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Septins Have a Dual Role in Controlling Mitotic Exit in Budding Yeast

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Summary

In *Saccharomyces cerevisiae*, the spindle position checkpoint ensures that cells do not exit mitosis until the mitotic spindle moves into the mother/bud neck and thus guarantees that each cell receives one nucleus [1–6]. Mitotic exit is controlled by the small G protein Tem1p. Tem1p and its GTPase activating protein (GAP) Bub2p/Bfa1p are located on the daughter-bound spindle pole body. The GEF Lte1p is located in the bud. This segregation helps keep Tem1p in its inactive GDP state until the spindle enters the neck. However, the checkpoint functions without Lte1p and apparently senses cytoplasmic microtubules in the mother/bud neck [7–9]. To investigate this mechanism, we examined mutants defective for septins, which compose a ring at the neck [10]. We found that the septin mutants *sep7*Δ and *cdc10*Δ are defective in the checkpoint. When movement of the spindle into the neck was delayed, mitotic exit occurred, inappropriately leaving both nuclei in the mother. In *sep7*Δ and *cdc10*Δ mutants, Lte1p is mislocalized to the mother. In *sep7*Δ, but not *cdc10*Δ, mutants, inappropriate mitotic exit depends on Lte1p. These results suggest that septins serve as a diffusion barrier for Lte1p, and that Cdc10p is needed for the septin ring to serve as a scaffold for a putative microtubule sensor.

Results and Discussion

Septin Mutants with Inappropriate Mitotic Exit

To identify proteins necessary to delay mitotic exit when the spindle fails to enter the mother/bud neck, we screened the Research Genetics collection of viable haploid deletion mutants for benomyl-sensitive growth; this search previously uncovered *bub2* [11]. Two septin mutants, *sep7*Δ and *cdc10*Δ, were identified. The three other vegetative septins in *Saccharomyces cerevisiae* are essential in this background and are therefore not in the collection.

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We assayed the spindle position checkpoint in these mutants with movies of living cells progressing through mitosis. The cells expressed GFP-Tub1p, allowing us to observe spindle position and spindle breakdown, which indicates mitotic exit. The strains carried a dynactin *arp1*Δ mutation to increase the frequency at which spindles delay movement into the neck. Under the microscope, we identified cells from an asynchronous log-phase culture that had elongated spindles in the mother. In an otherwise wild-type cell, mitotic exit is delayed until the spindle moves into the neck. When the checkpoint is defective, mitotic exit occurs before the spindle enters the neck. We define this as inappropriate mitotic exit.

As a negative control, 9% (6/67) of *arp1*Δ cells showed inappropriate mitotic exit (Figure 1A). In the remaining 91% (61/67) of cells, mitotic exit did not occur, and the spindle remained in the mother. Cells were only included in the latter category if the time-lapse record included a length of time sufficient for appropriate mitotic exit; this length of time is defined as the mean plus two standard deviations of the time from spindle elongation to spindle breakdown in cells undergoing appropriate mitotic exit. In many cells, the spindle entered the neck, and mitotic exit ensued. These cells were not counted and were not included in our analysis. Henceforth, all strains carry *arp1*Δ unless stated otherwise. As a positive control, 96% (22/23) of *bub2*Δ cells showed inappropriate mitotic exit (Figure 1A).

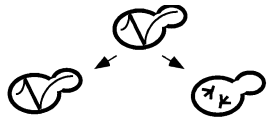
In a *cdc10*Δ strain, 97% (39/40) of cells showed inappropriate mitotic exit (Figure 1A), an essentially complete defect in the checkpoint, similar to that seen with loss of Bub2p. Among *sep7*Δ cells, 51% (42/83) showed inappropriate mitotic exit. Sep7p associates with Gin4p, a Nim1-related serine/threonine kinase [12–15]. Therefore, we examined *gin4*Δ and *sep7*Δ *gin4*Δ cells. A total of 18% (3/17) of *gin4* cells showed inappropriate mitotic exit, and this value was not significantly different from the one for wild-type control cells. However, 96% (23/24) of *sep7*Δ *gin4*Δ cells exhibited inappropriate mitotic exit (Figure 1A). Therefore, the spindle position checkpoint is more defective in the *sep7*Δ *gin4*Δ double mutant than in either single mutant; this finding suggests that both proteins prevent inappropriate mitotic exit, either by independent mechanisms or perhaps as two components of a large multiprotein complex.

