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Influence of the bud neck on nuclear envelope fission in *Saccharomyces cerevisiae*

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Abstract

Studies have shown that nuclear envelope fission (karyokinesis) in budding yeast depends on cytokinesis, but not distinguished whether this was a direct requirement, indirect, because of cell cycle arrest, or due to bud neck-localized proteins impacting both processes. To determine the requirements for karyokinesis, we examined mutants conditionally defective for bud emergence and/or nuclear migration. The common mutant phenotype was completion of the nuclear division cycle within the mother cell, but karyokinesis did not occur. In the *cdc24 swe1* mutant, at the non-permissive temperature, multiple nuclei accumulated within the unbudded cell, with connected nuclear envelopes. Upon return to the permissive temperature, the *cdc24 swe1* mutant initiated bud emergence, but only the nucleus spanning the neck underwent fission suggesting that the bud neck region is important for fission initiation. The neck may be critical for either mechanical reasons, as the contractile ring might facilitate fission, or for regulatory reasons, as the site of a protein network regulating nuclear envelope fission, mitotic exit, and cytokinesis. We also found that 77–85% of pairs of septin mutant nuclei completed nuclear envelope fission. In addition, 27% of *myo1*Δ mutant nuclei completed karyokinesis. These data suggested that fission is not dependent on mechanical contraction at the bud neck, but was instead controlled by regulatory proteins there.

Keywords

nuclear envelope; nuclear envelope fission; karyokinesis; budding yeast; *Saccharomyces cerevisiae*

INTRODUCTION:

In mammalian cells, nuclear envelope breakdown happens early in cell division, well separated from cytokinesis. In budding yeast and some other fungi, however, the nuclear envelope never breaks down, remaining intact throughout all stages of the life cycle including during cell fusion in mating and cell division (Byers 1981a; Wente *et al.* 1997). Therefore, in these fungi, nuclear envelope fission, also known as karyokinesis, is required for completion of cell division. Karyokinesis is likely to be carefully coordinated with other major cell division events, occurring prior to or concurrent with mitotic exit and cytokinesis. Moreover, karyokinesis may be spatially restricted to insure the even distribution nuclear envelope between the two daughter nuclei.

Unlike the growing body of data on the molecular control of mitotic exit and cytokinesis, the process of karyokinesis in budding yeast is poorly understood. Thought to be coupled to cytokinesis, karyokinesis has been shown to occur within minutes of contraction of the actomyosin ring (Lippincott and Li 2000). However, it was not known what aspects of cytokinesis were critical for karyokinesis. Some studies have documented defects or delays in karyokinesis in certain cytokinesis mutants, such as *iqg1*- cells and Δ*hof*1 cells (Lippincott and Li 2000). However, other studies have found, using anti-nuclear envelope protein antibodies in indirect immunofluorescence experiments, that karyokinesis can occur in four different septin mutants also defective in cytokinesis (Copeland and Snyder 1993). It is unclear whether or not the karyokinesis requirement for cytokinesis reflects a cell cycle link, a structural requirement for the bud neck, or a mechanical requirement for actomyosin ring contraction.

The bud neck between the mother and the daughter bud is a special place. In budding yeast, the future site of cytokinesis is determined early in the cell cycle, occurring at the site of bud formation, which becomes the mother-bud junction. The bud neck is required for the localization of many proteins including those regulating cytokinesis like cell cycle proteins, septins and actomyosin ring components. Therefore, the bud neck acts as a critical staging area for proteins contributing to the control of septum formation, plasma membrane separation and cytoplasmic division in *Saccharomyces cerevisiae* (Balasubramanian *et al.* 2004; Wolfe and Gould 2005; Wloka and Bi 2012). In addition to having a high concentration of proteins controlling cytokinesis, the bud neck also retains proteins directing the morphogenesis checkpoint and late mitotic events (Cid *et al.* 2002). The close spatial and temporal concurrence between karyokinesis and cytokinesis has suggested that they might be mechanistically coupled. However, the nature of the relationship between karyokinesis and cytokinesis has not yet been determined. It is possible that closure of the actin-myosin ring provides a mechanical role for cytokinesis in nuclear envelope separation, or cell signaling factors localized to the bud neck may serve as regulatory factors for karyokinesis. Moreover, it is possible that nuclear envelope fission may be directly regulated by the cell division cycle.

Migration of the nucleus through the bud neck is a critical event for completion of a normal cell cycle in budding yeast. Nuclear positioning and migration is dependent on astral microtubules, motor proteins such as Dhc1p/Dyn1p, and other proteins such as Kar9p (Palmer *et al.* 1992; Eshel *et al.* 1993; Li *et al.* 1993; Yeh *et al.* 1995; Carminati and Stearns 1997; Shaw *et al.* 1997b; Miller *et al.* 1998; Miller and Rose 1998; Beach *et al.* 2000; Yeh *et al.* 2000; Yamamoto and Hiraoka 2003). For a review, see (Kusch *et al.* 2003; Pringle 2006). Nuclear migration mutants provide a useful tool to examine nuclear envelope fission due to binucleate and multinucleate cells generated by impaired nuclear positioning.

Binucleate and/or multinucleate cells are not only found in nuclear migration mutants. Mutations affecting the morphogenesis checkpoint also generate multinucleate cells. Blocking bud formation and simultaneously preventing activation of the morphogenesis checkpoint, as occurs in the *cdc24–1 swe1*Δ mutant, generates cells with active nuclear division, but without bud formation, at non-permissive temperature (Sia *et al.* 1996). Cdc24p, a guanine nucleotide exchange factor, is critical for the establishment of polarized

growth necessary for bud formation, and the *cdc24–1* temperature sensitive mutant is defective in bud formation and nuclear division at non-permissive temperature (Adams *et al.* 1990). Swe1p, a homolog of Wee1, is a part of the morphogenesis checkpoint (Sia *et al.* 1996; Lew 2003; Keaton and Lew 2006; Pringle 2006; Howell and Lew 2012). Deletion of *SWE1* results in cells that are defective in delaying the cell cycle in response to defects in bud formation or the actin cytoskeletal network (Sia *et al.* 1996).

The bud neck is marked by the presence of the septin scaffolding proteins. The septins were discovered as temperature-sensitive mutations in four genes (*cdc3*, *10*, *11*, and *12*) that cause defects in cytokinesis, forming long chains of cells (Hartwell 1971; Byers and Goetsch 1976). Of the seven known budding yeast septins, five are expressed during vegetative growth (Cdc3p, Cdc10p, Cdc11p, Cdc12p, Shs1/Sep7p). The mitotic septins form a ring-like structure at the base of the bud neck, and act as a platform for proteins regulating cytokinesis and mitotic exit, as well as functioning as a diffusion barrier (Gladfelter *et al.* 2001; Versele and Thorner 2005; Pringle 2006)]. The septins colocalize at the future bud site from before the emergence of the bud until the completion of cytokinesis (Haarer and Pringle 1987; Ford and Pringle 1991; Kim *et al.* 1991). *SHS1/SEP7* is not required for septin ring formation in wild type cells, although cytokinesis is impaired without it (Mino *et al.* 1998; Iwase *et al.* 2007). Septins also act as a diffusion barrier between the mother and daughter cell (Barral *et al.* 2000; Takizawa *et al.* 2000). Septins have been shown to help establish boundaries for key proteins required for cytokinesis and to restrict protein movement within the plasma membrane and endoplasmic reticulum between the mother and daughter (Dobbelaere and Barral 2004; Luedeke *et al.* 2005; Caudron and Barral 2009; McMurray and Thorner 2009b). Many cell cycle and cytokinesis regulatory proteins are localized to the septin rings, including mitotic kinases like Cdc5p, Cdc15p, and Dbf2p, that may have an overlapping role in controlling cytokinesis (Cid *et al.* 2002; McMurray and Thorner 2009b).

