Characterization of the roles of Blt1p in fission yeast cytokinesis

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ABSTRACT Spatial and temporal regulation of cytokinesis is essential for cell division, yet the mechanisms that control the formation and constriction of the contractile ring are incompletely understood. In the fission yeast \textit{Schizosaccharomyces pombe} proteins that contribute to the cytokinetic contractile ring accumulate during interphase in nodes—precursor structures around the equatorial cortex. During mitosis, additional proteins join these nodes, which condense to form the contractile ring. The cytokinesis protein Blt1p is unique in being present continuously in nodes from early interphase through to the contractile ring until cell separation. Blt1p was shown to stabilize interphase nodes, but its functions later in mitosis were unclear. We use analytical ultracentrifugation to show that purified Blt1p is a tetramer. We find that Blt1p interacts physically with Sid2p and Mob1p, a protein kinase complex of the septation initiation network, and confirm known interactions with F-BAR protein Cdc15p. Contractile rings assemble normally in \textit{blt1}\textsuperscript{Δ} cells, but the initiation of ring constriction and completion of cell division are delayed. We find three defects that likely contribute to this delay. Without Blt1p, contractile rings recruited and retained less Sid2p/Mob1p and Clp1p phosphatase, and β-glucan synthase Bgs1p accumulated slowly at the cleavage site.

INTRODUCTION

The fission yeast \textit{Schizosaccharomyces pombe} uses a highly conserved mechanism to assemble and constrict a cytokinetic contractile ring composed of actin filaments and myosin-II to divide by medial fission (Pollard and Wu, 2010). Preparation for cytokinesis begins during interphase, when key signaling proteins accumulate around the cell equator in cortical structures called interphase nodes. These node proteins include kinases Cdr1p, Cdr2p, and Wee1p, putative RhoGEF Gef2p, kinesin-like protein Klp8p, anillin-related protein Mid1p, and the presumed scaffolding protein Blt1p (Paoletti and Chang, 2000; Morrell \textit{et al}., 2004; Martin and Berthelot-Grosjean, 2009; Moseley \textit{et al}., 2009; Guzman-Vendrell \textit{et al}., 2013). At the onset of mitosis, interphase nodes mature into cytokinesis nodes upon recruitment of IQGAP protein Rng2p, myosin-II heavy chain Myo2p and light chains Rlc1p and Cdc4p, F-BAR protein Cdc15p, and formin Cdc12p (Wu \textit{et al}., 2003, 2006; Almonacid \textit{et al}., 2011; Laporte \textit{et al}., 2011; Padmanabhan \textit{et al}., 2011). Interactions between myosin-II and actin filaments polymerized by Cdc12p condense nodes into the contractile ring (Vavylonis \textit{et al}., 2008).

After contractile ring assembly, a signal transduction pathway called the septation initiation network (SIN) compacts the ring, initiates contractile ring constriction, and promotes the onset of separation during cytokinesis (Hachet and Simanis, 2008; Krapp and Simanis, 2008). The SIN uses a protein phosphorylation cascade that activates the NDR-family kinase Sid2p and its regulatory subunit Mob1p at the cleavage site (Sparks \textit{et al}., 1999; Hou \textit{et al}., 2000; Salimova \textit{et al}., 2000). Among the Sid2p kinase substrates is the Cdc14-family phosphatase Clp1p, which concentrates in the nucleus during interphase but moves to kinetochores, the mitotic spindle, and the cleavage site during mitosis (Clifford \textit{et al}., 2008). Sid2p phosphorylation of Clp1p contributes to its retention in the cytoplasm (Chen \textit{et al}., 2008). At the contractile ring, Clp1p dephosphorylates Cdc15p, causing conformation changes that contribute to compaction of the contractile ring and progression of cytokinesis (Clifford \textit{et al}., 2008; Roberts-Galbraith \textit{et al}., 2010). In
RESULTS

Localization of Blt1p across the cell cycle

We localized Blt1p and other cytokinesis proteins in live cells throughout the cell cycle by tagging each protein with a fluorescent protein in its native genomic locus. Each strain also expressed Sad1p–red fluorescent protein (RFP) or Sad1p–cyan fluorescent protein (CFP) to mark spindle pole bodies (SPBs), which we used to establish a time course within the cell cycle in which SPB separation at the onset of anaphase marks time zero (Wu et al., 2003). Events that occur before SPB separation have negative time values, and events after SPB separation have a positive time values.