Localization of Lte1-3GFP in Septin Mutants

The septin ring functions as a diffusion barrier between the mother and bud [16, 17]. Lte1p is mislocalized in the septin mutant *cdc12-6* [18–20]. We hypothesized that *sep7*Δ or *cdc10*Δ mutations might allow Lte1p into the mother and may thus activate Tem1p prematurely. In addition, the septin ring functions as a scaffold for proteins at the neck [10], and our previous studies suggested the existence of a microtubule sensor at the neck. We hypothesized further that loss of Sep7p or Cdc10p might disrupt the scaffold for such a sensor.

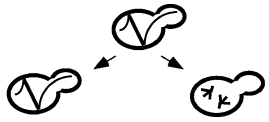
The diffusion model predicts that Lte1p should mislo-

A



Strain	No Mitotic Exit %(n)	Inappropriate Mitotic Exit %(n)
arp1Δ	91% (61)	9% (6)
arp1Δ bub2Δ	4% (1)	96% (22)
arp1Δ sep7 Δ	49% (41)	51% (42)
arp1Δ gin4Δ	82% (14)	18% (3)
arp1Δ sep7 Δ gin4Δ	4% (1)	96% (23)
arp1Δ cdc10Δ	3% (1)	97% (39)

B



Strain	No Mitotic Exit %(n)	w/LTE1	Inappropriate Mitotic Exit %(n)	w/LTE1
arp1Δ lte1Δ	82% (42)	91%	18% (9)	9%
arp1Δ sep7Δ lte1Δ	91% (29)	49%	9% (3)	51%
arp1Δ sep7Δ gin4Δ lte1Δ	77% (23)	4%	23% (7)	96%
arp1Δ cdc10Δ lte1Δ	25% (12)	3%	75% (36)	97%

Figure 1. Inappropriate Mitotic Exit in Septin Mutants

(A) The spindle position checkpoint was completely lost in *bub2*Δ, *sep7*Δ *gin4*Δ, and *cdc10*Δ cells, was partly defective in *sep7*Δ cells, and was unaffected in *gin4*Δ cells. All strains carried *arp1*Δ to delay spindle movement into the neck. The percentage of cells with misaligned spindles that exited mitosis without moving the spindle into the neck (Inappropriate Mitotic Exit) and the percentage cells with misaligned anaphase spindles that remained in mitosis (No Mitotic Exit) are shown. Cells were categorized as "No Mitotic Exit" if they remained in mitosis for greater than the mean plus two standard deviations of the time from the start of spindle elongation to spindle breakdown in cells with spindles that did enter the neck. Those times were as follows (mean ± SD): control, 24 ± 5 min (n = 39); *bub2*Δ, 17 ± 2 min (n = 10); *sep7*Δ, 22 ± 4 min (n = 17); *gin4*Δ, 27 ± 6 min (n = 21); *sep7*Δ *gin4*Δ, 24 ± 4 min (n = 37); *cdc10*Δ, 22 ± 5 min (n = 48).

(B) Dependence of inappropriate mitotic exit on Lte1p. Cells were assayed as in (A). The times for appropriately positioned spindles to progress through mitosis (mean ± SD) were: *lte1*Δ, 22 ± 4 min (n = 17); *sep7*Δ *lte1*Δ, 30 ± 6 min (n = 4); *sep7*Δ *gin4*Δ *lte1*Δ, 32 ± 10 min (n = 36); *cdc10*Δ *lte1*Δ, 22 ± 4 min (n = 70). Strains shown in this figure are *arp1*Δ (YJC2409), *arp1*Δ *bub2*Δ (YJC2667), *arp1*Δ *sep7*Δ (YJC2414), *arp1*Δ *gin4*Δ (YJC2411), *arp1*Δ *sep7*Δ *gin4*Δ (YJC2721), *arp1*Δ *cdc10*Δ (YJC2488), *arp1*Δ *lte1*Δ (YJC2714), *arp1*Δ *sep7*Δ *lte1*Δ (YJC2767), *arp1*Δ *sep7*Δ *gin4*Δ *lte1*Δ (YJC2759), and *arp1*Δ *cdc10*Δ *lte1*Δ (YJC2785).

