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ZDS1 and *ZDS2*, Genes Whose Products May Regulate Cdc42p in *Saccharomyces cerevisiae*

ERFEI BI and JOHN R. PRINGLE*
Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599

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A genetic screen for GTPase-activating proteins (GAPs) or other negative regulators of the Rac/Rho family GTPase Cdc42p in *Saccharomyces cerevisiae* identified *ZDS1*, a gene encoding a protein of 915 amino acids. Sequence from the yeast genome project identified a homolog, *ZDS2*, whose predicted product of 942 amino acids is 38% identical in sequence to Zds1p. Zds1p and Zds2p have no detectable homology to known Rho-GAPs or to other known proteins. However, by several assays, it appears that overexpression of either Zds1p or Zds2p decreases the level of Cdc42p activity. Deletion analysis also suggests that Zds1p and Zds2p are at least partially overlapping in function. Deletion of *ZDS2* produced no obvious phenotype, and deletion of *ZDS1* produced no obvious phenotype other than a mild effect on cell shape. However, the *zds1 zds2* double mutant grew slowly with an apparent mitotic delay and produced elongated cells and buds with other evidence of abnormal morphogenesis. A glutathione *S*-transferase–Zds1p fusion protein that fully complemented the double mutant localized to presumptive bud sites and the tips of small buds. The similarity of this localization to that of Cdc42p suggests that Zds1p may interact directly with Cdc42p. As *ZDS1* and *ZDS2* have recently been identified also by numerous other groups studying a wide range of biological phenomena, the roles of Cdc42p in intracellular signaling may be more diverse than has previously been appreciated.

In the yeast *Saccharomyces cerevisiae*, the *CDC42* and *CDC24* gene products play a central role in the establishment of cytoskeletal and growth polarity during budding (12, 22, 73). Both genes were originally identified by temperature-sensitive-lethal mutants that arrest as large, round, unbudded cells at a restrictive temperature because they continue growth and the nuclear cycle but are unable to polarize their cell surface growth to produce buds (1, 2, 32, 69, 74, 75, 95, 96). Subsequent molecular analyses revealed that Cdc42p is a low-molecular-weight GTPase in the Rho/Rac family (38) and that Cdc24p is a guanine nucleotide exchange factor for Cdc42p (66, 107). Thus, activation of Cdc42p by Cdc24p-stimulated guanine nucleotide exchange is thought to promote the polarization of the cytoskeleton toward an appropriate point on the cell surface (12, 22, 47, 73).

The promotion of polarized cytoskeletal assembly by Cdc42p appears to be mediated in part by a pair of related protein kinases, Cla4p and Ste20p, that interact with, and are apparently activated by, Cdc42p (19, 57, 93, 105). At a restrictive temperature, a *cla4^{ts} ste20*Δ strain appears to undergo normal initial polarization of both actin and the neck filament-associated septin proteins; however, as the bud begins to grow, the septins disperse over the growing bud instead of forming a tight ring at the mother-bud neck (19). This phenotype might result either from a subtle defect in the organization of the actin cytoskeleton or from a failure of some late step in the organization of the septins at the presumptive bud site. The effectors through which Cdc42p promotes the initial organization of actin and the septins at the presumptive bud site have not yet been identified.

cdc24 and *cdc42* mutants are also defective in mating (79, 93, 104, 105). This defect presumably results in part from an involvement of Cdc24p and Cdc42p in the polarization of partner cells toward each other that is necessary for cell fusion (14). However, recent evidence suggests that these proteins are also involved in the transmission of the mating pheromone signal (93, 98, 105). This role also appears to involve activation by Cdc42p of Ste20p, which in turn activates the MAP kinase cascade headed by Ste11p and thus produces the various aspects of the pheromone response (33, 45).

Close homologs of Cdc42p have been found in many different organisms, including *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens* (13, 24, 39, 52, 63, 67, 91). These homologs are all 80 to 85% identical in amino acid sequence, and the genes from other organisms can complement *cdc42* mutations in *S. cerevisiae*, suggesting that the mechanisms involving Cdc42p have been closely conserved. *S. pombe* Cdc42p appears to play roles in polarity establishment and in mating that are similar to its roles in *S. cerevisiae* and also involve interaction with a Ste20p-related protein kinase (11, 59, 63, 71). In mammalian cells, Cdc42 may have a variety of biological roles: recent studies have implicated human Cdc42Hs in progression through the G_1 phase of the cell cycle (70), in the organization of the actin cytoskeleton and establishment of cell polarity (12, 23, 43, 68, 80, 99), in regulation of phosphoinositide signaling (106), and in regulation of transcriptional activation by at least two pathways (18, 35, 64). These effects are probably also mediated at least in part by the interaction of Cdc42Hs with protein kinase effectors. Two protein kinases, a tyrosine kinase (ACK) and a Ste20p-related serine/threonine kinase (PAK), were found to bind to Cdc42Hs, and PAK was shown to be activated by this interaction (56, 57, 60). However, the immediate targets of these protein kinases have not yet been identified.

Elucidation of the roles of Cdc42p in yeast and other systems will require identification not only of the effector pathways but also of the factors that regulate the GTPase cycle, and hence the activity, of Cdc42p. In addition to guanine nucleotide exchange factors, at least two other types of proteins are known to regulate the function of Rho/Rac family GTPases. Guanine nucleotide dissociation inhibitors (GDIs) inhibit GDP dissoci-

* Corresponding author. Mailing address: Department of Biology, CB 3280, University of North Carolina, Chapel Hill, NC 27599-3280. Phone: (919) 962-2293. Fax: (919) 962-0320.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference or source
C276-4A	**a** (prototroph)	102
YPH500	α *trp1 leu2 ura3 his3 lys2 ade2*	92
YEF473	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2*	This study[a]
YEF473A	**a** *trp1 leu2 ura3 his3 lys2*	Segregant from YEF473
YEF316	**a** *trp1 leu2 ura3 his3 lys2 ade2 cdc24-10*	This study[b]
YEF323	α *trp1 leu2 ura3 his4 ade2 cdc24-12*	This study[b]
YEF327	α *trp1 leu2 ura3 his4 ade2 cdc24-13*	This study[b]
DJTD2-16D	α *trp1 leu2 ura3 his4 gal2 cdc42-1*	38
YML12	**a** *leu2 ura3 cdc3-3*	This laboratory
YEF1223	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2 bem3Δ::HIS3/bem3Δ::HIS3 rga1Δ::HIS3/ rga1Δ::HIS3*	7
YEF844	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2 ZDS1/zds1Δ::HIS3*	See text
YEF875	**a** *trp1 leu2 ura3 his3 lys2 zds1Δ::HIS3*	Segregant from YEF844
YEF877	α *trp1 leu2 ura3 his3 lys2 zds1Δ::HIS3*	Segregant from YEF844
YEF864	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2 zds1Δ::HIS3/zds1Δ::HIS3*	YEF875 × YEF877
YEF938	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2 ZDS2/zds2Δ::HIS3*	See text
YEF950	α *trp1 leu2 ura3 his3 lys2 zds2Δ::HIS3*	Segregant from YEF938
YEF1023	**a**/α *trp1/trp1 leu2/leu2 ura3/ura3 his3/his3 lys2/lys2 ZDS1/zds1Δ::HIS3 ZDS2/zds2Δ::HIS3*	YEF875 × YEF950
YEF1074	**a** *trp1 leu2 ura3 his3 lys2*	Segregant from YEF1023
YEF1075	α *trp1 leu2 ura3 his3 lys2 zds1Δ::HIS3*	Segregant from YEF1023
YEF1076	**a** *trp1 leu2 ura3 his3 lys2 zds2Δ::HIS3*	Segregant from YEF1023
YEF1077	α *trp1 leu2 ura3 his3 lys2 zds1Δ::HIS3 zds2Δ::HIS3*	Segregant from YEF1023
YEF1016	**a** *trp1 leu2 his3 lys2 zds1Δ::HIS3 ura3:URA3:ZDS1:GST*	See text
YEF1017	α *trp1 leu2 his3 lys2 zds1Δ::HIS3 ura3:URA3:ZDS1:GST*	See text
YEF1020	**a**/α *trp1/trp1 leu2/leu2 his3/his3 lys2/lys2 zds1Δ::HIS3/zds1Δ::HIS3 ura3:URA3:ZDS1:GST/ura3:URA3:ZDS1:GST*	YEF1016 × YEF1017
YEF1110	α *trp1 leu2 his3 lys2 zds1Δ::HIS3 zds2Δ::HIS3 ura3:URA3:ZDS1:GST*	See text

[a] Constructed by diploidizing (using *HO* on a plasmid [34]) a segregant from C276-4A × YPH500.

[b] Derived by several crosses from the original *cdc24* mutant strains isolated in the screens described by Adams et al. (1) (*cdc24-10*) and by Harris and Pringle (30) (*cdc24-12* and *cdc24-13*).

ation and GTPase activity and regulate the association of the prenylated GTPases with membranes (9, 29, 31). A Rho-GDI (Rdi1p) has been identified in *S. cerevisiae* (61, 73), but it is not yet known whether it influences the function of Cdc42p in vivo. In contrast, GTPase-activating proteins (GAPs) stimulate the intrinsic GTPase activity of the GTPase and thus function, at least in part, as negative regulators (9). GAPs gain additional interest from the possibility that they may be effectors, as well as negative regulators, of the associated GTPases (9, 44, 73, 80). Because *CDC24* and *CDC42* are essential genes (17, 38, 66) and GTPase-defective Cdc42p is lethal (109), the GAP-stimulated conversion of Cdc42p-GTP to Cdc42p-GDP seems likely to be essential as well. Recent work has identified two apparent GAPs for *S. cerevisiae* Cdc42p, Bem3p and Rga1p, but cells are still viable when either or both of the corresponding genes are deleted (98, 107, 108). Thus, there may be one or more additional Cdc42p-GAPs in *S. cerevisiae* that have yet to be identified. To explore this possibility, we designed a genetic screen to search for Cdc42p-GAPs (or other negative regulators of Cdc42p). In this report, we characterize one gene identified in this screen and a homolog identified by searching sequences deposited by the genome project.

MATERIALS AND METHODS

Genetic and recombinant DNA methods. Standard methods of yeast genetics (28) and recombinant DNA manipulation (87) were used except as noted below. Restriction enzymes, exonuclease III, and Vent DNA polymerase were obtained from New England Biolabs (Beverly, Mass.). Oligonucleotide primers were purchased either from the University of North Carolina Pathology Department (Chapel Hill, N.C.) or from Integrated DNA Technologies (Coralville, Iowa). Sequential deletions of DNA to be sequenced were made by using exonuclease III; sequencing was by the dideoxy chain termination method. For physical mapping of cloned genes, ^{32}P-labeled DNA fragments were used to probe a filter containing an ordered set of yeast genomic DNA λ clones (American Type Culture Collection, Rockville, Md.).