Blt1p concentrated in cortical nodes along with kinase Cdr2p–monomeric enhanced green fluorescent protein (mEGFP) and anillin-like Mid1p–monomeric yellow fluorescent protein (mYFP) during interphase (Figure 1, −90 ± 10 to −15 ± 4 min; Wu et al., 2003; Moseley et al., 2009; Saha and Pollard, 2012). Cdr2p disappeared from nodes early in mitosis (+6 ± 2 min), but Blt1p and Mid1p remained in nodes and were joined by formin Cdc12p-mYFP (+0 ± 3 min) and F-BAR protein Cdc15p-mYFP (−5 ± 2 min) before being incorporated into fully formed contractile rings (+25 ± 3 min; Wu et al., 2003; Moseley et al., 2009). Mid1p-mYFP disappeared from the contractile ring before the onset of ring constriction (+28 ± 3 min), but Blt1p-mEGFP remained in the ring with Cdc12p-mYFP and Cdc15p-mYFP until the completion of ring constriction (+62 ± 6 min; Wu et al., 2003). The majority of Blt1p-mEGFP remained at the division site along with Bgs1p-mEGFP after completion of septation (+68 ± 5 min; Cortes et al., 2002), before relocalizing to cortical nodes at the middle of the cell (+90 ± 10 min).

addition, SIN signaling regulates the accumulation of (1,3)β-d-glucan synthase Bgs1p/Cps1p in the plasma membrane adjacent to the contractile ring, where the enzyme synthesizes the primary septum (Liu et al., 1999; Le Goff et al., 1999; Cortes et al., 2002; Jin et al., 2006). Synthesis of the primary septum in the cleavage furrow contributes to the inward force driving constriction of the contractile ring (Liu et al., 1999; Proctor et al., 2012). Although much is known about the assembly of interphase and cytokinesis nodes and SIN signaling, the mechanisms coordinating cytokinesis are poorly understood.

Blt1p is intriguing because it is one of the few proteins present in structures related to cytokinesis throughout the entire cell cycle. Although not essential for viability, Blt1p contributes to anchoring interphase node proteins Gef2p and Mid1p in the cell cortex (Moseley et al., 2009; Ye et al., 2012; Guzman-Vendrell et al., 2013; Jourdain et al., 2013), and all three proteins are incorporated in the contractile ring, whereas other interphase node proteins disperse into the cytoplasm (Moseley et al., 2009). This persistence in the contractile ring suggested that Blt1p might have additional, unidentified functions later in mitosis.

We report that cells lacking Blt1p assemble contractile rings normally but initiate and complete cleavage later than do wild-type cells. We identify roles for Blt1p in recruiting Sid2p/Mob1p protein kinase complex, phosphatase Clp1p, and glucan synthase Bgs1p to the cleavage site. Without Blt1p, all of these proteins accumulate slowly around the middle of the cell, likely contributing to the delay in cytokinesis.

