Introduction

The budding yeast *Saccharomyces cerevisiae* is a compelling model for the study of cell division because of its intrinsic geometric constraints: the daughter cell is formed by polarized growth at a specified site on the cortex of the mother, and the junction between the bud and mother, termed the bud neck, is the eventual site of cytokinesis. The success of mitosis, therefore, depends on positioning the mitotic spindle through the bud neck so that genomes lie on either side. Spindle positioning is accomplished by cytoplasmic microtubules that project outward from the spindle pole bodies (SPBs; the yeast centrosome equivalent) and interact with molecular motors at the cell cortex (Moore and Cooper, 2010). These interactions orient the spindle along the bud–mother axis and pull one end of the spindle through the neck with force provided by the microtubule motor dynein and its activator dynactin.

When the mechanisms that position the spindle are impaired, a cell may enter mitosis without moving the spindle into the bud neck. Under these conditions, however, the cell will remain in anaphase until the alignment of the spindle is corrected and one SPB moves through the bud neck. This delay is caused by a cell cycle checkpoint known as the spindle position checkpoint (SPC), which inhibits the mitotic exit network (MEN), thereby preventing the deactivation of cyclin-dependent kinase (Bardin et al., 2000; Bloecher et al., 2000; Daum et al., 2000; Pereira et al., 2000).

The activity of the SPC must be coordinated with mother–daughter polarity to prevent mitotic exit when the entire spindle is within the mother compartment and to permit mitotic exit once one SPB moves through the bud neck. This coordination depends on a Ras-like GTPase, Tem1, which localizes to the SPBs and activates the MEN (Bardin et al., 2000; Molk et al., 2004). Tem1 is negatively regulated by a bipartite putative GTPase-activating protein complex, Bub2–Bfa1, which also localizes to the SPBs (Pereira et al., 2000; Geymonat et al., 2002; Ro et al., 2002). The association of Bub2–Bfa1 with the SPBs is critical for SPC function, and it is regulated by another SPC component, the protein kinase Kin4 (Maekawa et al., 2007; Caydasi and Pereira, 2009). Tem1 is positively regulated by the putative guanine nucleotide exchange factor, Lte1, which localizes to the bud (Shirayama et al., 1994). Lte1 polarity is critical for the integrity of the SPC; mutations that allow Lte1 to access the mother compartment also disrupt the SPC (Bardin et al., 2000; Pereira et al., 2000; Castillon et al., 2003). Although genetic data indicate that Lte1 activates Tem1, the precise function of Lte1 has not been characterized. Importantly, Lte1 has not been shown to provide exchange activity toward Tem1.
(Geymonat et al., 2009). Together, these results support a model in which Tem1 activity depends on the location of the SPBs; only when an SPB enters the bud does Tem1 encounter its activator and mitotic exit commence.

Although this model is consistent with the observed correlation between spindle position and the timing of mitotic exit, it does not explain similar correlations observed in mutants that lack Lte1. LTE1 is necessary for mitotic exit at low temperatures (<18°C), but lte1Δ-null mutant cells are viable at higher temperatures and also undergo mitotic exit after one SPB moves through the bud neck with kinetics similar to wild-type cells (Shirayama et al., 1994; Adames et al., 2001). SPC activity must therefore be coordinated with mother–daughter polarity via mechanisms that do not involve Lte1.

Several lines of evidence indicate that interactions between cytoplasmic microtubules and the bud neck are essential for this coordination. First, disrupting cytoplasmic microtubule interactions with the bud neck by mutations or laser cutting destabilizes the SPC (Adames et al., 2001; Moore et al., 2009). Second, the SPC depends on the network of septin filaments at the bud neck (Castillon et al., 2003). Third, interactions between cytoplasmic microtubules and unidentified factors associated with the bud neck and/or bud cortex regulate the dynamics of Bub2–Bfa1 association with the SPBs (Pereira et al., 2001; Fraschini et al., 2006; Caydasi and Pereira, 2009; Monje-Casas and Amon, 2009). These results indicate that signaling factors at the bud neck promote SPC activity while the spindle is within the mother compartment.

To elucidate this signaling pathway, we screened mutants of bud neck–localized proteins for a loss-of-SPC phenotype. We find that the protein kinase Elm1 is necessary for the SPC and that this function requires its kinase activity and localization to the bud neck. Furthermore, our results indicate that the function of Elm1 in the SPC is independent of previously identified roles. Elm1 is closely related to the kinases Sak1 and Tos3, and we show that sak1 and tos3 mutants also exhibit disruption-of-SPC phenotypes, albeit with decreased penetrance. Finally, we provide evidence that the role of Elm1 in the SPC is to activate the Kine4 kinase. These findings uncover a novel step in the SPC and suggest how a polarized regulator may influence asymmetric SPC activity.

Results

ELM1 prevents spindle disassembly in the mother compartment

To identify factors at the bud neck that contribute to the SPC, we screened deletion mutants of neck-localized proteins for a loss-of-SPC phenotype. We combined these mutations with null mutations in dynemin–dynactin to disrupt spindle positioning, and we recorded time-lapse videos of GFP-labeled microtubules to monitor spindle morphology as an indicator of mitotic exit. We found that elm1Δ mutant cells did not prolong mitosis when the spindle failed to move through the bud neck; instead, these spindles disassembled within the mother, resulting in binucleate mothers and anucleate daughters (Fig. 1 A). This phenotype is the defining characteristic of SPC loss-of-function mutants, such as kin4Δ (Fig. 1 B; Blocher et al., 2000; Pereira et al., 2000; Castillon et al., 2003; D’Aquino et al., 2005; Pereira and Schiebel, 2005; Nelson and Cooper, 2007).

Elm1 is a multifunctional kinase with roles in several signaling pathways. To determine whether the SPC phenotype of elm1Δ mutants could be attributed to defects in these pathways, we examined strains bearing mutations in known Elm1 substrates combined with the arplΔ mutation, which abolishes dynein–dynactin function. One substrate of Elm1 is Snf1, the yeast AMP-activated protein kinase (AMPK; Hong et al., 2003; Sutherland et al., 2003). We analyzed snf1Δ mutants in our SPC integrity assay and found no detectable loss of SPC integrity (Fig. 1 B); thus, the function of Elm1 in the SPC does not involve AMPK/Snf1.

