma Chemical Co. (St. Louis, Mo.).

**Media, growth conditions, and strains.** Conditions for the growth and maintenance of bacterial and yeast strains have been described previously (23, 25, 38). The permissive and restrictive temperatures for growth of temperature-sensitive mutants were 23 and 37°C, respectively. *Escherichia coli* HB101 was routinely used as a plasmid host and *E. coli* CJ236 and MV1190 were used in site-directed mutagenesis experiments (22). The *S. cerevisiae* strains used were TD4, *MAT***a** *ura3 his4 leu2 trp1 gal2* (20); DJTD2-16A, *MAT***a** *cdc42-1 ura3 his4 leu2 trp1 gal2* (20); DJMD4-30B, *MAT***a** *ura4 asp5 his3 ilv5 leu2 GAL2* (20); DC5, *MAT***a** *his3 leu2*

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*gal2* (6); and DJMD22-3B, *MATa cdc42-1 his4 leu2 trp1 GAL2* (20).

**DNA manipulations.** Standard procedures were used for recombinant DNA manipulations (25), *E. coli* and yeast transformations (25, 38), and plasmid isolation from *E. coli* (5). Site-directed mutagenesis (22) was performed with the MUTA-GENE kit (Bio-Rad Laboratories, Richmond, Calif.), with an M13mp19 phage containing *CDC42Sc* on a 1.8-kb *Sca*I-*Xba*I fragment (20) as the starting template. A mutation producing a *Nde*I site at the *CDC42Sc* start codon was constructed (this change does not affect complementation of the *cdc42-1$^{ts}$* mutation), and this template was used to generate single mutants; single-mutant templates were used to generate double mutants. The mutagenic oligonucleotides are GGTCTTCCACCATATGCAAACGC (*Nde*I site; the underlined C and T are both A in the wild-type sequence), GGTGATGTTGCTGTTG (Gly to Val-12; the underlined T is G in the wild-type sequence), CGGCCGGTCTAGAAG (Gln to Leu-61; the underlined T is A in the wild-type sequence), GCAGATTGCTCTAAGG (Asp to Ala-118; the underlined C is A in the wild-type sequence), and CCTGT TATCAAGAAAAGTAAAAAATCTACAATTTTGTAGTC (Cys to Ser-188; the underlined C is G in the wild-type sequence). To confirm the presence of desired mutations and the absence of any spurious mutations, we determined the sequence of the entire coding region for each mutant gene by M13 dideoxy sequencing (35).

**Plasmid constructions.** Plasmids YEp351, YEp51, YEp103 (*CDC42Sc*), pBM272, and pRS315 have been described elsewhere (18, 20, 21, 40). The 1.7-kb *Xho*I-*Hin*dIII fragments from M13mp19 phage containing single mutant and wild-type genes were inserted into *Sal*I-*Hin*dIII-digested YEp351. YEp-derived 1.7-kb *Bam*HI-*Hin*dIII fragments were inserted into *Bam*HI-*Hin*dIII-digested pRS315 to generate YCp-based plasmids. To construct p*GAL* plasmids, a 1.4-kb *Hpa*I fragment containing upstream *CDC42Sc* sequences (20) was deleted from the YCp-based plasmids. A 685-bp *Eco*RI-*Hin*dIII fragment containing the divergent *GAL1* and *GAL10* promoters derived from pBM272 (21) was blunt ended with the Klenow fragment of DNA polymerase I and inserted into *Hpa*I-digested "promoterless" YCp-based plasmids. YCp-based plasmids containing double mutant genes were constructed by inserting the 1.7-kb *Xho*I-*Hin*dIII fragment from M13mp19 phage containing double mutant genes into *Xho*I-*Hin*dIII-digested pRS315.

**Peptide synthesis and immunological techniques.** The Cdc42Sc-specific peptide NVFDEAIVAALEPPVIK (in standard one-letter code) corresponding to residues 165 to 181 was synthesized as described previously (8) and conjugated to ovalbumin. This peptide-ovalbumin conjugate (200 μg) was mixed 1:1 with Freund complete adjuvant and used to immunize New Zealand White rabbits intramuscularly. Weekly booster injections were given subcutaneously for 5 to 6 weeks by using 100 μg of conjugate mixed 1:1 with Freund incomplete adjuvant, and sera were collected weekly thereafter. Affinity-purified antibodies were generated by binding crude immune sera to nitrocellulose strips containing the Cdc42Sc protein overproduced in *E. coli* (29a) and eluting Cdc42-specific antibodies with a low-pH glycine wash (15). Antibody-Cdc42Sc protein complexes were visualized on Western blots by using horseradish peroxidase-conjugated secondary antibodies as described (15). Whole-cell lysates were prepared by spheroplasting with zymolyase (ICN Biochemicals, Costa Mesa, Calif.), followed by boiling in sodium dodecyl sulfate (SDS) loading buffer (15).