FIGURE 1: Localization of Blt1p across the cell cycle relative to other node and contractile ring proteins. Time series of reversed contrast fluorescence micrographs taken at 2-min intervals and displayed at 15- or 30-min intervals of one representative series from ≥20 wild-type cells examined expressing Blt1p-mEGFP, Cdr2p-mEGFP, Mid1p-mYFP, Cdc12p-mYFP, Cdc15p-mYFP, or Bgs1p-mEGFP. Time is in minutes, with spindle pole body separation defined as time zero. Arrow indicates Mid1p-mYFP in the nucleus. Scale bar, 2.5 μm.
Hydrodynamic analysis showed that native Blt1p is a tetramer in equilibrium with its 78-kDa subunits. Purified Blt1p eluted from a calibrated Superdex 200 column with a partition coefficient of 0.145, corresponding to a Stokes radius of 68 Å and a diffusion coefficient of $3.25 \times 10^{-8}$ cm$^2$/s (Figure 2B and Table 1). This Stokes radius is much larger than that of serum albumin (35 Å), which has a subunit molecular weight (66 kDa) similar to that of Blt1p (Erickson, 2009). This suggested that Blt1p forms homo-oligomers or is highly asymmetric. Sedimentation velocity analytical ultracentrifugation showed that purified Blt1p is a hydrodynamically well-behaved oligomer. At 10 μM virtually all Blt1p sedimented at 13.6 S, whereas at low concentrations (0.1 μM) an additional, more slowly sedimenting species was present at 6.3 S (Figure 2C). We also determined the diffusion coefficient (D) for both species from boundary spreading of the sedimenting protein (Table 1). The S and D values gave molecular weights of $75 \text{ kDa}$ for the 6-S species, consistent with the size of the Blt1p monomer, and $343 \text{ kDa}$ for the 14-S species (Figure 2C and Table 1), indicating that Blt1p forms homotetramers. The frictional coefficient of 1.3 indicates that the Blt1p tetramer is a slightly asymmetric molecule.

**Blt1p ensures proper timing for onset of contractile ring constriction**

Careful quantitative analysis revealed no differences in contractile ring assembly between wild-type and bt1Δ cells in spite of the prominance of Blt1p in interphase and cytokinesis nodes. Supplemental Figures S1 and S2 document that myosin-II mEGFP-Myo2p, unconventional myosin-II Myp2p-mYFP, and Cdc15p-mEGFP each appeared in nodes and contractile rings at the same times in wild-type and bt1Δ cells. Furthermore, the numbers of these proteins in rings and the time required for ring formation were normal in bt1Δ cells (see Supplemental Results). Measurements of Rlc1p, the regulatory light chain for both type II myosins, Myo2p and Myp2p (Le Goff et al., 2000; Naqvi et al., 2000), showed that it appears in the contractile ring in two waves, the first consisting of $\sim 4000$ molecules, associated with Myo2p (Supplemental Figures S1F and S2B), and the second consisting of $\sim 2000$ molecules, associated with Myp2p (Supplemental Figure S2, B and E) in both wild-type and bt1Δ cells. The first cytokinesis defect to appear in bt1Δ cells during the cell cycle was a 10-min delay in the onset of contractile ring constriction due to mislocalization of Myo2p-mYFP (Le Goff et al., 2000; Naqvi et al., 2000), showed that it appears in the contractile ring in two waves, the first consisting of $\sim 4000$ molecules, associated with Myo2p (Supplemental Figures S1F and S2B), and the second consisting of $\sim 2000$ molecules, associated with Myp2p (Supplemental Figure S2, B and E) in both wild-type and bt1Δ cells. The timing of anaphase A, anaphase B, and telophase age of 68 ± 5 min in wild-type cells to 79 ± 5 min in bt1Δ cells (Figure 3B). The timing of anaphase A, anaphase B, and telophase was normal in bt1Δ cells (Supplemental Figure S3).