Elm1 also functions in a signaling cascade that controls morphogenesis by coupling bud growth with the G2/M transition. In this pathway, Elm1 phosphorylates Hsl1, promoting its interaction with Hsl7 (Sztotnicki et al., 2008). The Hsl1–Hsl7 complex recruits the Wee1-family kinase Swe1 to the bud neck (Longtine et al., 2000), where it is deactivated via phosphorylation by Cdc5/polo kinase (Sakchai et al., 2004). We hypothesized that the disruption of the SPC in elm1Δ mutants could be attributed to increased Swe1 activity. We tested this by first examining whether loss of Hsl1 disrupted the SPC similar to elm1 mutants. To the contrary, hsl1Δ mutants exhibited no detectable defect in SPC integrity (Fig. 1 B). Next, we examined SPC integrity in the presence of a stabilized mutant of Swe1, swe1Δ1 (McMillan et al., 2002). This mutant showed a slight disruption of the SPC but was not significantly different from wild-type cells (P = 0.4; Fig. 1 B). To test whether Swe1 was necessary for SPC failure in elm1Δ mutants, we combined the swe1Δ- and elm1Δ-null mutations. These cells exhibited checkpoint failure at a rate similar to elm1Δ mutants (P = 0.64; Fig. 1 B). Furthermore, the swe1Δ mutant alone exhibited impaired SPC integrity in the presence of wild-type ELM1, but this phenotype was less penetrant than elm1Δ (P = 0.03; Fig. 1 B). We conclude that the loss-of-SPC phenotype in elm1Δ mutants cannot be attributed to enhanced Swe1 activity.

Elm1-family kinases (EFKs) exhibit varying effects on SPC integrity

Elm1, Sak1, and Tos3 comprise the EFKs, which exhibit overlapping roles in the phosphoregulation of AMPK/Snf1 along with sequence similarity within the kinase domains (Hunter and Plowman, 1997; Hong et al., 2003; Sutherland et al., 2003; Rubenstein et al., 2006). We found that null mutants of SAK1 and TOS3 exhibit intermediate levels of SPC impairment (Fig. 1 C), indicating that each of the EFKs is important for the integrity of the SPC.

To determine whether the kinase activities of the EFKs are important for their function in the SPC, we introduced point mutations in ELM1, SAK1, and TOS3 designed to abrogate kinase activity (Blacketer et al., 1993; Nath et al., 2003). In each case, kinase-defective alleles exhibited defects in SPC integrity that were identical to the deletion mutants (Fig. 1 C). Thus, kinase activity appears to be necessary for the function of the EFKs in the SPC.
Next, we performed epistasis experiments to determine whether the EFKs exert redundant functions for the SPC. First, we generated sak1Δ tos3Δ double mutants in haploid cells; these exhibited a level of SPC integrity similar to either mutant alone (P = 0.34 compared with sak1Δ; P = 0.25 compared with tos3Δ; Fig. 1 C), suggesting that Sak1 and Tos3 are not functionally redundant and may act in the same pathway. The complete loss of SPC integrity in elm1Δ haploid mutants prevents the analysis of additive effects when combined with sak1Δ and tos3Δ. Furthermore, diploid strains heterozygous for elm1Δ did not exhibit defects in SPC integrity and did not confer an additive defect when combined with the homozygous null alleles of sak1 and tos3 (unpublished data). Thus, we cannot determine whether the function of Sak1 or Tos3 overlaps with Elm1. Nevertheless, our results suggest a prominent and necessary role for Elm1 in the SPC; in contrast, Sak1 and Tos3 serve minor roles and may act in a common pathway.

Structure function and localization analysis

Given the similarity among the EFKs, we asked what features of the Elm1 protein might confer its pronounced role in the SPC. The kinase domains of Elm1, Sak1, and Tos3 show sequence similarity (Elm1: 51% similarity to Sak1 and 53% similarity to Tos3), but the regions carboxy terminal to the kinase domains are highly divergent. A previous study demonstrated that the carboxy-terminal region of Elm1 was not necessary to regulate AMPK/Snf1; however, this region was essential for the unique function of Elm1 in regulating morphogenesis (Rubenstein et al., 2006). To test whether this carboxy-terminal region was required for the SPC, we generated a truncated allele similar to the one described by Rubenstein et al. (2006). The allele was expressed from the endogenous locus. This mutant, elm1ΔK117R, completely lost SPC integrity (Fig. 1 C). Therefore, the carboxy-terminal region of Elm1 confers a function that is necessary for the SPC.

We considered that the localization of EFKs within the cell may be important for the SPC. Consistent with previous findings, we observed fluorescently tagged Elm1 at the bud neck in dividing cells (Fig. 2 A; Bouquin et al., 2000). Elm1 was also found at the neck in mitotic cells with aberrantly positioned spindles, indicating that Elm1 is enriched at the neck when the SPC is active (Fig. 2 B). In contrast, neither Sak1 nor Tos3 exhibited enrichment at the neck (Fig. S1). These results suggest that the presence of Elm1 at the bud neck may be important for its function in the SPC.

The bud neck is surrounded by a network of septin filaments, which provide a scaffold for signaling proteins and may act as a diffusion barrier for components of the mother and bud cytosol (Barral et al., 2000; Takizawa et al., 2000). We examined the distribution of Elm1 at the neck closely to determine its location relative to the septin network. Consistent with previous results, Elm1 colocalized with the septin network, labeled by Cdc3, during bud growth, but Elm1 was absent once the septins split into two rings, which occurs during cytokinesis (Kim et al., 1991; Bouquin et al., 2000; Lippincott et al., 2001). In large-budded cells with an hourglass-shaped septin network, Elm1 occupies a narrow band within the septin ring and proximal to the mother side of the neck (Fig. 2 C).
may have access to components of the mother cytoplasm.

Elm1 is present on the mother side of the bud neck, where it

exerts an additional role in the early step in the MEN pathway (Hartwell et al., 1973; Mah et al., 2001), and we tested whether this block increases the frequency of anaphase spindles in the mother compartment of

The SPC prolongs mitosis by inhibiting the activity of the MEN; therefore, we reasoned that Elm1 could either be important for inhibition of the MEN or for a later event within the MEN (Fig. 3 A). To determine whether Elm1 functions upstream of the MEN, we examined elmlΔ mutants under conditions in which the initiation of MEN signaling is abrogated. The temperature-sensitive cdc15-2 mutant disables the Cdc15 kinase, thereby blocking an early step in the MEN pathway (Hartwell et al., 1973; Mah et al., 2001), and we tested whether this block increases the frequency of anaphase spindles in the mother-compartment of elmlΔ mutant cells. When Cdc15 function was disrupted by shifting to the restrictive temperature (37 °C), elmlΔ cdc15-2 dyn1Δ cells accumulated in anaphase, and the percentage of cells with intact mitotic spindles within the mother compartment increased to a level similar to that of cdc15-2 dyn1Δ cells expressing wild-type

ELM1 (P = 0.66; Fig. 3 B). Loss-of-function mutations in bub2Δ, which are known to abolish the SPC, yielded results similar to the elmlΔ mutant (Fig. 3 B). We also tested the sak1Δ and tos3Δ mutants in this assay; however, our results were inconclusive because the decreased penetrance of these mutants failed to produce a phenotype distinguishable from the cdc15-2 dyn1Δ control cells (Fig. 3 B). We conclude that mitotic exit within the mother compartment of elmlΔ mutants requires the function of Cdc15, consistent with Elm1 acting upstream of the MEN as part of the SPC.