In this work, we used nuclear migration mutants, bud formation mutants and septin mutants to dissect out what parts of cytokinesis were necessary for karyokinesis. We also studied what role the bud neck played, mechanical or regulatory, in karyokinesis.

MATERIALS AND METHODS:

Strains and yeast methods

Budding yeast strains used in this study can be found in Table 1. Strains were grown according to standard methods, using YPD media for strains with no markers or integrated markers, and minimal media supplemented with the necessary amino acids for strains carrying Sec63-GFP, a NE-ER marker, also known as pMR5029 (Melloy *et al.* 2007)(Melloy *et al.* 2009). The nuclear migration mutants, *tub2–401*, *kar9Δ* and *dhc1Δ*, carrying pMR5029, were grown overnight at room temperature and then incubated at 18–20° C for approximately four hours before observation to exacerbate the nuclear migration defect in these cells. The mutants were also studied at 25° C as a control. The wild type control (MS7617) with pMR5029 was also observed at 18° C and 25° C. The *cdc24–1swe1Δ* mutant, carrying pMR5029, was grown overnight at room temperature, then shifted to 37° C for 4–6 hours before observation. Wild type controls grown at either 25° C or 37° C were also studied. For shift down experiments, *cdc24–1swe1Δ* mutant cells incubated for 4–6

hours at 37 C were then shifted to room temperature for 1–1.5 hours before time-lapse microscopy was conducted. Time points were taken every 7 minutes.

For the *cdc3–3* and *cdc12–6* septin mutants, carrying pMR5029, cultures were grown overnight at room temperature and then shifted to 37° C for approximately 4 hours before being imaged at room temperature. Time points were taken every 7 minutes. For longer time-lapse experiments, when pER-GFP was observed in combination with Tub1-GFP (pMR5508), time points were taken every 20 minutes and observed during the temperature shift to 37° C. Cells were maintained at 37° C using the DeltaT microscope stage heater and objective heater (Bioptechs, Butler, PA). *Myo1*Δ mutant cells were observed at room temperature after an overnight incubation. The wild type control YEF473A was used in karyokinesis counts for the septin and *myo1*Δ mutant strains.

To observe nuclei, cells were stained with Hoechst 33342 (H3570; Invitrogen, Carlsbad, CA) for 15 minutes before microscopic observation. Some DNA staining was also done using DAPI (32670; Sigma-Aldrich, St. Louis, MO).

Fluorescence Microscopy

Budding yeast cells were imaged using either the Delta Vision Fluorescence Microscopy System (Applied Precision, Issaquah, WA) fitted with a Cool SNAP HQ CCD camera (Photometrics, Tucson, AZ) or a Leica Microsystems DM5500A microscope (Bannockburn, IL) linked to an ORCA-ER CCD camera (Hamamatsu, Japan).

For both systems, a 100X oil immersion objective was used. At various time points, images were collected using a GFP (FITC) filter set and a DNA (DAPI/A4) filter set. Reference images were also taken using DIC. For z stacks, images were captured at 0.5 micrometer intervals over 5 micrometers. Images captured on the Delta Vision system were analyzed and deconvolved using softWoRx (Applied Precision), while the images captured using the Leica Microsystems apparatus were analyzed using Leica Application Suite, Advanced Fluorescence software.

RESULTS:

In the first part of our study, we wanted to examine nuclear envelope fission in the context of nuclear migration mutants in which division of the nuclear contents occurs in the mother cell, due to the migration failure. We used several nuclear migration mutants, including *kar9*Δ, *dhc1*Δ, and *tub2–401*, by transforming them with a nuclear envelope-endoplasmic reticulum marker (Melloy *et al.* 2007). All of the mutants formed binucleate or multinucleate cells under non-permissive temperature conditions (Sullivan and Huffaker 1992; Miller and Rose 1998).

All three mutants were grown at permissive temperature overnight, and then shifted to non-permissive temperature to enrich for the population of binucleate cells arising from nuclear migration failure. The cells contained a GFP-tagged nuclear envelope-ER marker (green in images) and the cells were stained with a DNA dye (blue). In the *tub2–401*, *kar9*Δ, and *dhc1*Δ mutants, binucleate cells were detected by the DNA stain, but the nuclear envelopes

were still connected, as seen with the GFP marker (indicated by arrows in Figure 1). We counted cells of each genotype, including wild type, at both permissive and non-permissive temperature. In the cells observed with two or more nuclei in the mother cell, 88–100% of the cells had connected nuclear envelopes (Table 2). In the remaining cells, the orientation of the cells in the image prevented a conclusive statement on any connection between the nuclei. These results suggested that karyokinesis required either cell cycle progression, cleavage by the cytokinetic furrow, or the regulatory influence of the bud neck.

To distinguish among the possible mechanisms for karyokinesis, we examined nuclear envelope fission in a mutant defective in both bud emergence and the morphogenesis checkpoint, *cdc24–1 swe1*Δ (Sia *et al.* 1996). At the non-permissive temperature, these mutants cannot form a bud because of a lack of functional Cdc24p. However, the nuclear cycle continues in the mother cell because of the lack of Swe1p, generating multinucleate cells after several hours at non-permissive temperature. The bud emergence defect is reversible; after return to lower temperatures, the *cdc24–1 swe1*Δ mutant begins budding (Sia *et al.* 1996). By studying the double mutant at non-permissive temperature and upon shifting down to permissive temperature, *cdc24–1 swe1*Δ, one can follow the nuclear cycle and nuclear envelope fission independent of any cell cycle delay triggered through the morphogenesis checkpoint.

As shown in Figure 2A and 2B, binucleate and multinucleate cells formed in the *cdc24–1 swe1*Δ mutant at the non-permissive temperature. Among the cells examined, 98% of the cells with two or more nuclei had connected nuclear envelopes; no wild type cells were found at a comparable stage (Table 3). We then asked whether nuclear fission occurred after the cells were shifted back to permissive temperature, to allow cell cycle and bud emergence to occur. If cell cycle progression alone regulates karyokinesis, then it should occur for all nuclei regardless of their cellular position. Alternatively, if the bud neck is required then, karyokinesis should only occur on nuclei that pass through the neck.