**Genetic and physical interactions of Blt1p with SIN components Mid1p and Sid2p**

Because we found no evidence that the defect in the onset of contractile ring constriction in bt1Δ cells was due to mislocalization of

| A. Gel filtration | Stokes radius (Å) | Diffusion coefficient (cm$^2$/s) |
|------------------|------------------|---------------------------------|
| Blt1p oligomer   | 68               | $3.3 \times 10^{-8}$            |

| B. Sedimentation velocity, analytical ultracentrifugation | Sedimentation coefficient (s$^{-1}$) | Diffusion coefficient (cm$^2$/s) | Calculated molecular weight (g/mol) | $f/f_0$  |
|----------------------------------------------------------|--------------------------------------|---------------------------------|-----------------------------------|---------|
| Blt1p monomer                                            | 6.3 $\times 10^{-13}$                | $7.5 \times 10^{-8}$            | 75,457                            | 1.2     |
| Blt1p oligomer                                           | 13.6 $\times 10^{-13}$               | $3.6 \times 10^{-8}$            | 343,245                           | 1.3     |

**TABLE 1:** Hydrodynamic properties of Blt1p-hexahistidine.
cytokinesis node proteins, we sought other proteins that interact with Blt1p by screening for decreased viability at 25, 30, or 36°C of double mutants of blt1Δ with mutations of other cytokinesis genes in strains with the same auxotrophic markers (Table 2 and Supplemental Figure S4). Our quantitative analysis identified synthetic interactions between blt1Δ and genes for multiple components of the SIN, including the terminal components of the signaling pathway, mob1Δ and sid2Δ (Figure 4A). The NDR-family kinase Sid2p and its accessory protein Mob1p regulate contractile ring constriction and septation (Sparks et al., 1999; Hou et al., 2000; Salimova et al., 2000).

![FIGURE 3: The onset and completion of contractile ring constriction are delayed in blt1Δ cells relative to wild-type cells. Times are in minutes, with spindle pole body separation defined as time zero. (A) Time series of fluorescence micrographs at 8-min intervals of (top) wild-type blt1+ cells and (bottom) blt1Δ cells expressing Rlc1p-mEGFP (green) to mark nodes and contractile rings and Sad1p-RFP (red) to mark spindle pole bodies. (B) Time courses of the accumulation of cells ±1 SD (○, ●) beginning contractile ring constriction and (□, ■) completing septation. Open symbols are 22 wild-type cells, and filled symbols are 27 blt1Δ cells expressing Rlc1p-mEGFP. Asterisks indicate time points at which the mean values of the two cell types differed with p < 0.0001. Scale bar, 5 μm.]