Two distinct biochemical pathways have been proposed to regulate the SPC: one attenuates the MEN activator Lte1 (Nelson and Cooper, 2007), and the other promotes the activity of the MEN inhibitor Bub2–Bfa1 (D’Aquino et al., 2005; Pereira and Schiebel, 2005; Maekawa et al., 2007; Chan and Amon, 2009). We performed a series of experiments to determine whether Elm1 functions upstream of Lte1 or Bub2–Bfa1. First, we asked whether Elm1 inhibits the activity of Lte1, in which case the SPc phenotype of elmlΔ mutants would be caused by hyper-active Lte1. We assayed elmlΔ lte1Δ double mutants for SPC integrity. We measured the frequency of multibudded cells, which are generated when cells exit mitosis with the spindle in the mother compartment and proceed to enter the next cell division without undergoing septation. Compared with the time-lapse video assay, this assay allows for rapid analysis of a greater number of cells. We found that elmlΔ lte1Δ double mutants generated multiple buds to the same extent observed in the elmlΔ single mutant (Fig. 3 C). Likewise, the defects of sak1Δ and tos3Δ mutants were not affected by the loss of Lte1. We also compared the localization of Lte1 in wild-type cells and elmlΔ mutants. In both cases, Lte1 was enriched in the bud, with no foci or accumulation of Lte1 seen in the mother compartment (Fig. S2). These data indicate that the role of the EFKs in the SPC is independent of Lte1.

Next, we asked whether Elm1 acts via regulation of Bub2–Bfa1. The SPC promotes the activity of Bub2–Bfa1 through the Kin4 kinase (Pereira and Schiebel, 2005; Maekawa et al., 2007). Whereas loss of Kin4 disables the SPC, overexpression of Kin4 constitutively inhibits mitotic exit, resulting in growth arrest (D’Aquino et al., 2005; Pereira and Schiebel, 2005). We examined whether Elm1 is necessary for the function of Kin4 using the growth arrest induced by Kin4 overexpression as a measure of activity. We found that elmlΔ mutants rescued the growth inhibition of Kin4 overexpression, similar to bub2Δ and bfa1Δ controls (Fig. 3 D). In contrast, sak1Δ and tos3Δ mutants were inhibited, similar to wild-type cells, and triple mutants lacking all three EFKs grew similar to the elmlΔ single mutant (Fig. 3 D).

Because the function of Kin4 is to activate Bub2–Bfa1, we next tested whether Elm1 was necessary for the function of Bfa1. Bfa1 overexpression, like Kin4 overexpression, inhibits mitotic exit and causes growth arrest (Li, 1999). Overexpression of Bfa1 inhibited the growth of elmlΔ mutants, similar to wild-type cells, indicating that Elm1 is not downstream of Bfa1 (Fig. 3 D). Furthermore, the Bfa1 overexpression phenotype was not suppressed by the loss of the two other EFKs, the loss of Bub2 or Kin4, or the simultaneous loss of Elm1 and Kin4 (Fig. 3 D).
Together, these results suggest that Elm1 is necessary for the function of Kin4 and that Elm1 functions upstream of Bfa1 in the SPC.

If Elm1 acts in a common pathway with Kin4 and Bfa1, the overexpression of Elm1 might be expected to hyperactivate the SPC and delay mitotic exit. We tested this hypothesis using growth assays and cell cycle analysis under conditions in which ELM1 was ectopically expressed at high levels. Neither assay showed evidence of impaired mitotic exit; overexpression of ELM1 did not suppress growth, nor did it cause an accumulation of mitotic cells over time (Fig. S3). These results indicate that, unlike Kin4 and Bfa1, increased levels of Elm1 activity are not sufficient to inhibit mitotic exit.

**Elm1 is not necessary for the spindle assembly checkpoint (SAC)**

In addition to the SPC, Bub2 and Bfa1 function in cell cycle checkpoints that respond to spindle assembly errors and DNA damage (Hoyt et al., 1991; Li, 1999; Wang et al., 2000; Hu et al., 2001). We tested whether Elm1 might also have checkpoint functions beyond the SPC. Treatment with nocodazole destabilizes the yeast microtubule cytoskeleton and triggers cell cycle arrest by the SAC. Whereas wild-type cells delay cell cycle progression in the presence of nocodazole, SAC mutants (mad2Δ, bfa1Δ, and bub2Δ) complete the cell cycle and enter another round of division, forming an additional bud (Fig. 4). We found that elm1Δ mutants delay cell cycle progression in nocodazole (Fig. 4). Consistent with previous results, kin4Δ mutants also exhibit cell cycle delay in nocodazole (Fig. 4; D’Aquino et al., 2005; Pereira and Schiebel, 2005). This suggests that Elm1, like Kin4, is not necessary for the SAC. We did, however, observe an increase in the frequency of multibudded elm1Δ and kin4Δ cells after several hours in nocodazole. Although the basis for this increase is not clear, we have observed similar effects in other mutants that promote the activity of the MEN (unpublished data). We conclude that Elm1 functions primarily in the SPC.