By time-lapse microscopy (n= 4 movies), we observed that the nucleus that passed through the bud neck underwent fission, while the nuclei remaining in the cell body did not (Supplemental literature). A series of eight images in one of the time-lapse analyses are shown in Figure 2C. The position of the nuclear envelope as it passes through the bud neck is noted with an arrow. After several time points, one can see the occurrence of nuclear envelope fission in the part of the nuclear envelope present in the bud neck. However, for the other nuclei retained in the cell body karyokinesis had not occurred (arrows in the bottom half of the images). These results suggested that cell cycle progression is not sufficient for karyokinesis, but requires the presence of the bud neck. In principal, the bud neck may act either mechanically to facilitate karyokinesis, or proteins localized to the bud neck may regulate fission. When the bud neck is formed, once the nuclear envelope passed through the neck, it can undergo karyokinesis.

Upon identifying the bud neck region as important for nuclear envelope fission, we next tested whether nuclear envelope fission can occur in mutants that are defective for constructing the septin scaffold necessary for protein localization required for mitotic exit and cytokinesis. Passage through the bud neck may be needed in a mechanical or regulatory

way. Disrupting the septin scaffold may reveal if there is a mechanical requirement for bud neck constriction to facilitate karyokinesis. However, if karyokinesis proceeds in the septin mutant, then this would indicate that regulatory proteins are still present and are sufficient for karyokinesis to occur.

To test the requirement for the bud neck, we used two temperature-sensitive septin mutants, *cdc12–6* and *cdc3–3*. Using the GFP nuclear envelope marker and DNA stain, we observed distinct nuclei surrounded by their own nuclear envelopes, even at the non-permissive temperature where cytokinesis failed. Time-lapse analysis of nuclear envelope fission in a *cdc12–6* mutant is shown in Figure 3A. Karyokinesis occurred between time-points 2 and 3, as evidenced by the break in the nuclear envelope. We also studied karyokinesis using Tub1-GFP as a marker for the mitotic spindle in longer time-lapse studies (Figure 3B). We observed the formation and elongation of the mitotic spindle within the nuclear envelope, followed by karyokinesis. Both telophase and karyokinesis occurred despite the presence of a functional bud neck. Examination of static images of live cells containing the pER-GFP marker, showed that at the non-permissive temperature, 77–85% of pairs of nuclei underwent nuclear envelope fission in septin mutants (Table 4). After longer incubations (e.g. 6 hours) at the non-permissive temperature, nuclear fission rose to 100% in the septin mutants (data not shown). These results demonstrated that the structure of the bud neck, per se, is not required for nuclear fission.

Since recent studies indicated that a partially functional actomyosin ring may be still present in a septin mutant, we next observed karyokinesis in a *myo1*Δ mutant lacking the budding yeast Type II myosin (Bi *et al.* 1998; Feng *et al.* 2015). Karyokinesis counts were taken on four different occasions. Individual cells were difficult to observe because mutant cells in this strain background tended to clump together as noted previously (Bi *et al.* 1998) Cells were not sonicated to separate so as not to disturb the karyokinesis observation. However, karyokinesis was observed in at least 27% of the medium to large-budded cells (Table 4).

One possibility for the observation that karyokinesis occurs in the septin mutants, but not in nuclear migration or bud emergence mutants, is that there is not sufficient space to fully elongate the mitotic spindle within a single cell. Karyokinesis might then occur in septin mutants because they form long chains of cells with sufficient space for full spindle elongation. Nuclear fission might then result from the rupture of hyper-elongated nuclei. If so, then we would expect that the occurrence of karyokinesis in the septin mutants would be correlated with nuclear length. We saw no major difference in the distance between nuclei for nuclei that had undergone fission and those that had not (Figure 3D). For example, the distance between septin mutant nuclei that had not undergone fission had a range of 5–11 micrometers. The distance between septin mutant nuclei that had undergone fission had a range of 5–15 micrometers, with over 75% of the nuclei falling in the 7–11 micrometer range (Figure 3D). In addition, our time-lapse analysis using Tub1-GFP in the septin mutants indicated that spindle elongation can still occur in these cells.

Karyokinesis occurring in the septin mutants suggests that the bud neck is not mechanically required for nuclear fission. Presumably proteins normally required at the bud neck may be present but dispersed in the cells. We therefore examined the integrity of the septin ring in

the *cdc12–6* and *cdc3–3* mutants using a Shs1/SEP7-GFP marker (Iwase *et al.* 2007). Our results indicated that the septin ring was indeed dispersed, as evidenced by a diffuse Shs-GFP signal (arrows in Figure 4A). We studied the Shs1/Sep7-GFP signal in wild type, *cdc3–3* mutant, and *cdc12–6* mutant cells and saw abnormal Shs1/Sep7-GFP localization along the cortex of the cell or at the end of a cell projection in both septin mutants, but not in the wild type cells (Figure 4B). This supported previous work indicating that the septin ring is not intact in *cdc12* mutants (Kim *et al.* 1991; Barral *et al.* 2000; Dobbelaere *et al.* 2003). These data indicated that without the diffusion barrier or protein scaffold of the septins present, nuclear envelope fission could still be successfully triggered by proteins presented in the cell, but not concentrated at the bud neck by an intact septin ring. Also, the bud neck does not fulfill a mechanical requirement for karyokinesis. Cell cycle progression involving division of the nuclear contents is not sufficient to trigger karyokinesis, and failure of cytokinesis is not enough to stop karyokinesis.

DISCUSSION:

Karyokinesis can be uncoupled from cytokinesis

Ordinarily, nuclear envelope fission is connected to passage through the bud neck. Using more than one way to generate binucleate/multinucleate cells, we detected connected nuclear envelopes, suggesting a defect common to any type of budding yeast cell mutant lacking a bud neck. However, septin mutants, with a failure of cytokinesis, still undergo nuclear envelope fission most of the time. In our septin mutant studies, we used live cell imaging monitored over longer periods of time, in contrast to previous studies noting a lack of karyokinesis in a higher percentage of fixed septin mutant cells (Copeland and Snyder 1993). In addition, even in a *myo1*Δ mutant, karyokinesis can still occur.

Previous studies indicated a temporal relationship between karyokinesis and cytokinesis (Lippincott and Li 2000). Our studies indicate that although the timing of the two processes is very close, cytokinesis may fail and yet karyokinesis goes forward in the right regulatory environment. Many proteins can be found at the bud neck, and could potentially regulate karyokinesis independently of cytokinesis. In the case of a septin mutant where bud neck proteins are dispersed, karyokinesis can still occur without a functional bud neck. In contrast, in elongated cell types such as in pseudohyphal growth, the nucleus still divides at the bud neck (Kim and Rose 2015).