**Photomicroscopy methods.** Procedures for fixing cells with formaldehyde, examining cell morphologies, staining cell-wall chitin with Calcofluor, staining nuclei with DAPI, and anti-actin immunofluorescence have been described previously (34). Photomicrographs were obtained with an Olympus BH-2 epifluorescence microscope containing Hoffman Modulation Contrast optics.

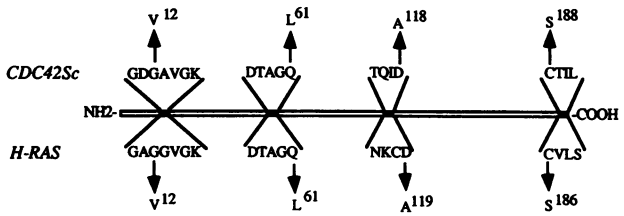


FIG. 1. Comparison of mutations in *CDC42Sc* (20) to mutations in *H-ras* (7) that alter either the GTP-binding/hydrolysis of p21$^{H\text{-}ras}$ (Val-12, Leu-61, Ala-119) or isoprenylation (Ser-186).

## RESULTS

**Mutations in predicted GTP-binding and hydrolysis domains.** On the basis of the conservation between the Cdc42Sc and Ras proteins in areas implicated in GTP binding and hydrolysis, we made three mutations in *CDC42Sc* based on analogous mutations in human *H-ras* that affect GTP binding and hydrolysis and confer a transforming phenotype (3) (Fig. 1). The mutant genes were inserted into the low-copy-number (YCp) vector pRS315 and introduced into the *cdc42-1$^{ts}$ leu2* haploid strain DJTD2-16A at 23°C. Plasmids containing the *CDC42*$^{Val\text{-}12}$, *CDC42*$^{Leu\text{-}61}$, and *CDC42*$^{Ala\text{-}118}$ alleles gave 100- to 1,000-fold fewer Leu$^+$ transformants than the control plasmids pRS315 or YCp(*CDC42Sc*) (Table 1), suggesting that the mutant gene products result in dominant lethality in *cdc42-1$^{ts}$* cells at the permissive temperature. Similar results were obtained when these mutant genes were carried on the high-copy-number plasmid YEp351 (data not shown).

The effect of the mutant alleles on low-copy-number plasmids was also tested in the wild-type *CDC42Sc* strain TD4. The *CDC42*$^{Val\text{-}12}$ and *CDC42*$^{Leu\text{-}61}$ alleles again

TABLE 1. Transformation of yeast by various plasmid-based *CDC42Sc* alleles[a]

| Plasmid | Genotype of recipient strain[b] | | Phenotype |
|---|---|---|---|
| | *cdc42-1$^{ts}$* | *CDC42* | |
| YCp(*CDC42*$^{Val\text{-}12}$) | − | − | Dominant lethal |
| YCp(*CDC42*$^{Leu\text{-}61}$) | − | − | Dominant lethal |
| YCp(*CDC42*$^{Ala\text{-}118}$) | − | +/− | Dose dependent dominant lethal |
| YCp(*cdc42*$^{Ser\text{-}188}$) | +(*ts*) | + | Nonfunctional |
| YCp(*CDC42Sc*) | + | + | Functional |
| YCp | +(*ts*) | + | |
| YCp(*cdc42*$^{Val\text{-}12,Ser\text{-}188}$) | +(*ts*) | + | Nonfunctional |
| YCp(*cdc42*$^{Leu\text{-}61,Ser\text{-}188}$) | +(*ts*) | + | Nonfunctional |
| YCp(*cdc42*$^{Ala\text{-}118,Ser\text{-}188}$) | +(*ts*) | + | Nonfunctional |

[a] Data are from at least three independent transformation experiments.

[b] Symbols: + represents 100- to 1,000-fold more transformants per microgram of DNA than −; +/− represents 10-fold fewer transformants per microgam of DNA than control plasmids. *ts* indicates no growth at 36°C, the *cdc42-1$^{ts}$* restrictive temperature, and a nonfunctional protein. The *cdc42-1$^{ts}$* strain is DJTD2-16A; the *CDC42* strain is TD4.

showed a very low frequency of transformants, indicating dominant lethality (Table 1). In contrast, the frequency of transformants with the *CDC42*$^{Ala-118}$ allele was only 10-fold lower than that of control plasmids (Table 1). Similar transformation frequencies were obtained when these alleles were carried on high-copy-number plasmids and when these plasmids were tested in a *cdc42-1*$^{ts}$ strain carrying the wild-type *CDC42Sc* gene on a high-copy-number plasmid (data not shown). YEp(*CDC42*$^{Ala-118}$) transformants grew at the same rate as cells containing YEp(*CDC42Sc*); however, microscopic examination showed that 5 to 10% of the cells were abnormally large in the population of cells (200 cells were counted) carrying YEp(*CDC42*$^{Ala-118}$) versus 0 to 1% in the population of cells carrying YEp(*CDC42Sc*).

