Assembly of normal actomyosin rings in the absence of Mid1p and cortical nodes in fission yeast

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Cytokinesis in many eukaryotes depends on the function of an actomyosin contractile ring. The mechanisms regulating assembly and positioning of this ring are not fully understood. The fission yeast *Schizosaccharomyces pombe* divides using an actomyosin ring and is an attractive organism for the study of cytokinesis. Recent studies in *S. pombe* (Wu, J.Q., V. Sirotkin, D.R. Kovar, M. Lord, C.C. Beltzner, J.R. Kuhn, and T.D. Pollard. 2006. *J. Cell Biol.* 174:391–402; Vavylonis, D., J.Q. Wu, S. Hao, B. O'Shaughnessy, and T.D. Pollard. 2008. *Science.* 319:97–100) have suggested that the assembly of the actomyosin ring is initiated from a series of cortical nodes containing several components of this ring. These studies have proposed that actomyosin interactions bring together the cortical nodes to form a compacted ring structure. In this study, we test this model in cells that are unable to assemble cortical nodes. Although the cortical nodes play a role in the timing of ring assembly, we find that they are dispensable for the assembly of orthogonal actomyosin rings. Thus, a mechanism that is independent of cortical nodes is sufficient for the assembly of normal actomyosin rings.

Introduction

In many eukaryotes, physical division of the cell depends on the function of an actomyosin ring (Schroeder, 1968; Fujiwara and Pollard, 1976; Mabuchi and Okuno, 1977). Forces generated upon actomyosin interactions coupled with the delivery of new membranes (and cell walls in some cases) lead to the bisection of the mother cell into two daughters (Fujiwara and Pollard, 1976; Balasubramanian et al., 2004; Glotzer, 2004). The actomyosin rings are assembled in a plane perpendicular to the axis of chromosome segregation. How the actomyosin ring components organize into a ring structure that is perpendicular to the plane of chromosome segregation is only poorly understood.

In recent years, *Schizosaccharomyces pombe*, which divides using an actomyosin ring, has emerged as an attractive model for the study of cytokinesis (Balasubramanian et al., 2004; Wolfe and Gould, 2005). In fission yeast, an actomyosin ring is assembled early in mitosis and constricts after mitotic exit (Marks et al., 1986; Fankhauser et al., 1995; McCollum et al., 1995; Wu et al., 2006). Filamentous actin (F-actin) in the ring has been shown to assemble from a leading cable (Chang et al.,

1997; Chang, 1999; Arai and Mabuchi, 2002). Recent studies have led to the proposal that actomyosin ring assembly instead is initiated from multiple cortical nodes, each containing two dimers of the formin Cdc12p, anillin-related protein Mid1p, type II myosins, the FCH-BAR (Fer/Cip4 homology–Bin-amphiphysin-Rvs) domain protein Cdc15p, and the IQGAP (IQ motif–containing GTPase-activating protein)-related protein Rng2p (Wu et al., 2006; Vavylonis et al., 2008). Nodelike structures have also been detected from several other studies (Bahler et al., 1998; Wong et al., 2002; Motegi et al., 2004). Mid1p plays a key role in the cortical anchoring of these nodes because loss of Mid1p function eliminates the assembly of detectable nodes (Wu et al., 2006). Computational modeling experiments consistent with experiments of Daga and Chang (2005) have revealed that forces generated by type II myosins combined with a search and capture mechanism help compact nodes placed within a 8-μm zone into a proper ring (Daga and Chang, 2005; Vavylonis et al., 2008). Although the node-based ring assembly mechanism is very attractive, a prediction that arises from this model

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is that cells containing fewer or no nodes should be unable to assemble normal actomyosin rings.

In this study, we have characterized the process of actomyosin ring assembly in cells lacking Mid1p and detectable nodes. We find that cells lacking Mid1p and detectable nodes can assemble normal-looking actomyosin rings, albeit with a delayed kinetics. Thus, a cortical node–independent mechanism is sufficient for the assembly of properly organized actomyosin rings.

Results and discussion

Cortical nodes are not detected in cells lacking Mid1p

In *S. pombe*, Wu et al. (2006) have shown that Mid1p assembles first into nodes and recruits other proteins (including type II myosin and its light chain Rlc1p, formin Cdc12p, FCH-BAR protein Cdc15p, and IQGAP-Rng2p) into nodes (Motegi et al., 2000, 2004; Paoletti and Chang, 2000). Fig. 1 A shows examples of Rlc1p-3GFP nodes in wild-type cells and their absence in *mid1Δ* cells, which is consistent with experiments of Wu et al. (2006). Furthermore, as shown by Wu et al. (2006), we have found that Cdc15p organizes into a few nodelike structures (15 ± 7 nodes/cell; *n* = 17; Fig. 1 A) and that the number and intensity of these structures is increased (45 ± 18 nodes/cell; *n* = 22; Fig. 1 A) upon treatment with latrunculin A (LatA), which prevents actin polymerization (Ayscough et al., 1997). We have also been able to observe colocalization of Cdc15p-GFP nodes with Rlc1p-mCherry nodes in LatA-treated cells (Fig. 1 B). As shown by Wu et al. (2006), we have found that organization of Cdc15p into nodes in untreated and LatA-treated cells depended on Mid1p function. Although we have been able to detect Rlc1p and Cdc15p in cortical nodes, we were unable to detect the formin Cdc12p in cortical nodes in unperturbed cells, which is consistent with studies by Chang (1999) and Yonetani et al. (2008). Nevertheless, as described by Wu et al. (2006), we did detect Cdc12p nodes upon LatA treatment (Fig. 1 A). Again, organization of Cdc12p nodes in LatA-treated cells depended on Mid1p function. Approximately 51% of Cdc12p nodes (*n* = 270) generated by LatA treatment colocalized with Rlc1p nodes (Fig. 1 C). We have also found that Rlc1p nodes are not detected in mutants defective in the fission yeast Polo kinase Plo1p (unpublished data), which is a known regulator of fission yeast Mid1p (Bahler et al., 1998). Collectively, these studies established that some actomyosin ring components (such as Rlc1p and Cdc15p) are detected in nodes in unperturbed cells, whereas others (such as Cdc12p) are detected in nodes only upon LatA treatment. Furthermore, our experiments as well as other studies (Motegi et al., 2004; Wu et al., 2006) have established that the organization of cortical nodes depends on Mid1p function.