**Does Elm1 influence the localization of Kin4?**

Kin4 localizes to the mother cell cortex, SPBs, and bud neck, and its function in the SPC requires exchange between these sites (D’Aquino et al., 2005; Pereira and Schiebel, 2005; Maekawa et al., 2007; Chan and Amon, 2009). To determine whether Elm1 regulates the localization of Kin4, we visualized Kin4 in living cells by generating a functional fusion of dimeric RFP (tdimer2) to the carboxy terminus of the endogenous gene. Although we did not observe a distinct enrichment of the and a 10-fold dilution series was spotted onto media selective for plasmid retention. Plates contained either galactose to induce expression or glucose to inhibit expression. Strains containing empty vector are shown as controls. Strains: wild type (wt), yJC2295; elm1Δ, yJC5254; tos3Δ, yJC6419; sak1Δ, yJC6492; elm1Δ sak1Δ tos3Δ, yJC6474; bub2Δ, yJC5251; bfa1Δ, yJC6447; kin4Δ, yJC6448; and kin4Δ elm1Δ, yJC6573. Plasmids: pGAL-KIN4, pBJ1651; pGAL-BFA1, pBJ1652; and vector, pBJ216. Values are the means of five counts of at least 50 cells. Error bars are the standard error of the means.

---

**Figure 3. Elm1 functions in the SPC.** (A) Diagram of SPC regulation of mitotic exit. (B) Inappropriate mitotic exit in EFK mutants requires Cdc15 activity. Asynchronous cultures were grown to mid-log phase at 23°C, diluted 1:10 into new media, and incubated at either 23°C or 37°C for 3.5 h. SPC activity was measured by scoring the percentage of cells with intact mitotic spindles (>2 µm) within the mother compartment, based on GFP-labeled microtubules. Strains: dyn1Δ cdc15-2, yJC6380; bub2Δ dyn1Δ cdc15-2, yJC6497; elm1Δ dyn1Δ cdc15-2, yJC6926; sak1Δ dyn1Δ cdc15-2, yJC6493; and tos3Δ dyn1Δ cdc15-2, yJC7083. (C) Failure of the SPC (i.e., inappropriate mitotic exit) in EFK mutants does not require Lte1. Indicated strains expressing either wild-type LTE1 or the lte1Δ-null mutant were arrested at START by treatment with α-factor and released into fresh media at 23°C. After 3 h, the percentage of cells exhibiting multiple buds, which is indicative of checkpoint failure, was determined. Strains: dyn1Δ, yJC4078; dyn1Δ lte1Δ, yJC7066; dyn1Δ elm1Δ, yJC7067; dyn1Δ elm1Δ lte1Δ, yJC7068; dyn1Δ sak1Δ, yJC7071; dyn1Δ sak1Δ lte1Δ, yJC7072; dyn1Δ tos3Δ, yJC7069; and dyn1Δ tos3Δ lte1Δ, yJC7070. (D) Elm1 is required for the growth inhibition caused by Kin4 overexpression. High-copy plasmids containing KIN4 or BFA1 under the control of a galactose-inducible promoter were transformed into the indicated strain background,
Kin4-tdimer2 signal on the mother cortex in our experiments, we did detect Kin4 at the SPB in the mother compartment during mitosis (Fig. 5 A). When the mitotic spindle was entirely within the mother, Kin4 prominently localized to both SPBs, consistent with previous studies (Fig. 5 A; Pereira and Schiebel, 2005; Maekawa et al., 2007). In elm1Δ mutants, Kin4 was present at the SPB in the mother compartment during mitosis (Fig. 5 B). We conclude that Elm1 is not necessary for the localization of Kin4 to the SPBs.

We then measured the fluorescence intensity of the Kin4-tdimer2 signal at the neck in wild-type and elm1Δ mutant cells. Kin4-tdimer2 fluorescence was enriched at the bud neck in cells with mitotic spindles in the mother compartment, suggesting that Kin4 associates with the neck when the SPC is active (Fig. 5 C). Once cells exited mitosis, which is indicated by the disassembly of the spindle, the enrichment of Kin4 at the neck increased 10-fold (Fig. 5 A and D). In elm1Δ cells, Kin4 was not enriched at the bud neck when the spindle was in the mother, nor did we detect Kin4 at the neck in postmitotic elm1Δ cells (Fig. 5 B and E). These results suggest that Elm1 may contribute to the accumulation of Kin4 at the bud neck.

Is Elm1 necessary for activation of the Kin4 kinase?

Next, we asked whether Elm1 is important for activation of the Kin4 kinase. Elm1 activates the kinases Snf1 and Hsl1 by phosphorylating a threonine residue within the activation loop of the kinase domain (Fig. 6 A; Hong et al., 2003; Sutherland et al., 2003; Szotnicki et al., 2008). The analogous region of Kin4 contains a threonine at position 209, and preventing phosphorylation of this residue by substitution with alanine ablates Kin4 kinase activity and function in the SPC (D’Aquino et al., 2005; Maekawa et al., 2007). We therefore hypothesized that Elm1 may activate the Kin4 kinase by phosphorylating residue T209.

To determine whether Elm1 is necessary for the phosphorylation of Kin4, we first compared the phosphorylation of Kin4 in wild-type and elm1Δ mutants. Consistent with previous results, we observed Kin4 from extracts of asynchronously growing wild-type cells migrating as a primary band with a faint slower-migrating species on 1D SDS-PAGE (Fig. 6 B, lane 1; D’Aquino et al., 2005). The slower-migrating species was enriched when cells were arrested in metaphase by nocodazole treatment (Fig. 6 B, lane 3). In separate experiments, the slower-migrating species was depleted by treatment with phosphatase, confirming that this band represents phosphorylated Kin4 (unpublished data). The slower-migrating species was not detected in extracts from elm1Δ mutant cells, even during metaphase arrest (Fig. 6 B, lanes 2 and 4). Thus, phosphorylated species of Kin4 are diminished in the absence of Elm1, consistent with the notion that Elm1 phosphorylates Kin4.

If the critical function of Elm1 in the SPC is to phosphorylate T209 of Kin4, constitutive phosphorylation of this residue might obviate the need for Elm1. To test this hypothesis, we generated alleles of KIN4 that either mimic the negative charge of phosphorylation (kin4Δ-T209D) or prevent phosphorylation (kin4Δ-T209A). Overexpression of phosphomimetic Kin4-T209D strongly inhibited cell growth in a wild-type strain background, indicating that this mutant acts as a functional Kin4 kinase (Fig. 6 C). Furthermore, growth inhibition by Kin4-T209D overexpression required the downstream target of the Kin4 kinase, indicating that Kin4-T209D acts downstream of Elm1 in the SPC.
kinase, Bfa1, confirming that Kin4-T209D functions by activating the SPC (Fig. 6 C). In contrast, overexpression of the phosphoablated Kin4-T209A mutant did not inhibit growth, confirming that phosphorylation at T209 is necessary for function (Fig. 6 C; D’Aquino et al., 2005; Maekawa et al., 2007). To test whether Elm1 is necessary for the function of Kin4-T209D, we repeated the overexpression experiment in elm1Δ mutants. Overexpression of Kin4-T209D inhibited the growth of elm1Δ mutants, similar to wild-type cells (Fig. 6 C). Thus, Elm1 is not necessary for the function of Kin4 when T209 is constitutively phosphorylated, consistent with the hypothesis that Elm1 phosphorylates T209 of Kin4.