| S. pombe mutation | Generic name            | 25°C       |                  | 30°C       |                  | 36°C       |                  |
|-------------------|-------------------------|------------|-----------------|------------|-----------------|------------|-----------------|
|                   |                         | Mutant     | + blt1Δ         | Mutant     | + blt1Δ         | Mutant     | + blt1Δ         |
| Wild type         |                         | 100        | 96 ± 5          | 100        | 85 ± 10         | 100        | 81 ± 6          |
| cdc11-19          | SIN scaffold protein    | 95 ± 9     | 93 ± 3          | 97 ± 4     | 88 ± 10         | 22 ± 11    | 13 ± 9          |
| cdc14-118         | SIN component           | 80 ± 5     | 60 ± 16         | 62 ± 8     | 24 ± 11         | 0 ± 0      | 0 ± 0           |
| cdc15-127         | F-BAR protein           | 96 ± 3     | 91 ± 4          | 90 ± 5     | 81 ± 23         | 12 ± 5     | 8 ± 7           |
| cdc16-116         | SIN GAP                 | 88 ± 8     | 58 ± 14         | 11 ± 3     | 2 ± 1           | 0 ± 0      | 0 ± 0           |
| cdc2-M26          | Cycillin-dependent kinase 1 | 77 ± 5    | 71 ± 7          | 67 ± 2     | 58 ± 7          | 61 ± 9     | 52 ± 6          |
| cdc25-22          | Phosphatase             | 87 ± 7     | 81 ± 9          | 60 ± 10    | 31 ± 15         | 9 ± 2      | 6 ± 4           |
| cdc3-6            | Profilin                | 85 ± 12    | 70 ± 10         | 65 ± 2     | 52 ± 15         | 1 ± 1      | 1 ± 2           |
| cdc4-8            | Myosin-II light chain   | 91 ± 7     | 82 ± 11         | 77 ± 9     | 56 ± 15         | 72 ± 11    | 51 ± 13         |
| cdc7-24           | SIN kinase              | 72 ± 14    | 65 ± 6          | 53 ± 12    | 34 ± 13         | 0 ± 0      | 0 ± 0           |
| cdc8-27           | Tropomyosin             | 95 ± 6     | 77 ± 12         | 86 ± 5     | 56 ± 6          | 0 ± 0      | 0 ± 0           |
| cps1-119          | (1,3)β-D-Glucan synthase | 90 ± 3    | 83 ± 8          | 60 ± 3     | 53 ± 11         | 2 ± 1      | 1 ± 1           |
| mid1Δ             | Anillin                 | 33 ± 11    | 17 ± 7          | 36 ± 3     | 15 ± 7          | 15 ± 8     | 10 ± 6          |
| mob1-R4           | SIN regulatory subunit   | 45 ± 5     | 25 ± 14         | 21 ± 7     | 11 ± 6          | 0 ± 0      | 0 ± 0           |
| myo2-E1           | Myosin-II heavy chain   | 83 ± 12    | 67 ± 8          | 51 ± 9     | 30 ± 11         | 2 ± 1      | 3 ± 3           |
| plo1-24C          | Polo kinase             | 79 ± 6     | 65 ± 4          | 78 ± 8     | 49 ± 9          | 12 ± 6     | 12 ± 8          |
| mg2-D5            | IQGAP                   | 93 ± 8     | 88 ± 7          | 80 ± 7     | 70 ± 4          | 15 ± 6     | 11 ± 9          |
| sid1-125          | SIN kinase              | 57 ± 12    | 55 ± 9          | 41 ± 8     | 16 ± 10         | 0 ± 0      | 0 ± 0           |
| sid2-250          | SIN Ndr-family kinase   | 63 ± 6     | 40 ± 19         | 11 ± 8     | 6 ± 4           | 0 ± 0      | 0 ± 0           |
| sid4-A1           | SIN scaffold protein     | 72 ± 11    | 21 ± 9          | 1 ± 2      | 2 ± 2           | 0 ± 0      | 0 ± 0           |
| spg1-106          | SIN GTPase              | 30 ± 10    | 13 ± 12         | 11 ± 3     | 5 ± 2           | 0 ± 1      | 0 ± 0           |

*Percentage growth shown on plates at 25°C for 48 h, 30°C for 36 h, or 36°C for 36 h.

TABLE 2: Genetic interactions with blt1Δ.
A

| Condition          | Cell Count (10^4) |
|--------------------|------------------|
| wildtype           | 25C: 10^4, 30C: 10^4, 36C: 10^4 |
| blt1Δ              | 25C: 10^4, 30C: 10^4, 36C: 10^4 |
| mob1-R4            | 25C: 10^4, 30C: 10^4, 36C: 10^4 |
| sid2-250/blt1Δ     | 25C: 10^4, 30C: 10^4, 36C: 10^4 |

B

| Condition          | Status |
|--------------------|--------|
| Blt1p-6xHis        | +      |
| MBP-6xHis          | -      |

C

Mob1-mEGFP

D

Percent of Cells

E

Molecules of Mob1p at Cleavage Furrow

F

Sid2-mEGFP

G

Percent of Cells

H

Molecules of Sid2p at Cleavage Furrow
The growth defects of point mutants mob1-R4 and sid2-250 were more severe when combined with blt1Δ at both 25 and 30°C (Figure 4A and Supplemental Figure S4).

Pull-down assays confirmed physical interactions between Blt1p and these SIN components; Blt1p pulled down from lysates 42% of Mob1p-mEGFP and 22% of Sid2p-mEGFP, as well as 23% of Cdc15p-mEGFP (Figure 4B), as described previously (Moseley et al., 2009). Ni-NTA beads lacking Blt1p did not pull down any of these fluorescent fusion proteins from lysates (Figure 4B). Mob1p and Sid2p interact with each other, and both are essential, so we could not determine whether Blt1p interacts with just one or both of Mob1p and Sid2p.