Candidate regulatory proteins localized to the bud neck region by septins that may control karyokinesis

Many classes of regulatory proteins may play a role in nuclear envelope fission. Some candidate proteins are cell cycle checkpoint proteins already known to control at least one event near the end of cell division, including cytokinesis or mitotic exit. One class includes the mitotic exit network proteins. The mitotic exit network has an overlapping role in both mitotic exit and cytokinesis. The MEN proteins themselves localize to the bud neck via the septins near the end of cell division (Jimenez *et al.* 1998; Lippincott *et al.* 2001; Luca *et al.* 2001; Menssen *et al.* 2001; Song and Lee 2001; Surana *et al.* 2002; Seshan and Amon 2004). In the basidiomycete, *Ustilago maydis*, which undergoes an “open” mitosis unlike budding

yeast, a Tem1p-like protein is involved in nuclear envelope breakdown during mitosis (Straube *et al.* 2005). In addition, the anaphase-promoting complex (APC/C) is thought to play a role in a "semi-open" mitosis in *Schizosaccharomyces japonicus* by marking Oar2 for destruction. Oar2 helps regulate the production of new membrane phospholipids (Aoki *et al.* 2013). Ibd1p (also known as Bfa1p) has been shown to promote binucleate and anucleate cell formation when overexpressed (Lee *et al.* 1999). Bfa1p is involved in the spindle checkpoint pathway and may work in conjunction with the checkpoint protein Bub2p (Li 1999). Bub2p may also be important in regulating nuclear envelope fission, given its many roles in regulating the end of the cell cycle. Overexpression of Bub2p has also been linked to cytokinesis defects (Park *et al.* 2009).

A second class of candidate proteins includes the septin-dependent kinases such as Hsl1p and Gin4p. Defects in ER compartmentalization in an *hsl1*Δ*gin4*Δ double mutant suggest that the septin-dependent kinases play a role in directing other membrane movements just prior to cytokinesis (Luedeke *et al.* 2005). Select septin mutants (*cdc10*Δ *and sep7/shs1*Δ) display perturbed mitotic exit and mislocalization of Lte1p (Hofken and Schiebel 2002; Seshan *et al.* 2002; Castillon *et al.* 2003). Recent studies also suggest that Hsl7 and the Elm1 kinase may actually sense bud emergence and interact with septins differently after bud emergence (Kang *et al.* 2016). However, the function of these kinases would presumably be impaired in the septin mutants, where karyokinesis still occurs.

Mechanics of karyokinesis

The mechanism of karyokinesis remains unclear. Proteins may act to catalyze fission by effecting membrane curvature, either through generating mechanical force, participating in a protein scaffold assembly that could force curvature of the outer and inner envelopes, or by mediating insertion of additional phospholipids in the membrane, or some combination of these functions (Shibata *et al.* 2009). Septin filaments have been shown to affect membrane curvature in liposomes (Tanaka-Takiguchi *et al.* 2009). Managing membrane dynamics as the nuclear envelope goes through the major shape changes of a "closed" mitosis might involve the conserved proteins participating in homotypic ER membrane fusion such as Sey1p, Dsl1p, Lnp1, and reticulons (Chen *et al.* 2013b; Rogers *et al.* 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES:

Adams AEM, Johnson DI, Longnecker RM, Sloat BF and Pringle JR, 1990 CDC42 and CDC43, two additional genes involved in budding and the establishment of polarity in the Yeast *Saccharomyces cerevisiae*. J Cell Biol 111: 131–142.2195038

Aoki K, Shiwa Y, Takada H, Yoshikawa H and Niki H, 2013 Regulation of nuclear envelope dynamics via APC/C is necessary for progression of semi-open mitosis in *Schizosaccharomyces japonicus*. Genes to Cells 18: 733–752.23786411

Balasubramanian MK, Bi E and Glotzer M, 2004 Comparative analysis of cytokinesis in budding yeast, fission yeast, and animal cells. Current Biology 14: R806–R818.15380095

Barral Y, Mermall V, Mooseker MS and Snyder M, 2000 Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Molecular Cell 5: 841–851.10882120

Beach DL, Thibodeaux J, Maddox P, Yeh E and Bloom K, 2000 The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. Current Biology 10: 1497–1506.11114516

Bi E, Maddox P, Lew DJ, Salmon ED, McMillan JN , 1998 Involvement of an Actomyosin Contractile Ring in Saccharomyces cerevisiae Cytokinesis. The Journal of Cell Biology 142: 1301–1312.9732290

Byers B, 1981a Cytology of the yeast life cycle, pp. 59–96 in The molecular biology of the yeast Saccharomyces: Life Cycle and Inheritance. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Byers B, and Goetsch L, 1976 A highly ordered ring of membrane-associated filaments in budding yeast. J Cell Biol 69: 717–721.773946

Carminati JL, and Stearns T, 1997 Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. J Cell Biol 138: 629–641.9245791

Castillon GA, Adames NR, Rosello CH, Seidel HS, Longtine MS , 2003 Septins have a dual role in controlling mitotic exit in budding yeast. Current Biology 13: 654–658.12699621

Caudron F, and Barral Y, 2009 Septins and the lateral compartmentalization of eukaryotic membranes. Developmental Cell 16: 493–506.19386259

Chen S, Novick P and Ferro-Novick S, 2013b ER structure and function. Current Opinion in Cell Biology 25: 428–433.23478217

Cid VJ, Jimenez J, Molina M, Sanchez M, Nombela C , 2002 Orchestrating the cell cycle in yeast: sequential localization of key mitotic regulators at the spindle pole and the bud neck. Microbiology 148: 2647–2659.12213912

Copeland CS, and Snyder M, 1993 Nuclear pore complex antigens delineate nuclear envelope dynamics in vegetative and conjugating *Saccharomyces cerevisiae*. Yeast 9: 235–249.8488725

Dobbelaere J, and Barral Y, 2004 Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. Science 305: 393–396.15256669

Dobbelaere J, Gentry MS, Hallberg RL and Barral Y, 2003 Phosphorylation-dependent regulation of septin dynamics during the cell cycle. Developmental Cell 4: 345–357.12636916

Eshel D, Urrestarazu LA, Vissers S, Jauniaux J-C, van Vliet-Reedijk JC , 1993 Cytoplasmic dynein is required for normal nuclear segregation in yeast. Proc Nat Acad Sci 90: 11172–11176.8248224

Feng Z, Okada S, Cai G, Zhou B and Bi E, 2015 Myosin−II heavy chain and formin mediate the targeting of myosin essential light chain to the division site before and during cytokinesis. Molecular Biology of the Cell 26: 1211–1224.25631819

Ford SK, and Pringle JR, 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: 281–292.1934633

Gladfelter AS, Pringle JR and Lew DJ, 2001 The septin cortex at the yeast mother-bud neck. Curr Opin Microbiol 4: 681–689.11731320

Haarer BK, and Pringle JR, 1987 Immunofluorescence localization of the Saccharyomyces cerevisiae gene product to the vicinity of the 10-nm filaments in the mother-bud neck. Mol Cell Biol 7: 3678–3687.3316985

Hartwell LH, 1971 Genetic control of the cell division cycle in yeast IV. Genes controlling bud emergence and cytokinesis. Experimental Cell Research 69: 265–276.4950437

Hofken T, and Schiebel E, 2002 A role for cell polarity proteins in mitotic exit. EMBO J 21: 4851–4862.12234925

Howell AS, and Lew DJ, 2012 Morphogenesis and the Cell Cycle. Genetics 190: 51–77.22219508

Iwase M, Luo J, Bi E and Toh-e A, 2007 Shs1 plays separable roles in septin organization and cytokinesis in *Saccharomyces cerevisiae*. Genetics 177: 215–229.17603111

Jimenez J, Cid VJ, Cenamor R, Yuste M, Molero G , 1998 Morphogenesis beyond cytokinetic arrest in Saccharyomyces cerevisiae. J Cell Biol 143: 1617–1634.9852155