Media and growth conditions. Standard yeast (85) and *Escherichia coli* (87) media were used except as noted. The rich, buffered medium YM-P (48) was used routinely for growth of yeast in liquid culture. Glucose (2%) was used as carbon source except for induction of gene expression under *GAL* promoter control, for which 2% glucose, 2% raffinose, and/or 2% galactose were used as specified in Results. Yeast strains were grown at 23°C except where noted otherwise.

Strains and plasmids. *E. coli* DH12S (Gibco BRL, Gaithersburg, Md.) was used routinely as a plasmid host. *E. coli* MC1066a (*leuB600 trpC9830 pyrF74*::Tn*5* Kan^r *ara hsdR hsdM$^+$ srl*::Tn*10 recA13*) was used to select plasmids carrying the yeast *LEU2* gene (88). Yeast strains are listed in Table 1.

Plasmid pRS316-CDC42 was constructed by cloning a ~1.7-kb *Bam*HI-*Hin*dIII *CDC42*-containing fragment from YEp351-CDC42 (109) into the corresponding sites of pRS316 (*CEN URA3*) (92). pPB547 (2μm *LEU2*) carries the full-length *BEM3* gene (4, 107). YEp13-RGA1 was constructed by cloning a ~6.0-kb *Hin*dIII *RGA1*-containing fragment from YEp24-RGA1 (98) into the *Hin*dIII site of YEp13 (2μm *LEU2*) (83). YEp13-ZDS2 carries full-length *ZDS2* in YEp13 (72). pYESR-55 (*CEN URA3*) carries a truncated allele of *ZDS2* (encoding Met-372 to Ile-942) under *GAL1* promoter control (72). YEplac-ZDS1* was constructed by cloning a ~2.5-kb *Bam*HI-*Sph*I fragment that contains the entire insert DNA from the original *ZDS1* plasmid, YEp13-ZDS1* (see Results), into the corresponding sites of YEplac181 (2μm *LEU2*) (27). The YEp13 genomic DNA library contains yeast *Sau*3A fragments inserted at the *Bam*HI site of YEp13 (20). The YCp50-LEU2 genomic DNA library contains size-fractionated yeast *Sau*3A fragments (9 to 12 kb) inserted at the *Bam*HI site of YCp50-LEU2, a derivative of the *CEN*-based plasmid YCp50 (84) in which *URA3* has been replaced by *LEU2* (97).

For mutagenesis of *ZDS1*, a ~5.6-kb *Bam*HI-*Sal*I fragment that carries the full-length gene was cloned from YCp-ZDS1 (see Results) into the corresponding sites of pALTER-1 (Promega, Madison, Wis.). The *Bam*HI site was then destroyed by fill-in with the DNA polymerase Klenow fragment, yielding plasmid pALTER-ZDS1. Mutagenesis was then performed as recommended by Promega. To delete *ZDS1*, two mutagenic primers were used to introduce *Bam*HI sites directly before the start codon and directly after the stop codon. For the 5′ region, the primer sequence was 5′-TAG CTG TCT TTT ATA GGA TCC ATG TCC AAT AGA GAT-3′; for the 3′ region, the primer sequence was 5′-CAA CAA CAA CCC TGA GGA TCC AAA GGC TTC AGG CTG CA-3′ (the *Bam*HI sites are underlined). The entire *ZDS1* gene was then replaced by the *HIS3* gene carried on a *Bam*HI fragment derived from YDp-H (6), yielding plasmid pALTER-zds1Δ::HIS3. This plasmid was cut with *Sma*I (site in the vector sequences) and *Sal*I (site 463 bp downstream of the *ZDS1* stop codon),

and the fragment carrying the deletion construct was isolated and transformed into diploid strain YEF473, selecting stable His$^+$ transformants, to generate strain YEF844.

To construct the *ZDS1* overexpression plasmid, the 5′-region primer described above was used to introduce a *Bam*HI site directly before the start codon in pALTER-ZDS1, yielding plasmid pALTER-ZDS1NB. The 318-bp *Eco*RI-*Bam*HI fragment upstream of *ZDS1* was then replaced by a ~0.7-kb *Eco*RI-*Bam*HI fragment from pRS316-GAL1/10 (25) that carries the *GAL1* promoter and its upstream activation sequence (40), resulting in plasmid pALTER-GAL1-ZDS1. A ~3.9-kb *Eco*RI-*Sal*I fragment of this plasmid that contains *GAL1-ZDS1* was then cloned into YEp352 (2μm *URA3*) (36), yielding plasmid YEp352-GAL1-ZDS1.

To construct a *GST-ZDS1* fusion plasmid, a ~3.2-kb *Bam*HI-*Sal*I fragment of plasmid pALTER-ZDS1NB was cloned into the corresponding sites of pEGKT (2μm *URA3*) (65), yielding plasmid pEGKT-ZDS1, which encodes an in-frame glutathione *S*-transferase (GST)–Zds1p fusion protein under control of the *GAL1/10* upstream activation sequence and *CYC1* promoter. *ZDS1* was also tagged immediately after the start codon with an in-frame GST *Not*I cassette (20), using a *Not*I site that had been introduced into pALTER-ZDS1 by using the primer 5′-TAG CTG TCT TTT ATA ATG AGC GGC CGC TCC AAT AGA GAT AAC GAG-3′ (the *Not*I site is underlined). A ~4.3-kb *Eco*RI-*Sal*I fragment that carries *ZDS1*:*GST* (including 313 bp upstream of the *ZDS1* start codon) was then cloned from the resulting plasmid into the integrative plasmid YIplac211 (*URA3*) (27), yielding plasmid YIplac211-ZDS1:GST. This plasmid was linearized within *URA3* by digestion with *Apa*I and transformed into YEF875 and YEF877, selecting stable Ura$^+$ transformants, to generate strains YEF1016 and YEF1017, respectively. The *Apa*I-digested construct was also transformed into strain YEF1077 to generate strain YEF1110.

A complete deletion of *ZDS2* was constructed by using the PCR method described by Baudin et al. (3). Two primers were used to amplify the *HIS3* gene from pRS303 (92) together with flanking sequences derived from the immediately upstream and downstream regions of *ZDS2*. The forward primer was 5′-ATC ACG TTT GCA CTA TAG ACT GAA TTT AAA TTA GAA TTT TGA TTG TAC TGA GAG TGC ACC-3′, and the reverse primer was 5′-CCT TAT ATA GGT ATC TAT CAA TCT TGT AAA CAG TTA TGA GCT GTG CGG TAT TTC ACA CCG-3′ (*ZDS2* flanking sequences are underlined). The amplified fragment was then transformed directly into strain YEF473, selecting stable His$^+$ transformants, to generate strain YEF938.

Genetic screen. To isolate possible negative regulators of Cdc42p, strain YEF327 containing plasmid pRS316-CDC42 was transformed with the YEp13 genomic DNA library. Transformants were grown on SD-Ura-Leu plates at 23°C, replicated multiple times on YPD plates to reduce the numbers of cells transferred, and eventually replicated onto SD-Ura-Leu plates containing 1 M sorbitol and incubated at 37°C. Colonies that failed to grow at 37°C contained candidate plasmids, which were recovered into *E. coli* and retested in the same assay to confirm the phenotype.

Morphological observations. Overall cell morphologies were observed by differential interference contrast (DIC) microscopy on cells that had been lightly sonicated to disperse clumps (76). In some cases, cells that had been fixed with 3.7% formaldehyde were digested with 35 U of lytic enzyme (ICN Biochemicals, Cleveland, Ohio) per ml for 40 min at 37°C; this procedure separates cells that have completed cytokinesis even if the cell wall events necessary for cell division are not complete (76). Chitin was visualized by staining with Calcofluor (Sigma, St. Louis, Mo.) (77). Indirect immunofluorescence microscopy was performed essentially as described previously (77). Rabbit polyclonal antibodies against GST (Molecular Probes, Eugene, Oreg.) were affinity purified on nitrocellulose strips containing GST-Cdc24p (7) and then used to visualize GST-tagged Zds1p. Goat polyclonal antiactin antibodies (supplied by J. A. Cooper, Washington University) were used to visualize actin structures. A rat monoclonal antitubulin antibody (YOL1/34; Accurate Chemical and Scientific, Westbury, N.Y.) was used to visualize microtubule structures (2, 41, 42). Rabbit polyclonal antibodies against Cdc11p (26) were used to visualize septin localization. The secondary antibodies used were goat anti-rabbit immunoglobulin G (IgG) conjugated with BODIPY (Molecular Probes, Inc.), donkey anti-goat IgG conjugated with rhodamine, and rabbit anti-rat IgG conjugated with fluorescein isothiocyanate (Jackson Immunoresearch Laboratories, West Grove, Pa.). Nuclei were visualized by staining with 1 μg of bisBenzimide (Sigma) per ml, which was included in the mounting medium. Fluorescence microscopy was performed with a Nikon Microphot SA microscope, using a 60× Plan-apo objective.

Nucleotide sequence accession number. The *ZDS1* sequence has been assigned GenBank/EMBL accession number L42821.

RESULTS

Identification of *ZDS1*. A genetic screen for negative regulators of Cdc42p was designed on the basis of the observation that multicopy *CDC42* can suppress temperature-sensitive *cdc24* mutations (5). It seemed likely that increasing the dosage of a gene encoding a negative regulator of Cdc42p would eliminate this suppression. To test this possibility, multicopy plasmids carrying *BEM3* and *RGA1*, which encode putative GAPs for Cdc42p (98, 107, 108), were transformed into strain YEF327 (*cdc24-13*) harboring pRS316-CDC42, and the transformants were plated on SD-Leu-Ura plates at 23°C and replicated onto SD-Leu-Ura plates with 1 M sorbitol at 37°C. (The sorbitol improves the suppression of *cdc24* by *CDC42* [5].) As expected, colonies containing either the *BEM3* or *RGA1* plasmid did not grow, whereas colonies containing the vector alone did grow (Fig. 1A).