Next, we tested whether constitutive phosphorylation of Kin4 at T209 restored SPC function in elm1Δ mutants. For this, we constructed a phosphoimetic kin4-T209D allele at the chromosomal KIN4 locus, replacing the wild-type allele. Cells expressing kin4-T209D grew normally and did not exhibit prolonged mitoses (Fig. S4). We assayed SPC integrity by measuring the frequency of multinucleate cells and found that these were rare in the kin4-T209D mutant, similar to the level seen for wild-type KIN4 (Fig. 6 D; P = 0.84 when comparing ELM1 KIN4 cells with ELM1 kin4-T209D cells). This suggests that Kin4-T209D promotes the proper function of the SPC. When combined with the elm1Δ mutation, kin4-T209D suppressed the generation of multinucleate cells, indicating a restoration of SPC activity (Fig. 6 D). The frequency of multinucleate elm1Δ kin4-T209D cells was slightly greater than that of wild-type cells, but not significantly different (P = 0.14). Thus, constitutive phosphorylation of Kin4 at residue T209 eliminates the requirement for Elm1 in the SPC.

Is the accumulation of Elm1 at the bud neck necessary for the SPC?
Having identified a role for Elm1 in the SPC, we returned to the question of how the association of Elm1 with the bud neck might influence this function. Elm1 is enriched at the neck when the SPC is active (Fig. 2) and during nocodazole arrest (not depicted). We showed that Elm1Δ420, which retains the kinase domain but lacks the carboxy-terminal domain, is deficient in the SPC (Fig. 1 C). We found that Elm1Δ420-tdimer2 showed no fluorescence accumulation at the bud neck, suggesting that the carboxy-terminal domain is necessary to target Elm1 to the neck (Fig. 7 A). We also considered that truncation of the carboxy-terminal domain may disrupt other functions of Elm1 critical for the SPC. To assess the functionality of Elm1Δ420-tdimer2, we tested whether overexpression might restore function. Indeed, overexpressed Elm1Δ420-tdimer2 did support Kin4’s growth inhibition activity (Fig. 7 B), and it suppressed the accumulation of multiple microtubule-organizing centers (MTOCs) seen in elm1Δ dyn1Δ–null mutants (Fig. 7 C).

Next, we asked whether targeting the Elm1 kinase domain to the bud neck might rescue its function in the SPC. To test this, we created chimeras by fusing Elm1Δ420 to the neck-localized proteins Bni4 and Cdc4. Expression of either chimera rescued the elongated cell morphology phenotype of Elm1 mutants, indicating that both are at least partially functional. Both chimeras exhibited localization to the neck, but only Bni4-elm1Δ420-tdimer2 was enriched at the neck during mitosis (Fig. 7, D and E; and not depicted). Furthermore, only Bni4-elm1Δ420-tdimer2 supported Kin4 growth inhibition activity (Fig. 7 B) and suppressed the generation of multi-MTOC cells in an elm1Δ dyn1Δ–null mutant background (Fig. 7 C). These results indicate that accumulation of Elm1 at the bud neck promotes its function in the SPC.
The accumulation of Elm1 at the bud neck promotes its function in the SPC. (A) The localization of Elm1 to the bud neck requires its carboxy-terminal region. Tandem RFP/tdimer2 was integrated behind codon 420 of ELM1. Cells also express GFP-Tub1. Images were collected on a wide-field microscope. Strain: yC6854. [B] Kin4 activity is supported by overexpressed elm1Δ420 or chimeric Bni4-elm1Δ420. Strains with high-copy plasmids containing KIN4 under the control of a galactose-inducible promoter and 10-fold dilution series were spotted onto media selective for plasmid retention and containing either galactose to induce expression or glucose to inhibit expression. Strains containing empty vector are shown as controls. Strains: wild type [wt], yCJ2295; elm1Δ, yCJ5254; elm1Δ420-tdimer2, yCJ6849; pGAL-elm1Δ420-tdimer2, yCJ7286; BNI4-elm1Δ420-tdimer2, yCJ7292; and KCC4-elm1Δ420-tdimer2, yCJ7293. Plasmids: pGAL-KIN4, pBJ1651 and vector, pBJ216. (C) Multi-MTOC assay for SPC function. Mother compartment is indicated by light gray bars, and multiple MTOCs resulting from checkpoint failure are indicated by dark gray bars. Values are the means of 10 counts of at least 50 cells from two separate experiments. Error bars are the standard error of the means. Strains: dyn1Δ, yCJ5603; dyn1Δ elm1Δ, yCJ7079; dyn1Δ gal1Δ-elm1Δ420-tdimer2, yCJ7299,7300; dyn1Δ elm1Δ BNI4-elm1Δ420-tdimer2, yCJ7296,7297; and dyn1Δ elm1Δ KCC4-elm1Δ420-tdimer2, yCJ7298. [D] Bni4-elm1Δ420-tdimer2 localizes to the bud neck when the mitotic spindle is in the mother compartment. Cells are dyn1Δ mutants that also express GFP-Tub1. Images were collected on a wide-field microscope. Arrowhead points to Bni4-elm1Δ420-tdimer2 at the bud neck. Strain: yCJ7296. [E] Quantification of fluorescence intensities for Elm1-tdimer2 and Bni4-elm1Δ420-tdimer2 across the bud neck. Fluorescence intensities were measured [see Materials and methods] in cells with mitotic spindles in the mother based on GFP-Tubulin. Plotted values are the means from 12 cells for each strain. Solid lines indicate outlines of cells. Strains: Elm1-tdimer2, yC6852 and Bni4-elm1Δ420-tdimer2, yCJ7296. a.u., arbitrary units. Bars, 2 µm.

Discussion

In a previous study, we demonstrated that the activity of the SPC depends on the septin network at the bud neck, and, based on these results, we proposed that the neck serves as a platform for signaling mechanisms that inhibit mitotic exit when the spindle is delayed within the mother compartment (Castillon et al., 2003). This model could explain why movement of one spindle pole beyond the neck and into the bud is a critical event: this allows MEN components at that pole to escape the inhibitory activity of the SPC. In the current study, we seek to identify molecules involved in the sensing or signaling mechanism at the neck, using localization to the neck and requirement for SPC activity as criteria for our screen. Elm1 meets these criteria, and our results indicate that Elm1 contributes a specific function to the SPC that is independent of its known roles in the activation of AMPK/Snfl and the regulation of Swe1.