Kang H, Tsygankov D and Lew DJ, 2016 Sensing a bud in the yeast morphogenesis checkpoint: a role for Elm1. Molecular Biology of the Cell 27: 1764–1775.27053666

Keaton MA, and Lew DJ, 2006 Eavesdropping on the cytoskeleton: progress and controversy in the yeast morphogenesis checkpoint. Curr Opin Microbiol 9: 540–546.17055334

Kim HB, Haarer BK and Pringle JR, 1991 Cellular morphogenesis in the *Saccharyomyces cerevisiae* cell cycle: localization of the *CDC3* gene product and the timing of events at the bud site. J Cell Biol 112: 535–544.1993729

Kim J, and Rose MD, 2015 Stable Pseudohyphal Growth in Budding Yeast Induced by Synergism between Septin Defects and Altered MAP-kinase Signaling. PLOS Genetics 11: e1005684.26640955

Kusch J, Liakopoulos D and Barral Y, 2003 Spindle asymmetry: a compass for the cell. Trends in Cell Biology 13: 562–569.14573349

Lee J, Hwang H-S, Kim J and Song K, 1999 Ibd1p, a possible spindle pole body associated protein, regulates nuclear-division and bud separation in *Saccharomyces cerevisiae*. Biochimica et Biophysica Acta 1449: 239–253.10209303

Lew DJ, 2003 The morphogenesis checkpoint: how yeast cells watch their figures. Curr Opin Cell Biol 15: 648–653.14644188

Li R, 1999 Bifurcation of the mitotic checkpoint pathway in budding yeast. Proc Nat Acad Sci 96: 4989–4994.10220406

Li Y-Y, Yeh E, Hays T and Bloom K, 1993 Disruption of mitotic spindle orientation in a yeast dynein mutant. Proc Nat Acad Sci 90: 10096–10100.8234262

Lippincott J, and Li R, 2000 Nuclear envelope fission is linked to cytokinesis in budding yeast. Experimental Cell Research 260: 277–283.11035922

Lippincott J, Shannon KB, Shou W, Deshaies RJ and Li R, 2001 The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. J Cell Sci 114: 1379–1386.11257003

Luca FC, Mody M, Kurischko C, Roof DM, Giddings TH , 2001 Saccharomyces cerevisiae Mob1p is required for cytokinesis and mitotic exit. Mol Cell Biol 21: 6972–6983.11564880

Luedeke C, Frei SB, Sbalzarini I, Schwarz H, Spang A , 2005 Septin-dependent compartmentalization of the endoplasmic reticulum during yeast polarized growth. J Cell Biol 169: 897–908.15967812

McMurray MA, and Thorner JW, 2009b Septins: molecular partitioning and the generation of cellular assymetry. Cell Division 4: 18.19709431

Melloy P, Shen S, White E, McIntosh JR and Rose MD, 2007 Nuclear fusion during yeast mating occurs by a three-step pathway. J Cell Biol 179: 659–670.18025302

Melloy P, Shen S, White E and Rose MD, 2009 Distinct roles for key karyogamy proteins during yeast nuclear fusion. Mol Biol Cell 17: 3778–3782.

Menssen R, Neutzner A and Seufert W, 2001 Asymmetric spindle pole localization of yeast Cdc15 kinase links mitotic exit and cytokinesis. Current Biology 11: 345–350.11267871

Miller RK, Heller KM, Frisen L, Wallack DL, Loayza D , 1998 The kinesin-related motor proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. Mol Biol Cell 9: 2051–2068.9693366

Miller RK, and Rose MD, 1998 Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J Cell Biol 140: 377–390.9442113

Mino A, Tanaka K, Kamei T, Umikawa M, Fujiwara T , 1998 Shs1p: a novel member of septin that interacts with Spa2p, involved in polarized growth in *Saccharomyces cerevisiae*. Biochem Biophys Res Comm 251: 732–736.9790978

Palmer RE, Sullivan DS, Huffaker TC and Koshland D, 1992 Role of astral microtubules and actin in spindle orientation and migration in the budding yeast, *Saccharomyces cerevisiae*. J Cell Biol 119: 583–593.1400594

Park SY, Cable AE, Blair J, Stockstill KE and Shannon KB, 2009 Bub2 regulation of cytokinesis and septation in budding yeast. BMC Cell Biology 10: 43.19490645

Pringle JR, 2006 Cytoskeleton and Morphogenesis, pp. 211–241 in Landmark Papers in Yeast Biology, edited by Linder P, Shore D and Hall MN Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rogers JV, McMahon C, Baryshnikova A, Hughson FM and Rose MD, 2014 ER-associated retrograde SNAREs and the DSL1 complex mediate an alternative, Sey1p-independent homotypic ER fusion pathway. Mol Biol Cell 14-07-1220.

Seshan A, and Amon A, 2004 Linked for life: temporal and spatial coordination of late mitotic events. Current Opinion in Cell Biology 16: 41–48.15037303

Seshan A, Bardin AJ and Amon A, 2002 Control of Lte1 localization by cell polarity determinants and Cdc14. Current Biology 12: 2098–2110.12498684

Shaw SL, Yeh E, Maddox P, Salmon ED and Bloom K, 1997b Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. J Cell Biol 139: 985–994.9362516

Shibata Y, Hu J, Kozlov MM and Rapoport TA, 2009 Mechanisms shaping the membranes of cellular organelles. Annu Rev Cell Dev Biol 25: 329–354.19575675

Sia RAL, Herald HA and Lew DJ, 1996 Cdc28 tyrosine phosphorylation and the morphogenesis checkpoint in budding yeast. Mol Biol Cell 7: 1657–1666.8930890

Song S, and Lee KS, 2001 A novel function of *Saccharomyces cerevisiae CDC5* in cytokinesis. J Cell Biol 152: 451–469.11157974

Straube A, Weber I and Steinberg G, 2005 A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. EMBO J 24: 1674–1685.15861140

Sullivan DS, and Huffaker TC, 1992 Astral microtubules are not required for anaphase B in Saccharomyces cerevisiae. J Cell Biol 119: 379–388.1400581

Surana U, Yeong FM and Lim HH, 2002 MEN, destruction and separation: mechanistic links between mitotic exit and cytokinesis in budding yeast. BioEssays 24: 659–666.12111726

Takizawa P, DeRisi JL, Wilhelm JE and Vale RD, 2000 Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science 290: 341–344.11030653

Tanaka-Takiguchi Y, Kinoshita M and Takiguchi K, 2009 Septin-mediated uniform bracing of phospholipid membranes. Current Biology 19: 140–145.19167227

Versele M, and Thorner JW, 2005 Some assembly required: yeast septins provide the instruction manual. Trends in Cell Biology: 414–424.16009555

Wente SR, Gasser SM and Caplan AJ, 1997 The nucleus and nucleocytoplasmic transport in *Saccharomyces cerevisiae*, pp. 471–546 in The molecular and cellular biology of the yeast Saccharomyces, edited by Pringle JR, Broach JR and Jones EW Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Wloka C, and Bi E, 2012 Mechanisms of cytokinesis in budding yeast. Cytoskeleton 69: 710–726.22736599

Wolfe BA, and Gould KL, 2005 Split decisions: coordinating cytokinesis in yeast. Trends in Cell Biology 15: 10–18.15653073

Yamamoto A, and Hiraoka Y, 2003 Cytoplasmic dynein in fungi: insights from nuclear migration. J Cell Sci 116: 4501–4512.14576344

Yeh E, Skibbens RV, Cheng JW, Salmon ED and Bloom K, 1995 Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae* 130: 687–700.