Cells lacking Mid1p and detectable nodes assemble normal actomyosin rings upon inhibition of division septum synthesis

Cells defective for Mid1p or Plo1p have been shown to assemble abnormal actomyosin rings (Chang et al., 1996; Sohrmann et al., 1996; Bahler et al., 1998), which at first glance is consistent with the predictions of recent studies (Wu et al., 2006; Vavylonis et al., 2008). We have found that the abnormal rings in *mid1* and *plo1* mutant cells can become stabilized as a result of septum assembly before ring compaction. Fig. 2 A shows examples of F-actin distribution in wild type, cells of three different mutant alleles of *mid1* (*mid1*-18, *mid1*-ΔNES, and *mid1Δ*), and *plo1*-1 cells. Unlike in mitotic wild-type cells, F-actin rings in mitotic *mid1* and *plo1*-1 mutants are detected at various angles (Fig. 2 A; Bahler et al., 1998). To eliminate any confusion in scoring phenotypic effects that might arise from the assembly of division septum along improperly organized rings, we inactivated Cps1p, a 1,3-β-glucan synthase involved in septum assembly (Liu et al., 1999), in cells defective in Mid1p or Plo1p function. Surprisingly, >85% of *cps1*-191 *mid1*-18, *cps1*-191 *mid1*-ΔNES, *cps1*-191 *mid1Δ*, and *cps1*-191 *plo1*-1 cells assembled normal and orthogonal actomyosin rings (Fig. 2, B and C). Rings in *cps1*-191 *mid1*-18, *cps1*-191 *mid1*-ΔNES, *cps1*-191 *mid1Δ*, and *cps1*-191 *plo1*-1 cells were positioned at abnormal sites, which is consistent with a role for Mid1p and Plo1p in actomyosin ring positioning.

It remained possible that inactivation of Cps1p function led to the restoration of medial nodes by unknown mechanisms. To test this possibility, *cps1*-191 and *cps1*-191 *mid1Δ* cells were fixed and stained with antibodies against tubulin and the actomyosin ring protein Cdc4p, a light chain for Myo2p (Fig. 2 D). Cdc4p was readily detected in medial nodes in 30/54 *cps1*-191 cells with short spindles, whereas the rest contained fully formed actomyosin rings. Such organization of Cdc4p was not observed in *cps1*-191 *mid1Δ* cells (36/53 contained no obvious structures, and 17/53 contained disorganized cables), ruling out the possibility that normal ring assembly in *cps1*-191 *mid1Δ* cells is caused by the restoration of medial nodes. These observations established that Mid1p and detectable nodes are not required for assembly of orthogonal myosin and F-actin rings. These observations also established that the nonorthogonal rings become fixed at improper angles caused by septum assembly before ring compaction in *mid1* and *plo1* mutants.

To gain further insight into the mechanism of ring assembly in the absence of Mid1p (and nodes), we imaged ring assembly in *cps1*-191 *mid1*-18 expressing Rlc1p-GFP as a ring marker. As previously reported by others and us (Bahler et al., 1998; Huang et al., 2007), the assembly of myosin II bundles initiated at nonmedial sites (Fig. 2 E). Interestingly, these myosin II bundles eventually organized into orthogonal rings (Fig. 2 E). These experiments established that in the absence of Mid1p (and detectable nodes), myosin II bundles assemble initially from random locations and at various angles and that they eventually compact into orthogonal rings.

Mid1p and cortical nodes play an important role in the kinetics of actomyosin ring assembly in early stages of mitosis