Elm1 is involved in organizing the septin network at the bud neck (Bouquin et al., 2000; Gladfelter et al., 2004); therefore, the disruption of the SPC in elm1Δ mutants could be an indirect effect of altering septin organization. Our results argue against this possibility. Elm1 contributes to septin organization via a pathway that includes the protein kinase Gin4 and the cyclin-binding protein Nap1 (Gladfelter et al., 2004). Loss of this pathway, however, does not account for the elm1Δ phenotype. We previously showed that gin4Δ mutants exhibit a mild SPC phenotype (Castillon et al., 2003). We have also found that loss-of-function mutations in nap1 exhibit a similarly mild SPC phenotype (unpublished data). These intermediate phenotypes stand in contrast to the total loss of SPC function in elm1-null mutants. Furthermore, the SPC relies on the septin network to restrict Lte1 to the bud (Castillon et al., 2003), but the disruption of the SPC in elm1Δ mutants is not attributable to aberrant Lte1 activity (Figs. 3 C and S2). Together, these data support our conclusion that Elm1 has a primary role in the SPC that is separate from its role in septin organization.

We propose that the role of Elm1 in the SPC is to activate the Kin4 kinase (Fig. 8). Elm1 is necessary for the function of Kin4 in our epistasis experiments (Fig. 3 D), suggesting that Elm1 either activates Kin4 or functions downstream between Kin4 and Bfa1. Previous studies have shown that Kin4 kinase is activated by phosphorylation of residue T209 (D’Aquino et al., 2005; Cayadasi et al., 2010). We found that phosphorylated isoforms of Kin4 were absent or greatly diminished in elm1Δ mutants (Fig. 6 B). Furthermore, Elm1 was not necessary for the function of phosphomimetic Kin4-T209D, indicating that Elm1 acts upstream of Kin4 phosphorylation (Fig. 6 C). Consistent with this notion, phosphomimetic Kin4-T209D restored SPC integrity in elm1Δ mutants (Fig. 6 D). Therefore, Elm1 appears to activate Kin4 by phosphorylating residue T209. Our results are consistent with those of Cayadasi et al. (2010), who further demonstrate that Elm1 directly phosphorylates Kin4 at T209.
This mechanism is akin to the documented roles of Elm1 in activating AMPK/Snf1 and Hsl1 by phosphorylating residues analogous to T209 of Kin4 (Fig. 6 A; Hong et al., 2003; Sutherland et al., 2003; Szkotnicki et al., 2008).

In addition to Elm1, we found that loss-of-function mutations in the related kinases Sak1 and Tos3 also disrupt the integrity of the SPC; however, the effects of these mutations were less penetrant than those observed for \textit{elm1Δ}, indicating a lesser role in the SPC (Fig. 1 C). The differences in penetrance between the \textit{elm1Δ}, \textit{sak1Δ}, and \textit{tos3Δ} mutants may be explained by each kinase targeting discrete substrates in redundant pathways or exhibiting differential activity toward a common substrate. At this point, we cannot discriminate between these possibilities. Elm1, Sak1, and Tos3 are known to serve overlapping roles in regulating the yeast AMPK/Snf1, and each is sufficient to phosphorylate the activation loop threonine of AMPK/Snf1 (Hong et al., 2003; Sutherland et al., 2003). Thus, it remains possible that Sak1 and Tos3 can activate Kin4, but at a lower rate than Elm1.

Given the redundant biochemical activity of the EFKs, why does Elm1 exhibit a more pronounced role in the SPC? The answer is likely to involve the carboxy-terminal region of Elm1, which is not conserved in Sak1 or Tos3. This region of Elm1 is not necessary for the redundant function of Elm1 in regulating Snf1 (Rubenstein et al., 2006) nor the phosphorylation of Kin4, which is not conserved in Sak1 or Tos3. This region of Elm1 is required for a threshold of Elm1 kinase activity at the neck to function (Maekawa et al., 2007; Chan and Amon, 2009). Based on these data, we conclude that Elm1 does not act in a sensor mechanism that detects aberrant spindle position.

We favor an alternative model in which Elm1 is one component in a network that creates a region of high SPC activity within the mother compartment. Two critical features of this network are the localization and activity of Kin4. Kin4 localizes to the mother cortex and bud neck before mitosis and accumulates at the SPB in the mother compartment during mitosis with concomitantly decreased localization at the cortex and neck (D’Aquino et al., 2005; Pereira and Schiebel, 2005). Preventing exchange between these sites by constitutively targeting Kin4 to the cortex or SPBs disrupts SPC activity, suggesting that Kin4 must visit both sites to function (Maekawa et al., 2007; Chan and Amon, 2009). We propose that exchange is necessary for Kin4 to be activated by Elm1 at the bud neck before being targeted to the SPBs by an independent regulatory module involving PP2A-Rts1 (Chan and Amon, 2009). Our findings also reveal that the localization of Elm1 to the bud neck is critical for Kin4 activation, even when high levels of Kin4 are present in the cell (Fig. 7 B). Why is the localization of Elm1 critical? Because SPC activity can be restored by elevating expression levels of the Elm1 kinase domain or by exogenously targeting this domain to the neck (Fig. 7 C), we hypothesize that the SPC requires a threshold of Elm1 kinase activity at the neck to function. It is tempting to speculate that Elm1 could be linked at the neck to additional SPC-regulating mechanisms that control Kin4 localization and sense spindle positioning errors; further study will be required.

