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Genetic Interactions among Regulators of Septin Organization

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Septins form a cortical scaffold at the yeast mother-bud neck that restricts the diffusion of cortical proteins between the mother and bud and serves as a signaling center that is important for governing various cell functions. After cell cycle commitment in late G_1, septins are assembled into a narrow ring at the future bud site, which spreads to form a mature septin hourglass immediately after bud emergence. Although several septin regulators have been identified, it is unclear how they cooperate to assemble the septin scaffold. We have examined septin localization in isogenic strains containing single or multiple mutations in eight septin organization genes (*CDC42*, *RGA1*, *RGA2*, *BEM3*, *CLA4*, *GIN4*, *NAP1*, and *ELM1*). Our results suggest that these regulators act largely in parallel to promote either the initial assembly of the septin ring (*CDC42*, *RGA1*, *RGA2*, *BEM3*, and *CLA4*) or the conversion of the ring to a stable hourglass structure at the neck (*GIN4*, *NAP1*, and *ELM1*). Aberrant septin localization patterns in mutant strains could be divided into apparently discrete categories, but individual strains displayed heterogeneous defects, and there was no clear-cut correspondence between the specific mutations and specific categories of defect. These findings suggest that when they are deprived of their normal regulators, septin scaffolds collapse into a limited repertoire of aberrant states in which the nature of the mutant regulators influences the probability of a given aberrant state.

The septins are a family of evolutionarily conserved filament-forming proteins that were first identified in cytokinesis-defective mutants of *Saccharomyces cerevisiae* (17). Septins form a cortical scaffold that promotes the localized assembly of various other proteins, including proteins involved in cytokinesis, cell wall deposition, cell cycle control, and bud site selection (15, 23). In mammalian cells, septins are often colocalized with actin filaments and have been found in association with proteins that are important for exocytosis (35). Through the recruitment of various proteins to specific parts of the cell cortex, septins are thought to demarcate cortical domains (e.g., the cleavage furrow or synaptic membranes) for specialized functions.

Since most, if not all, septin functions appear to involve the targeting of other proteins to the cortical sites populated by the septins, it is of great interest to elucidate how the septins themselves are directed to assemble at those sites and how they are organized into an effective scaffold. In *S. cerevisiae*, the septins form a discrete ring at the cortical site of future bud emergence which expands to form an hourglass-shaped collar at the mother-bud neck once the bud has emerged. The collar remains at the neck until cytokinesis, when the collar splits into two larger and fainter rings that mark the division site on newborn cells, and the rings then disassemble (usually) prior to the next round of bud emergence (15, 22). These changes in the shape of the septin structure throughout the cell cycle correspond to changes in the dynamics of septin proteins, as measured by fluorescence recovery after photobleaching (9, 11). Septins within the new rings in unbudded cells are exchangeable, whereas septins within the hourglass collars are immobile until the collar splits into two rings, when the septins are once again exchangeable. Recent work has identified a limited but growing number of proteins that are required to promote this pattern of septin organization during the cell cycle.

Assembly of the initial septin ring requires the small GTPase Cdc42p and its guanine nucleotide exchange factor Cdc24p. Point mutations in the effector domain of Cdc42p cause improper assembly of the septin ring, generating unstable and/or abnormally large (in diameter) rings (13). Those same point mutations impair GTP hydrolysis by Cdc42p, suggesting that Cdc42p must undergo cycles of GTP loading and hydrolysis to promote septin ring assembly. Such cycles involve the actions of three Cdc42p-directed GTPase-activating proteins (GAPs) (Rga1p, Rga2p, and Bem3p) with overlapping functions in septin ring assembly (9, 13, 29). The Cdc42p effector kinases Cla4p and Ste20p are also important for septin organization (with Cla4p playing the dominant role) (10, 18, 25, 37), perhaps via direct phosphorylation of septins (36). Studies using conditional mutants suggested that Cdc24p, Cdc42p, Ste20p, and Cla4p are specifically involved in the initial assembly of the septin ring (and/or spreading to form the septin hourglass) but are no longer required to maintain septin organization once the hourglass collar has been assembled (13, 18, 37).

In addition to Cdc42p and its associated regulators and effectors, several other proteins have been implicated in promoting septin organization. These include two protein kinases (Gin4p and Elm1p) and two protein phosphatases (Glc7p/PP1 and Rts1p/PP2A), which are themselves localized to the septin collar at specific times, and it has been suggested that phosphorylation and/or dephosphorylation of the septins themselves may regulate septin dynamics during the cell cycle (5, 7, 8, 11, 12, 20, 24, 33, 36). Two other proteins with no known

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TABLE 1. Yeast strains used for this study[a]