Yeh E, Yang C, Chin E, Maddox P, Salmon ED , 2000 Dynamic positioning of mitotic spindles in yeast: role of microtubule motors and cortical determinants. Mol Biol Cell 11: 3949–3961.11071919

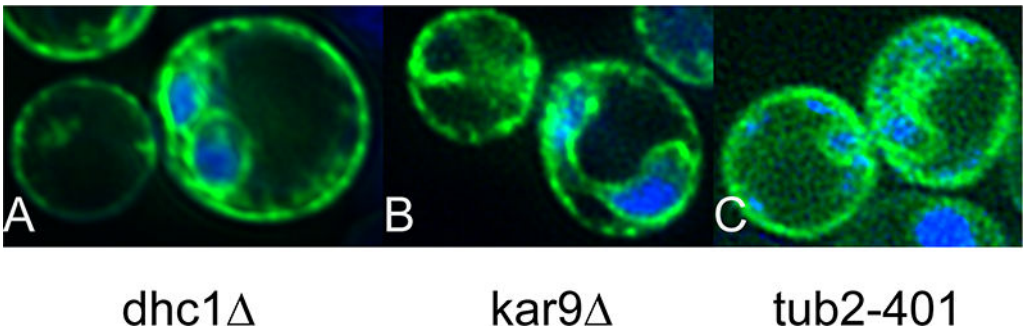

Figure 1: Karyokinesis in defective in nuclear migration mutants

Shown are three different nuclear migration mutants: *dhc1Δ* (a), *kar9Δ* (b), and *tub2–401* (c) with more than one nucleus in the mother cell. All three have connected nuclear envelopes as seen using a fluorescent marker for the nuclear envelope, pMR5029 (indicated by arrows). Green, Sec63-GFP, Blue, DNA

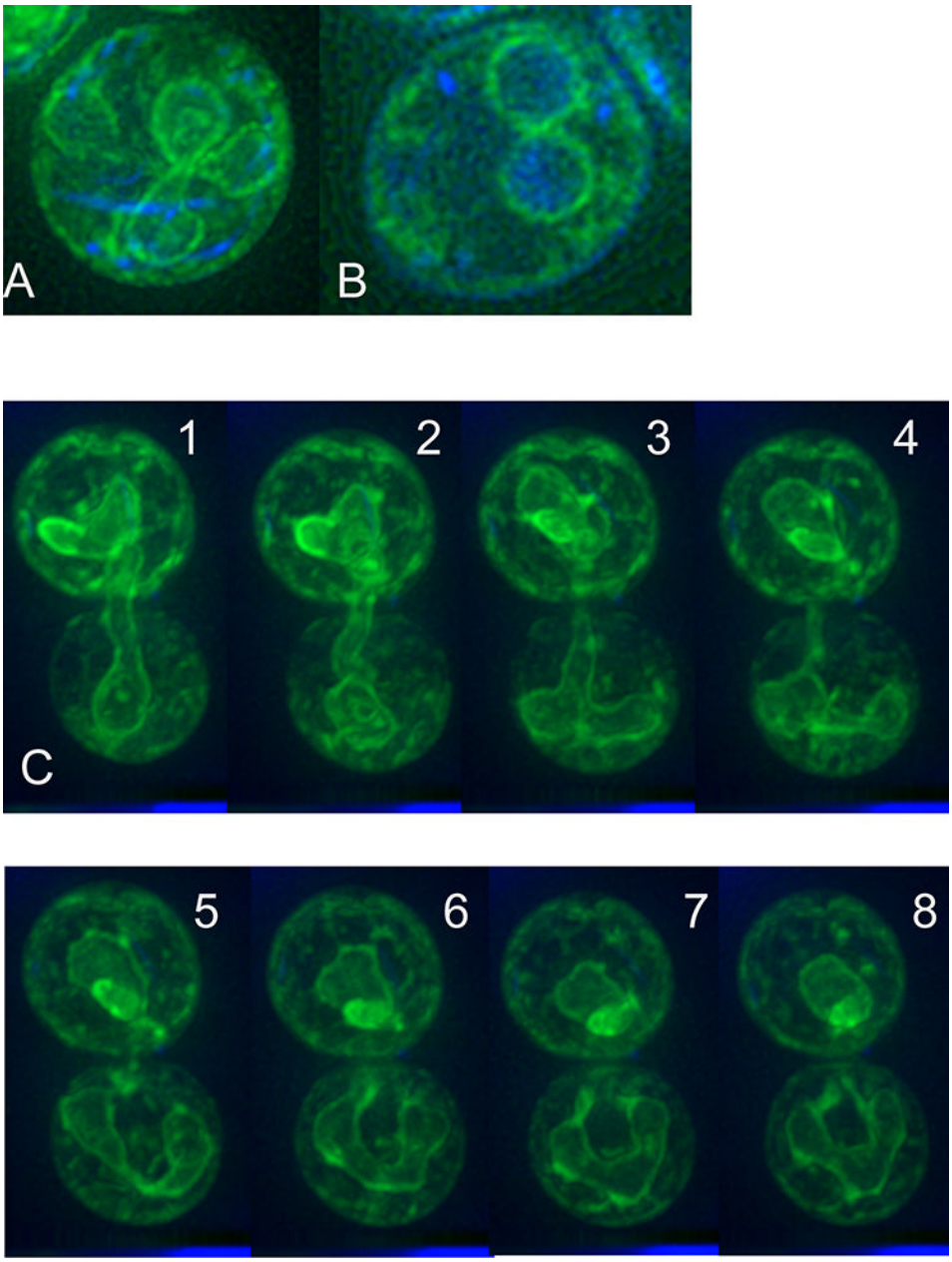

Figure 2: Karyokinesis failure in *cdc24–1swe1Δ* mutants at non-permissive temperature is reversed upon return to permissive temperature after passage of a nucleus through the bud neck

Shown in (A) and (B) are two examples of multinucleate *cdc24–1swe1Δ* cells at non-permissive temperature for 4–6 hours displaying connected nuclear envelopes (arrows). After the same incubation period at non-permissive temperature, shift down experiments were performed to watch the nuclear envelope during bud emergence. Shown in (C) is a time-lapse experiment (each time point is 7 minutes apart) following the nuclear envelope using pMR5029. Note that the nucleus that passes through the bud neck undergoes fission (arrows), while the nuclei still in the mother cell do not undergo fission (black and white arrowheads). Green, Sec63-GFP, Blue, DNA