To isolate additional negative regulators of Cdc42p, a YEp13-based genomic-DNA library was transformed into strain YEF327 harboring pRS316-CDC42, and ~8,000 transformants were screened for failure to grow at 37°C. Four plasmids that reproducibly conferred this phenotype were found. Although any gene whose overexpression is harmful at higher temperatures might give this phenotype, the cells containing any of the four identified plasmids were predominantly (>80%) large, round, and unbudded at 37°C, suggesting that the plasmid-borne genes might have interfered specifically with the function of Cdc24p and Cdc42p in polarity establishment. In addition, none of the four plasmids interfered with the growth of a wild-type strain at 37°C. Cross-hybridization experiments suggested that the four plasmids contained nonoverlapping inserts that were distinct from each other and from *BEM3* and *RGA1*. Hybridization of the inserts from three of the four plasmids to an ordered set of genomic λ clones (81) showed that one mapped to the right arm of chromosome XIII, between *ZRC1* and *DPD1* and close to the latter; one mapped to the right arm of chromosome XV, between *CDC31* and *KRE5* and close to the latter; and one mapped to the left arm of chromosome VII, between *TFG1* and *CDC20* and close to the former. The map position of the fourth isolate was determined by database search after partial sequence analysis. This isolate mapped to the right arm of chromosome IV, between *SUP80* and *STE5* and close to the latter. It seems clear that the screen was not saturated, as it failed to identify *BEM3* and *RGA1*, and each of the new sequences was isolated only once.

The gene derived from chromosome XIII was chosen for further study because it gave the strongest effect in the original assay (Fig. 1A); for reasons explained below (see Discussion), this gene was named *ZDS1*. To test further the possible relevance of *ZDS1* to the establishment of cell polarity, plasmids YEp13, pPB547 (*BEM3*), YEp13-RGA1, and YEp13-ZDS1* (the original *ZDS1* isolate) were transformed into strains YEF316 (*cdc24-10*), DJTD2-16D (*cdc42-1*), and YML-12 (*cdc3-3*). Transformants were streaked onto plates selective for the plasmids and incubated at the minimal restrictive temperature for each strain. *BEM3*, *RGA1*, and *ZDS1* were all found to reduce the restrictive temperatures of the *cdc24-10* (Fig. 1B) and *cdc42-1* (Fig. 1C) mutants but not of the *cdc3-3* mutant (which is defective in cytokinesis) (data not shown), suggesting that *ZDS1* specifically affects the genes involved in polarity establishment. In addition, although a wild-type strain harboring YEp13-ZDS1* appeared to have a normal growth rate at all temperatures tested, some cells were morphologically aberrant, being enlarged, elongated, and/or irregularly shaped, reminiscent of *cdc42-1* cells grown at semipermissive temperatures (data not shown). Such morphological aberrancies were more pronounced in *cdc24*^{ts} strains (especially a *cdc24-12* strain) harboring YEp13-ZDS1*; during growth at permissive temperatures, a large fraction (~80%) of the cells became very large, round, and unbudded (Fig. 1E). Such cells are morphologically similar to, but significantly larger than, cells of *cdc24*^{ts} and *cdc42*^{ts} strains after incubation at restrictive temperatures. The *cdc24-12* strain harboring a *BEM3* or *RGA1* plasmid displayed similar, but less pronounced, morphological aberrancies in

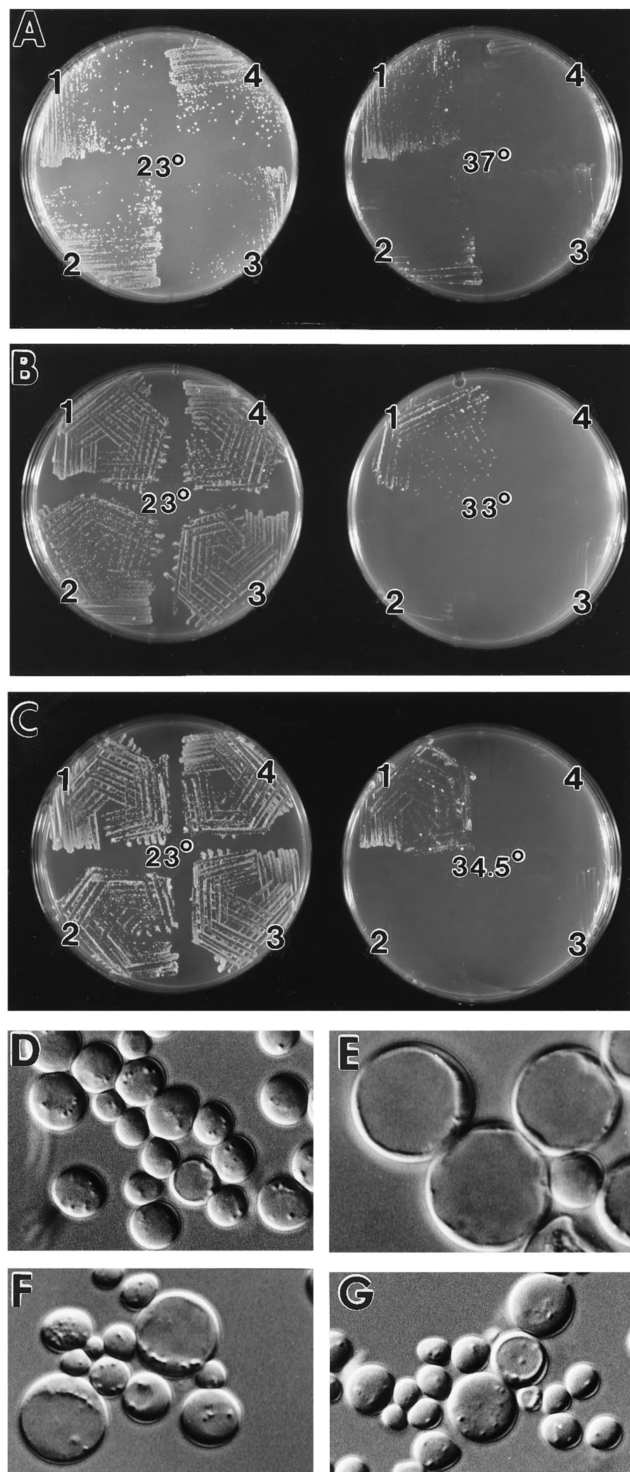

FIG. 1. Effects of overexpressing Zds1p* or one of the known Cdc42p-GAPs (Bem3p or Rga1p) in *cdc24^ts^* and *cdc42^ts^* mutants. Zds1p* is the truncated protein encoded by the original plasmid isolate YEp13-ZDS1* (see text). (A to C) The indicated strains transformed with (1) YEp13, (2) pPB547 (*BEM3*), (3) YEp13-RGA1, or (4) YEp13-ZDS1* were streaked on plates selective for the plasmids (SD-Leu or SD-Leu-Ura) and incubated at a permissive temperature (23°C) or at a minimal restrictive temperature for the strain in question, as indicated. The restrictive-temperature plate in panel A also contained 1 M sorbitol. (A) *cdc24-13* strain YEF327 harboring plasmid pRS316-CDC42. (B) *cdc24-10* strain YEF316. (C) *cdc42-1* strain DJTD2-16D. (D to G) *cdc24-12* strain YEF323 harboring plasmid YEp13 (D), YEp13-ZDS1* (E), pPB547 (F), or YEp13-RGA1 (G) was streaked on SD-Leu plates, incubated at 23°C for 3 to 4 days, and examined by DIC microscopy after resuspension in YM-P medium.

~10 to 15% of the cells (Fig. 1F and G). Taken together, the data suggest that Zds1p negatively regulates cell polarity by decreasing the function of Cdc42p.

Sequence analysis of *ZDS1* and identification of *ZDS2*. The entire ~2.2-kb insert from YEp13-ZDS1* was cloned as a ~2.5-kb *Bam*HI-*Sph*I fragment (*Bam*HI site from the insert-vector junction, *Sph*I site from the vector) into YEplac181. The resulting plasmid, YEplac-ZDS1*, was transformed into YEF327 (*cdc24-13*) harboring pRS316-CDC42 and into *cdc24-10*, *cdc42-1*, and *cdc3-3* strains. In all respects, the effects of this plasmid were the same as those of YEp13-ZDS1* (data not shown), indicating that the activity of interest was indeed contained on the ~2.2-kb fragment. Sequencing of this fragment revealed one large open reading frame that was apparently truncated at its 5′ end. Thus, a ~2.0-kb *Bam*HI-*Sal*I fragment was used as a hybridization probe to screen the YCp50-LEU2 library for a plasmid containing the full-length gene. One positive clone, YCp-ZDS1, was found to contain ≥3.5 kb upstream of the point at which the open reading frame in YEp13-ZDS1* was truncated. Sequencing of this upstream region by using appropriate sequential primers revealed that the complete *ZDS1* open reading frame is 2,745 bp in length (accession number L42821). (The additional sequence also showed that the *Bam*HI site that formed one vector-insert junction in YEp13-ZDS1* had been created by the ligation of *Bam*HI-digested vector and *Sau*3A-digested genomic DNA.) Subsequently, the sequence of this region was also released by the genome project (accession number Z49260 [55]). The sequences match perfectly over the open reading frame and for at least 744 bp upstream and 641 bp downstream. Sequence data from the genome project also revealed the existence of a *ZDS1* homolog (here designated *ZDS2*) on the opposite arm of chromosome XIII (accession number Z49210 [94]).

The predicted products of *ZDS1* and *ZDS2* are novel proteins of 915 and 942 amino acids, respectively (Fig. 2). Overall, the two proteins have 37.5% identity (41.6% similarity) in amino acid sequence after introduction of multiple gaps to maximize the alignment. The sequence similarity is concentrated in six highly homologous regions, five of them in the N-terminal halves of the proteins and one near the C termini (Fig. 2). Neither protein has significant homology to any known GAP, suggesting that Zds1p may negatively regulate Cdc42p by a different mechanism. The program TM-pred (37) predicts potential membrane-spanning domains near the C termini of Zds1p (residues 826 to 848) and Zds2p (residues 837 to 859). However, the presence of both charged residues and prolines within these regions makes the reliability of this prediction doubtful. The program of Lupas et al. (53) identifies two regions in Zds1p (residues 245 to 274, $P = 0.42$; residues 365 to 407, $P = 0.70$) and one in Zds2p (residues 32 to 73, $P = 0.82$) with some potential to form coiled coils. Interestingly, Zds1p contains a stretch of four tandemly repeated hexapeptides (residues 762 to 785, consensus VQXSAP) that is not conserved in Zds2p (Fig. 2). The functions of these several motifs are not known.