Strain	Relevant genotype	Source or reference
DLY1	**a** *bar1*	28
DLY2723	**a** *rga1*::*TRP1 rga2*::*URA3 bem3*::*LEU2*	13
DLY3067	**a** *bar1 cdc42*::*LEU2*::*GAL1p-CDC42*	14
DLY3341	**a** *rga1*::*TRP1 bem3*::*LEU2*	13
DLY3344	**a** *rga1*::*TRP1*	13
DLY3346	**a** *bem3*::*LEU2*	13
DLY3347	**a** *rga1*::*TRP1 rga2*::*URA3*	13
DLY3353	**a** *rga2*::*URA3*	13
DLY3361	**a** *rga2*::*URA3 bem3*::*LEU2*	13
DLY4223	**a**/α *cdc42*^{V36T,K94E}/*cdc42*::*LEU2*::*GAL1p-CDC42*	13
DLY4224	**a**/α *his2*::*cdc42*^{Y32H}::*HIS2*/*his2 cdc42*::*URA3*/*cdc42*::*LEU2*::*GAL1p-CDC42*	13
DLY4410	α *gin4-Δ9*	This study
DLY4596	**a** *gin4-Δ9 cdc42*::*LEU2*::*GAL1p-CDC42*	This study
DLY4686	**a** *gin4-Δ9 cla4*::*TRP1*	This study
DLY4687	**a** *elm1*::*URA3*	This study
DLY4712	**a** *cla4*::*TRP1 elm1*::*URA*	This study
DLY4714	**a** *gin4-Δ9 elm1*::*URA3*	This study
DLY4719	α *gin4-Δ9 rga1*::*TRP1*	This study
DLY4720	α *gin4-Δ9 rga1*::*TRP1 rga2*::*URA3*	This study
DLY4727	**a** *gin4-Δ9 elm1*::*URA3 cla4*::*TRP1*	This study
DLY4753	**a** *gin4-Δ9 rga1*::*TRP1 bem3*::*LEU2 rga2*::*URA3*	This study
DLY4759	**a** *elm1*::*URA3 rga1*::*TRP1*	This study
DLY4790	**a** *bar1nap1*::Kan^r	This study
DLY4848	**a** *gin4-Δ9 nap1*::Kan^r	This study
DLY4854	α *nap1*::Kan^r *cla4*::*TRP1*	This study
DLY4855	α *nap1*::Kan^r *cla4*::*TRP1 elm1*::*URA3*	This study
DLY4856	α *nap1*::Kan^r *cla4*::*TRP1 gin4-Δ9*	This study
DLY4857	α *gin4-Δ9 nap1*::Kan^r *elm1*::*URA3*	This study
DLY4858	**a** *nap1*::Kan^r *elm1*::*URA3*	This study
DLY4859	**a** *nap1*::Kan^r *cla4*::*TRP1 gin4-Δ9 elm1*::*URA3*	This study
DLY4881	α *nap1*::Kan^r *rga1*::*TRP1*	This study
DLY4887	α *nap1*::Kan^r *rga1*::*TRP1 bem3*::*LEU2*	This study
DLY4891	**a** *nap1*::Kan^r *rga1*::*TRP1 rga2*::*URA3*	This study
DLY4893	α *nap1*::Kan^r *rga1*::*TRP1 bem3*::*LEU2 rga2*::*URA3*	This study
DLY4931	α *elm1*::*URA3 rga1*::*TRP1 rga2*::*URA3*	This study
DLY4932	**a** *elm1*::*URA3 rga2*::*URA3 bem3*::*LEU2*	This study
MOSY23	**a** *cla4*::*TRP1 cdc42*::*LEU2*::*GAL1p-CDC42*	26
MOSY148	**a** *bar1cla4*::*TRP1*	This study

[a] All strains are in the BF264-15Du (27) background (*ade1 his2 leu2-3,112 trp1-1 ura3Δns*).

catalytic activity (Bni5p, which is colocalized with septins, and Nap1p, which binds to Gin4p) also contribute to septin organization (19, 21, 25). When the genes encoding any of these proteins are deleted, septin localization and cell morphology are aberrant, although mutant cell populations exhibit heterogeneous septin localization defects and the specific roles of the proteins in promoting septin organization or septin dynamics remain undefined.

In *cdc42*^{Y32H}, *gin4*Δ, *cla4*Δ, and *nap1*Δ mutants, a septin cortex usually forms at the neck but appears aberrant (fainter, more irregular, "fuzzy," or organized into a set of separate parallel septin bars along the mother-bud axis) (13, 24, 25). In *cdc42*^{V36T,K94E}, *cdc42*^{V36G}, *rga1*Δ *rga2*Δ *bem3*Δ *ste20*Δ *cla4-as3*, and *elm1*Δ mutants, caps or ectopic rings of septin staining appear at the bud tip or within the bud, often in addition to fainter septin staining at the neck (8, 9, 13, 33, 37). It is unclear whether these different classes of phenotypes are intrinsic to the different mutants or are contributed by the different strain backgrounds in which they have been studied. In addition, most analyses have focused on septin phenotypes in budded cells, and it is not clear whether the initial septin ring formed in unbudded cells is normal or aberrant in the mutants. To circumvent these problems, we have directly compared septin localization in a panel of isogenic mutants with mutations in eight septin organization genes (*CDC42*, *RGA1*, *RGA2*, *BEM3*, *CLA4*, *GIN4*, *NAP1*, and *ELM1*), as well as isogenic strains containing combinations of mutations. The analysis supports a distinction between mutants that affect the initial septin ring and those that only affect the hourglass collar. We observed only a few instances of epistasis, with most mutants displaying additive or occasionally synergistic effects when they were combined. These findings suggest that most of the identified septin regulators act in parallel to promote either initial ring assembly or conversion of the septin ring to an hourglass.