**A mutation in the putative isoprenylation site.** Cys-to-Ser mutations in the conserved -Cys-Xaa-Xaa-Xaa (Xaa is any amino acid) carboxyl-terminal sequence of human and yeast *RAS* (Fig. 1) abolish isoprenylation, membrane localization, and oncogenic potential (3). We therefore made the analogous change and inserted the *cdc42*$^{Ser-188}$ gene into YEp and YCp plasmids. These plasmids resulted in a transformation frequency of the *cdc42-1*$^{ts}$ and *CDC42Sc* strains that was similar to that of the control plasmids. The *cdc42*$^{Ser-188}$ allele was incapable of complementing the *cdc42-1*$^{ts}$ allele at the restrictive temperature of 36°C (Table 1), indicating that the *cdc42*$^{Ser-188}$ allele is a recessive, nonfunctional allele. This result suggests that proper membrane localization is necessary for *CDC42Sc* function.

**Effect of *cdc42*$^{Ser-188}$ mutation on GTP-binding and hydrolysis mutations.** Three double-mutant alleles, *cdc42*$^{Val-12,Ser-188}$, *cdc42*$^{Leu-61,Ser-188}$, and *cdc42*$^{Ala-118,Ser-188}$ (Fig. 1), were constructed to test the effect of the presumptive mislocalization caused by the *cdc42*$^{Ser-188}$ mutation on the lethality associated with the Val-12, Leu-61, and Ala-118 mutations. YCp plasmids containing the double-mutant alleles were capable of transforming both wild-type and *cdc42-1*$^{ts}$ strains at the same frequency as control plasmids but were incapable of complementing the *cdc42-1*$^{ts}$ allele (Table 1). The *cdc42*$^{Ser-188}$ mutation is therefore capable of suppressing the dominant lethality associated with the *CDC42*$^{Val-12}$, *CDC42*$^{Leu-61}$, and *CDC42*$^{Ala-118}$ alleles and suggests that the proper localization of the mutant proteins is necessary for them to exert their lethal effects.

**Expression of *CDC42Sc* mutant alleles under *GAL* promoter control.** The wild-type and four single-mutant genes were placed under the control of a galactose-inducible promoter (*GAL1* or *GAL10*) on YCp plasmids. These constructs allow growth on glucose medium and determination of the mutant terminal phenotypes after induction on galactose medium (Fig. 2 and 3). These p*GAL* plasmids were introduced into the *cdc42-1*$^{ts}$ strain DJMD22-3B at 23°C on glucose (repressing) medium (Fig. 2A). Cells containing p*GAL-CDC42Sc*, p*GAL-cdc42*$^{Ser-188}$, or YEp51 (a *LEU2 GAL10* control plasmid) grew on medium containing 2% galactose (inducing conditions), but cells containing p*GAL-CDC42*$^{Val-12}$, p*GAL-CDC42*$^{Leu-61}$, or p*GAL-CDC42*$^{Ala-118}$ could not grow under these inducing conditions (Fig. 2B). This result is consistent with the dominant lethality seen with plasmids containing these mutations in *cdc42-1*$^{ts}$ cells. Similar results were obtained when these plasmids were introduced into the wild-type strain DJMD4-30B (data not shown).

Increased expression of Cdc42 proteins was seen after a shift to inducing conditions in Western blot analysis with Cdc42Sc-specific antibodies (Fig. 4A). The multiple protein bands recognized by the Cdc42Sc-specific antibodies may be different modified forms of the protein, which is an interesting possibility because the Cdc42Hs/G25K protein is phosphorylated in vitro by the epidermal growth factor receptor tyrosine kinase (17). The mobility rate of the Ser-188 protein is slower than that of the wild-type protein, suggesting that the Ser-188 protein may be unprocessed (see Discussion). The lesser amount of Cdc42 protein in *cdc42-1*$^{ts}$ cells than in wild-type cells (Fig. 4B) may contribute to the dominant lethality of *CDC42*$^{Ala-118}$ observed in *cdc42-1*$^{ts}$ but not wild-type cells. Whereas the *CDC42*$^{Ala-118}$ allele was not lethal in wild-type cells when expressed under its own promoter on a YCp plasmid (Table 1), it was lethal when expressed under *GAL* promoter control on a YCp plasmid, suggesting that it is a dose-dependent dominant-lethal allele.