To determine whether the full-length Zds1p has the same effects as the truncated protein (amino acids 409 to 915) encoded by the original YEp13-ZDS1* clone, a ~5.6-kb *Bam*HI-*Sal*I fragment carrying the full-length gene was subcloned from YCp-ZDS1 to the high-copy-number plasmid YEplac181 and tested in the assays shown in Fig. 1A to C. The full-length gene had effects similar to, but somewhat weaker than, those of the truncated allele (data not shown). We also tested the effects of full-length (plasmid YEp13-ZDS2) and truncated (plasmid pYESR-55; Fig. 2) *ZDS2* in the assays shown in Fig. 1A to C. Both plasmids had little or no effect on the suppression of

```
                                        Zds1p   ......
                                        Zds2p 1 MVLMED 6
  1 MSNRDNESMLR....TTSSDKAIASQR............DKRKSEVLIAA 34
  7 MQNKDGHNTVENSSGGTDSNNNIQMRRMRKTQLSKKELFEKRKSDVLIAA 56

 35 QSLDNEIRSVKNLKRLSIGSMDLLIDPELDIKFGGESSGRRSWS...... 78
 57 KSLDTEIQNVKNLKRLSIGSMDLVIDPELEFKVNSRNSYSSDSSKESLQE 106

 79 ................GTTSSSASMPSDTTTVNNTRYSDPTPLENLHGRG 112
107 SLHEENIIRSEQKEEQGSEDNDAYEEGDATNVDDS..IDITQTEYLHDEE 154

113 NSGIE.....SSNKTKQGNYLGIKKGVHSPSRKLNANV............ 145
155 TLEKEKIIRNASSSTSSSARVTSRNRRLSGVKTLAHDVVLDVENDHDSKM 204

146 ..LKKNLLWVPANQHPNVKPDNFLELVQDTLQNIQLSDNGEDNDGNSNEN 193
205 VDLTQNLLWVPADQHPNVKPENYLELIQDTLQNIQIS...........TN 243

194 NDIEDNGEDKESQSYENKENNTINLNRGLSRHGNASLIRRPSTLRRSYTE 243
244 QDIDEN.......KLELGNNHVIS.....NRKRTGSVVRRPSRLKTSYTK 281

244 FDDNEDDDNKGDSASETVNKVEERISKIKERPVSLRDITEELTKISNSAG 293
282 FDDEPPLADKPQEGEIQVDKRISSSDIKTIRSVSLKEITEELTKISNNAG 331

294 LTDNDAITLARTLSMAGSYSDKKDQPQPEGHYDEGDIGFSTSQANTLDDG 343
332 LTDSDAVTLARSLSMSGSFTNE...............SLHLNGNHTENDN 366

344 EFASNMPINNTMTWPERSSLRRSRFNTYRIRSQEQEKEVEQSVDEMKNDD 393
367 EFASNMFNETGLTIPERSSLRRSKFNTYKIRLEGSSLPQAVKLNSLMN.. 414

394 EERLKLTKNTIKVEIDPHKSPFRQQDEDSENMSSPGSIGDFQDIYNHYRQ 443
415 ............IQTNDNRRSASSPASYTQVPQEQASLNDFHEIFDHYRR 452

444 SSGEWEQEMGIEKEAEEVPVKVRNDTVEQDLELREGTTDMVKPSATDDNK 493
453 TSTDWSTE.......NEKYVDSTNYYSDEEDLTHASISQESSLLSTDSSN 495

494 ETKRHRRRNGWTWLNNKMSREDDNEENQGDDENEENVDSQRME.....LD 538
496 NSVLIKPHNTGSMISEKLDQHVSSSEKSNTNNSEANHGWSWLNSSNGSLN 545

539 NSKKHYISLFNGGEKTEV..SNKEEMNNSSTSTATSQTRQKIEKTFANLF 586
546 ANEQTYQQLTDDEDDEECVDNEKADFVNLSVSRRAKSTKRASERINHSKN 595

587 RRKP...HHKHDASS..SPSSSPSSSPSIPNNDAVHVRVRKSKKLGNKSG 631
596 RHSPIFQIHSEEAKSVVITPSVVSSSESQPSKPTAPAVVEKKVELPTDTQ 645

632 REPVEPIVLRNRPRPHRHHHSRHGSQKISVKTLKDSQPQQ..QIPLQPQL 679
646 ASTHKKNSLEKRLAKLFKRKQHNGTCKSDVKVIKKSVKKELKKKASHSSL 695

680 EGAIEIEKKEESDSESLPQLQPAVSVSSTKSNSRDREEEEAKKKNKKRSN 729
696 SKFRKSPKKKPQEAEVERPSSPTKTITTEDIDTASVIEPEVRSSNA..ST 743

730 TTEISNQQHSKHVQKENTDEQKAQLQ.APAQEQVQTSVPVQASAPVQNSA 778
744 LLPDSHTSHSSEFVVETISELDGDDSFDISGGDVNYDVEVHSSISRDTTA 793

779 PVQTSAPVEASAQTQAPAAPPLKHTSILPPRKLTFADVKKPDKPNSPVQF 828
794 GLEE....DIGAEREDNTSPTAPQISTLPPRKLTFEDVVKPDYSNAPIKF 839

829 TDSAFGFPLPLLTVSTVIMFDHRLPINVERAIYRLSHLKLSNSKRGLREQ 878
840 TDSAFGFPLPMITNSTVIMFDHRLGINVERAIYRLSHLKLSDPGRELRQQ 889

879 VLLSNFMYAYLNLVNHTLYMEQVAHDKEQQQQQQQP*............ 915
890 VLLSNFMYSYLNLVNHTLYMEQVGTGDIAFNGDSALGMMDKNDSDGTILI 939

    ...        Zds1p
940 PDI* 942   Zds2p
```

FIG. 2. Alignment of the predicted amino acid sequences of Zds1p and Zds2p. The four hexapeptide repeats in Zds1p are underlined. Arrows mark the first amino acids of the truncated Zds1p* and Zds2p* proteins (as encoded by plasmids YEp13-ZDS1* and pYESR-55, respectively).

cdc24-13 by multicopy *CDC42*. However, they did reduce the restrictive temperatures of *cdc24-10* and *cdc42-1* strains (data not shown), suggesting that Zds1p and Zds2p can share at least some functions.

Characterization of single and double mutants. Precise deletions of *ZDS1* and *ZDS2* were constructed as described in Materials and Methods. The success of the constructions was confirmed by Southern blot analysis on the transformed diploids and haploid segregants (data not shown). For both genes, tetrad analysis of heterozygous deletion strains yielded four viable spores per tetrad, and His^+:His^- segregated 2:2. Thus, neither gene is essential for cell viability. Haploid *zds1*Δ::*HIS3* strains grew as rapidly as wild-type strains at several temperatures between 18 and 37°C. Although a few (~5%) cells in the growing population were elongated in shape (Fig. 3B), there were no apparent defects in mating ability or budding pattern (data not shown). Moreover, a homozygous *zds1* deletion strain, YEF864, had no obvious defect in growth rate, budding pattern, or sporulation (data not shown). Similarly, deletion of *ZDS2* produced no obvious phenotype in haploid cells (Fig. 3C and data not shown; homozygous *zds2* deletion diploid cells were not examined).

To examine whether *ZDS1* and *ZDS2* are functionally redundant, we constructed a double mutant by crossing strains YEF875 (*zds1*Δ::*HIS3*) and YEF950 (*zds2*Δ::*HIS3*). An apparent tetratype tetrad (3 His^+:1 His^-) was obtained, and the genotypes of the four segregants were determined by Southern blot analysis (data not shown). The double-mutant segregant grew significantly more slowly than did the wild-type or single-mutant segregants (data not shown). In addition, the double-mutant cells were consistently (>90%) aberrant morphologically (Fig. 3D). In most cases, an elongated bud with constrictions at intervals along its length was attached to a large mother cell. The phenotypes of the double mutant were largely or fully complemented by *ZDS1* in either a low-copy-number (YCp-ZDS1) or a high-copy-number (YEplac-ZDS1) plasmid or by *ZDS2* in a high-copy-number plasmid (YEp13-ZDS2). (*ZDS2* was not tested in a low-copy-number plasmid.) The high-copy-number plasmid carrying truncated *ZDS1* (YEp13-ZDS1*; see above) partially complemented the double-mutant phenotypes (i.e., most cells had normal morphology, but some still had elongated buds), suggesting that both the N-terminal and C-terminal halves of Zds1p are required for its full function. Taken together, the data suggest that Zds1p and Zds2p have functions in cellular morphogenesis that are at least partially redundant.

The viability and elongated cell shape of the *zds1 zds2* mutant suggested that the machinery for polarized growth was functional, and perhaps even hyperactive, in the absence of Zds1p and Zds2p function. To examine this issue further, the double mutant and the control strains were stained with Calcofluor to visualize the patterns of chitin deposition and with antiactin antibodies to visualize the organization of the actin cytoskeleton. The *zds1* and *zds2* single mutants had patterns of chitin distribution similar to that of wild-type cells; that is, chitin was localized to the mother-bud necks and bud scars (data not shown). In the double mutant, chitin was localized to the mother-bud necks, the bud scars, and the constrictions along the elongated buds, often in bands that appeared broader than those in wild-type cells (Fig. 3E). Thus, localized