**Timely localization of Mob1p and Sid2p to the contractile ring depends on Blt1p**

The presence of Blt1p influenced the relocation of Mob1p-mEGFP and Sid2p-mEGFP from SPBs to the contractile ring during cytokinesis. Mob1p and Sid2p concentrate in SPBs throughout the cell cycle, but unlike other SIN proteins, both move to the contractile ring during mitosis (Sparks et al., 1999; Hou et al., 2000, 2004; Salimova et al., 2000; McCormick et al., 2013). In wild-type cells, Mob1p appeared around the equator at time +21 ± 2 min, increased to a peak average of 1500 ± 180 molecules at +36 min, and departed by +53 ± 2 min (Figure 4, C–E). In blt1Δ cells, Mob1p arrived at the equator at time +24 ± 2 min and initially accumulated at the same rate as in wild-type cells but then slowed and peaked at 1200 ± 170 molecules at +36 min and departed earlier at +46 ± 2 min (Figure 4, C–E). Ring constriction began in wild-type cells at +28 min, when 1200 ± 270 molecules of Mob1p were present in the ring, but was delayed until +38 min in blt1Δ cells, when the ring contained an average of 1200 ± 210 molecules of Mob1p (Figure 4E).

Similar to Mob1p, Sid2p appeared in the contractile ring at about the same time in wild-type cells (+23 ± 2 min; departing by +62 ± 3 min) and blt1Δ cells (+26 ± 2 min; departing by +58 ± 3 min; Figure 4, F and G), but the peak number of Sid2p molecules was much lower (1000 ± 240 ± 40 min) in blt1Δ cells than in wild-type cells (1700 ± 280 ± 40 min; Figure 4H). Rings in wild-type cells accumulated 1000 ± 270 molecules of Sid2p at the time they began to constrict at +28 min, whereas rings in blt1Δ cells did not begin to constrict until +38 min with an average of 1000 ± 270 molecules of Sid2p (Figure 4H). Wild-type and blt1Δ cells have the same total number of Mob1p or Sid2p molecules (Supplemental Figure S5, A and B). Thus, contractile rings in both wild-type and blt1Δ cells begin to constrict when they accumulate ~1000 active Sid2p/Mob1p kinase complexes. This suggests that a threshold level of kinase activity might trigger constriction.

To test this hypothesis, we varied the total numbers of Mob1p-mYFP and Sid2p-mYFP in cells using the 3nmt promoter and its repressor, thiamine, to control their expression. Without thiamine, the 3nmt promoter produced wild-type levels of both proteins, but growth in liquid culture for 10 h with 15 μM thiamine modestly reduced the total number of Mob1p-mYFP from 35,100 ± 600 to 23,300 ± 500 molecules/cell and the total number of Sid2p-mYFP from 31,300 ± 2000 to 21,500 ± 1100 molecules/cell (Supplemental Figure S5, A, B, E, and F). This mild repression of either Sid2p or Mob1p had no effect on recruitment of Mob1p-YFP or Sid2p-mYFP to SPBs or the timing of anaphase A, anaphase B, or telophase (Supplemental Figure S7, A–D). This is expected from the micromolar cytoplasmic concentrations of Mob1p and Sid2p and the high affinity of their receptor, Cdc11p, on SPBs (McCormick et al., 2013). However, mild depletion of Mob1p and Sid2p delayed ring constriction 7–8 min to the time when the rings contained 1000 molecules of Mob1p-mYFP and Sid2p-mYFP, the same numbers at the onset of constriction in unrepressed cells (Supplemental Figure S6, D and F). This indicates that the unknown contractile ring receptor for Mob1p/Sid2p has a lower affinity than Cdc11p in SPBs. Thus, under three different conditions, contractile rings began to constrict when ~1000 molecules of Sid2p/Mob1p accumulated in the ring.