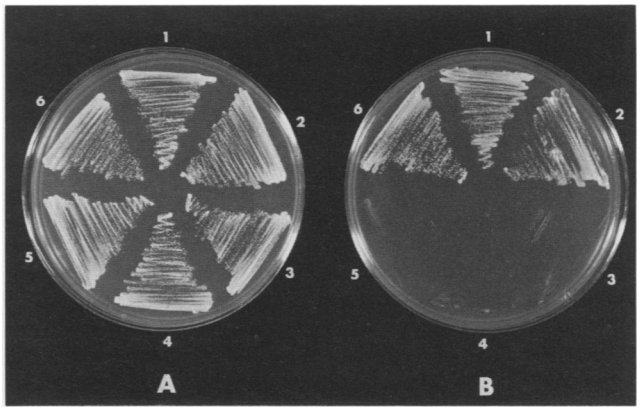


FIG. 2. Growth of cells containing p*GAL* mutant plasmids. p*GAL* plasmids were introduced into yeast strain DJMD22-3B by selection for Leu$^+$ transformants at 23°C. Transformants were then streaked onto -Leu minimal-medium plates that used either 2% glucose (A) or 2% galactose (B) as the sole carbon source, and the plates were incubated at 23°C. Sectors contain cells with the following plasmids: 1, YEp51; 2, p*GAL-CDC42Sc*; 3, p*GAL-CDC42*$^{Val-12}$; 4, p*GAL-CDC42*$^{Leu-61}$; 5, p*GAL-CDC42*$^{Ala-118}$; 6, p*GAL-cdc42*$^{Ser-188}$. Identical results were obtained when these plasmids were introduced into the wild-type strain DJMD4-30B (data not shown).

Cells containing p*GAL-CDC42Sc* and p*GAL-cdc42*$^{Ser-188}$ (Fig. 3C) grown on 2% galactose displayed wild-type cellular morphologies with respect to the parameters described below, except that cells containing p*GAL-CDC42Sc* exhibited a random budding pattern (20). Cells containing either p*GAL-CDC42*$^{Val-12}$ or p*GAL-CDC42*$^{Leu-61}$ grown on 2% galactose were arrested as a morphologically heterogeneous population; 70% of the cells had greatly elongated buds, multiple buds, and/or amorphous shapes and 30% had wild-type morphologies (Fig. 3A, D, and E). Of the aberrant cells, 30% had a single elongated bud and 70% had multiple buds and amorphous shapes.

Because of the involvement of *CDC42Sc* in controlling cellular morphogenesis, we examined the morphological characteristics of cell size, actin and chitin localization, and nuclear DNA content of these dying cells. The large size of the *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ mutant mother cells suggests that the normal ability to localize new cellular growth to the bud has been compromised in favor of mother cell growth (see Discussion). In normal cells, actin-containing structures are observed both as cortical spots within the growing bud and as actin cables within the mother and growing bud (2). Aberrant actin-containing structures were present in the *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ cells (Fig. 3D), with actin cortical spots localized to one or more of the buds