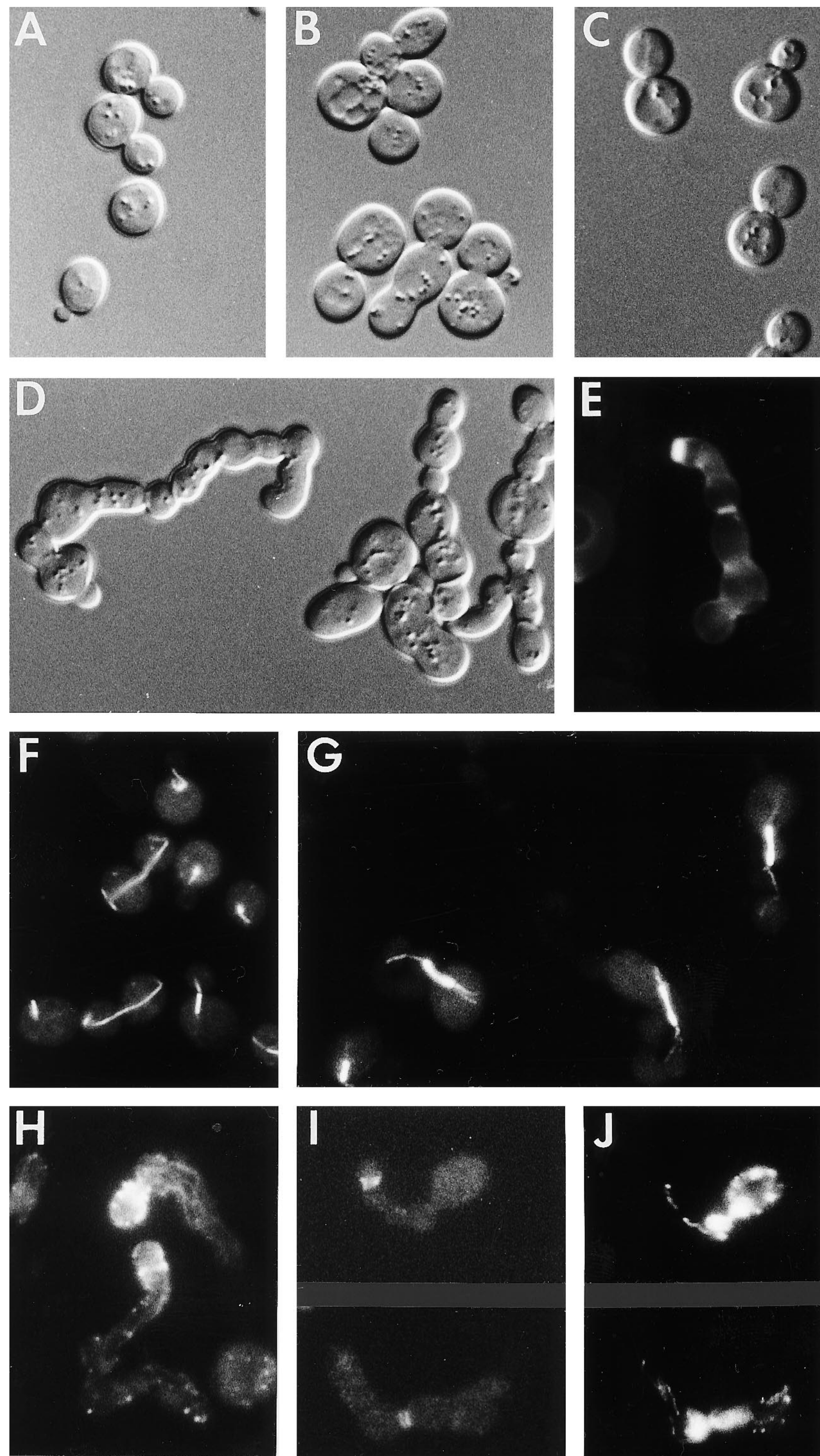

FIG. 3. Phenotypic analysis of *zds1*, *zds2*, and *zds1 zds2* mutants. Exponentially growing cells of wild-type strain YEF1074 (A and F), *zds1*Δ::*HIS3* strain YEF1075 (B), *zds2*Δ::*HIS3* strain YEF1076 (C), and *zds1*Δ::*HIS3 zds2*Δ::*HIS3* strain YEF1077 (D, E, and G to J) were observed by DIC (A to D) or fluorescence (E to J) microscopy. (E) Localization of chitin by staining with Calcofluor. (F and G) Immunofluorescence localization of microtubules. (H) Immunofluorescence localization of actin. (I and J) Cells double stained for Cdc11p (I) and for DNA (J).

cell wall deposition could occur in the absence of Zds1p and Zds2p function, although the patterns were somewhat abnormal. Actin organization in the single mutants appeared essentially normal: cortical spots were concentrated in small buds and at the tips of larger buds, and actin cables in the cytoplasm ran from the mother cell into the bud (data not shown). In the double mutant, actin patches were highly concentrated at the tips of the elongated buds, and actin cables were oriented

along these buds (Fig. 3H). Taken together, the data on cell shape, chitin deposition, and actin organization indicate that cell polarization occurs, and may indeed be somewhat accentuated, in the absence of Zds1p and Zds2p function.

To determine if loss of Zds1p and/or Zds2p affected the nuclear cycle, DNA was stained with bisBenzimide and spindles were visualized by antitubulin immunofluorescence. The single mutants did not differ detectably from the wild-type control strain; cells were observed at different stages of the nuclear cycle in the expected frequencies (Fig. 3F and data not shown). In contrast, most cells of the double mutant had a single mass of DNA. Some cells contained two closely spaced masses of DNA, but cells containing well-separated chromosome sets were rare. Microtubule staining showed that most (~70%) cells had short or partially elongated spindles (Fig. 3G), suggesting that the double mutant might have a mitotic delay.

From the DIC images of the *zds1 zds2* mutant (Fig. 3D), it did not appear that the constrictions along the elongated cells and buds represented sites of completed cell division. This conclusion was confirmed by showing that the morphology changed little when fixed cells were treated with cell wall-digesting enzymes, a procedure that separates cells that have completed cytokinesis even if cell division is not complete (see Materials and Methods). To examine further the nature of the constrictions and the distribution of potential division sites, the *zds1 zds2* mutant and control strains were stained with antibodies specific for Cdc11p, one of the septin proteins that is involved in cytokinesis (26, 50). The single mutants did not differ detectably from wild type; Cdc11p was localized to presumptive bud sites, to mother-bud necks, and to recent division sites (data not shown). Cells of the double mutant typically displayed strong septin staining at one of the constrictions, most commonly the constriction between the terminal compartment of the elongated bud and the rest of the cell body (Fig. 3I); sometimes weaker septin staining was observed at one or more additional constrictions. In most (≥85%) of the cells, the position of the nucleus did not correspond to that of the constriction showing strong septin staining (Fig. 3I and J, upper cell). Such cells presumably cannot complete division successfully, which may explain why the double mutant grows so slowly. Other cells (≤15%) showed correlated septin and nuclear staining (Fig. 3I and J, lower cell) and could presumably divide successfully to maintain growth of the population. Some (~5%) of the cells displayed septin staining at the tip of the terminal compartment or in a ring or patch on one side of the cell body (data not shown). Thus, in the absence of Zds1p and Zds2p function, septins can localize to specific regions of the cell. However, the septin structures are often abnormal or mispositioned with respect to the nucleus, resulting, presumably, in problems in cell division.

Effects of *ZDS1* overexpression. Plasmid YEp352-GAL1-ZDS1 (see Materials and Methods) was used to examine the effects of *ZDS1* overexpression in diploid (YEF473) and haploid (YEF473A) cells. When *ZDS1* was induced by galactose, cells of both strains were typically larger and sometimes rounder than when *ZDS1* was not induced (Fig. 4A to D). In the diploid cells, the buds produced during *ZDS1* induction were approximately normal (or perhaps slightly rounder than normal) in shape (Fig. 4B). In the haploid cells, the buds produced during *ZDS1* induction typically were abnormal in shape (Fig. 4D). The enlarged cells observed under inducing conditions displayed generally bright (although not perfectly uniform) staining with Calcofluor (data not shown), indicating that chitin deposition was delocalized. In addition, the cortical actin spots appeared to be randomly distributed in the enlarged cells (Fig. 4F; cf. the uninduced cells in Fig. 4E), and ≥15% of these cells were binucleate (Fig. 4H). Taken together, the results suggest that overexpression of *ZDS1* interfered with the establishment of cell polarity, resulting in failure, delay (relative to the nuclear cycle), or abortion of budding events, consistent with the hypothesis that Zds1p is a negative regulator of polarity establishment. Similar results were obtained when *ZDS1* was overexpressed in the *bem3/bem3 rga1/rga1* strain YEF1223 (data not shown), indicating that the effects of *ZDS1* overexpression are not mediated by a pathway dependent on the GAPs Bem3p and Rga1p.

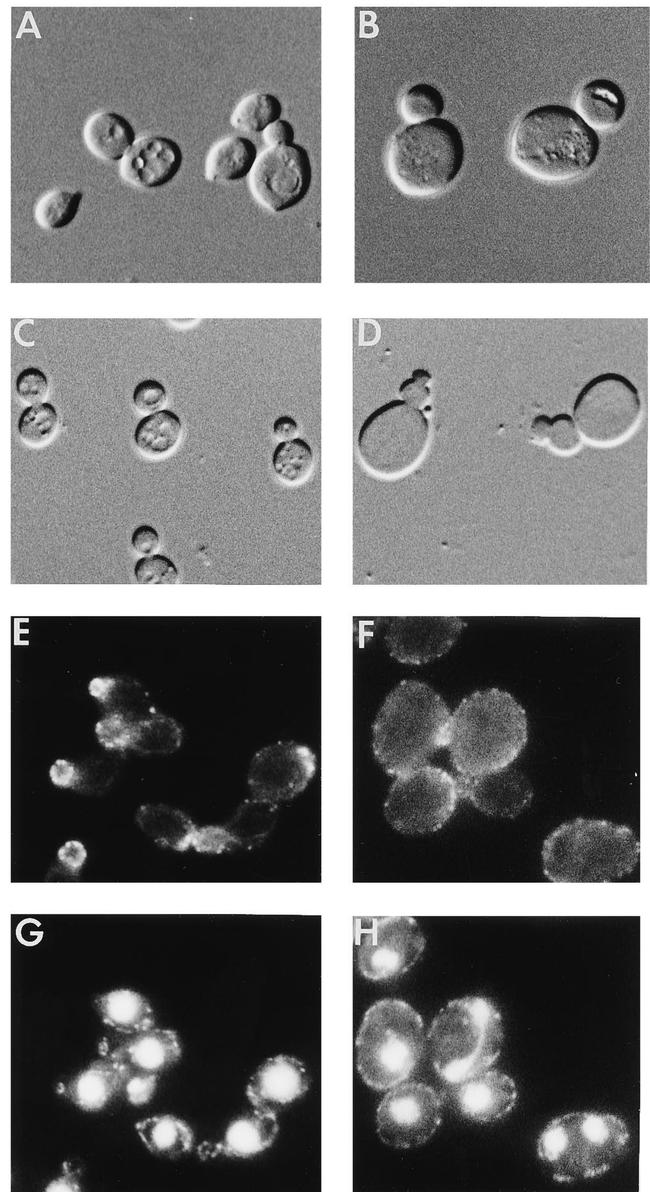

FIG. 4. Effects of overexpressing *ZDS1*. Wild-type strains YEF473 (A, B, and E to H) and YEF473A (C and D) containing plasmid YEp352-GAL1-ZDS1 were grown in SD-Ura with 2% glucose overnight, washed three times with either SD-Ura with 2% raffinose (noninducing) or SD-Ura with 2% raffinose and 2% galactose (inducing) medium, and then resuspended in the noninducing (A, C, E, and G) or inducing (B, D, F, and H) medium for 8.5 h at 23°C. Cells were then observed by DIC microscopy (A to D) or by fluorescence microscopy after double staining for actin (E and F) and DNA (G and H).