MATERIALS AND METHODS

Yeast strains. The *S. cerevisiae* strains used for this study are listed in Table 1. All of the strains were in the BF264-15Du strain background (*ade1 his2 leu2-3,112 trp1-1 ura3Δns*), which has frequently been used for cell cycle studies and morphogenesis studies by the Reed, Cross, Wittenberg, Lew, and other laboratories (27). This strain grows robustly at temperatures up to 39°C. The generation of *cdc42*::*GAL1p-CDC42*::*LEU2* (14), *cdc42*^{V36T,K94E}, *cdc42*^{Y32H}, and *rga2*::*URA3* (13), *rga1*::*TRP1* and *bem3*::*LEU2* (4), *cla4*::*TRP1* (3), *gin4-Δ9* (24), *nap1*::Kan^r (25), and *elm1*::*URA3* (6) alleles was described previously. Standard media and methods were used for yeast manipulations (16), and all multiple

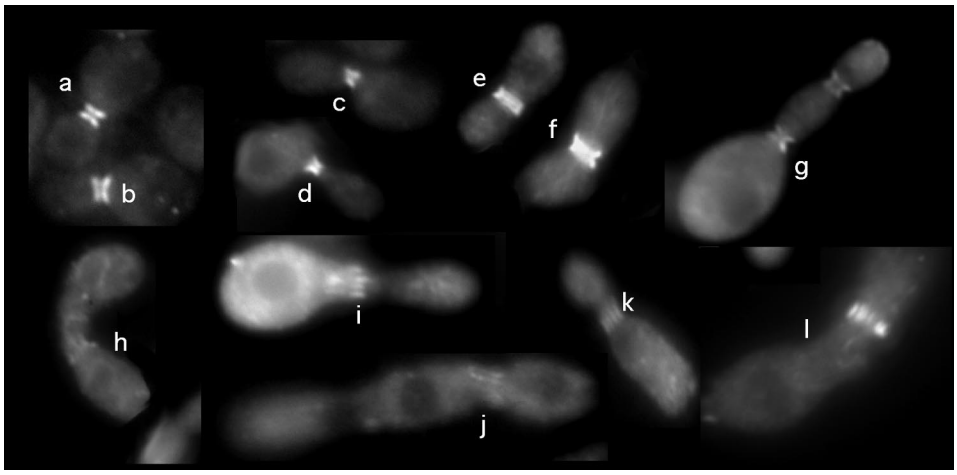

FIG. 1. Septin phenotypes in budded cells. Cells from the strains indicated below were grown to exponential phase in yeast extract-peptone-dextrose (YEPD) at 30°C, fixed, and processed for the visualization of septin localization by use of an anti-Cdc11p antibody. The illustrative cells in the figure display a normal septin hourglass (a and b; strain DLY1), irregular neck staining (c and d; strain DLY4790), wide necks (e and f; strain MOSY148), an ectopic septin hourglass in the elongated bud in addition to a faint neck hourglass (g; strain DLY4223), faint or absent septin staining and a bent or broad neck (h and j; strain DLY4859), and septin bars at aberrantly shaped necks (i and k; strain DLY4224) or displaced into the bud (l; strain DLY4224).

mutant combinations were generated by crosses between mutants in the same strain background. Plasmids pMOSB55, -56, and -57 are CEN URA3 vectors containing *CDC42*, *cdc42*^{Y32H}, and *cdc42*^{V36T,K94E} mutants, respectively. Expression was driven from the *CDC42* promoter (26).

Microscopy. Fixation, septin staining by standard indirect immunofluorescence with commercially available anti-Cdc11p antibodies, and microscopic analysis were performed as previously described (14).

RESULTS

Septin phenotypes in single-mutant strains. The phenotypes of septin organization mutants in the strain background used are illustrated in Fig. 1 and quantitated in Table 2. Septin staining using anti-Cdc11p antibodies revealed a variety of defects, which were divided into four categories for the purposes of quantitation (Table 2). Cells displaying a wild-type septin hourglass at the neck were scored as "neck-normal" (Fig. 1, cells a and b), and cells that lacked all septin staining were scored as "absent" (Fig. 1, cells h and j). Cells were scored as "neck-abnormal" when septin staining was prominent at the neck but was either fainter than that of the wild type (Fig. 1, cells c, i, and k), lacked crisp boundaries (Fig. 1, cells c, d, and i), exhibited a set of parallel bars along the mother-bud axis (Fig. 1, cells i, k, and l), or displayed some combination of these features. Cells were scored as mislocalized when septin staining displayed a cap or ring (Fig. 1, cells g and l) of septin staining away from the neck (usually at or near the bud tip), even if the same cell also displayed septin staining at the neck. In addition to these variations in septin localization, cells often displayed cell morphology defects, including wide necks (Fig. 1, cells e, f, and j), elongated buds (Fig. 1, cells g, i, j, k, and l), narrow or kinked necks (Fig. 1, cells c, d, h, and k), or some combination of these features.