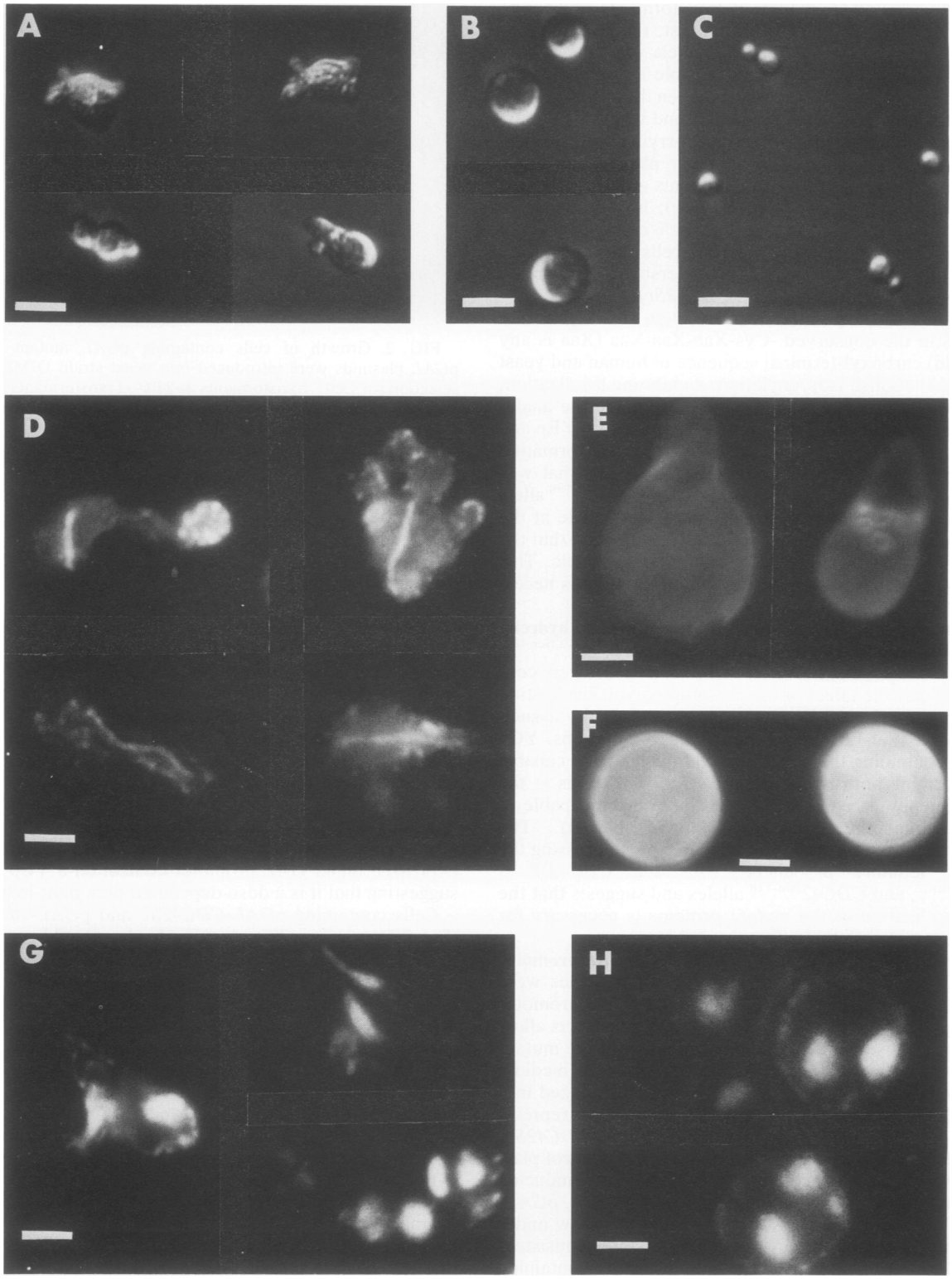


FIG. 3. Morphological characterization of cells containing p*GAL-CDC42* mutant plasmids. DJMD22-3B cells containing p*GAL* plasmids were grown to early log phase in -Leu minimal liquid media plus 2% raffinose. Cells were washed twice in sterile water and resuspended in the same media containing 2% galactose. Cells were allowed to continue growing for 15 h before being fixed with formaldehyde. (A to C) Hoffman Modulation Contrast photomicrographs of cells containing p*GAL-CDC42*$^{Val\text{-}12}$ (A), p*GAL-CDC42*$^{Ala\text{-}118}$ (B), and p*GAL-cdc42*$^{Ser\text{-}188}$ (C). (D) Fluorescence micrograph of cells containing p*GAL-CDC42*$^{Val\text{-}12}$ stained with anti-actin antibodies to visualize microfilaments. (E and F) Fluorescence micrographs of cells containing p*GAL-CDC42*$^{Val\text{-}12}$ (E) and p*GAL-CDC42*$^{Ala\text{-}118}$ (F) stained with Calcofluor to visualize chitin deposition. (G and H) Fluorescence micrographs of cells containing p*GAL-CDC42*$^{Val\text{-}12}$ (G) and p*GAL-CDC42*$^{Ala\text{-}118}$ (H) stained with DAPI to visualize nuclear DNA. Bars: panels A through C, 15 μm; panels D through H, 4 μm.

