The Role of Cdc42 in Signal Transduction and Mating of the Budding Yeast Saccharomyces cerevisiae* 

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The small G-protein Cdc42 functions in many eukaryotic signal transduction pathways. In the budding yeast Saccharomyces cerevisiae, cells with defective Cdc42 fail to induce mating-specific genes in response to mating factor and to adopt the proper morphology for conjugation. Here we show that the failure of mating factor-induced transcription is largely the indirect result of arrest at a specific cell cycle position and/or the accumulation of high levels of the Cln1/2-Cdc28 kinase, a known repressor of mating factor signal transduction. Cdc42-defective cells with restored transcriptional induction have a partially restored mating ability but are still defective in the morphological response to mating factor. These results show that Cdc42 is not required for transduction of the mating factor signal per se but that it is essential for proper mating factor-induced morphogenesis.

Cdc42 is a member of the Rho family of small G-proteins, which is essential for many signal transduction and morphogenic processes in eukaryotic cells (1, 2). Some of its biological effects result from the binding to and the activation of members of the p21-activated kinase (PAK)3 family of protein kinases (3). Like other G-proteins, Cdc42 can exist in an active, GTP-bound state and an inactive, GDP-bound state (4). In the budding yeast Saccharomyces cerevisiae, the transition from inactive to active state is catalyzed by the guanyl exchange factor Cdc24 (5), and several GTP-ase activating proteins are thought to promote the formation of the inactive GDP-Cdc42 state (5, 6). The GTP-bound form of Cdc42 can interact with the yeast PAK family members Ste20 and Cla4 (7–9). Ste20 by itself is essential for mating factor signal transduction, haploid invasive growth, and pseudohyphal growth functions of Ste20, but they have normal in vitro kinase activity and are proficient in mating functions (13, 14). Apparently, the interaction of Cdc42 with Ste20 is required for some but not for all Ste20 functions.

Because the Cdc42-Ste20 interaction is not required for mating factor signal transduction, it is unclear why cells with temperature-sensitive cdc42 and cdc24 alleles are defective in mating factor-induced transcription and other mating functions (7, 11, 15). One possibility is that Cdc42 interacts with a factor other than Ste20 that is essential for mating factor signal transduction. This idea is supported by the recent finding in mammalian cells that Cdc42 does not only interact with PAKs but also with the MEKK1 and MEKK4 protein kinases (16), which have homologs in yeast. Another potential explanation for the signal transduction defect of cdc42 and cdc24 cells at restrictive temperature is based on the following observations: (a) the signaling defect of cdc42 and cdc24 cells is observed most strongly in cells that are fully arrested at the cdc24/cdc42 block (7, 11), (b) it has been shown that high levels of Cln1/2-Cdc28 kinase accumulate at such blocks (17), and (c) we have previously shown that high levels of Cln1/2-Cdc28 can repress the mating factor signal transduction pathway (18). We tested whether the signaling defect of cdc24 and cdc42 cells was due to repression of mating factor signaling by the high levels of Cln1/2-Cdc28 kinase at the cdc42/cdc24 block.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions—The genotypes of yeast strains used in this study are given in Table I. All strains were isogenic to BP264–15D (orp1-1a leu2-3, 112 ura3 ade1 his2) or, in the case of cdc24-1 and cdc2-1 strains, backcrossed to the BP264–15D strain background at least five times. Strains were constructed by standard methods, and cells were grown in YEP (yeast extract peptone) media with 2% dextrose or 3% galactose as described (19). Treatment of cells with mating factor was at concentrations of 0.1 μM or higher. Quantitative mating assays were carried out essentially as described previously (18).

Northern and Morphological Analysis—Northern analysis was as described (18). Cell morphology was examined by phase contrast optics, and negatives of pictures were scanned into a digital format. Contrast and brightness were further adjusted using the Adobe Photoshop computer program.

RESULTS

Repression of Mating Factor Signal Transduction by Cln1/2-Cdc28 Kinase—To test whether the defect in signal transduction activity observed at a cdc24 or cdc2 block could be due to repression of the mating factor signaling pathway by the G1 cyclins CLN1 and CLN2, we compared the signal transduction activity under restrictive conditions of cdc42 and cdc24 strains with and without CLN1/2. As shown before (7, 11), cells with temperature-sensitive alleles of cdc42 and cdc24 at restrictive temperature showed little transcriptional induction by mating factor of the reporter gene FUS1 (Fig. 1A). Deletion of CLN1...
and CLN2 in the cdc42 and cdc24 strains resulted in a significant increase in signaling activity at restrictive temperature; cdc24 or cdc42 cells without CLN1/2 had about a 3-fold increase in signal transduction activity when compared with cells with CLN1/2 (Fig. 1A). Some defect in signal transduction activity, however, was still observed. At permissive temperature none of these strains showed a major defect in signaling activity (Fig. 1A). These observations suggest that part of the signaling defect at the cdc42 and cdc24 arrest points is due to the presence of CLN1 and CLN2.