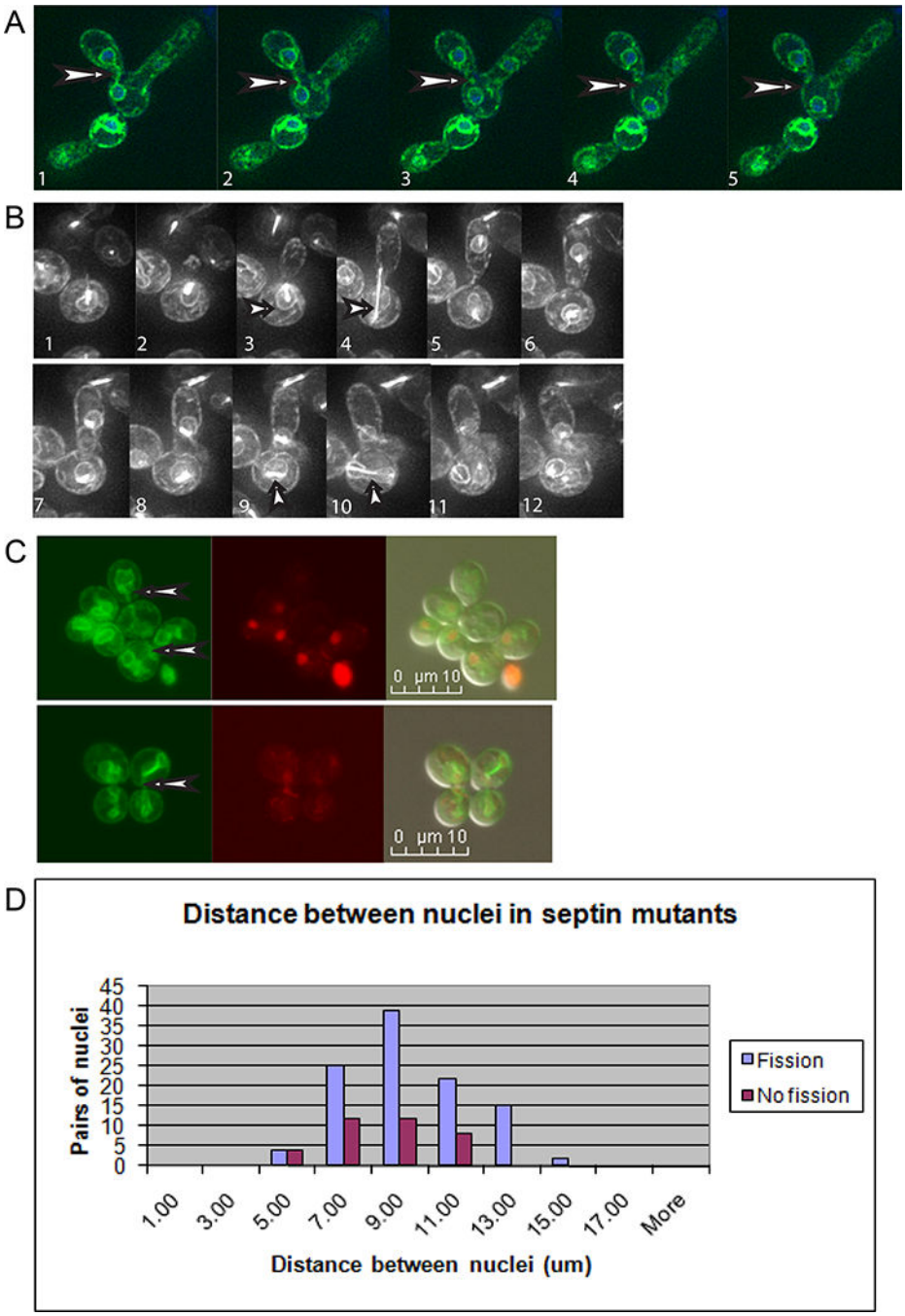

Figure 3: Karyokinesis occurs in septin mutants failing to undergo cytokinesis

Shown in (A) is a time-lapse experiment using *cdc12–6* carrying the pMR5029 marker. After four hours at non-permissive temperature, long chains of cells have formed with multiple nuclei. Time points were taken every 7 minutes. One can see that the nucleus passing through the bud neck undergoes karyokinesis during the time-lapse (indicated by arrow). Green, Sec63-GFP, Blue, DNA. Shown in (B) is the same strain but also carrying the Tub1-GFP marker. Time points were taken every 20 minutes. One can see spindle elongation happening between time points 3 and 4 as indicated by the arrows, and a karyokinesis event

happening by time point 5. In addition, one can also see spindle elongation happening between time points 9 and 10 as indicated by arrows, followed by a karyokinesis event by time point 11. In (C) are representative images of the *myo1*Δ strain carrying the pMR5029 marker. Arrows indicate the nuclear envelope. Note one cell has completed karyokinesis in the top panel. GFP (left panel), DNA (middle panel), merge (right panel). Analysis of the distance between nuclei in relation to fission or no fission in the mutants is shown in (D).