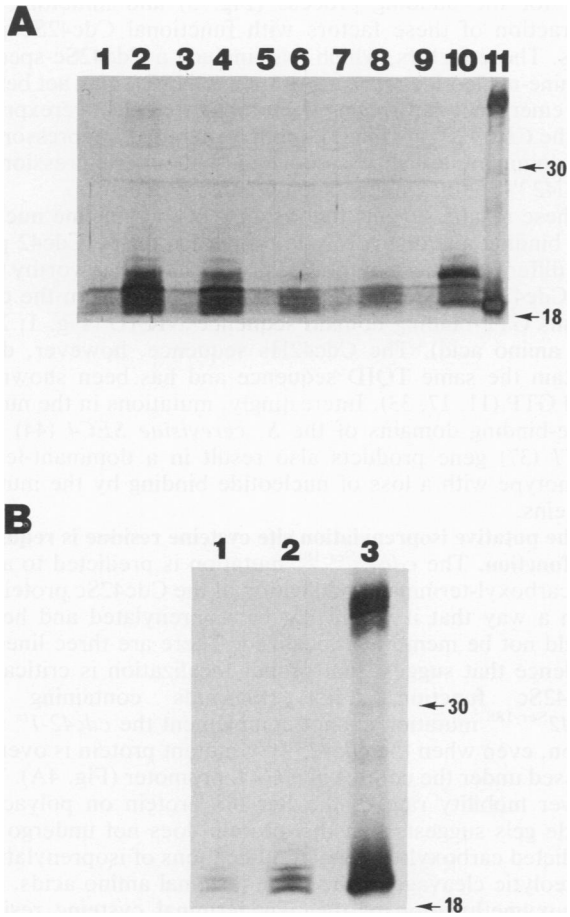


FIG. 4. (A) Western blot analysis of mutant Cdc42Sc proteins. DJMD4-30B cells containing p*GAL-CDC42Sc* (lanes 1 and 2), p*GAL-CDC42*$^{Val-12}$ (lanes 3 and 4), p*GAL-CDC42*$^{Ala-118}$ (lanes 5 and 6), p*GAL-CDC42*$^{Leu-61}$ (lanes 7 and 8), and p*GAL-cdc42*$^{Ser-188}$ (lanes 9 and 10) were grown at 23°C in -Leu minimal media plus 2% raffinose to mid-log phase, washed once with sterile water, and resuspended in the same media plus 2% galactose. Lysates were made from cells that had been galactose induced for 0 h (lanes 1, 3, 5, 7, and 9) or 15 h (lanes 2, 4, 6, 8, and 10). Equal amounts of protein (~50 μg) were loaded in each lane except lane 8 (~25 μg), run on an SDS-12.5% polyacrylamide gel, and transferred to nitrocellulose. The filter was probed with crude anti-Cdc42Sc primary antibodies (1/200 dilution) and horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (1/500 dilution). Anti-Cdc42Sc antibodies recognize an ~21-kDa protein from *E. coli* cells that overproduce the wild-type Cdc42Sc protein (lane 11) (29a) along with an ~38-kDa protein. (B) Western blot analysis of Cdc42-1 and wild-type proteins. Lysates were made from *cdc42-1*$^{ts}$ (strain DJMD22-3B; lane 1) and *CDC42Sc* (strain DC5; lane 2) cells grown at 23°C to mid-log phase. Equal amounts of protein were loaded in each lane and run and transferred as in panel A. Lane 3 is the same as lane 11 in panel A. The filter was probed with affinity-purified anti-Cdc42 antibodies (1/50 dilution) and horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (1/500 dilution). Mobilities of protein molecular mass markers (in kilodaltons) are indicated by arrows.

and thick actin bars present in ~60% of the cells. These actin bars were significantly larger than normal actin cables, and their orientation varied from cell to cell, with some oriented towards a bud and others away from a bud.

In normal cells, chitin is found at the mother-bud junction in a ringlike structure, which is left behind on the mother cell as a "bud scar" after cytokinesis and cell septation (42). Chitin is somewhat delocalized in the *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ cells (Fig. 3E), with a variable amount of chitin localized to the mother-bud junction, a structure which is not well defined in some cells and absent in others. Approximately 20% of these mutant cells are multinucleate (Fig. 3G), with up to four nuclei present in some cells. The concentrated DAPI staining in these cells is associated with antitubulin antibody staining, but the less intense DAPI staining does not seem to have microtubules associated with it (data not shown; see Discussion).

Cells containing p*GAL-CDC42*$^{Ala-118}$ were arrested with 80% large, round, unbudded cells (Fig. 3B), a morphology similar to that of *cdc42-1*$^{ts}$ mutant cells grown at 36°C (1). Delocalized chitin deposition (Fig. 3F), multinucleated cells (Fig. 3H), and delocalized actin staining (data not shown) were also similar with respect to *cdc42-1*$^{ts}$ arrested cells. In ~10% of these cells, we observed thick actin bars similar to those found in *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ cells (Fig. 3D).

## DISCUSSION

Cellular polarity is manifested in at least two ways during the *S. cerevisiae* cell cycle: (i) selection of a nonrandom bud site at one of the two cell poles and (ii) localized growth at the bud site resulting in the appearance and selective growth of the bud. The *CDC42Sc* gene product is involved in the control of cellular polarity and its predicted amino acid sequence exhibits a high degree of amino acid similarity to that of members of the *ras* superfamily of low-molecular-weight GTP-binding proteins. The Cdc42Hs protein has amino acid residues identical to those of Cdc42Sc in the GTP-binding domains and has been shown to bind and hydrolyze GTP (11, 17, 33). We have analyzed the effect of *CDC42Sc* mutations in these conserved domains.