Although normal actomyosin rings were assembled in *cps1*-191 *mid1Δ* cells, the assembly process appeared to be slower. To characterize ring assembly in *cps1*-191 *mid1Δ* cells, we imaged
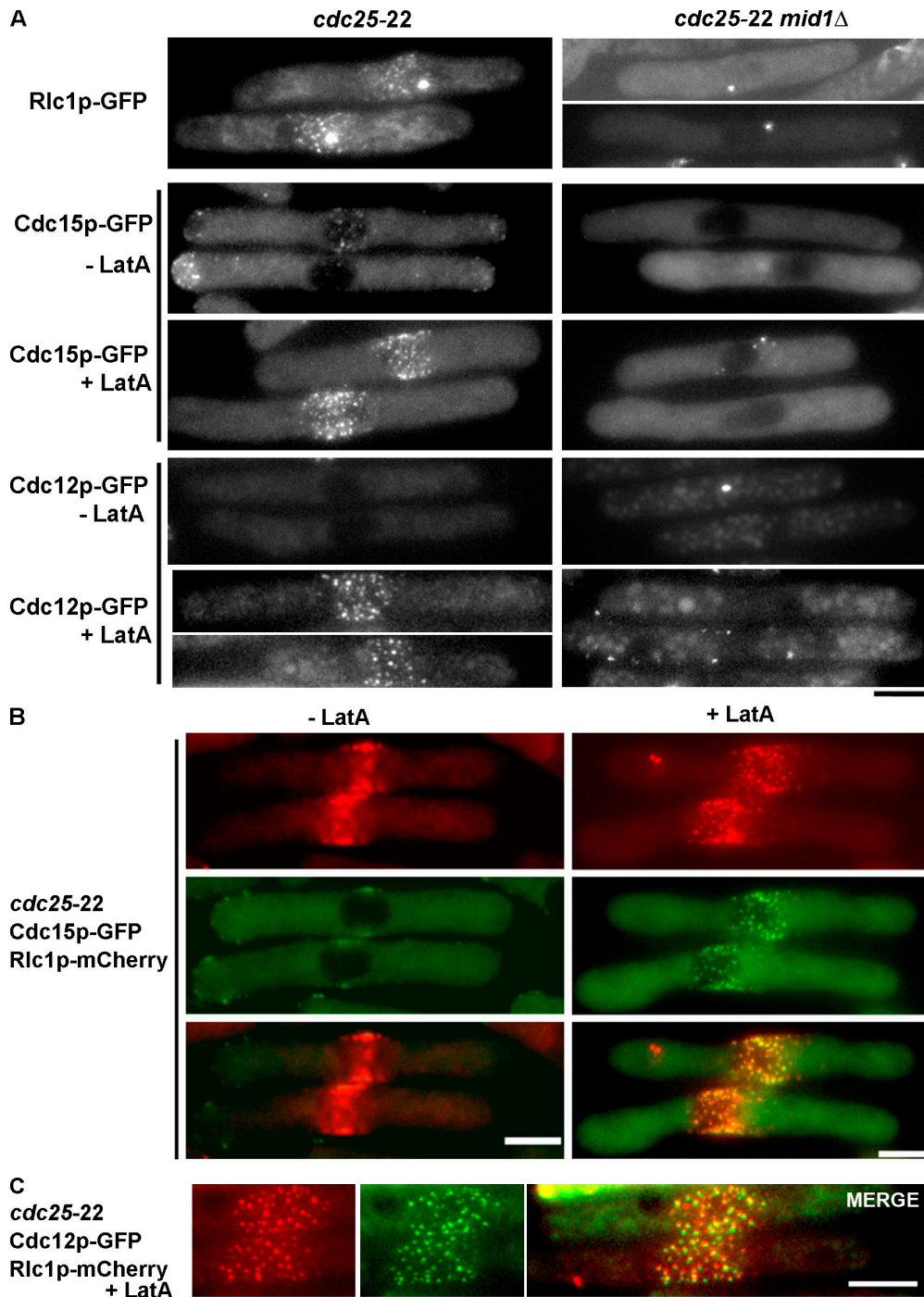
Figure 1. **Membrane-associated nodes of Rlc1p, Cdc15p, and Cdc12p are not detected in cells lacking Mid1p.** (A) *cdc25*-22 and *cdc25*-22 *mid1*Δ cells expressing Rlc1p-GFP, Cdc15p-GFP, or Cdc12p-GFP were arrested at the G2/M boundary by incubation at the restrictive temperature of 36°C. Cells were shifted to the permissive temperature of 24°C, and images were captured 30–45 min after a shift down. In some instances, as indicated, LatA was added to the culture to facilitate visualization of the cortical nodes of Cdc15p and Cdc12p. (B and C) *cdc25*-22 cells expressing Rlc1p-mCherry and Cdc15p-GFP (B) or Rlc1p-mCherry and Cdc12p-GFP (C) were cultured as described in A and treated with LatA, and images were captured. Bars, 5 μm.

dynamics of myosin II ring assembly and mitosis simultaneously in cells expressing Rlc1p-GFP and Pcp1p-GFP (a spindle pole body [SPB] marker; Flory et al., 2002). In *cps1*-191 mutants (Fig. 3 A), myosin II ring assembly was initiated soon after separation of SPBs at prometaphase. Organization of a myosin II ring was completed in <10 min (9.7 ± 1.4 min; *n* = 7 cells) from the time of SPB separation. In contrast, although myosin II cable