Mating Factor Signal Transduction in cdc42 and cdc24 Cells Blocked the START Cell Cycle Position (20)—Mutant cdc42 and cdc24 cells arrest at a specific cell cycle position as large unbudded cells (20). It is at this position that the signaling defect of these cells was observed (7, 11). We wanted to eliminate potential cell cycle effects that might contribute to the defect in signal transduction activity. The possibility of such cell cycle effects is suggested by observations in cdc34-1 mutant cells, which like cdc24 and cdc42 arrest at a post-START cell cycle position (20). Just like cdc24 and cdc42 cells, cdc34-1 blocked at restrictive temperature are defective in pheromone signal transduction activity (data not shown). Unlike cdc42 and cdc24, however, the defect in signal transduction activity of these cdc34 cells could not be alleviated by deletion of CLN1 and CLN2 (data not shown), indicating that besides CLN1/2-mediated repression of signal transduction, another effect, possibly related to the post-START cell cycle position, strongly contributes to the signaling defect of cdc34-1 cells. To test for potential cell cycle position effects that might contribute to the signaling defect of cdc24 and cdc42 cells, we used cln−cdc24-1 GAL1::CLN1 and cln−cdc42-1 GAL1::CLN1 cells. These cells were grown on galactose medium and then arrested at START by addition of glucose, which results in CLN deprivation by repression of the GAL1 promoter (21). Cdc24 or Cdc42 were then inactivated by elevation of the temperature, and mating factor signal transduction activity was determined by monitoring FUS1 transcriptional induction. At this different cell cycle position, signal transduction activity of the cdc42 and cdc24 mutant cells was similar to that of wild type cells at both permissive (data not shown) and restrictive temperature (Fig. 1B). The same cells kept in galactose and arrested at the cdc arrest points (with high CLN1 expression levels) were strongly defective in signal transduction activity (Fig. 1B). Deletion of STE5 (a gene required for mating factor signal transduction (22)) in cln−cdc24-1 GAL1::CLN1 cells eliminated all mating factor-induced signal transduction activity of CLN1-blocked cells at restrictive temperature (data not shown). This indicates that transcriptional induction of the FUS1 reporter gene by mating factor is not due to some artifactual activation but occurs through bona fide activation of the mating factor signal transduction pathway. Taken together, these data demonstrate that the previously observed signaling defect of cdc24 and cdc42 cells can be explained largely by cell cycle position effects in combination with high level CLN1/2-associated kinase activity.

In Vivo, CLN2 Appears to Contribute More to Repression than CLN1—Our observations on the signaling defects of cdc24 and cdc42 cells allowed us to test the in vivo contribution of

### Table I

Strains Genotype

| Strain   | Genotype                          |
|----------|-----------------------------------|
| BOY931   | MATa bar1                         |
| BOY1248  | MATa bar1 cdc24-1                 |
| BOY1235  | MATa bar1 cln1 cln2 cdc24-1       |
| BOY1251  | MATa bar1 cdc42-1                 |
| BOY1240  | MATa bar1 cln1 cln2 cdc24-1       |
| BOY836   | MATa bar1 cln1 cln2 cln3          |
| BOY1074  | MATa bar1 cln1 cln2 cln3::URA3 cdc24-1 leu2::LEU2::GAL1::CLN1 |
| BOY1076  | MATa bar1 cln1 cln2 cln3::URA3 cdc42-1 leu2::LEU2::GAL1::CLN1 |
| BOY921   | MATa bar1                         |
| BOY1001  | MATa bar1 cdc24-1                 |
| BOY1005  | MATa bar1 cln1 cdc24-1            |
| BOY1004  | MATa bar1 cln2 cdc24-1            |
| BOY1007  | MATa bar1 cln1 cln2 cdc24-1       |
It has been observed that phogenesis—transduction (18). The observation that reduce mating factor signal transduction activity. The can, when expressed from their own promoter, by themselves (Fig. 1). Although these observations are made in a somewhat artificial situation, this indicates that both CLN1 and CLN2 can, when expressed from their own promoter, by themselves reduce mating factor signal transduction activity. The observation that CLN2 seems more effective in repression (Fig. 1C) might help explain why expression of CLN2 from the GAL1 promoter is particularly effective in repression of signal transduction (18).