**Mutations predicted to reduce GTPase result in dominant lethality.** The *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ mutations are analogous to oncogenic mutations in *H-ras* that have decreased intrinsic GTPase activity, thereby locking the mutant proteins in an "activated" GTP-bound state. The *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ mutations are dominant lethal to both wild-type and *cdc42-1*$^{ts}$ alleles. This dominant-negative phenotype suggests that these activated proteins may be irreversibly associated with cellular factors necessary for the budding process (Fig. 5), thereby preventing functional Cdc42Sc proteins from interacting with them. Such cellular factors, which could include a Cdc42Sc-specific GTPase-activating protein (GAP), may act after bud emergence (downstream effectors) since cells containing these mutations on plasmids usually die with either multiple buds or an elongated single bud (Fig. 3A and D). Suppressors of the dominant lethality associated with overexpression of *CDC42*$^{Val-12}$ and *CDC42*$^{Leu-61}$ may define such downstream effectors.

The multiple, abnormally shaped buds on these dying cells suggest that an activated Cdc42Sc protein cannot regulate proper bud emergence (i.e., more than one bud is formed on the same mother cell) and proper bud morphogenesis. The large size of the mutant mother cells compared with wild-type cells and with some of the abnormal buds suggests that these cells have lost the ability to maintain proper cell polarity with respect to directing new cell growth to the emerging bud rather than the mother cell. The large size of the mother cells might also result from a nonspecific cellular pathology.

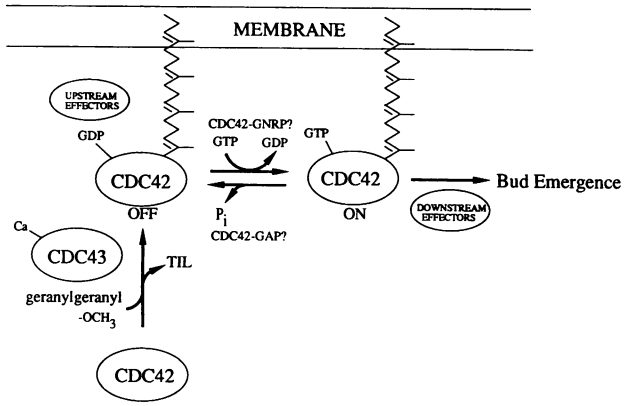


FIG. 5. A model for the function of Cdc42Sc in controlling bud emergence. GNRP, guanine nucleotide release protein; GAP, GTPase activating protein. See text for details.

Actin-containing structures were aberrant in these mutant cells (Fig. 3D). Cortical spots were localized asymmetrically to one or more of the buds, and thick actin bars were randomly oriented within the mother cell and sometimes within the bud. Similar thick bars have been observed in temperature-sensitive actin mutants (28), profilin-deficient mutants (14), tropomyosin-deficient mutants (24), some *sac* mutants (29), and upon overproduction of the Abp1 actin-binding protein (10). This phenotype therefore suggests that normal actin assembly in these mutant cells is either unstable or improperly organized.

These mutant cells are often multinucleate, as observed by DAPI staining (Fig. 3G) and anti-tubulin antibody staining (data not shown). Low-intensity DAPI staining that was not associated with anti-tubulin antibody staining was also observed. This could be due either to an excess of mitochondria in these cells or to fragmented nuclear DNA.

Chitin is delocalized in these cells (Fig. 3E), but not to the same extent as in *cdc42-1*$^{ts}$ mutants (1), *CDC42*$^{Ala\text{-}118}$ mutants (Fig. 3F), or actin mutants (28). There does seem to be some normal chitin localization to the mother-bud junction, although this is variable from cell to cell. Chitin production may be decreased in these cells or correct localization may be partially impaired, possibly because of the disruption of actin microfilaments.

**A mutation predicted to alter nucleotide binding results in an unexpected phenotype.** The *H-ras*$^{Ala\text{-}119}$ mutation leads to a transforming phenotype similar to that of the *ras* mutants described above and to a defect in guanine nucleotide binding (3). This mutant protein is thought to be in an activated state judging from its increased GDP dissociation rate coupled with a high GTP-GDP ratio within the cell, thereby resulting in a higher probability of the protein being in a GTP-bound state. If the analogous mutation in *CDC42Sc* had a similar effect, it would be predicted to show the same phenotype as in the *CDC42*$^{Val\text{-}12}$ and *CDC42*$^{Leu\text{-}61}$ mutants. The *CDC42*$^{Ala\text{-}118}$ terminal phenotype, however, is the same as the *cdc42-1*$^{ts}$ (1) and null (20) mutant phenotypes (i.e., loss of function), suggesting that the Ala-118 mutant protein is in an inactivated form rather than an activated form. The *CDC42*$^{Ala\text{-}118}$ allele is not simply a null allele, because it showed dominant lethality to *cdc42-1*$^{ts}$ at permissive temperatures and dose-dependent dominant lethality to the wild type. This dominant-negative phenotype may be due to inactivated Ala-118 protein binding to cellular factors necessary for the budding process (Fig. 5) and inhibiting the interaction of these factors with functional Cdc42Sc proteins. These factors, which may include a Cdc42Sc-specific guanine-nucleotide releasing protein (GNRP), may act before bud emergence (upstream effectors), since cells overexpressing the *CDC42*$^{Ala\text{-}118}$ allele cannot form buds. Suppressors of the dominant lethality associated with overexpression of *CDC42*$^{Ala\text{-}118}$ may define such upstream effectors.