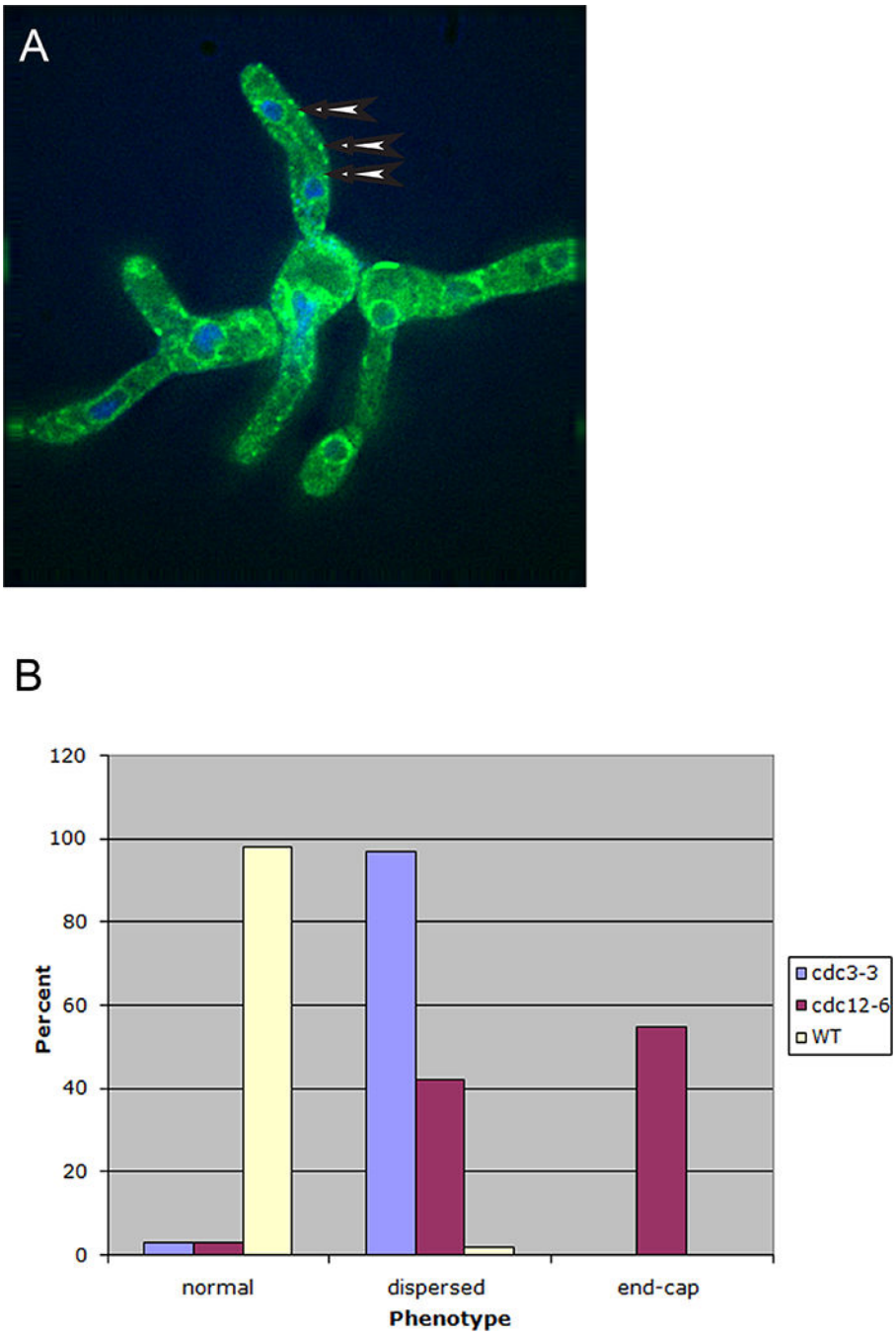

Figure 4: No intact septin ring is detected in temperature-sensitive septin mutants

Shown in (A) are *cdc12–6* cells at non-permissive temperature expressing a SHS-GFP marker. In (B), the SHS-GFP localization is quantified, with the majority of *cdc12–6* mutants either displaying dispersed SHS-GFP signal or an end cap-like structure. (indicated by arrows).

Table 1:

Strains and Plasmids

Strain	Genotype	Source or Reference
MS22	*MATa, trp1-Δ1, lys2–801, ade2–101, canS*	Rose lab
MS7617	Same as MS22 except contains pMR5029	Rose lab
MS52	MATα, leu2–3, 112, trp1-Δ1, ura3–52	Rose lab
MS7619	Same as above except contains pMR5029	Rose lab
MY 3044 (CuY67–2)	*MATa, ura3–52, lys2–801, his4–539, tub2–401*	T. Huffaker
MY8488	*MATa, ura3–52, lys2–801, his4–539, tub2–401, TRP1::URA3,* contains pMR5029	Rose lab
MS4262	*MATα, DHC1::URA3, leu2–3, 112, trp1-Δ1, ura3–52*	Rose lab
MS7683	Same as above except contains pMR5029	Rose lab
MS4313	*MATa, KAR9-Δ1::LEU2, leu2–3, 112, ura3–52, trp1-Δ1*	Rose lab
MS8420	Same as above except contains pMR5029	Rose lab
MY8569 (DLY690)	*MATa, ura3Δns, leu2–3, 112, trp1-Δ1, his2, bar1, cdc24–1, SWE1::LEU2*	D. Lew
MY8644	*MATa, ura3Δns, leu2–3, 112, trp1-Δ1, his2, bar1, cdc24–1, SWE1::LEU2* contains pMR5029	Rose lab
MY8566 (DLY1)	*MATa, ura3Δns, leu2–3, 112, trp1-Δ1, his2, bar1*	D. Lew
MY11675	Same as above except contains pMR5029	Rose lab
MY9161 (YEF743)	*MATa,cdc12–6, leu2, ura3*	E. Bi
MY9179	*MATa,cdc12–6, leu2, ura3, TRP1::LEU2* contains pMR5029	Rose lab
MY9162 (YEF739)	*MATa, cdc3–3, leu2, ura3*	E. Bi
MY9175	*MATa, cdc3–3, leu2, ura3, TRP1::URA3* contains pMR5029	Rose lab
MY9491	*MATa, cdc3–3, leu2, ura3, TRP1::URA3, SHS-GFP-URA3*	Rose lab
MY9492	*MATa, cdc12–6, leu2, ura3, TRP1::LEU2, SHS1-GFP-LEU2*	Rose lab
YEF473a	*MATa, his3, leu2, lys2, trp1, ura3*	E. Bi
yTM 231	Same as above except contains pMR5029	this work
YEF1804	*MATa, his3, leu2, lys2, trp1, ura3, myo1Δ::KanMX6*	E. Bi
yTM 232	Same as above except contains pMR5029	this work
Plasmid	**Relevant Markers**	
pMR5029	pER-GFP marker, Sec63-GFP in pRS414 (*TRP1*)	Rose lab
pMR5487 (pM-6)	Integrating plasmid with SHS1-GFP (*LEU2*)	Iwase and Toh-e
pMR5488 (pM-7)	Integrating plasmid with SHS1-GFP (*URA3*)	Iwase and Toh-e
pMR5508	GFP-Tub1 in pRS305	Rose lab

Table 2:

Nuclear envelope fission defects in nuclear migration mutants

Genotype	Temperature (° C)	≥ 2 nuclei in unbudded to medium budded cell connected inconclusive*		Total # of binucleate cells observed	Total # of cells observed
tub2–401	18–20	16 (94%)	1	17	265
tub2–401	25	3 (100%)	0	3	53
dhc1Δ	18–20	68 (88%)	9	77	933
dhc1Δ	25	2 (100%)	0	2	34
kar9Δ	18–20	10 (91%)	1	11	2202
WT	18	1 (100%)	0	1	122
WT	23	1 (100%)	0	1	128

* may still be connected but unclear from Z stack

Table 3:

Nuclear envelope fission defects in *cdc24swe1* mutants

Genotype	Temperature (° C)	≥ 2 nuclei in unbudded to medium budded cell connected inconclusive*		Total # of binucleate cells observed	Total # of cells observed
cdc24swe1 Sec63-GFP	4–6 hrs, 37	28 (100%)	0	28	60
cdc24swe1 Sec63-GFP with Hoechst staining	4–6 hrs, 37	16 (94%)	1	17	94
Wild type (DLY1) Sec63-GFP	5 hrs, 37	0 (0%)	0	0	22

*May still be connected but unclear from Z stack or static images

Table 4:

Nuclear envelope fission in septin and *myo1*Δ mutants

Genotype	Temperature (° C)	≥ 2 nuclei * connected	not connected	Number of nuclei pairs
cdc3–3	25	25 (47%)	28 (53%)	53
cdc12–6	25	17 (52%)	16 (48%)	33
WT	25	19 (46%)	22 (54%)	41
cdc3–3	4hrs, 37	27 (15%)	148 (85%)	175
cdc12–6	4hrs, 37	50 (23%)	170 (77%)	220
WT	4hrs, 37	11 (44%)	14 (56%)	25
*myo1*Δ	25	37 (73%)	14 (27%)	51

* In wildtype or mutants at room temperature, cells counted were medium to large-budded cells that had completed anaphase with typically one pair of nuclei. In the septin mutants at non-permissive temperature, more than one pair of nuclei might be detected in the mutant cell body.