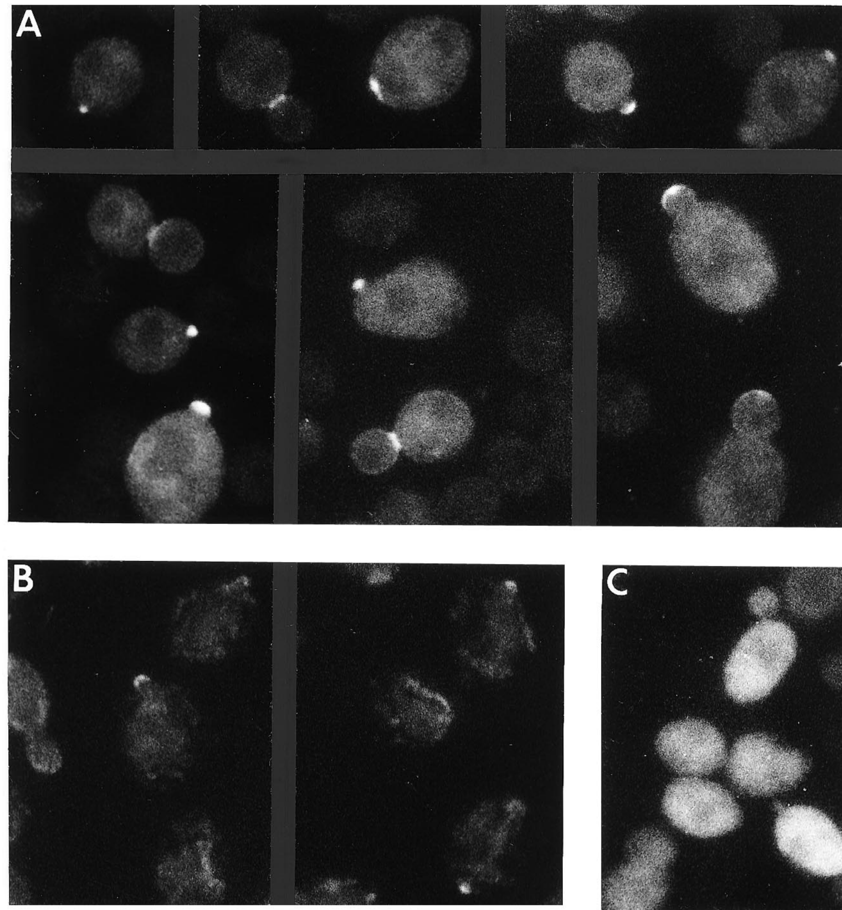

FIG. 5. Immunolocalization of GST-tagged Zds1p, using affinity-purified anti-GST antibodies (see Materials and Methods). (A) Strain YEF864 (*zds1*Δ::*HIS3*/*zds1*Δ::*HIS3*) harboring plasmid pEGKT-ZDS1. The GST-Zds1p fusion protein was induced as described in the text. (B) Strain YEF1020 (*zds1*Δ::*HIS3*/*zds1*Δ::*HIS3 ura3*:*URA3*:*ZDS1*:*GST*/*ura3*:*URA3*:*ZDS1*:*GST*). (C) Strain YEF864 harboring plasmid pEGKT (encoding GST not fused to Zds1p) after induction as for panel A.

Localization of Zds1p. Zds1p and Zds2p might interact directly with Cdc42p and/or other components of the morphogenetic machinery. Alternatively, the effects described above might be secondary (or even less direct) consequences of the actual primary activities of Zds1p and Zds2p. In an attempt to illuminate this issue, we used GST-tagged Zds1p to localize this protein. Two different constructs were used (see Materials and Methods for details). Plasmid pEGKT-ZDS1 encodes a fusion of GST to the N terminus of full-length Zds1p under control of the *GAL1/10* upstream activation sequence (see Materials and Methods). When introduced into the *zds1 zds2* strain YEF1077, this plasmid largely complemented the growth rate and morphological phenotypes during growth in SD-Ura medium containing 2% glucose (for repression of the fusion gene), 2% glucose and 2% galactose (for weak induction of the fusion gene), or 2% raffinose and 2% galactose (for strong induction of the fusion gene). To localize the fusion protein, plasmid pEGKT-ZDS1 was transformed into strain YEF864 (*zds1/zds1*), and the fusion protein was induced by growth for ~12 h in SD-Ura with 2% raffinose and 2% galactose; 2% glucose was then added to repress fusion protein expression for ~6 h before the cells were collected for immunofluorescence analysis using GST-specific antibodies (see Materials and Methods). (This growth regimen was adopted because under repressing or weakly inducing conditions, too little fusion protein was produced for reliable localization, whereas with strong induction, the cells grew slowly, perhaps because of a synergistic effect of the overexpression of GST-Zds1p and the poor carbon source.) Under these conditions, GST-Zds1p was localized predominantly to the tips of small and medium-sized buds and to bright patches on unbudded cells (Fig. 5A). Because of their singularity (one per cell), location near the poles of the ellipsoidal cells in this bipolar-budding strain, and similarity to the patches at the tips of small buds, the patches on unbudded cells seem likely to represent incipient bud sites. Cells with larger buds had weaker or no staining of their bud tips, but a few percent of such cells were stained at the mother-bud neck (Fig. 5A). No staining of nuclei was observed. When GST alone was expressed in strain YEF864 (by introducing plasmid pEGKT), it was dispersed in the cytoplasm (Fig. 5C).

The localization of GST-Zds1p observed in cells containing plasmid pEGKT-ZDS1 might be an artifact resulting from high levels of expression of the fusion protein. To address this possibility, we constructed strain YEF1020, a *zds1/zds1* strain homozygous for an insertion of plasmid YIplac211-ZDS1:GST at the *URA3* locus (see Materials and Methods). In this strain, a GST-Zds1p fusion protein should be produced under the control of the *ZDS1* promoter. When this same construct was integrated at the *URA3* locus of the *zds1 zds2* double-mutant strain YEF1077, producing strain YEF1110, the growth rate and morphology of the integrants were indistinguishable from those of wild type at 23 and 37°C, whereas integrants with the

YIplac211 vector alone integrated at *URA3* retained the mutant phenotypes (data not shown). Immunofluorescence observations on strain YEF1020 showed signals similar to those seen with YEF864 harboring pEGKT-ZDS1 (Fig. 5B), although in a smaller fraction of the cells; visualization of the signal in strain YEF1020 was improved by reducing the time of fixation (45 min rather than the 2.5 h used with strain YEF864 harboring pEGKT-ZDS1). No signal was observed at mother-bud necks in strain YEF1020, possibly just because the GST immunofluorescence signal was much weaker than that in cells carrying plasmid pEGKT-ZDS1 (see Discussion). In any case, the similarity of bud-tip and presumptive-bud-site staining in strain YEF1020 and in strain YEF864 harboring pEGKT-ZDS1 suggests that this represents a bona fide aspect of the localization of functional Zds1p.

DISCUSSION

In a genetic screen for GAPs or other negative regulators of Cdc42p, we identified the *ZDS1* gene. Several observations suggest that overexpression of *ZDS1* decreases the level of Cdc42p activity. First, introduction of a *ZDS1* plasmid reduces or eliminates the suppression of a *cdc24^ts^* allele by multicopy *CDC42*, and the resulting strains contain predominantly large, round, unbudded cells after incubation at a restrictive temperature. Second, *ZDS1*-containing plasmids also reduce the restrictive temperature for *cdc24^ts^* and *cdc42^ts^* mutants, and a *cdc24* mutant harboring a *ZDS1* plasmid contains mostly large, round, unbudded cells even during growth at a permissive temperature. In all of these respects, the effects of overexpressing *ZDS1* parallel (but are in some respects more extreme than) the effects of overexpressing *BEM3* or *RGA1*, two genes whose products appear to be bona fide GAPs for Cdc42p (98, 107, 108). Finally, overexpression of *ZDS1* in wild-type cells also appears to cause a partial loss of cell polarization.

Sequence from the genome project revealed the existence of a *ZDS1* homolog, *ZDS2*; the *ZDS1* and *ZDS2* gene products have multiple blocks of strong sequence homology. In addition, two lines of evidence suggest that Zds1p and Zds2p can share at least some functions: overexpression of Zds2p has some of the same effects as overexpression of Zds1p, and a *zds1 zds2* double mutant has a more extreme phenotype than either of the single mutants. Moreover, the elongated growth form and strongly polarized actin cytoskeleton of the double mutant suggest that Cdc42p may be hyperactive, or otherwise improperly regulated, in the absence of Zds1p and Zds2p activity. This phenotype resembles, at least to a first approximation, that of cells expressing a $CDC42^{G12V}$ allele (109), and the differences could reflect the unequal consequences of a simple loss of GTPase activity on one hand versus a loss of particular regulatory factors on the other. Other aspects of the double-mutant phenotype are difficult to interpret in detail. However, the abnormalities in chitin deposition and the lack of correspondence between septin localization and nuclear position can also be interpreted as reflecting abnormal control of morphogenesis, and the apparent delay in nuclear division might reflect the operation of a morphogenesis checkpoint (46).

Interestingly, the truncated *ZDS1* allele isolated in the original screen, which encodes only amino acids 409 to 915 of the full-length protein, partially rescued the phenotypes of the double mutant and had a somewhat stronger effect than the full-length gene in the original assays. These observations suggest that the activity of Zds1p may be carried in its C-terminal portion whereas the N-terminal portion is regulatory, although further structure-function studies of both Zds1p and Zds2p will be necessary to clarify this issue.

While our studies were in progress, *ZDS1* and/or *ZDS2* were identified independently by numerous other laboratories using an astonishingly wide variety of screens (hence the abbreviation *ZDS*, for zillion different screens). These included suppressor and synthetic-lethal screens beginning with particular alleles of *CDC28*, which encodes the yeast cyclin-dependent kinase (8, 103); screens for suppressors of mutations in *SIN4* and *MCM1*, which encode global regulators of transcription (89, 103); screens for suppressors of an *ssd1 hht1* (histone H3) double mutant (54); screens for genes whose overexpression can inhibit pheromone signal transduction (72); screens for suppressors of mutations in one of the genes encoding casein kinase II, an essential enzyme that appears to have a variety of physiological substrates (62); a screen for suppressors of a mutation in the mRNA-capping enzyme (90); a screen for suppressors of mutations in the translation initiation factor eIF4A (49); a screen for suppressors of mutations in *CDC20*, whose product is involved in nuclear division (16); a two-hybrid screen for proteins interacting with Snf1p, a protein kinase that regulates transcription in response to changes in carbon source (51); and two different screens designed to detect proteins interacting with telomeres (86). Although the inhibition of pheromone signaling by overexpression of *ZDS2* might well result from a decrease in Cdc42p activity, the other genetic interactions described above are not so easily rationalized on this basis, at least in terms of the known functions of Cdc42p. Thus, one possible interpretation of the available data is that the apparent effects on Cdc42p activity, and hence on cellular morphogenesis, that we have described in this report should be regarded as indirect effects of proteins that function primarily as regulators of transcription, mRNA stability, translation, protein turnover, or some other global process.