Some general features emerged from the analysis of these isogenic strains. Firstly, the phenotypes were heterogeneous in that the different cells of a single mutant strain could display different categories of defect. Secondly, different mutants displayed characteristic propensities to display a particular defect (e.g., *gin4* mutants exhibited septin bars at a higher frequency than other mutants, whereas *elm1* and *cla4* mutants exhibited mislocalized septins at higher frequencies than other mutants) (Table 2). Thirdly, there was a correlation such that cells displaying mislocalized septins frequently displayed elongated buds. In addition, cells displaying aberrant neck septin phenotypes often had misshapen (aberrantly wide, narrow, and/or kinked) necks.

Initial assembly of septin ring. We also examined the morphology of the septin rings formed prior to bud emergence in a variety of mutant strains. For this analysis, cells from mostly unbudded stationary-phase cultures were inoculated into fresh medium and fixed at 4 h to enrich for cells that were about to bud (in some cases, these observations were confirmed by using synchronized cells isolated by centrifugal elutriation from growing populations). As previously reported (13), different *cdc42* alleles produced either normal-looking (*cdc42*^{V36T,K94E}) or large-diameter (*cdc42*^{Y32H}) initial septin rings. Whereas single mutants lacking individual Cdc42p-directed GAPs displayed normal rings, triple-mutant *rga1*Δ *rga2*Δ *bem3*Δ cells displayed a spectrum of phenotypes ranging from wild-type rings to very faint or broken-looking rings (Fig. 2). *gin4*Δ, *nap1*Δ, and *elm1*Δ mutants formed septin rings that appeared indistinguishable from the wild type, whereas *cla4*Δ mutants formed appreciably larger rings (Fig. 2). These phenotypes support the hypothesis that Cdc42p, the GAPs, and Cla4p are all important for the proper assembly of the initial ring.

Genetic interactions among septin organization mutants. Previous studies examined genetic interactions among mutations in genes encoding Cdc42p-directed GAPs (9, 13, 29) and among mutations in *GIN4*, *NAP1*, and *CLA4* genes (25, 34). In both cases, progressively more penetrant and severe phenotypes were observed in strains containing more mutations. We confirmed that *gin4*, *nap1*, and *cla4* mutations also caused progressively more penetrant phenotypes in our strain back-

TABLE 2. Frequencies of different septin localization defects in mutant strains[a]

Strain	Relevant genotype	% of cells with defect			
		Normal neck	Abnormal neck	Mislocalized	Absent
DLY4790	*nap1*	41	59	0	0
MOSY148	*cla4*	16	70	9	5
DLY4410	*gin4*	20	80	0	0
DLY4687	*elm1*	9	74	9	8
DLY3344	*rga1*	90	9	0	1
DLY3353	*rga2*	93	6	0	1
DLY3346	*bem3*	93	6	0	1
DLY4854	*nap1 cla4*	17	79	1	4
DLY4848	*nap1 gin4*	3	77	6	14
DLY4714	*gin4 elm1*	1	84	4	11
DLY4858	*nap1 elm1*	7	81	2	10
DLY4712	*cla4 elm1*	2	54	24	20
DLY4686	*cla4 gin4*	4	54	16	26
DLY4881	*nap1 rga1*	25	66	8	1
DLY4719	*gin4 rga1*	3	79	17	1
DLY4759	*elm1 rga1*	3	63	15	19
DLY3347	*rga1 rga2*	55	28	10	7
DLY3341	*rga1 bem3*	74	16	8	2
DLY3361	*rga2 bem3*	81	15	1	3
DLY4856	*nap1 cla4 gin4*	0	57	20	23
DLY4727	*cla4 gin4 elm1*	0	53	16	31
DLY4855	*cla4 elm1 nap1*	0	64	8	28
DLY4857	*nap1 gin4 elm1*	0	93	6	7
DLY4891	*nap1 rga1 rga2*	5	80	9	6
DLY4887	*nap1 rga1 bem3*	5	68	13	14
DLY4720	*gin4 rga1 rga2*	4	70	14	12
DLY4931	*elm1 rga1 rga2*	6	54	13	27
DLY4932	*elm1 rga2 bem3*	3	45	38	14
DLY2723	*rga1 rga2 bem3*	27	29	32	11
DLY4859	*nap1 gin4 elm1 cla4*	0	43	22	35
DLY4893	*nap1 rga1 rga2 bem3*	1	49	15	35
DLY4753	*gin4 rga1 rga2 bem3*	1	24	17	58
*[b]	*elm1 rga1 rga2 bem3*				

[a] At least 200 cells were scored for each strain.
[b] Segregants of this genotype were identified by accounting for marker segregation in multiple tetrads. Spores germinated and produced a few very large elongated cells but did not form colonies. This genotype was synthetically lethal.

ground (Table 2). Moreover, we found that combining mutations in any of these genes with mutations in the GAP-encoding *RGA1*, *RGA2*, and *BEM3* genes also yielded progressively more penetrant phenotypes (Table 2). For simplicity, we will refer to these effects as additive, although the fact that different cells in an individual strain displayed heterogeneous categories and severities of defect makes it impossible to reduce these phenotypes to a quantifiable septin defect parameter. Nevertheless, these additive genetic interactions do rule out the model that any set of these septin regulators acts in a simple linear regulatory pathway.