calize to the mother in the mutants. To test this prediction, we localized Lte1p by tagging the endogenously expressed copy of Lte1p at its C terminus with three tandem copies of GFP. Lte1-3GFP functioned normally (see the Experimental Procedures). Lte1-3GFP localized as punctae at the cortex of the bud in control cells, as expected [1, 2] (Figure 2A). These punctae were mobile, moving at ~1 μm/s (see Movie 1 in the Supplemental Data available with this article online). The mother did not contain any punctae, and the level of diffuse cytoplasmic staining in the mother was similar to the level of autofluorescence (Figure 2A).

In *sep7*Δ cells, Lte1-3GFP also localized predominantly as cortical punctae in the bud. A few cortical punctae were present in the mother, and the level of fluorescence in the cytoplasm of the mother appeared elevated (Figure 2A). Lte1-3GFP localized similarly in *sep7*Δ *gin4*Δ and *cdc10*Δ cells. To quantitate the level of Lte1-3GFP in the mother, we determined the fluorescence per pixel of the mother from digital images of cells with small to medium buds. The level of Lte1-3GFP was significantly higher in *sep7*Δ and *cdc10*Δ mothers than in wild-type or *arp1*Δ mothers (Figure 2B). *sep7*Δ *gin4*Δ mothers had even higher levels of Lte1-3GFP that were significantly greater than the levels in *sep7*Δ and *cdc10*Δ mothers.

Our assay for inappropriate mitotic exit provides results in the form of percentages of cells, so we analyzed the Lte1p-3GFP level of the mother in a similar manner. We determined what percentage of mother cells had fluorescence values greater than those for control cells without Lte1p-3GFP (Figure 2C). *sep7*Δ, *sep7*Δ *gin4*Δ, and *cdc10*Δ strains all had greater numbers of cells with significantly high levels of Lte1-3GFP in the mother compared to wild-type and *arp1*Δ strains. Therefore, these results are consistent with the Lte1p diffusion model. Because Lte1-3GFP punctae are mobile, they may diffuse along the cortex from the bud into the mother and then disassemble, as has been reported for actin patches [16, 17].

Dependence of Inappropriate Mitotic Exit on Lte1p

Another prediction of the diffusion model is that removing Lte1p from the cell should suppress inappropriate mitotic exit in the septin mutants. Conversely, if the septin's function is that of a scaffold for a microtubule sensor at the neck, then loss of Lte1p should not suppress inappropriate mitotic exit. These alternatives are not mutually exclusive. If a septin has both functions, then loss of Lte1p may lead to partial suppression.

To test these possibilities, we deleted *LTE1* in *sep7*Δ, *sep7*Δ *gin4*Δ, and *cdc10*Δ strains. We performed the same assay for inappropriate mitotic exit described above. The assays were done at 30°C because deleting *LTE1* did not affect the timing of mitosis or mitotic exit at this temperature (see the Experimental Procedures).

In a *sep7*Δ *lte1*Δ strain, 9% (3/32) of cells with delayed spindle movement into the neck exhibited inappropriate mitotic exit, and this value was less than the value of 51% (42/83) in *sep7*Δ *LTE1* cells (Figures 1A and 1B). Furthermore, 23% (7/30) of *sep7*Δ *gin4*Δ *lte1*Δ cells