assembly was initiated soon after SPB separation in *cps1*-191 *mid1*Δ cells (Fig. 3 B), organization of normal myosin II rings was accomplished only after full separation of the SPBs to the opposite ends of the cell. Starting at the point of SPB separation, organization of normal myosin II rings took at least twice as long (32.3 ± 19.5 min; *n* = 8 cells) in *cps1*-191 *mid1*Δ cells.
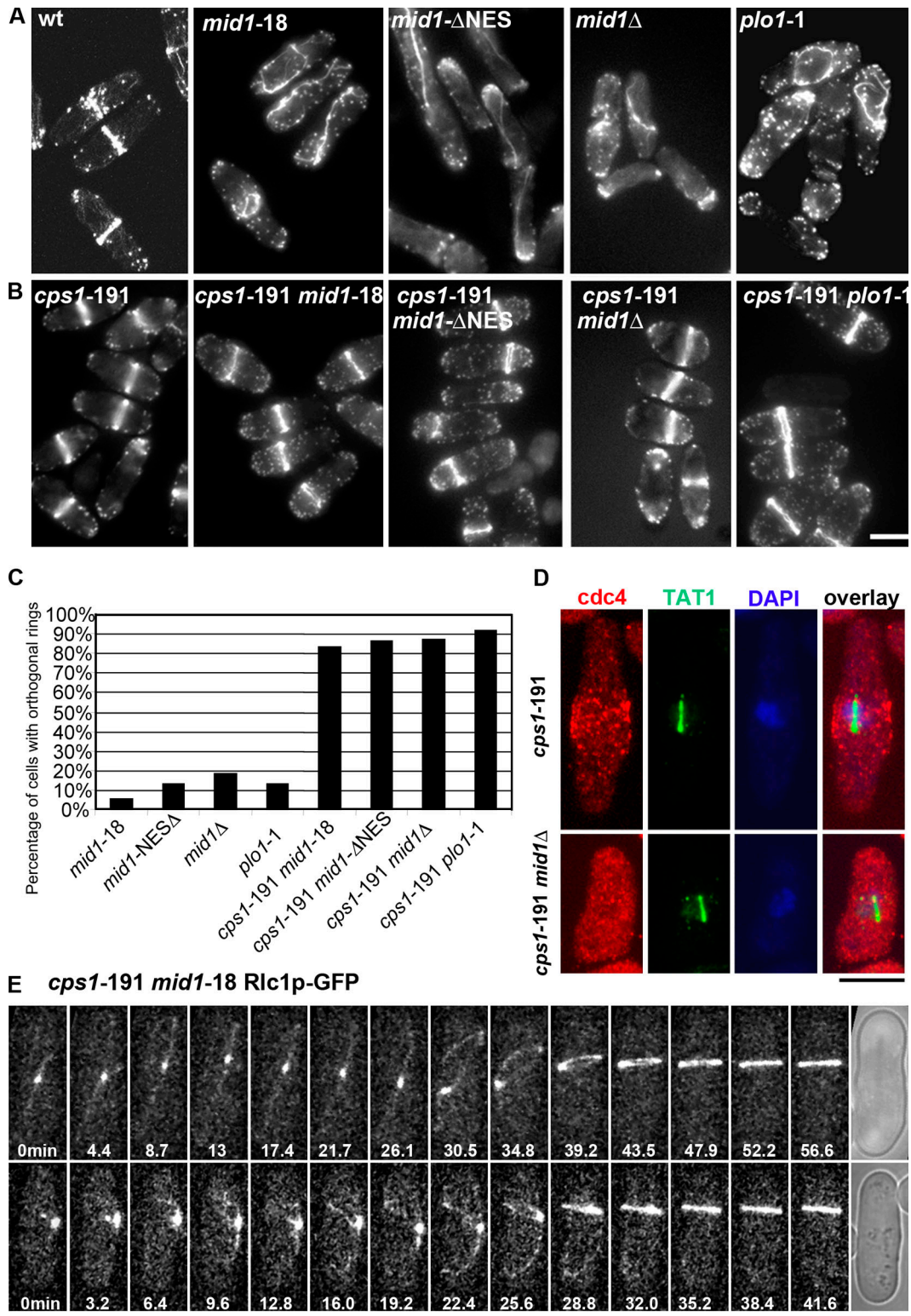
Figure 2. **Orthogonal actomyosin rings assemble with high efficiency in *mid1* and *plo1* mutants.** (A and B) Cells of the indicated genotypes were shifted to 36°C, fixed, and stained with Alexa Fluor 488 phalloidin to visualize F-actin rings. (C) Quantitation of cells with orthogonal rings. At least 500 cells were scored for each genotype. (D) Nodes are not restored in *cps1*-191 *mid1Δ* mutants. *cps1*-191 and *cps1*-191 *mid1Δ* cells were fixed and stained with Tat1 antibodies (tubulin), Cdc4p antibodies, and DAPI (nuclei). (E) Randomly oriented myosin II bundles eventually organize into orthogonal myosin II rings in *cps1*-191 *mid1*-18 cells. *cps1*-191 *mid1*-18 cells expressing Rlc1p-GFP were imaged by confocal microscopy at 36°C. The elapsed time is shown in minutes. wt, wild type. Bars, 5 μm.
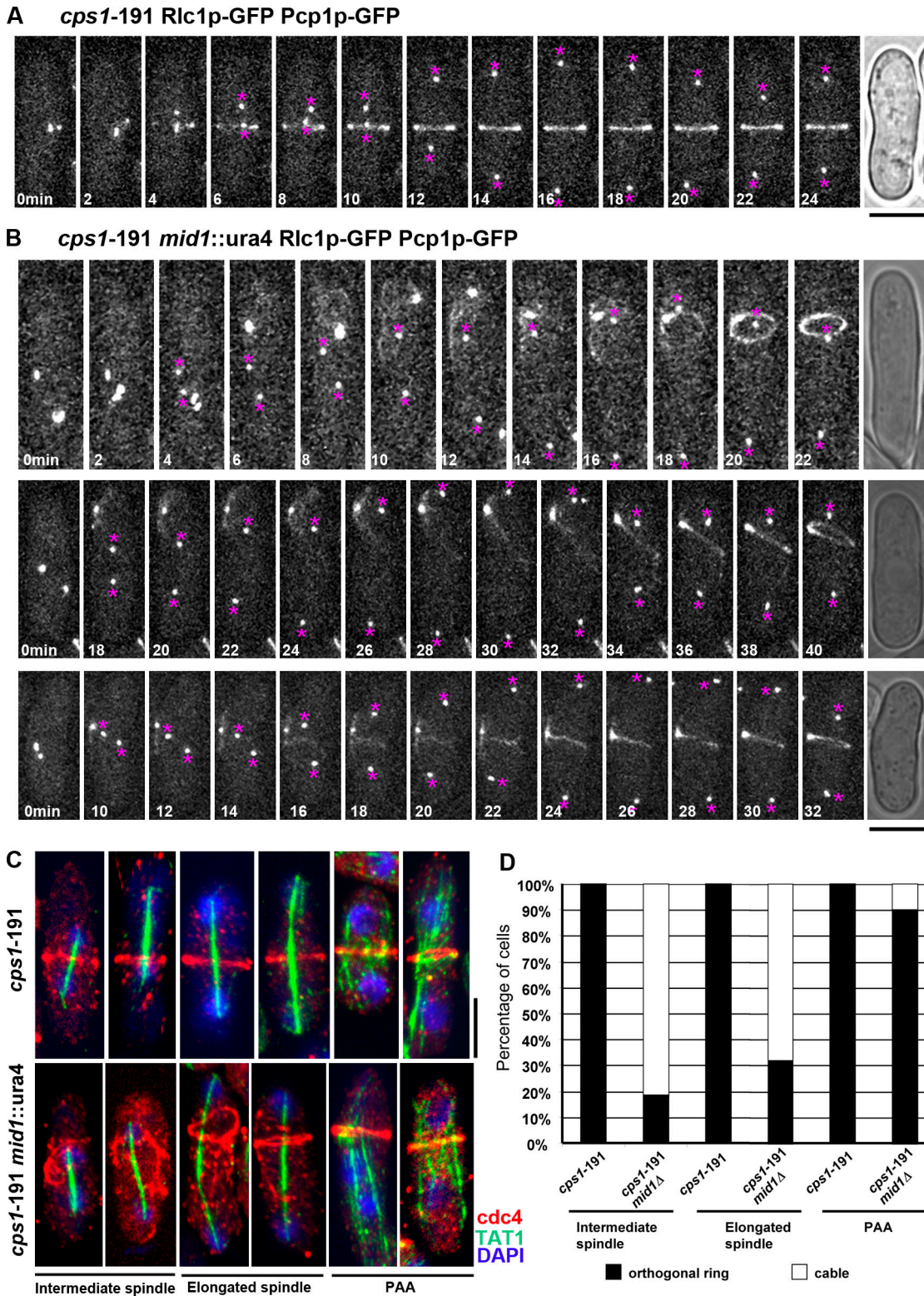
Figure 3. **Mid1p and associated medial nodes are required for the organization of actomyosin rings in early mitosis.