Materials and methods

Yeast strains and manipulation

Chemicals and reagents were obtained from Sigma-Aldrich or Thermo Fisher Scientific unless stated otherwise. General yeast manipulation, media, and transformation were performed by standard methods (Amberg et al., 2005). Strains and plasmids are listed in Table S1. Gene deletions were constructed by PCR product-mediated transformation (Petracek and Longtine, 2002). Fluorescent chimeras of Elm1, Kin4, Sak1, and Tos3 were generated by fusion of PCR-amplified cassettes to the 3′-end of the chromosomal locus (Shelf and Thorn, 2004). Fusion proteins were assayed for functionality by scoring the fidelity of the SPC in \textit{arg1Δ} haploids that expressed tagged versions of each fusion. To construct Elm1Δ240, the tdimer2 fluor-marker cassette was integrated behind codon 420 of chromosomal Elm1. For overexpression of Elm1 or \textit{elm1Δ420-tdimer2}, the \textit{TRP1-PγADP cassette (Petracek and Longtine, 2002)} was integrated at the 5′-end, replacing the endogenous promoter. To construct plasmids bearing \textit{kin4} mutations, substitution mutations were introduced into \textit{pB1651} by site-directed mutagenesis and verified by DNA sequencing. To generate \textit{kin4Δ209D} at the endogenous chromosomal locus, we used a site-specific genomic mutagenesis strategy (Gray et al., 2005). In brief, the URA3 marker was amplified from pKS306 with oligonucleotides containing flanking sequences homologous to the \textit{Kin4} locus and integrated into a wild-type strain. The URA3 marker was then excised by transformation with a...
PCR product containing point mutations and flanking the KIN4 sequence. Excision of the URA2 marker was verified by PCR of genomic DNA. The presence of the T209D substitution and absence of other mutations were confirmed by sequencing the genomic locus. Western blot analysis confirmed that Kin4-T209D is expressed at levels similar to wild-type Kin4. To construct Bn4-elm1::URA3::GFP from a lambda library with flanking homology to the 3' end of the BNI4 or KCC4 loci. Integration was confirmed by diagnostic PCR and sequencing; expression was confirmed by detection of a tdimer2::RFP signal at the bud neck.

Plasmids used in this study were provided by the following people: Dr. D. Lew (Duke University, Durham, NC) provided the sww1Δ1 expression plasmid (pBJ1492/pMJ1139). Dr. J. Pringle (Stanford University, Stanford, CA) provided the CDC3-GFP expression plasmid (pBJ1488). Dr. K. Lee (National Institutes of Health, Bethesda, MD) provided the TUB1-GFP::LEU3 integration plasmid (pBJ1351/pSK1050). Drs. A. Khmelinskii and E. Schiebel (Universitat Heidelberg, Heidelberg, Germany) provided the mCherry-TUB1::URA3 plasmid (pAK011).

Assay for SPC integrity
The integrity of the SPC was analyzed by time-lapse microscopy of GFP-labeled microtubules in living cells as previously described (Castillon et al., 2003). In brief, living cells from asynchronous cultures grown to early log phase were suspended in nonfluorescent medium, mounted on a slide, and sealed beneath a coverslip with paraffin wax. Images were captured at 30°C on a microscope (BX52; Olympus) equipped with a 1.35 N.A. 100X PlanApo objective lens and a camera (CoolSNAP HQ; Roper Industries) using QED software. Images were processed using Photoshop (Adobe) to adjust levels and image (National Institutes of Health) to apply color lookup tables.

Fluorescence microscopy
For still wide-field images, cells were suspended in nonfluorescent medium at 25°C, and images were collected on an inverted fluorescence microscope (IX70; Olympus) with a 1.40 N.A. 100X PlanApo objective lens and a camera (CoolSNAP HQ; Roper Industries) using QED software. Images were processed using Photoshop (Adobe) to adjust levels and image (National Institutes of Health) to apply color lookup tables.

Fluorescence intensities were measured across a 2 × 1-μm region of interest, perpendicular to the bud neck and centered on the smallest diameter of the cell. The mean signal intensities for each pixel column along the axis perpendicular to the neck were measured using the Plot Profile function of ImageJ. Normalized values were determined by first subtracting the mean of the first and last points on the axis and then dividing by the maximum intensity within the region. Elm1-tdimer2 fluorescence was measured in large-budded mitotic cells, evident by single rings of Cdc3-GFP, Bni4-GFP, or Kcc-GFP. Kin4-tdimer2 fluorescence was measured in mitotic and postmitotic cells, indicated by the morphology of spindles labeled with GFP-tubulin.

Overexpression analysis
For the cell growth assay, liquid cultures of cells carrying high-copy 2μ plasmids with indicated genes under galactose-inducible promoters were grown to early log phase and then were arrested at START with 2% galactose and 2% raffinose to induce expression. Samples were collected at 30-min intervals, fixed in 3.7% formaldehyde and 100 mM potassium phosphate for 5 min, and washed once with quencher solution (100 mM potassium phosphate, 0.1% Triton X-100, and 10 mM ethanalamine) and once with 100 mM potassium phosphate. The proportion of mitotic cells was determined by the presence of anaphase-length spindle (>2 μm) based on fluorescence imaging of GFP-labeled microtubules.

Western blotting
Kin4 tagged with a 13myc epitope at the endogenous chromosomal locus was detected in lysates of cells grown asynchronously or arrested in metaphase. Asynchronous cells were grown to mid-log phase. For metaphase arrest, cells were grown to early log phase and then treated with 15 μg/ml nocodazole for 2 h. Cell number was normalized by OD600. 5% trichloracetic acid was added to each culture and mixed for 10 min. Cells were then pelleted and washed with cold acetone. Cell pellets were resuspended in equal parts lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 2.75 mM DTT) and supplemented with yeast protease inhibitor cocktail and acid-washed glass beads and were lysed by bead beating for six cycles of 2 min each. Crude lysates were supplemented with Laemmli SDS-PAGE buffer, boiled, spun briefly, and then run on 8% SDS-PAGE and transferred to a nitrocellulose membrane. Blots were probed with mouse anti-13myc 9E10 (Covance) at 1:1,000.

Online supplemental material
Fig. S1 shows the localization of Sak1 and Tos3. Fig. S2 shows Ure1 localization in elm1Δ mutants. Fig. S3 shows that ELM1 overexpression does not inhibit mitotic exit. Fig. S4 shows that phosphomimetic Kin4-T209D is not sufficient to prolong mitosis. Table S1 shows strains and plasmids used in this study and in four figures. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201006092/DC1.

We are grateful to Drs. Scott Nelson and Mark Longtine and other members of the Cooper laboratory for advice and suggestions.

This research was supported by a grant from the National Institutes of Health to J.A. Cooper (GM47337). J.K. Moore was supported by a postdoctoral fellowship from the Molecular Oncology program of the Siteman Cancer Center at Washington University, funded by the National Institutes of Health (T32CA113275).