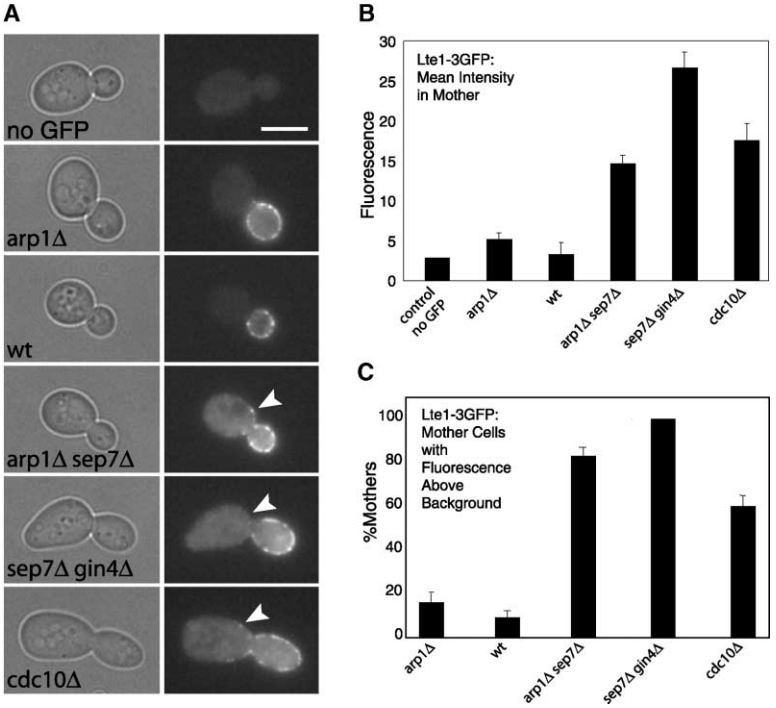


Figure 2. Lte1p Distribution in Septin Mutants

(A) Lte1p localization was examined in wild-type, *arp1*Δ, *arp1*Δ *sep7*Δ, *sep7*Δ *gin4*Δ, and *cdc10*Δ cells expressing endogenously tagged Lte1-3GFP by wide-field epifluorescence. Cells with no GFP were also assayed as a control. Arrowheads mark examples of punctae in the mother cells. The scale bar represents 5 μm.

(B) The intensity of Lte1-3GFP in mother cells of septin mutants was measured. Values are the mean fluorescence per pixel (arbitrary units) in the mother of small to medium budded cells. Error bars indicate SEM. A background value of 35 units was subtracted from all values. The number of cells measured is as follows: control, 20; *arp1*Δ, 20; wild-type, 77; *arp1*Δ *sep7*Δ, 25; *sep7*Δ *gin4*Δ, 43; and *cdc10*Δ, 54.

(C) Percentage of cells with mother fluorescence above background in septin mutants. The brightness of images, acquired as above, was adjusted until the level of autofluorescence was near zero in control cells. Mother cells visible after adjustment were scored as fluorescent mothers above background. Error bars indicate SEP. The number of cells scored is as follows: *arp1*Δ, 16; wild-type, 12; *arp1*Δ *sep7*Δ, 63; *sep7*Δ *gin4*Δ, 51; and *cdc10*Δ, 48. Strains shown in this figure are no GFP (*arp1*Δ) (YJC2409), wild-type *LTE1-3GFP* (YJC3108), *arp1*Δ *LTE1-3GFP* (YJC3042), *arp1*Δ *sep7*Δ *LTE1-3GFP* (YJC3041), *sep7*Δ *gin4*Δ *LTE1-3GFP* (YJC3109), and *cdc10*Δ *LTE1-3GFP* (YJC3107).

exited mitosis inappropriately, and this value was less than 91% in *sep7*Δ *gin4*Δ *LTE1* cells (Figures 1A and 1B). Therefore, loss of Lte1p largely suppressed the defect of *sep7*Δ and *sep7*Δ *gin4*Δ mutants in the spindle position checkpoint, and this finding is consistent with the Lte1p diffusion model.

In a *cdc10*Δ *lte1*Δ strain, 75% (36/48) of cells displayed inappropriate mitotic exit (Figure 1C) compared to 97% (39/40) in a *cdc10*Δ *LTE1* strain. Although deletion of *LTE1* had a small but statistically significant effect ($p < 0.05$) on the ability of *cdc10*Δ mutants to delay mitotic exit, most of the cells exited mitosis inappropriately. Thus, Cdc10p has an Lte1-independent role in the checkpoint, suggesting that Cdc10p may be necessary for sensing microtubules in the neck.