** (A and B) *cps1*-191 and *cps1*-191 *mid1*Δ cells expressing Rlc1p-GFP (myosin II ring) and Pcp1p-GFP (SPBs) were imaged by time-lapse microscopy. The SPBs are marked with pink asterisks. The elapsed time is shown in minutes. (C) *cps1*-191 and *cps1*-191 *mid1*Δ cells were fixed and stained with antibodies against tubulin and Cdc4p. Representative images of cells with intermediate length and elongated spindles as well as those with postanaphase arrays (PAA) are shown. (D) Quantitation of orthogonal rings and randomly oriented cables in *cps1*-191 and *cps1*-191 *mid1*Δ cells at various stages of mitosis. At least 50 cells were scored in each category. Bars, 5 µm.
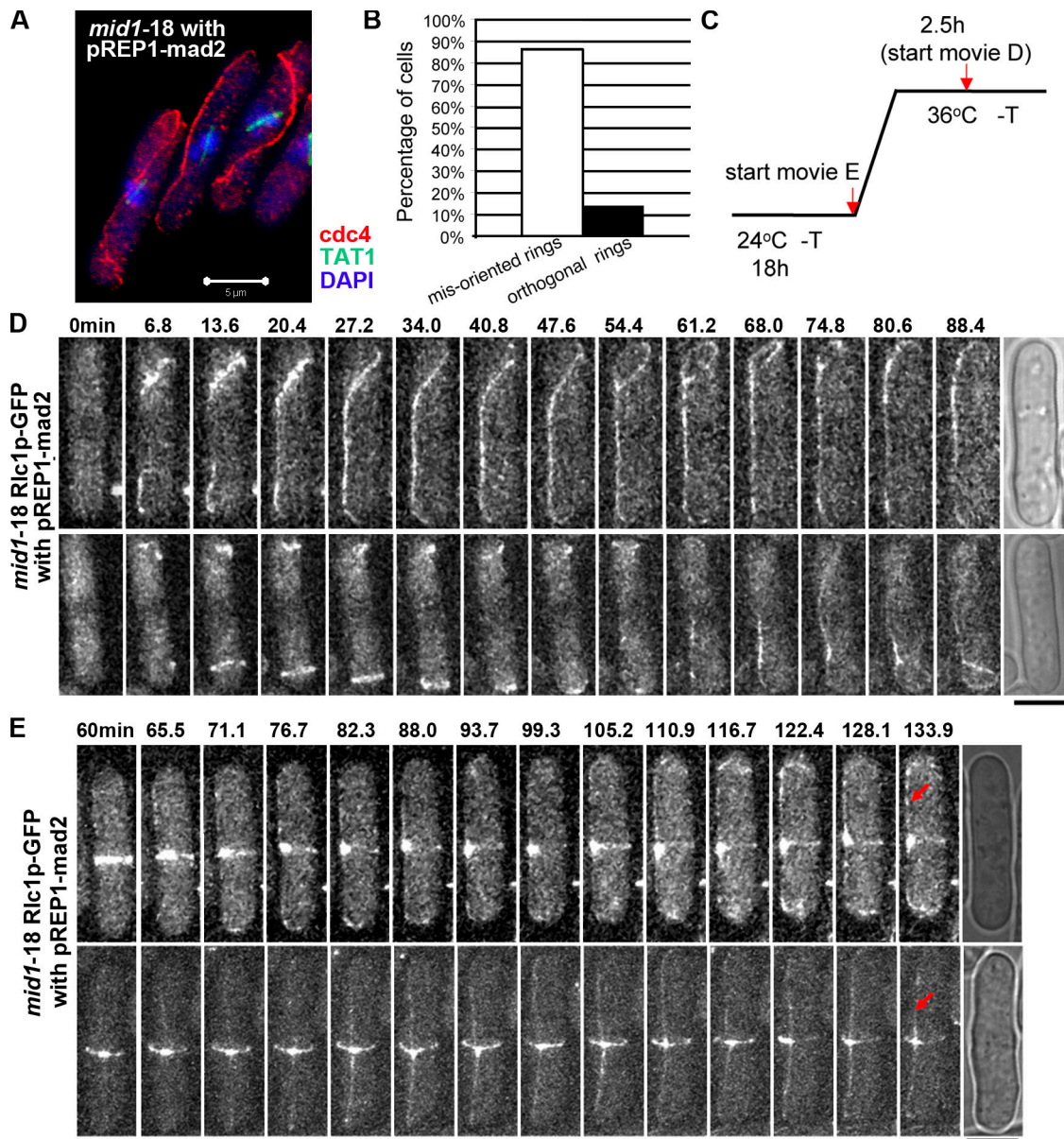
Figure 4. **Mid1p and cortical nodes are important for orthogonal ring assembly in early mitosis.** (A) *mid1*-18 cells expressing Rlc1p-GFP were arrested in metaphase by overproduction of the mitotic checkpoint protein Mad2p expressed under control of the thiamine-repressible *nmt1* promoter. Cells were grown in the absence of thiamine for 18 h at 24°C, shifted to 36°C for 3.5 h, and fixed and stained with Tat1 antibodies (tubulin), Cdc4p antibodies (rings), and DAPI (nuclei). (B) Quantitation of orthogonal rings and misoriented rings in metaphase-arrested *mid1*-18 cells. (C) Schematic representation of imaging experiments in D and E. (D) Time-lapse imaging of myosin II ring/cable assembly in metaphase-arrested *mid1*-18 cells. 0 min refers to the time when the imaging was initiated (2.5 h after the shift to 36°C). (E) Time-lapse imaging of unraveling of a preformed medial myosin II ring in a metaphase-arrested *mid1*-18 cell upon shift to the restrictive temperature (red arrows). Bars, 5 µm.