Genetic interactions between *elm1*Δ and other septin organization mutants have not been reported. We found that *elm1*Δ *cla4*Δ and *elm1*Δ *rga1*Δ strains displayed additive phenotypes (see above), growing progressively worse as *rga2* and *bem3* were also deleted (Table 2). In contrast, *elm1*Δ *gin4*Δ and *elm1*Δ *nap1*Δ strains appeared very similar to the *elm1*Δ single mutants (Fig. 3 and Table 2). Even the triple *elm1*Δ *gin4*Δ *nap1*Δ mutant was only marginally more severe than the *elm1*Δ single mutant (Fig. 3). These epistatic interactions are consistent with the view that Elm1p acts downstream of parallel pathways from Gin4p and Nap1p or that Elm1p is a key upstream activator of Gin4p and Nap1p (see Discussion).

The two *cdc42* alleles that we examined were part of a collection of point mutants that had altered residues in the Cdc42p effector loop (14). From that collection, these two were unique in showing defects in septin organization but not in actin organization (13, 14). Suppression analysis showed that the septin defects could be suppressed by elevated expression of the GAP Rga1p, and biochemical analysis indicated that both mutants were mildly defective in GTP hydrolysis, suggesting that their GTP hydrolysis defect might contribute to the septin defect (13). However, the detailed biochemical defects were distinct (see Discussion), as were the septin defects (*cdc42*^{V36T,K94E} mutants had mislocalized septins, whereas *cdc42*^{Y32H} mutants had large initial septin rings and neck-abnormal septins). These mutants display dosage-sensitive phenotypes (13), and when they are expressed from CEN ARS plasmids they display only mild (*cdc42*^{V36T,K94E}) or even undetectable (*cdc42*^{Y32H}) septin defects. In assessing the effect of combining these with other mutations in septin organization genes, we found that the two *cdc42* alleles displayed dramatic and allele-specific synergistic phenotypes when combined with *cla4*Δ or *gin4*Δ mutations (Fig. 4). In particular, *cdc42*^{V36T,K94E} *cla4*Δ mutants were synthetically lethal, whereas *cdc42*^{V36T,K94E} *gin4*Δ mutants were very similar to *gin4*Δ single mutants. In contrast, *cdc42*^{Y32H} *cla4*Δ mutants were very similar to *cla4*Δ single mutants, whereas *cdc42*^{Y32H} *gin4*Δ mutants exhibited a synergistic defect. The severely affected *cdc42*^{V36T,K94E} *cla4*Δ and *cdc42*^{Y32H}

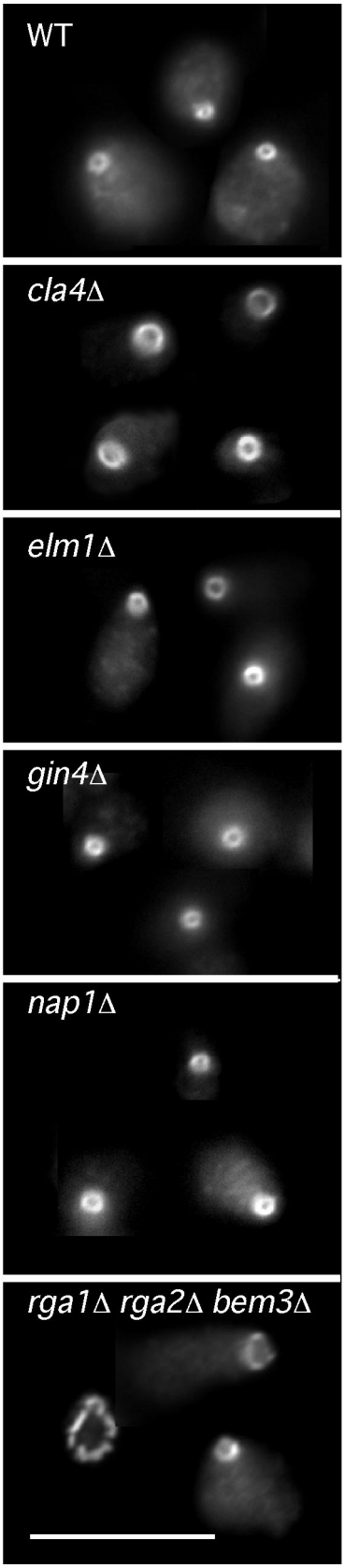

FIG. 2. Septin rings in unbudded cells. Cells from the strains indicated below were grown to stationary phase in YEPD at 30°C and then inoculated into fresh medium, grown for 4 h (at which point cells were entering the cell cycle), fixed, and processed for the visualization of septin localization by use of an anti-Cdc11p antibody. WT, strain DLY1; *cla4*Δ, strain MOSY148; *elm1*Δ, strain DLY4687; *gin4*Δ, strain DLY4410; *nap1*Δ, strain DLY4790; *rga1*Δ *rga2*Δ *bem3*Δ, strain DLY2723. Bar, 10 μm.