We hypothesized that Cdc10p might facilitate microtubule capture at the neck, because the *cdc12-6* mutant shows poor capture [21]. This hypothesis predicts that cytoplasmic microtubules extending from short spindles in *cdc10*Δ *lte1*Δ cells should be oriented toward the neck less often than in control cells. We found that the percentage of *cdc10*Δ cells with short spindles with cytoplasmic microtubules oriented toward the neck was not significantly different from that of control cells: 31% ± 4% SEP, $n = 122$, versus 36% ± 6% SEP, $n = 55$, respectively (SEP is standard error of proportion; $0.1 < p < 0.2$). Therefore, inappropriate mitotic exit in *cdc10*Δ *lte1*Δ cells appears not to be due to poor microtubule capture at the neck; this finding suggests that there may be loss of a microtubule sensor at the neck instead.

Two phenomena found to precede inappropriate mitotic exit in our previous studies, and in the few *lte1*Δ cells that did inappropriate mitotic exit in this study (Figure 1B), were loss of all cytoplasmic microtubules from the neck and abortive transient movements of the spindle into the neck [7]. Inappropriate mitotic exit occurred ~15 min after these phenomena. We reasoned that a wide neck, caused by a defective septin ring, might increase the probability of these phenomena and account for inappropriate mitotic exit in *cdc10*Δ *lte1*Δ cells. Among 36 movies of *cdc10*Δ *lte1*Δ cells with inappropriate mitotic exit, 22 provided clear views of individual cytoplasmic microtubules. In 32% (7/22), a microtubule was clearly present in the neck at all times, and the spindle never moved to within 0.5 μm of the neck. Therefore, inappropriate mitotic exit was not caused by movement of the spindle to the neck or loss of cytoplasmic microtubules from the neck. This further supports a role for Cdc10p in sensing microtubules in the neck.

Septin Ring Morphology

The septins Cdc3p, Cdc10p, Cdc11p, Cdc12p, and Sep7p form a ring at the mother/bud neck [12, 15], which prevents diffusion of some bud-polarized proteins into the mother [16, 17]. The septins also act as a scaffold for many other neck-residing proteins, many of which are distributed asymmetrically within the septin ring [10]. *sep7*Δ and *sep7*Δ *gin4*Δ mutants have a defective diffusion barrier for Lte1p and depend on Lte1p for their defect in preventing inappropriate mitotic exit. We reasoned that these mutants might have gross discontinuities in the septin ring. On the other hand, *cdc10*Δ mutants mislocalize Lte1p to a lesser extent than do *sep7*Δ or *sep7*Δ *gin4*Δmutants, and their checkpoint defect does not depend on Lte1p. We reasoned that *cdc10*Δ

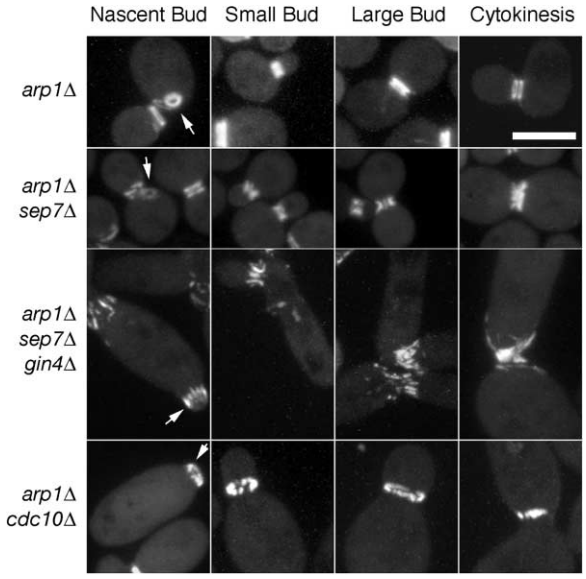


Figure 3. Septin Ring Morphology in Septin Mutants

Septin organization in wild-type, *sep7*Δ, *sep7*Δ *gin4*Δ, and *cdc10*Δ was examined by fluorescence microscopy of cells expressing Cdc3-GFP. Localization in nascent buds (arrows), small buds, medium buds, and at cytokinesis is shown. The scale bar represents 5 μm. Strains shown in this figure are *arp1*Δ "wild-type" (YJC2710), *arp1*Δ *sep7*Δ (YJC2711), *arp1*Δ *sep7*Δ *gin4*Δ (YJC2729), and *arp1*Δ *cdc10*Δ (YJC2835).