To further investigate the timing of myosin II ring organization in *cps1*-191 *mid1*Δ, we fixed and stained *cps1*-191 and *cps1*-191 *mid1*Δ cells with antibodies against tubulin and Cdc4p. Consistent with the time-lapse experiments, fully formed myosin II rings were detected in *cps1*-191 cells with intermediate length and elongated spindles as well as in cells with postanaphase microtubule arrays (Fig. 3, C and D). In contrast, in the *cps1*-191 *mid1*Δ cells, fully formed myosin II rings were observed in <20% of cells with intermediate length spindles and in <35% of cells with elongated spindles (Fig. 3, C and D). However, fully formed myosin II rings were detected in nearly 90% of *cps1*-191 *mid1*Δ cells (Fig. 3, C and D). These experiments

suggested that Mid1p and cortical nodes play a role in organizing actomyosin rings in early mitosis but that other mechanisms operate in later stages of mitosis to this end.

To independently test whether Mid1p was important for ring assembly in early mitotic cells, we arrested *mid1*-18 cells expressing Rlc1p-GFP at metaphase by overproduction of the mitotic checkpoint protein Mad2p (He et al., 1997). We performed three different experiments. First, we induced the expression of Mad2p, shifted cells to the restrictive temperature for *mid1*-18, and fixed and stained these cells with antibodies against Cdc4p, tubulin, and DAPI. In this experiment, we found that >85% of metaphase-arrested *mid1*-18 cells contained nonorthogonal
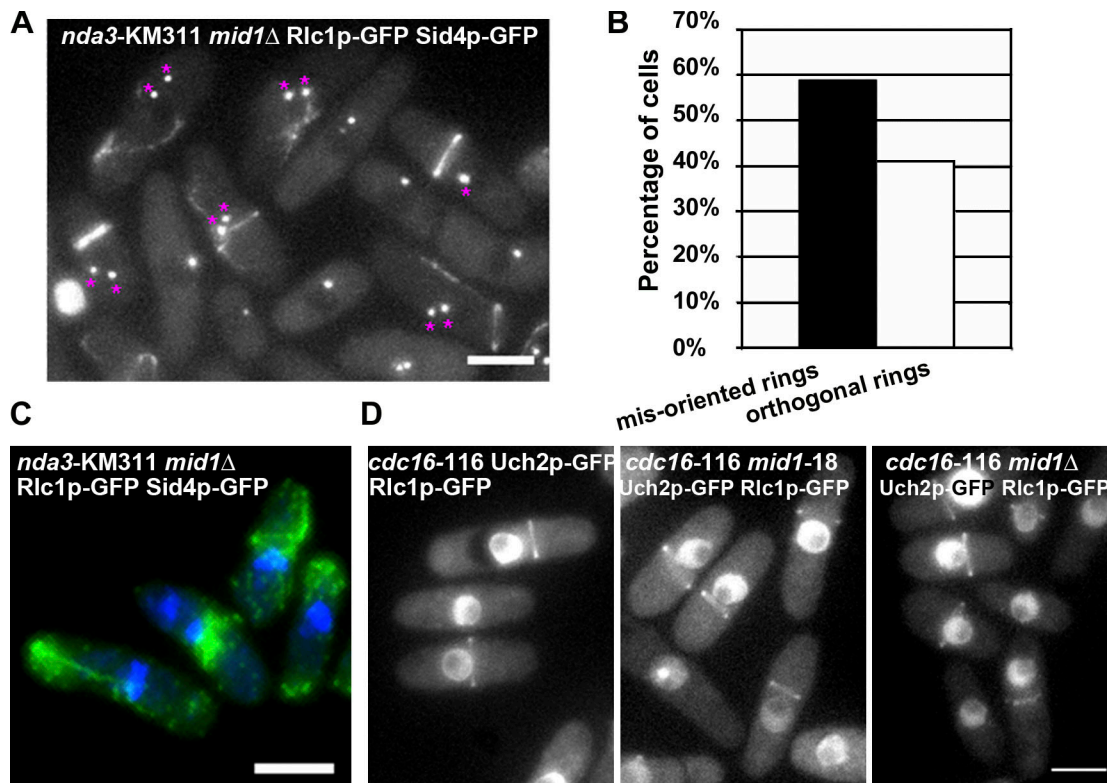
Figure 5. **Upon activation of the SIN, Mid1p and cortical nodes are not required for orthogonal ring assembly.** (A) *nda3*-KM311 *mid1Δ* cells expressing Rlc1p-GFP and Sid4p-GFP were cultured at 18°C for 6 h and imaged by fluorescence microscopy. The SPBs are marked with pink asterisks. (B) Quantitation of orthogonal rings and misoriented rings in *nda3*-KM311 *mid1Δ* cells. (C) Prometaphase-arrested *nda3*-KM311 *mid1Δ* cells were fixed and stained with Alexa Fluor 488 phalloidin and DAPI to visualize the F-actin cables and rings and the nuclei. (D) Upon SIN activation, orthogonal actomyosin rings assemble in the absence of Mid1p and cortical nodes. Cells of the indicated genotypes expressing Rlc1p-GFP and Uch2p-GFP were arrested in interphase by treatment with 12 mM HU for 6 h at 24°C and shifted to 36°C to inactivate Cdc16p function in the presence of HU, and images were captured. Bars, 5 μm.

myosin II cables (Fig. 4, A and B). Second, we imaged the process of myosin II ring assembly in metaphase-arrested *mid1*-18 cells (Fig. 4, C and D). We found that myosin II cables and bundles assembled efficiently in metaphase-arrested *mid1*-18 cells. However, these myosin II cables failed to become organized into rings even upon incubation for nearly 90 min (Fig. 4 D). Finally, we induced the expression of Mad2p, allowed myosin II ring assembly at the permissive temperature for *mid1*-18, and subsequently shifted cells to the restrictive temperature (Fig. 4, C and E). We found that shift of metaphase-arrested *mid1*-18 cells with a preexisting myosin II ring to the restrictive temperature led to unraveling and disassembly of this ring (Fig. 4 E, red arrows). These observations suggested that Mid1p and possibly the nodes were important for organization of actomyosin rings in early mitosis and that Mid1p function might be continuously required for the medial maintenance of the actomyosin ring until septation. The fact that metaphase-arrested unseptated *mid1*-18 cells failed to organize orthogonal rings ruled out the possibility that prevention of septation in *cps1*-191 *mid1⁻* mutants was solely responsible for actomyosin ring organization. Furthermore, this result suggested that some aspect of regulation occurring after metaphase is responsible for the organization of orthogonal rings in *cps1*-191 *mid1⁻* cells.