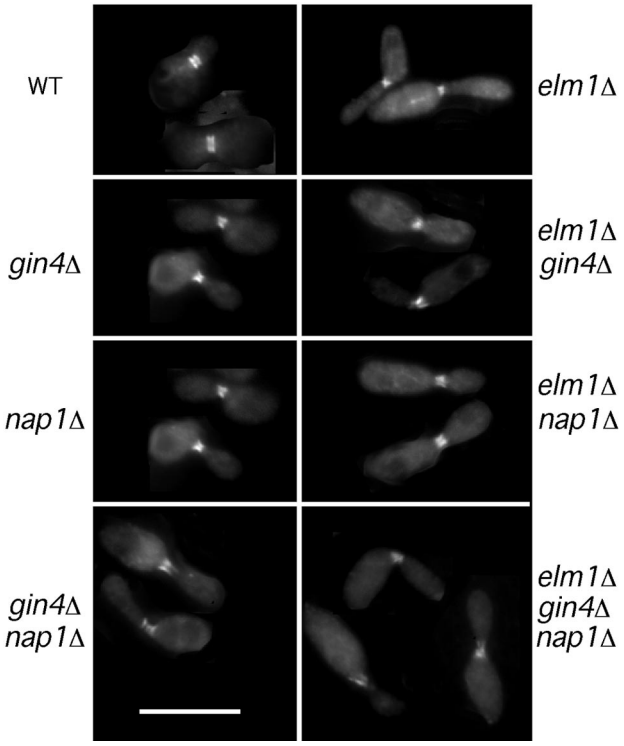

FIG. 3. Epistatic interactions among *elm1*, *gin4*, and *nap1* mutants. Cells from the strains indicated below were grown to exponential phase in YEPD at 30°C, fixed, and processed for the visualization of septin localization by use of an anti-Cdc11p antibody. WT, strain DLY1; *elm1*Δ, strain DLY4687; *gin4*Δ, strain DLY4410; *nap1*Δ, strain DLY4790; *elm1*Δ *gin4*Δ, strain DLY4714; *elm1*Δ *nap1*Δ, strain DLY4858; *gin4*Δ *nap1*Δ, strain DLY4848; *elm1*Δ *gin4*Δ *nap1*Δ, strain DLY4857. Bar, 10 μm.

*gin4*Δ mutants displayed large tubular multibranched cells (Fig. 4), with only patchy septin staining detectable (data not shown). The dramatic and distinct nature of the genetic interactions exhibited by the *cdc42* alleles strongly suggests that these mutants engender distinct sorts of defects in septin organization.

DISCUSSION

Most previous studies on septin organization have focused on individual regulators. The comparative analysis of a large panel of isogenic single- and multiple-mutant strains presented here highlights several heretofore underappreciated features of septin organization. The implications of these results are discussed below.

Pathways for septin organization? Our motivation for examining the effects of combining mutations in septin organization genes included the hope that regulatory pathways for