cells might not have gross defects in the septin ring but instead may lack a certain part of the septin ring, a part that serves as a scaffold for a sensor. To observe the organization of the septin ring, we expressed GFP-Cdc3 in each of the strains, which had no effect on cell morphology or timing of mitosis (data not shown).

Septins first appear as a single ring on the cell cortex at the site where the bud will emerge (Movies 2 and 3). As the bud grows, the ring widens along the mother/bud axis and assumes an hourglass shape [10] (Figure 3; Movies 2, 4, and 5). Upon cytokinesis, the hourglass separates into two rings, one going to the mother and one to the daughter (Figure 3; Movies 2 and 6). The mother and bud sides of the ring may be functionally distinct because some proteins localize to only one side [10].

In a *sep7*Δ mutant, many septin rings were aberrantly shaped (Figure 3; Movies 7–9). The septin ring was often broken and had one or more gaps. Also, the ring was sometimes disrupted more severely, appearing as longitudinally oriented bars or stripes in the neck. Sometimes, Cdc3-GFP was found in the bud, not the neck, in the shape of a ring or stripes (Figure 3). Control cells sometimes displayed a moderate number of rings with small discontinuities (Figure 3; Movies 4 and 6). Therefore, to quantitate abnormal ring morphology in a conservative manner, we restricted our definition of abnormal to the more severe defects of stripes in the neck, stripes in the bud, or rings in the bud. Based on this definition, 30% of *sep7*Δ mutants (n = 516) showed disrupted septin rings, compared to 0% (n = 316) in wild-type cells. These results are consistent with Lte1p diffusing from the bud into the mother through the fissures in the septin ring.

In *sep7*Δ *gin4*Δ cells, the septin ring, detected with Cdc3-GFP, showed prominent discontinuous overlapping stripes traversing the bud neck in all cells (Figure 3; Movie 10). Occasionally, Cdc3-GFP also localized to the bud in irregular patterns (Figure 3; Movies 11 and 12). In cells with multiple buds, the older, residual bud necks consisted almost exclusively of longitudinal stripes. Occasionally, these stripes were broken in the middle, producing two striped rings, one in the mother and one in the bud. This finding suggests that cytokinesis was in progress or was aborted (Movie 10). The exacerbated septin morphology defects in the *sep7*Δ *gin4*Δ mutant relative to the *sep7*Δ mutant parallels the more severe defect of the *sep7*Δ *gin4*Δ mutant relative to the *sep7*Δ mutant in keeping Lte1-3GFP in the bud and preventing inappropriate mitotic exit.

In *cdc10*Δ cells, Cdc3-GFP formed a relatively normal single ring early in the cell cycle (Figure 3; Movie 11). However, the ring did not widen or split into two rings. The persisting single ring was irregular in thickness and was found on the bud side of the neck or was displaced into the bud, away from the neck, in 81% of cells (Figure 3; Movies 12 and 13). In 19% of the cells, the ring was in the neck.

In time-lapse images, cell separation occurred on the mother side of the single ring, confirming that the ring was on the bud side of the neck (Movie 14). Thus, *cdc10*Δ mutants appear to have lost the mother portion of the septin ring, and this finding suggests that the putative microtubule sensor may reside there. *cdc10*Δ mutants also show premature disassembly of the septin ring before or near the time of mitotic exit, and this disassembly may abolish the function of the sensor (data not shown).

Supplemental Data

Supplemental Data including the Experimental Procedures, yeast strains, and movies cited in the text are available at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgments

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