To further assess whether Mid1p and the cortical nodes were important for ring assembly in metaphase, we arrested

nda3-KM311 and *nda3*-KM311 *mid1Δ* cells at prometaphase by a shift to 18°C. *nda3*-KM311 is a cold-sensitive mutant allele of the sole gene encoding the fission yeast β-tubulin (Hiraoka et al., 1984). *nda3*-KM311 cells arrest with an actomyosin ring (Chang et al., 1996). Interestingly, we found that nearly 59% of *nda3*-KM311 *mid1Δ* cells contained misoriented rings, whereas the rest contained orthogonal rings (Fig. 5, A–C; Rlc1p and Sid4p, an SPB component [Chang and Gould, 2000], are shown in A, whereas F-actin is shown in C). In contrast, rings in the *nda3*-KM311 cells were always orthogonal (unpublished data). These results furthered the notion that the function of Mid1p and cortical nodes was important for organization of normal actomyosin rings in early mitosis. The slightly higher proportion of orthogonal rings observed in *nda3 mid1Δ* cells might be caused by the prolonged block of *nda3*-KM311 cells at the restrictive temperature.

Activation of the septation initiation network (SIN) is sufficient for the assembly of orthogonal actomyosin rings in the absence of Mid1p and cortical nodes

Our experiments have led to the conclusion that in the absence of Mid1p and nodes, a second pathway can allow organization of actomyosin rings. Because rings in *mid1* mutants assembled later in mitosis, it was possible that some aspect of mitotic

progression promoted ring assembly in mutants such as the *cps1*-191 *mid1*Δ cells. Alternatively, because loss of Cps1p function leads to a prolonged maintenance of the SIN, it was possible that the SIN played a role in organization of actomyosin rings in the absence of Mid1p and the cortical nodes. The SIN is a signaling module that is activated upon passage through anaphase and plays a key role in maintenance and organization of actomyosin rings (Krapp et al., 2004; Wolfe and Gould, 2005). Previous experiments have shown that ectopic activation of the SIN led to the formation of actomyosin rings even in interphase cells (Cerutti and Simanis, 1999). Therefore, we tested whether activation of SIN in interphase-arrested *mid1*-18 and *mid1*Δ cells would lead to the formation of orthogonal actomyosin rings. We shifted *cdc16*-116, *cdc16*-116 *mid1*-18, and *cdc16*-116 *mid1*Δ cells expressing Rlc1p-GFP and Uch2p-GFP (as a nuclear marker) to the restrictive temperature for *cdc16*-116. Cdc16p is a GTPase-activating protein for Spg1p-GTPase, a key component of the SIN (Furge et al., 1998). *cdc16*-116 mutants maintain Spg1p in a GTP-bound form, leading to persistent SIN activation. The use of *cdc16*-116 also allowed us to distinguish whether SIN or some aspect of anaphase was important for ring assembly in *mid1* mutants. Interestingly, myosin II rings in interphase-arrested *cdc16*-116, *cdc16*-116 *mid1*-18, and *cdc16*-116 *mid1*Δ were all found to be orthogonal in orientation (Fig. 5 D). These experiments established that activation of SIN led to the organization of orthogonal actomyosin rings in the absence of Mid1p and cortical nodes. *mid1*-6 mutant cells deleted for *par1*, the gene encoding a regulatory subunit of phosphatase 2A, assemble orthogonal division septa (Le Goff et al., 2001). Although actomyosin rings were not looked at in this study, the fact that Par1p is a negative regulator of the SIN supports the idea that SIN activation helps establish orthogonal actomyosin rings. Additional genetic experiments (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200806151/DC1) provided evidence that organization of actomyosin bundles in *mid1* mutants and their reorganization in the *cps1*-191 background depended on the function of the SIN.

Our experiments with two mutants (*mid1* and *plo1*) lacking nodes have established that the accumulation of actomyosin ring components into nodes is not essential for organization of orthogonal rings, although Mid1p and the nodes are important for the kinetics of ring assembly. How do Mid1p and cortical nodes participate in actomyosin ring assembly? Previous experiments using electron microscopy of myosin SI decoration of F-actin filaments have shown that in early mitosis, the actomyosin ring is composed of two parallel bundles of F-actin organized together with other components of the ring (Kamasaki et al., 2007). It is possible that such an arrangement is achieved by actin filament cross-linking rather than by myosin II–based contractility, which depends on antiparallel actin filaments. Consistent with the idea that myosin II may not be fully active in early mitosis, Lord and Pollard (2004) have shown that the localization of the UCS (UNC-45/CRO1/SHE4) domain protein Rng3p, a fission yeast myosin II activator, occurs only after the completion of anaphase. Finally, actomyosin ring constriction (possibly via activation of myosin II–based contractility) occurs concomitant with the activation of SIN in anaphase B (Sparks et al., 1999). Collectively, previous work has shown that at the point of actomyosin ring assembly, myosin II might not be fully active (Lord and Pollard, 2004). It is possible that Mid1p and cortical nodes play a role in the organization of moderately or noncontractile actomyosin bundles into ring structures. Activation of myosin II (upon SIN activation and Rng3p localization) might be sufficient for the organization of orthogonal actomyosin rings even in the absence of Mid1p and cortical nodes. Further analysis of actomyosin ring assembly in *cps1*-191 *mid1*Δ cells should provide additional insights into the general mechanism of cytokinesis.