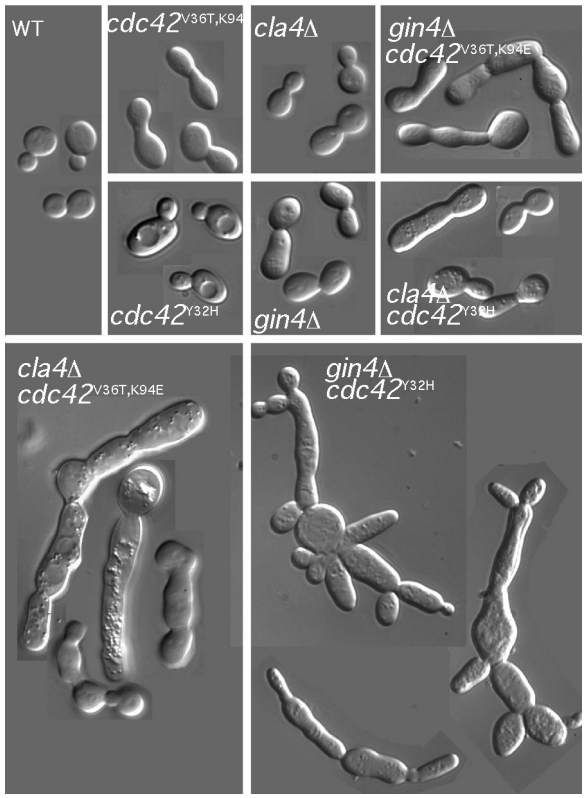

FIG. 4. Synthetic interactions among *cdc42*, *cla4*, and *gin4* mutants. Cells from the strains indicated below were grown at 30°C to exponential phase in minimal medium lacking uracil (to select for plasmid retention), fixed, and visualized by differential interference contrast microscopy. WT, strain DLY3067 carrying plasmid pMOSB55; *cla4*Δ, strain MOSY23 carrying plasmid pMOSB55; *gin4*Δ, strain DLY4596 carrying plasmid pMOSB55; *cdc42*^{V36T,K94E}, strain DLY3067 carrying plasmid pMOSB57; *cdc42*^{Y32H}, strain DLY3067 carrying plasmid pMOSB56; *cla4*Δ cdc42^{V36T,K94E}, strain MOSY23 carrying plasmid pMOSB57; *cla4*Δ cdc42^{Y32H}, strain MOSY23 carrying plasmid pMOSB56; *gin4*Δ cdc42^{V36T,K94E}, strain DLY4596 carrying plasmid pMOSB57; *gin4*Δ cdc42^{Y32H}, strain DLY4596 carrying plasmid pMOSB56.

septin organization would be revealed through epistatic interactions between mutants. However, in the large majority of cases, we observed additive effects of mutant combinations on septin ring organization, suggesting that most of the regulators act in parallel to promote septin ring integrity. The only exceptions were *elm1 gin4* and *elm1 nap1* mutants, which displayed phenotypes similar to that of the *elm1* single mutant. It is possible that these epistatic interactions reflect a regulatory pathway in which Gin4p and Nap1p act in parallel to activate Elm1p. Alternatively, Elm1p may activate both Gin4p and Nap1p. However, Gin4p and Elm1p are recruited to the septin cortex in a manner that is sensitive to defects in septin organization (25, 33), so it may be that the perturbation of septin organization in *elm1* mutants leads indirectly to a failure to recruit Gin4p to the septin cortex (or vice versa) and that Gin4p and Elm1p are thereby rendered less active. Thus, it remains possible that all of these factors act in parallel to promote septin organization and that the epistatic interactions reflect indirect effects rather than regulatory pathways linking these proteins.

We also observed two very severe (synthetic) genetic interactions between different alleles of *CDC42* and *gin4* or *cla4* mutants. The remarkable allelic specificity of these interactions strongly suggests that the *cdc42* mutants engender distinct sorts of defects in septin organization. We have reported that both alleles encode proteins with biochemical defects in GTP hydrolysis (13). However, Cdc42p^{V36T,K94E} displayed a defect in the intrinsic GTP hydrolysis rate which could still be stimulated by a recombinant Rga1p GAP domain, whereas Cdc42p^{Y32H} displayed a normal GTP hydrolysis rate which could no longer be stimulated by the GAP. It seems plausible that these biochemical differences underlie the phenotypic differences reported here. Alternatively, the alleles may impair distinct effector interactions in addition to their effect on GTP hydrolysis.

Assembly of the septin ring and septin hourglass. We examined the initial septin structures formed by unbudded cells emerging from stationary phase in a variety of mutant strains and found that *gin4*, *nap1*, and *elm1* mutants all made normal-looking initial rings, whereas *cla4* mutants made larger-diameter rings. In the *rga1 rga2 bem3* triple mutant, we observed a heterogeneous group of structures, including large rings and broken rings in which several separate strands of septins were arranged in a roughly circular array. The simplest interpretation of these findings is that Cla4p and the Cdc42p-directed GAPs contribute to the initial assembly of the septin ring in unbudded cells, whereas Gin4p, Nap1p, and Elm1p are not required for this step. (Recent studies concluded that Elm1p also plays roles in cell cycle commitment [30] and SNF1 regulation [31], but it is not clear whether or how those roles relate to Elm1p function in septin organization.) Coincident with (or shortly after) bud emergence, the septin ring spreads to form a collar, and it was in the budded collar that *gin4*, *nap1*, and *elm1* mutants displayed obvious defects. We suggest that Elm1p, Gin4p, and Nap1p act during or after bud emergence to promote the proper assembly and/or maintenance of the septin hourglass. Consistent with this view, Elm1p is only localized to the septin cortex after bud emergence (8), and even though Gin4p is recruited to the septins before bud emergence, it does not become fully active until later in the cell cycle (1).

Recent studies proposed two rather different models for the initial assembly of the septin ring. In one model, the septins are recruited directly into a ring structure (13). In the other model, septins are first recruited to a precursor cap structure which then converts to a ring (9). The latter model interprets the septin caps observed in mutant strains as a normally short-lived assembly intermediate whose conversion into a septin ring has been delayed. Our findings on *elm1* mutants, which often display caps in budded cells, are particularly interesting in this context. If the caps are early assembly intermediates, we would have expected to see caps in the unbudded *elm1* cells, which would eventually convert to rings after bud emergence. However, we actually observed normal septin rings in unbudded *elm1* cells, and caps developed only later, generally at the bud tip in cells with elongated buds. Many cells with such caps also had septin hourglass structures (albeit often fainter than wild-type structures) at the neck. These findings support the view

that caps arise as a secondary consequence of defects in the septin hourglass of budded cells and that they represent an abnormal septin structure rather than a "trapped" assembly intermediate (at least in *elm1* mutants).