Submitted: 15 June 2010
Accepted: 29 September 2010

References
Adames, N.R., J.R. Oberle, and J.A. Cooper. 2001. The surveillance mechanism of the spindle position checkpoint in yeast. J. Cell Biol. 153:159–168. doi:10.1083/jcb.153.1.159
Amberg, D.C., D. Burke, and J. Strathern. 2000. Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 203 pp.
Bardin, A.J., R. V西省廷, and A. Amon. 2000. A mechanism for coupling exit from mitosis to partitioning of the nucleus. Cell. 102:21–31. doi:10.1016/ S0092-8674(00)00007-6
Barral, Y., V. Merrell, M.S. Mooser, and M. Snyder. 2000. Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Mol. Cell. 5:841–851. doi:10.1016/S1097-2765(00)00234-X
Blacketer, M.J., C.M. Koehler, S.G. Coats, A.M. Myers, and P. Madura. 1993. Regulation of dimorphism in Saccharomyces cerevisiae: involvement of the novel protein kinase homolog Elmt1p and protein phosphatase 2A. Mol. Cell. Biol. 13:5567–5581.
Biec, A., G.M. Venturi, and K. Tatchell. 2000. Anaphase spindle position is monitored by the BUB2 checkpoint. Nat. Cell Biol. 2:556–558. doi:10.1038/35019601
Bouquin, N., Y. Barral, R. Courbéryette, M. Blondel, M. Snyder, and C. Mann. 2000. Regulation of cytokinesis by the Elm1 protein kinase in Saccharomyces cerevisiae. J. Cell Sci. 113:1435–1445.
Castillon, G.A., N.R. Adams, C.H. Rosello, H.S. Seidel, M.S. Longtine, J.A. Cooper, and R.A. Heil-Chapdelaine. 2003. Septins have a dual role in controlling mitotic exit in budding yeast. Curr. Biol. 13:654–658. doi:10.1016/S0960-9822(03)00247-1
Caydasi, A.K., and G. Pereira. 2009. Spindle alignment regulates the dynamic association of checkpoint proteins with yeast spindle pole bodies. Dev. Cell. 16:146–156. doi:10.1016/j.devcel.2008.10.013
Caydasi, A.K., B. Kurulmus, M.I. Orrico, A. Hofmann, B. Ibrahim, and G. Pereira. 2010. Elm1 kinase activates the spindle position checkpoint kinase Kin4. J. Cell Biol. 190:975–989. doi:10.1083/jcb.201006151

Chan, L.Y., and A. Amon. 2009. The protein phosphatase 2A functions in the spindle position checkpoint by regulating the checkpoint kinase Kin4. Genes Dev. 23:1639–1649. doi:10.1101/gad.1804609

D’Aquino, K.E., F. Monge-Casas, J. Paulson, V. Reiser, G.M. Charles, L. Lai, K.M. Shokat, and A. Amon. 2005. The protein kinase Kin5 inhibits exit from mitosis in response to spindle position defects. Mol. Cell. 19:223–234. doi:10.1016/j.molcel.2005.06.005

Daum, J.R., N. Gomez-Ospina, M. Winey, and D.J. Burke. 2000. The spindle checkpoint of Saccharomyces cerevisiae responds to separable microtubule-dependent events. Curr. Biol. 10:1375–1378. doi:10.1016/S0960-9822(00)00780-6

Fraschini, R., C. D’Ambrosio, M. Venturetti, G. Lucchini, and S. Piatti. 2006. Disappearance of the budding yeast Bub2–Bfa1 complex from the mother-bound spindle pole contributes to mitotic exit. J. Cell Biol. 172:335–346. doi:10.1083/jcb.200507162

Geymonat, M., A. Spanos, S.J. Smith, E. Wheatley, K. Rittinger, L.H. Johnston, C. D’Ambrosio, M. Venturetti, G. Lucchini, and S. Piatti. 2007. Disappearance of the budding yeast Bub2–Bfa1 complex from the mother-bound spindle pole contributes to mitotic exit. Mol. Biol. Cell. 18:3440–3450. doi:10.1091/mcb.E07-03-0242

Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. Determinants of Swe1p degradation in the yeast genome. Mol. Cell. Biol. 11:2609–2616. doi:10.1128/MCB.11.11.4049-4061.1991

Kozubowski, L., J.R. Larson, and K. Tatchell. 2005. Role of the septin ring in microtubule-dependent events. Curr. Biol. 15:1519–1532. doi:10.1016/j.molcel.2005.09.0708

Lippincott, J., B.K. Shannon, W. Shou, J.M. Stark, M.C. Schmidt, and D.G. Hardie. 2003. Elm1p is one of three upstream kinases for the Bub2p spindle checkpoint links nuclear migration with mitotic exit. Mol. Biol. Cell. 14:4124–4129. doi:10.1091/mbc.E03-02-0099

Malko, J., J.G. Evans, E.D. Salmon, D. Pellman, and K. Bloom. 2004. The differential roles of budding yeast Tem1p, Cdc15p, and Bub2p protein dynamics in mitotic exit. Mol. Biol. Cell. 15:1519–1532. doi:10.1091/mcb.E03-09-0708

Moore, J.K., and J.A. Cooper. 2010. Coordinating mitosis with cell polarity: Molecular motors at the cell cortex. Semin. Cell Dev. Biol. 21:283–293. doi:10.1016/j.semcdb.2010.01.020

Rubenstein, E.M., R.R. McCartney, and M.C. Schmidt. 2006. Regulatory domains of Snf1-activating kinases determine pathway specificity. Eukaryot. Cell. 5:620–627. doi:10.1128/EC.5.4.620-627.2006

Sakchaias, K., S. Asano, L.R. Yu, M.J. Shulewitz, C.J. Park, J.E. Park, Y.W. Cho, T.D. Veenstra, J. Thorner, and K.S. Lee. 2004. Coupling morphogenesis to mitotic entry. Proc. Natl. Acad. Sci. USA. 101:4124–4129. doi:10.1073/pnas.0400641101

Shirayama, M., Y. Matsu, and A. Toh-E. 1994. The yeast TEM1 gene, which encodes a GTP-binding protein, is involved in termination of M phase. Mol. Biol. Cell. 5:350–361. doi:10.1083/jcb.115.3.1140

Shyu, D., D.D. Loew, and J.D. Lew. 2000. The protein kinase Kin15 inhibits exit from mitosis in response to spindle position defects. Mol. Cell. 19:223–234. doi:10.1016/j.molcel.2005.06.005

Suzuki, H., S. Kondo, and T. Horigome. 1998. Coordinated exit from mitosis is required for chromosome segregation in budding yeast. Curr. Biol. 8:3440–3450. doi:10.1016/S0960-9822(00)00779-X

Elm1 regulates the SPC • Moore et al. 503