However, this interpretation appears difficult to reconcile with our data on Zds1p localization. Using *GST-ZDS1* fusions that rescue the phenotypes of the *zds1 zds2* double mutant, we have observed localization of the fusion protein to patches (presumably incipient bud sites) on unbudded cells and to the tips of small buds, the same regions to which Cdc42p itself has been localized (110). These data appear to provide strong support for the hypothesis that the primary activity of Zds1p (and, by extension, of Zds2p) involves a direct (or at least close) interaction with Cdc42p. How then might we rationalize the extraordinarily wide range of biological processes in which Zds1p and Zds2p have been implicated? One attractive possibility is that the role of Cdc42p in intracellular signaling is much broader than has been appreciated to date. Already the original concept of Cdc42p as an element signaling positional information to the cytoskeleton (1, 2, 38, 75) has been broadened to include the apparent role of Cdc42p in pheromone signaling (93, 98, 105). Moreover, in mammalian cells, Cdc42 has been implicated not only in control of organization of the actin cytoskeleton and cell polarization (12, 43, 68, 80, 99) but also in the regulation of transcription in response to external stimuli (18, 35, 64), in the control of phosphoinositide 3-kinase activity (106), and in the control of progression through the G_1 phase of the cell cycle (70). If Cdc42p in *S. cerevisiae* serves as the hub of a wide range of signaling pathways, the elements that interact with Cdc42p (such as, perhaps, Zds1p and Zds2p) might produce a wide range of biological effects when mutant or overproduced.

If Cdc42p in *S. cerevisiae* controls a wide range of signaling pathways, then it might do so through multiple effectors. It seems clear that at least some of the roles of Cdc42p (in both yeast and animal cells) are mediated by its activation of protein kinases of the Ste20p/Cla4p/PAK family (19, 57, 59, 60, 93, 105). In addition to Ste20p and Cla4p, *S. cerevisiae* contains at

least one other protein kinase in this family (accession number Z48149 [100]); the function of this protein is not known, but it shows two-hybrid interactions with Cdc42p (21) very similar to those reported previously for Ste20p and Cla4p (19, 93). These protein kinases may control various cellular processes either directly by phosphorylation of a variety of substrates or indirectly through their activation of one or more of the multiple, interrelated MAP kinase cascades (33, 45, 82), which may, in turn, regulate a variety of functions, including the transcription of various genes. Cdc42p may also have effectors other than the Ste20p/Cla4p/PAK family protein kinases. A particularly intriguing possibility is that the GAPs Bem3p and Rga1p function not just as negative regulators but also as effectors of Cdc42p function (73). If Cdc42p function is indeed mediated by multiple different effectors, this might explain the otherwise rather puzzling fact that the Cdc42p sequence has been so closely conserved (>80% sequence identity between yeasts and animals) over such a vast span of evolutionary time (39).

How might Zds1p and Zds2p interact with Cdc42p? The sequences have so far provided no real clues. No homology to known GAPs is detectable, and the Zds proteins do not contain the CRIB consensus sequence (10) found in many Cdc42p-interacting proteins. However, given the relatively weak homology among known and suspected Rho-GAPs (9, 44), it seems possible that not all Cdc42p-GAPs can be identified in this way, and it is unlikely that the CRIB consensus identifies more than a subset of Cdc42p-interacting proteins. An intriguing possibility is that the Zds proteins are involved in sequestering Cdc42p into different complexes that carry out different functions; the paradigm for this model would be the multiprotein complex that appears to be involved in transmission of the mating pheromone signal (15, 58, 78, 101). The different potential complexes might compete for the available Cdc42p, thus making a factor that promotes the formation of one complex a "negative regulator" of Cdc42p with regard to its function in other pathways. Further studies of the Zds proteins should illuminate these and perhaps other possibilities.

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REFERENCES

1. **Adams, A. E. M., D. I. Johnson, R. M. Longnecker, B. F. Sloat, and J. R. Pringle.** 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **111:**131–142.
2. **Adams, A. E. M., and J. R. Pringle.** 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98:**934–945.
3. **Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin.** 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:**3329–3330.
4. **Bender, A.** Personal communication.
5. **Bender, A., and J. R. Pringle.** 1989. Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the *ras*-related gene *RSR1*. Proc. Natl. Acad. Sci. USA **86:**9976–9980.
6. **Berben, G., J. Dumont, V. Gilliquet, P.-A. Bolle, and F. Hilger.** 1991. The YDp plasmids: a uniform set of vectors bearing versatile gene disruption cassettes for *Saccharomyces cerevisiae*. Yeast **7:**475–477.
7. **Bi, E., and J. R. Pringle.** Unpublished results.
8. **Blondel, M., and C. Mann.** Personal communication.
9. **Boguski, M. S., and F. McCormick.** 1993. Proteins regulating Ras and its relatives. Nature (London) **366:**643–654.
10. **Burbelo, P. D., D. Drechsel, and A. Hall.** 1995. A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. J. Biol. Chem. **270:**29071–29074.
11. **Chang, E. C., M. Barr, Y. Wang, V. Jung, H.-P. Xu, and M. H. Wigler.** 1994. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. Cell **79:**131–141.
12. **Chant, J., and L. Stowers.** 1995. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. Cell **81:**1–4.
13. **Chen, W., H. H. Lim, and L. Lim.** 1993. The *CDC42* homologue from *Caenorhabditis elegans*. Complementation of yeast mutation. J. Biol. Chem. **268:**13280–13285.
14. **Chenevert, J.** 1994. Cell polarization directed by extracellular cues in yeast. Mol. Biol. Cell **5:**1169–1175.
15. **Choi, K.-Y., B. Satterberg, D. M. Lyons, and E. A. Elion.** 1994. Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in *S. cerevisiae*. Cell **78:**499–512.
16. **Clark, D. J., and D. J. Burke.** Personal communication.
17. **Coleman, K. G., H. Y. Steensma, D. B. Kaback, and J. R. Pringle.** 1986. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation and characterization of the *CDC24* gene and adjacent regions of the chromosome. Mol. Cell. Biol. **6:**4516–4525.
18. **Coso, O. A., M. Chiariello, J.-C. Yu, H. Teramoto, P. Crespo, N. Xu, T. Miki, and J. S. Gutkind.** 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell **81:** 1137–1146.
19. **Cvrčková, F., C. De Virgilio, E. Manser, J. R. Pringle, and K. Nasmyth.** 1995. Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. Genes Dev. **9:**1817–1830.
20. **DeMarini, D. J., and J. R. Pringle.** Unpublished results.
21. **De Virgilio, C., and J. R. Pringle.** Unpublished results.
22. **Drubin, D. G., and W. J. Nelson.** 1996. Origins of cell polarity. Cell **84:**335–344.
23. **Dutartre, H., J. Davoust, J.-P. Gorvel, and P. Chavrier.** 1996. Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. J. Cell Sci. **109:**367–377.
24. **Eaton, S., P. Auvinen, L. Luo, Y. N. Jan, and K. Simons.** 1995. CDC42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. J. Cell Biol. **131:**151–164.
25. **Fares, H., and J. R. Pringle.** Unpublished results.
26. **Ford, S. K., and J. R. Pringle.** 1991. Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the *CDC11* gene product and the timing of events at the budding site. Dev. Genet. **12:**281–292.
27. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
28. **Guthrie, C., and G. R. Fink (ed.).** 1991. Methods in enzymology, vol. 194. Guide to yeast genetics and molecular biology. Academic Press, San Diego, Calif.
29. **Hancock, J. F., and A. Hall.** 1993. A novel role for RhoGDI as an inhibitor of GAP proteins. EMBO J. **12:**1915–1921.
30. **Harris, S. D., and J. R. Pringle.** 1991. Genetic analysis of *Saccharomyces cerevisiae* chromosome I: on the role of mutagen specificity in delimiting the set of genes identifiable using temperature-sensitive-lethal mutations. Genetics **127:**279–285.
31. **Hart, M. J., Y. Maru, D. Leonard, O. N. Witte, T. Evans, and R. A. Cerione.** 1992. A GDP dissociation inhibitor that serves as a GTPase inhibitor for the Ras-like protein CDC42Hs. Science **258:**812–815.
32. **Hartwell, L. H., J. Culotti, J. R. Pringle, and B. J. Reid.** 1974. Genetic control of the cell division cycle in yeast. Science **183:**46–51.
33. **Herskowitz, I.** 1995. MAP kinase pathways in yeast: for mating and more. Cell **80:**187–197.
34. **Herskowitz, I., and R. E. Jensen.** 1991. Putting the *HO* gene to work: practical uses for mating-type switching. Methods Enzymol. **194:**132–146.
35. **Hill, C. S., J. Wynne, and R. Treisman.** 1995. The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. Cell **81:**1159–1170.
36. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2:**163–167.
37. **Hofmann, K., and W. Stoffel.** 1993. A database of membrane spanning protein segments. Biol. Chem. Hoppe-Seyler **374:**166.
38. **Johnson, D. I., and J. R. Pringle.** 1990. Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. J. Cell Biol. **111:**143–152.
39. **Johnson, D. I., and J. R. Pringle.** 1995. Cdc42p, p. 283–287. *In* M. Zerial and L. Huber (ed.), Guidebook to the small GTPases. Oxford University Press, Oxford.
40. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent

GAL1-GAL10 promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**1440–1448.