Relationship between septin organization and cell shape. Within the panel of strains examined for this report, there was a general and striking correlation between defects in septin organization and alterations in cell shape. Previous studies documented the elongated bud phenotype in septin mutant strains and demonstrated that this phenotype involves deregulation of the cell cycle inhibitory kinase Swe1p (2, 17, 25). However, the range of morphological aberrations observed in the present study includes wide, narrow, kinked, and stretched-looking necks as well as unusually wide, narrow, and sometimes bent buds. These phenotypes go well beyond the simple elongated bud (with relatively normal neck) phenotype of cells lacking all organized septins, raising the question of whether there is a more complex link between septins and cell morphology. Because many of the mutations we examined are known to be pleiotropic, the possibility remains that the septin and cell shape abnormalities are separate and independent products of the mutations. However, some categories of septin defect were commonly associated with specific alterations in cell shape (septin bars with stretched necks or ectopic septin rings with elongated buds), suggesting that either misorganized septins can influence cell shape (beyond the bud-elongating effects of Swe1p) or cellular geometry can influence septin organization, as previously suggested (15, 32). In work to be reported elsewhere, we present evidence suggesting that both of these are true and that the interplay between cell shape and septin organization underlies the genesis of some of the septin phenotypes.

Interpretation of septin organization phenotypes. Genetic approaches to understanding septin organization seek to identify septin regulatory genes and, through analysis of their mutant phenotypes, to elucidate how those genes impact septin organization. Several apparently distinct categories of septin defect (e.g., parallel septin bars at the neck or ectopic septin caps at the bud tip) are encountered in the mutant strains, and these phenotypes have been instrumental in generating attractive hypotheses about the roles of some regulators in septin organization. The first study to describe the septin bar phenotype focused on the *gin4* strain, and Longtine et al. (24) suggested that Gin4p was important for cross-linking parallel septin filaments into a seamless collar at the neck. The localization of Gin4p to the septin collar throughout the budded phase of the cell cycle was certainly consistent with this inference (24). Support for the view that Gin4p acts as a septin cross-linker was provided by a recent study describing an increase in septin dynamics in both *gin4* and *cla4* mutant cells, suggesting that the role of Gin4p and Cla4p is to freeze the septins in place within the hourglass collar (11). Another recent study focused on the septin caps frequently observed in *cdc42*^{V36G} and *rga1 rga2 bem3* mutants, and Caviston et al. (9) suggested that the cap was a precursor step in assembling a septin ring (described above).

The hypotheses described above sought to provide a chain of causality in which the molecular defect stemming from a genetic lesion explained the details of a particular septin phenotype: the failure to cross-link parallel septin filaments at the necks of cells lacking Gin4p caused the filaments to form bundles which are seen as bars, whereas the failure to release septins from the cap in cells with delayed GTP hydrolysis by Cdc42p allowed the persistence of what would normally be a transient assembly intermediate. Surprisingly, however, we found that there was no clear-cut correspondence between a particular genetic lesion and a specific category of septin defect: different cells of a single mutant population often displayed different categories of septin defect, and many different mutant strains contained cells displaying any specified septin defect. Although biases could be identified such that some mutants (*gin4* and *cdc42*^{Y32H}) showed a higher propensity to develop septin bars while others (*elm1*, *cla4*, and *cdc42*^{V36T,K94E}) showed a higher propensity to develop septin caps, these biases were far from absolute. Moreover, a combination of two mutations that displayed only aberrant neck phenotypes, including septin bars, could yield a strain (e.g., *gin4 nap1*) that showed a significant frequency of mislocalized septin caps.

How can we account for the unexpectedly complex relationship between genotype and phenotype with regard to septin organization? We suggest that the phenotypes scored as different categories of septin defect reflect an intrinsic repertoire of alternate (aberrant) states of septin organization. By analogy to the alternative conformational states of individual proteins, these states might be viewed as local minima in the potential energy landscape for assemblies of septins and their partners. We suggest that specific mutations influence the probabilities that the wild-type septin hourglass in an individual cell will collapse to each of the several alternate states. Thus, a particular mutant population would display a characteristic distribution of cells exhibiting different septin states, and different mutant populations could contain cells exhibiting a specific septin state, as we observed. This state of affairs does not easily lend itself to a straightforward interpretation of the link between a mutant lesion and a specific aspect of septin organization, because the mutations influence the likelihood that septins will assume particular alternate states rather than dictating a unique outcome. Moreover, the influence of a specific mutation may include indirect effects of the mutation (e.g., through effects on cell shape or cell cycle) as well as any direct effects on septin organization. These considerations highlight the need to understand in much greater depth the genesis of particular septin phenotypes in mutant strains.

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