These results suggest that aspects of the guanine nucleotide binding and/or hydrolytic properties of the Cdc42 protein differ from those of the Ras protein. It is noteworthy that the Cdc42Sc sequence TQID-118 is different from the consensus GTP-binding domain sequence NKXD (Fig. 1; X is any amino acid). The Cdc42Hs sequence, however, does contain the same TQID sequence and has been shown to bind GTP (11, 17, 33). Interestingly, mutations in the nucleotide-binding domains of the *S. cerevisiae SEC4* (44) and *YPT1* (37) gene products also result in a dominant-lethal phenotype with a loss of nucleotide binding by the mutant proteins.

**The putative isoprenylation site cysteine residue is required for function.** The *cdc42*$^{Ser\text{-}188}$ mutation is predicted to alter the carboxyl-terminal modification of the Cdc42Sc protein in such a way that it would not be isoprenylated and hence would not be membrane localized. There are three lines of evidence that suggest that proper localization is critical to Cdc42Sc function. First, plasmids containing the *cdc42*$^{Ser\text{-}188}$ mutation cannot complement the *cdc42-1*$^{ts}$ mutation, even when the *cdc42*$^{Ser\text{-}188}$ mutant protein is overexpressed under the control of a *GAL* promoter (Fig. 4A). The slower mobility rate of the Ser-188 protein on polyacrylamide gels suggests that this protein does not undergo the predicted carboxyl-terminal modifications of isoprenylation, proteolytic cleavage of the three terminal amino acids, and carboxymethylation of the new terminal cysteine residue (36) that have been observed with the Cdc42Hs/G25K protein, which contains an all-*trans* geranylgeranyl cysteinyl methyl ester at its carboxyl terminus (45). Second, in double mutant constructs, the *cdc42*$^{Ser\text{-}188}$ mutation can suppress the lethality associated with the *CDC42*$^{Val\text{-}12}$, *CDC42*$^{Leu\text{-}61}$, and *CDC42*$^{Ala\text{-}118}$ mutations. Finally, overproduction of the Ser-188 mutant protein does not lead to abnormal positioning of budding sites (data not shown). This is in contrast to overproduction of the wild-type protein, which leads to a random budding pattern (20).

**Other components involved in controlling cell polarity.** The Cdc42Sc protein does not act alone in controlling cell polarity. Both *cdc43*$^{ts}$ (1) and *cdc24*$^{ts}$ (9, 41, 42) mutants have phenotypes identical to that of the *cdc42-1*$^{ts}$ mutant. The synthetic lethality of *cdc42*$^{ts}$ *cdc43*$^{ts}$ double mutants (1) and the multicopy suppression of a *cdc24*$^{ts}$ mutation by *CDC42Sc* and *CDC42Hs/G25K* (4, 27) suggest that the *CDC42Sc*, *CDC43*, and *CDC24* gene products interact within the cell.

The predicted amino acid sequence of *CDC43* (19, 29b) is similar to that of the *S. cerevisiae DPR1/RAM1* gene product (13), which is believed to be a component of the farnesyl-protein transferase enzyme complex that modifies the carboxyl termini of low-molecular-weight GTP-binding proteins (36, 43). Recently, we have shown that a *CDC43*-dependent geranylgeranyl-protein transferase activity exists in *S. cerevisiae* and that the *CDC42Sc* and *CDC42Hs* gene products are modified by this activity with the addition of a geranylgeranyl isoprene, presumably to the Cys-188 residue (12) (Fig. 5). The *CDC43* gene is identical to the *CAL1* gene (29b), which was defined by a $Ca^{2+}$-dependent mutation

(31), suggesting that calcium plays a role in Cdc43/Cal1 function and in the membrane localization of the Cdc42Sc protein. Calcium has also been implicated in the function of the *CDC24* gene product (26, 30, 32). It is unclear whether the *CDC24* gene product acts as an upstream or downstream effector of Cdc42Sc function.

Given the high degree of sequence similarity between *CDC42Sc* and *CDC42Hs/G25K*, it is likely that there are functional homologs of these potential *CDC42Sc*-interacting gene products in higher eukaryotes as well. Elucidation of the roles of these gene products in controlling cell polarity in *S. cerevisiae* should allow us to make extrapolations regarding their possible functions in the morphogenetic events of higher eukaryotes.

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