Materials and methods

Yeast strains and methodology

The fission yeast strains used in this study are listed in Table I. Strain Rlc1-3GFP-KanMX6 was provided by J.Q. Wu (Ohio State University, Columbus, OH), strain *mid1*-ΔNES was provided by F. Chang (Columbia University College of Physicians, New York, NY), and strain Cdc15p-GFP was provided by K. Gould (Howard Hughes Medical Institute, Nashville, TN). Strain Pcp1p-GFP was provided by S. Oliferenko (Temasek Life Sciences Laboratory, Singapore). To arrest cells in interphase, cells were first treated with 12 mM hydroxyurea (HU; Sigma-Aldrich) for 4 h and treated with the same amount of HU for an additional 2 h. Conventional fission yeast genetic and molecular biology techniques were performed as described previously (Moreno et al., 1991).

Microscopy

Cells were fixed and stained with DAPI, Alexa Fluor 488–conjugated phalloidin, Tat1 (provided by K. Gull, University of Manchester, England, UK), and Cdc4p primary antibodies and α-rabbit and α-mouse IgG conjugated with either Alexa Fluor 594 or 488 (Invitrogen) secondary antibodies as described previously (Moreno et al., 1991). Live cell imaging and fluorescence microscopy were performed as described previously (Tran et al., 2004). For time-lapse microscopy, cells were concentrated from early log-phase culture and spotted on a glass slide containing a yeast extract with supplements agarose pad. Microscopic images were captured using a fluorescence microscope (IX71; Olympus) equipped with a camera (CoolSNAP ES; Photometrics) and a Plan Apo 100× 1.45 NA objective lens (Olympus). For confocal microscopy and time-lapse microscopy, cells were observed under a confocal microscope (LSM 510; Carl Zeiss, Inc.) equipped with a Plan Apo 100 ×1.3 NA objective lens, a 405-nm diode laser (for DAPI excitation), a 488-nm argon laser (for GFP excitation), and a 543-nm HeNe laser (for Alexa Fluor 594 excitation). Confocal images were captured with LSM 510 software (Carl Zeiss, Inc.). The time-lapse analysis was conducted at 36°C. The temperature was controlled by an objective heater (Bioptechs). All of the images were analyzed with Metamorph software (MDS Analytical Technologies). All images in Figs. 1, 5, and 2 (A and B) were captured on a microscope (Olympus), whereas a confocal microscope (LSM 510) was used for the rest of the imaging experiments. All of the images were collected in 3D mode (0.5–0.6-μm step size) and are displayed as a maximum projection.

Online supplemental material

Fig. S1 shows actomyosin ring/bundle assembly and maintenance in the absence of Mid1p and that the cortical nodes depend on the SIN. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200806151/DC1.

Table I. **_S. pombe_ strains used in this study**

Strain number	Relevant genotype	Source
MBY102	_ade6_-210 _ura4_-D18 _leu1_-32 h+	Laboratory collection
MBY297	_mid1_-18, _leu1_-32, _ade6_-21X, h+	Laboratory collection
MBY461	_cps1_-191 _sid2_-250	Laboratory collection
MBY628	rlc1-GFP::ura4+ _cdc25_-22 h+	Laboratory collection
MBY1148	_cps1_-191 _ade6_-21x _ura4_-D18 _leu1_-32 h+	Laboratory collection
MBY1287	_mid1_-18 rlc1-GFP::ura4+ _ade6_-21x _leu1_-32 _ura4_-D18 h−	Laboratory collection
MBY1463	_plo1_-1, _leu1_-32, _ura4_-D18, _ade6_-21X, h−	Laboratory collection
MBY2016	_mid1_::ura4+, _leu1_-32, _ade6_-210, h−	Laboratory collection
MBY2019	_ade_-M216 _ura4_-D18 _leu1_-32 _dmf1_::ura4+ h- + pAP116 integrated (pmid1-dNES1+2 mid1 leu1+)	Laboratory collection
MBY2120	_cps1_-191 _clp1_::ura4+	Laboratory collection
MBY2378	_cdc25_-22 rlc-GFP::ura4+ _mid1_::ura4+	Laboratory collection
MBY2541	pCDL514 (pREP1-mad2) in MBY1287	Laboratory collection
MBY2591	_cps1_-191 rlc1-GFP::ura4+ _ura4_-D18 _leu1_-32 _ade6_-M21x h+	Laboratory collection
MBY2603	_cdc16_-116 uch2-GFP::ura4+ rlc-GFP::leu1+	This study
MBY2697	_cps1_-191 _mid1_-ΔNES1+2 leu+	This study
MBY3067	_mid1_-18 _clp1_::ura4+ rlc1-GFP::ura4+ _ura4_-D18 _leu1_-32 _ade6_-21x	Laboratory collection
MBY3503	_cdc16_-116 _mid1_-18 uch2-GFP::ura4+ rlc1-GFP::leu1+	This study
MBY3504	_cdc16_-116 _mid1_::ura4 uch2-GFP::ura4+ rlc1-GFP::leu1+	This study
MBY3957	_mid1_-18 _cps1_-191 rlc1-GFP::ura4+ h+	Laboratory collection
MBY4123	_cps1_-191 _mid1_::ura4+ rlc1-GFP h+	This study
MBY5242	_cps1_-191 _plo1_-1 rlc1-3GFP	This study
MBY5727	_cps1_-191 _mid1_::ura4 rlc1-GFP::ura+ pcp1-GFP::KanMX6	This study
MBY5730	cps1-191 rlc1-GFP::ura+ pcp1-GFP::KanMX6	This study
MBY5737	_cdc25_-22 cdc12-GFP::ura4+	This study
MBY5738	_cdc25_-22 _mid1_::ura4+ cdc12-GFP::ura4+	This study
MBY5739	_cdc25_-22 cdc15-GFP::KanMX6	This study
MBY5740	_cdc25_-22 _mid1_::ura4+ cdc15-GFP::KanMX6	This study

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