41. **Kilmartin, J. V., and A. E. M. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **98:**922–933.
42. **Kilmartin, J. V., B. Wright, and C. Milstein.** 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. J. Cell Biol. **93:**576–582.
43. **Kozma, R., S. Ahmed, A. Best, and L. Lim.** 1995. The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. Mol. Cell. Biol. **15:**1942–1952.
44. **Lamarche, N., and A. Hall.** 1994. GAPs for rho-related GTPases. Trends Genet. **10:**436–440.
45. **Levin, D. E., and B. Errede.** 1995. The proliferation of MAP kinase signaling pathways in yeast. Curr. Opin. Cell Biol. **7:**197–202.
46. **Lew, D. J., and S. I. Reed.** 1995. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. J. Cell Biol. **129:**739–749.
47. **Li, R., Y. Zheng, and D. G. Drubin.** 1995. Regulation of cortical actin cytoskeleton assembly during polarized cell growth in budding yeast. J. Cell Biol. **128:**599–615.
48. **Lillie, S. H., and J. R. Pringle.** 1980. Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. J. Bacteriol. **143:** 1384–1394.
49. **Linder, P.** Personal communication.
50. **Longtine, M. S., D. J. DeMarini, M. L. Valencik, O. S. Al-Awar, H. Fares, C. De Virgilio, and J. R. Pringle.** 1996. The septins: roles in cytokinesis and other processes. Curr. Opin. Cell Biol. **8:**106–119.
51. **Ludin, K., and M. Carlson.** Personal communication.
52. **Luo, L., Y. J. Liao, L. Y. Jan, and Y. N. Jan.** 1994. Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. Genes Dev. **8:**1787–1802.
53. **Lupas, A., M. Van Dyke, and J. Stock.** 1991. Predicting coiled coils from protein sequences. Science **252:**1162–1164.
54. **Lutz, K., B. A. Morgan, and M. M. Smith.** Personal communication.
55. **Lye, G., and C. M. Churcher.** Unpublished results.
56. **Manser, E., T. Leung, H. Salihuddin, L. Tan, and L. Lim.** 1993. A nonreceptor tyrosine kinase that inhibits the GTPase activity of $p21^{CDC42}$. Nature (London) **363:**364–367.
57. **Manser, E., T. Leung, H. Salihuddin, Z. Zhao, and L. Lim.** 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature (London) **367:**40–46.
58. **Marcus, S., A. Polverino, M. Barr, and M. Wigler.** 1994. Complexes between STE5 and components of the pheromone-responsive mitogen-activated protein kinase module. Proc. Natl. Acad. Sci. USA **91:**7762–7766.
59. **Marcus, S., A. Polverino, E. Chang, D. Robbins, M. H. Cobb, and M. H. Wigler.** 1995. Shk1, a homolog of the *Saccharomyces cerevisiae* Ste20 and mammalian $p65^{PAK}$ protein kinases, is a component of a Ras/Cdc42 signaling module in the fission yeast *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **92:**6180–6184.
60. **Martin, G. A., G. Bollag, F. McCormick, and A. Abo.** 1995. A novel serine kinase activated by rac1/CDC42Hs-dependent autophosphorylation is related to PAK65 and STE20. EMBO J. **14:**1970–1978.
61. **Masuda, T., K. Tanaka, H. Nonaka, W. Yamochi, A. Maeda, and Y. Takai.** 1994. Molecular cloning and characterization of yeast *rho* GDP dissociation inhibitor. J. Biol. Chem. **269:**19713–19718.
62. **McCann, R., and C. V. C. Glover.** Personal communication.
63. **Miller, P. J., and D. I. Johnson.** 1994. Cdc42p GTPase is involved in controlling polarized cell growth in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **14:**1075–1083.
64. **Minden, A., A. Lin, F.-X. Claret, A. Abo, and M. Karin.** 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81:**1147–1157.
65. **Mitchell, D. A., T. K. Marshall, and R. J. Deschenes.** 1993. Vectors for the inducible overexpression of glutathione S-transferase fusion proteins in yeast. Yeast **9:**715–723.
66. **Miyamoto, S., Y. Ohya, Y. Sano, S. Sakaguchi, H. Iida, and Y. Anraku.** 1991. A *DBL*-homologous region of the yeast *CLS4/CDC24* gene product is important for Ca^{2+}-modulated bud assembly. Biochem. Biophys. Res. Commun. **181:**604–610.
67. **Munemitsu, S., M. A. Innis, R. Clark, F. McCormick, A. Ullrich, and P. Polakis.** 1990. Molecular cloning and expression of a G25K cDNA, the human homolog of the yeast cell cycle gene *CDC42*. Mol. Cell. Biol. **10:**5977–5982.
68. **Nobes, C. D., and A. Hall.** 1995. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell **81:**53–62.
69. **Ohya, Y., S. Miyamoto, Y. Ohsumi, and Y. Anraku.** 1986. Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. J. Bacteriol. **165:**28–33.
70. **Olson, M. F., A. Ashworth, and A. Hall.** 1995. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. Science **269:**1270–1272.
71. **Ottilie, S., P. J. Miller, D. I. Johnson, C. L. Creasy, M. A. Sells, S. Bagrodia, S. L. Forsburg, and J. Chernoff.** 1995. Fission yeast $pak1^{+}$ encodes a protein kinase that interacts with Cdc42p and is involved in the control of cell polarity and mating. EMBO J. **14:**5908–5919.
72. **Porter, G., and B. Errede.** Personal communication.
73. **Pringle, J. R., E. Bi, H. A. Harkins, J. E. Zahner, C. De Virgilio, J. Chant, K. Corrado, and H. Fares.** 1995. Establishment of cell polarity in yeast. Cold Spring Harbor Symp. Quant. Biol. **60:**729–743.
74. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Life cycle and inheritance. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
75. **Pringle, J. R., S. H. Lillie, A. E. M. Adams, C. W. Jacobs, B. K. Haarer, K. G. Coleman, J. S. Robinson, L. Bloom, and R. A. Preston.** 1986. Cellular morphogenesis in the yeast cell cycle, p. 47–80. *In* J. Hicks (ed.), Yeast cell biology. Alan R. Liss, New York.
76. **Pringle, J. R., and J.-R. Mor.** 1975. Methods for monitoring the growth of yeast cultures and for dealing with the clumping problem. Methods Cell Biol. **11:**131–168.
77. **Pringle, J. R., R. A. Preston, A. E. M. Adams, T. Stearns, D. G. Drubin, B. K. Haarer, and E. W. Jones.** 1989. Fluorescence microscopy methods for yeast. Methods Cell Biol. **31:**357–435.
78. **Printen, J. A., and G. F. Sprague, Jr.** 1994. Protein-protein interactions in the yeast pheromone response pathway: Ste5p interacts with all members of the MAP kinase cascade. Genetics **138:**609–619.
79. **Reid, B. J., and L. H. Hartwell.** 1977. Regulation of mating in the cell cycle of *Saccharomyces cerevisiae*. J. Cell Biol. **75:**355–365.
80. **Ridley, A. J.** 1995. Rho-related proteins: actin cytoskeleton and cell cycle. Curr. Opin. Genet. Dev. **5:**24–30.
81. **Riles, L., J. E. Dutchik, A. Baktha, B. K. McCauley, E. C. Thayer, M. P. Leckie, V. V. Braden, J. E. Depke, and M. V. Olson.** 1993. Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics **134:**81–150.
82. **Roberts, R. L., and G. R. Fink.** 1994. Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. **8:**2974–2985.
83. **Rose, A. B., and J. R. Broach.** 1990. Propagation and expression of cloned genes in yeast: 2-μm circle based vectors. Methods Enzymol. **185:**234–279.
84. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:**237–243.
85. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
86. **Roy, N., and K. W. Runge.** Personal communication.
87. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
88. **Sandbaken, M. G., and M. R. Culbertson.** 1988. Mutations in elongation factor EF1-α affect the frequency of frameshifting and amino acid misincorporation in *Saccharomyces cerevisiae*. Genetics **120:**923–934.
89. **Schleiffer, A., and G. Ammerer.** Personal communication.
90. **Schwer, B., and S. Shuman.** Multicopy suppressors of temperature-sensitive mutations of yeast mRNA capping enzyme. Gene Expr., in press.
91. **Shinjo, K., J. G. Koland, M. J. Hart, V. Narasimhan, D. I. Johnson, T. Evans, and R. A. Cerione.** 1990. Molecular cloning of the gene for the human placental GTP-binding protein Gp (G25K): identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein CDC42. Proc. Natl. Acad. Sci. USA **87:**9853–9857.
92. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
93. **Simon, M.-N., C. De Virgilio, B. Souza, J. R. Pringle, A. Abo, and S. I. Reed.** 1995. Role for the Rho-family GTPase Cdc42 in yeast mating-pheromone signal pathway. Nature (London) **376:**702–705.
94. **Skelton, J., and C. M. Churcher.** Unpublished results.
95. **Sloat, B. F., A. Adams, and J. R. Pringle.** 1981. Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. **89:**395–405.
96. **Sloat, B. F., and J. R. Pringle.** 1978. A mutant of yeast defective in cellular morphogenesis. Science **200:**1171–1173.
97. **Spencer, F., and P. Hieter.** Personal communication.
98. **Stevenson, B. J., B. Ferguson, C. De Virgilio, E. Bi, J. R. Pringle, G. Ammerer, and G. F. Sprague, Jr.** 1995. Mutation of *RGA1*, which encodes a putative GTPase-activating protein for the polarity-establishment protein Cdc42p, activates the pheromone-response pathway in the yeast *Saccharomyces cerevisiae*. Genes Dev. **9:**2949–2963.
99. **Stowers, L., D. Yelon, L. J. Berg, and J. Chant.** 1995. Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. Proc. Natl. Acad. Sci. USA **92:**5027–5031.
100. **Vandenbol, M., P. Durand, D. Potetelle, and F. Hilger.** Unpublished results.
101. **Whiteway, M. S., C. Wu, T. Leeuw, K. Clark, A. Fourest-Lieuvin, D. Y. Thomas, and E. Leberer.** 1995. Association of the yeast pheromone re-

sponse G protein βγ subunits with the MAP kinase scaffold Ste5p. Science **269:**1572–1575.

102. **Wilkinson, L. E., and J. R. Pringle.** 1974. Transient G1 arrest of *S. cerevisiae* cells of mating type α by a factor produced by cells of mating type **a**. Exp. Cell Res. **89:**175–187.
103. **Yu, Y., Y. W. Jiang, R. J. Wellinger, K. Carlson, J. M. Roberts, and D. J. Stillman.** 1996. Mutations in the homologous *ZDS1* and *ZDS2* genes affect cell cycle progression. Mol. Cell. Biol. **16:**5254–5263.
104. **Zahner, J. E., and J. R. Pringle.** Unpublished results.
105. **Zhao, Z.-S., T. Leung, E. Manser, and L. Lim.** 1995. Pheromone signalling in *Saccharomyces cerevisiae* requires the small GTP-binding protein Cdc42p and its activator *CDC24*. Mol. Cell. Biol. **15:**5246–5257.
106. **Zheng, Y., S. Bagrodia, and R. A. Cerione.** 1994. Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85*. J. Biol. Chem. **269:**18727–18730.
107. **Zheng, Y., R. Cerione, and A. Bender.** 1994. Control of the yeast bud-site assembly GTPase Cdc42. Catalysis of guanine nucleotide exchange by Cdc24 and stimulation of GTPase activity by Bem3. J. Biol. Chem. **269:** 2369–2372.
108. **Zheng, Y., M. J. Hart, K. Shinjo, T. Evans, A. Bender, and R. A. Cerione.** 1993. Biochemical comparisons of the *Saccharomyces cerevisiae* Bem2 and Bem3 proteins. Delineation of a limit Cdc42 GTPase-activating protein domain. J. Biol. Chem. **268:**24629–24634.
109. **Ziman, M., J. M. O'Brien, L. A. Ouellette, W. R. Church, and D. I. Johnson.** 1991. Mutational analysis of *CDC42Sc*, a *Saccharomyces cerevisiae* gene that encodes a putative GTP-binding protein involved in the control of cell polarity. Mol. Cell. Biol. **11:**3537–3544.
110. **Ziman, M., D. Preuss, J. Mulholland, J. M. O'Brien, D. Botstein, and D. I. Johnson.** 1993. Subcellular localization of Cdc42p, a *Saccharomyces cerevisiae* GTP-binding protein involved in the control of cell polarity. Mol. Biol. Cell **4:**1307–1316.