Cdc42 Function Is Required for Mating Factor-induced Morphogenesis—It has been observed that cdc42 and cdc24 cells at restrictive temperature are defective in mating factor-induced morphogenesis and have a strong mating defect (15, 23–25). It is unclear, however, whether this reflects a genuine requirement for Cdc42 function in morphogenesis and mating or whether these observations are an indirect result of the strong signal transduction defect at the cdc24/cdc42 blocks. Because inactivation of Cdc42 function in START-arrested cells did not result in a major signal transduction defect, we could separate direct from indirect effects and directly examine the requirement of Cdc42 for mating factor-induced morphogenesis and mating. We used thermosensitive cdc24 mutants because the cdc24-1 allele has been observed to be “tighter” than the cdc42-1 allele (Ref. 17 and data not shown); it is therefore expected to be better suited for these experiments that require extended incubations at restrictive temperature.

To test the involvement of Cdc24 in mating factor-induced morphogenesis, cln− cdc24 GAL1::CLN1 cells were arrested at START by CLN deprivation, and a portion of the cells was then shifted to the restrictive temperature of 36 °C. Fig. 2 shows the morphology of START-arrested cells with and without mating factor treatment. In response to mating factor, both wild type and cdc24 cells at permissive temperature formed the typical mating projections called “shmoos.” At the elevated temperature, however, only the wild type cells formed mating projections, whereas the cdc24 cells in the presence of mating factor displayed a round, nonpolarized morphology. Because START-arrested cdc24 cells at 36 °C are proficient in signal transduction activity, this shows that Cdc24 and by inference Cdc42 are required for proper mating factor-induced morphogenesis.

We then tested the mating ability of cdc24 cells in which mating factor signal transduction activity was restored by CLN deprivation. As shown in Fig. 3, cdc24 cells blocked at restrictive temperature displayed a marked mating defect, although in our strain background and under these experimental conditions, the defect did not appear as large as reported previously by others (15). In cdc24 cells in which signal transduction activity was restored by prior CLN deprivation, there was a partial restoration of the mating defect at restrictive temperature. The cdc24 cells at a CLN block still appeared to have a somewhat reduced mating efficiency when compared with wild type cells.

**DISCUSSION**

We demonstrate that repression of the mating factor signal transduction pathway by the Cln1/2-cdc28 kinase in combination with cell cycle position effects can largely explain the previously observed signaling defect of cdc24 and cdc24 cells blocked at restrictive temperature (7, 11). There clearly is no absolute requirement for Cdc42 or Cdc24 in mating factor signal transduction. This is consistent with the observations that there is no requirement for an interaction of Cdc42 with Ste20 for mating factor signal transduction (11, 13, 14). Moreover, these data show that there is not some other step in the mating factor signal transduction route that requires Cdc42 function.

Even though cdc24 cells that were arrested at START by CLN deprivation had normal mating factor signal transduction activity, they failed to form projections for conjugation. This requirement of Cdc42 and Cdc24 in morphogenesis during the sexual cycle of budding yeast is in keeping with the crucial importance of these proteins in morphogenesis during the vegetative cell cycle (23–25) and with their morphogenic role in
other eukaryotes (1, 2). An involvement of Cdc24/Cdc42 specifically in mating factor-induced morphogenesis is also supported by a recent study describing the generation of CDC24 alleles that are only defective in mating factor-induced morphogenesis, whereas other vegetative and sexual functions of Cdc24 are unaffected (26). When compared with activation of PAK family members from other organisms (7, 27), Cdc42 binding to the budding yeast Ste20 or Cla4 kinases results only in, at best, a moderate stimulation of kinase activity (7, 28), whereas for Ste20 it appears to be critical for in vivo localization of the kinase (13, 14). The primary mode of activation of Cla4 or Ste20 by Cdc42 may be localization of the kinases to the proper site for function rather than stimulation of in vivo kinase activity. Such a localization mechanism accounts for a large part of the activation of Raf kinase by Ras (29, 30).

The mating defect of cdc24 cells at restrictive temperature could be partially overcome by blocking cells at a different cell cycle position with low CLN kinase activity. Because cells have normal mating factor signal transduction activity at that position, this suggests that part of the previously observed mating defect of cdc24 cells (15) can be attributed to a signal transduction defect. That mating in these cells was not as efficient as that of wild type cells, might be due to the fact that they failed to form projections for conjugation. This indicates that the poor mating phenotype of cdc24 cells that was observed previously can be attributed to a combination of signal transduction and morphogenic defects.

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