**Gef2p is not required for localization of Mob1p and Sid2p to the contractile ring**

Because localization of Gef2p to interphase nodes and to the cleavage site depends on Blt1p (Moseley et al., 2009; Ye et al., 2012; Jourdain et al., 2013), and since Gef2p is proposed to regulate the SIN (Ye et al., 2012), we expected that a shortage of equatorial Gef2p in the blt1Δ cells might explain the slow accumulation of Mob1p and Sid2p in rings. Consistent with previous studies, contractile rings in blt1Δ cells accumulated fewer molecules of Gef2p (peak of 480 ± 150 molecules at +36 min) than wild-type cells (peak of 1300 ± 300 molecules at +40 min) (Supplemental Figure S8).

Nevertheless, Mob1p and Sid2p both accumulated normally in rings of gef2Δ cells. The average times of Mob1p appearance (+21 ± 2 min wild type; +20 ± 3 min gef2Δ), peak numbers of Mob1p molecules at the cleavage site (1600 ± 200 wild type; 1300 ± 300 gef2Δ), and leaving times (+53 ± 2 min wild type; +55 ± 3 min gef2Δ) did not differ significantly in the two strains.
equatorial ring at time +6 ± 2 min and remained until +29 ± 2 min in wild-type cells and appeared in the ring from time +6 ± 2 min until +32 ± 5 min in blt1Δ cells (Figure 5, A and B).

The absence of Blt1p delays the synthesis of the primary septum in the cleavage furrow

Because the accumulation of (1,3)β-D-glucan synthase Bgs1p/Cps1p at the cleavage site depends on SIN activity (Le Goff et al., 1999; Liu et al., 1999, 2000; Cortes et al., 2002), we investigated the behavior of Bgs1p in blt1Δ cells. Wild-type and blt1Δ cells have the same total number of molecules of this transmembrane enzyme (Supplemental Figure S5D), and Bgs1p-mEGFP began to accumulate around the equator (+16 ± 2 min) in wild-type and +17 ± 1 min) in blt1Δ cells. Nevertheless, cells without Blt1p had four defects in the regulation of Bgs1p. First, the concentration of Bgs1p into a narrow ring was delayed from +27 ± 2 min in wild-type cells until +32 ± 2 min in blt1Δ cells (Figure 6, A and B). Second, the peak number of Bgs1p molecules at the cleavage site was reduced from 11,800 ± 2600 molecules at +42 min in wild-type cells to 8000 ± 1200 molecules at +64 min in blt1Δ cells (Figure 6D). Third, constriction of contractile rings was delayed from +28 min when wild-type cells accumulated 8200 ± 1200 molecules of Bgs1p at the cleavage site to +38 min in blt1Δ cells when 7700 ± 600 molecules of Bgs1p-mEGFP...
Blt1p is not required for contractile ring assembly

The presence of Blt1p in interphase nodes suggested that it might recruit other proteins to cytokinesis nodes or contractile rings, but the only example we found was the known dependence of Gef2p localization in interphase nodes on Blt1p (Moseley et al., 2009; Ye et al., 2012; Guzman-Vendrell et al., 2013; Jourdain et al., 2013). We measured a 60% decrease in Gef2p in cytokinesis nodes and contractile rings in \textit{blt1} cells but found that Gef2p is not required for normal rates of accumulation or disappearance of Mob1p/Sid2p in contractile rings. Although Gef2p disappears earlier than normal from the division site in \textit{blt1} cells, cells without Gef2p divide normally (Ye et al., 2012). In the absence of Blt1p, Mid1p mediates the localization of Gef2p to cytokinesis nodes and the contractile ring (Guzman-Vendrell et al., 2013), but it is unclear what retains Gef2p at the ring after Mid1p departs in \textit{blt1} cells.

Our quantitative measurements showed that Blt1p is not required to recruit myosin-II (Myo2p and Rlc1p) or the F-BAR protein Cdc15p to contractile rings or unconventional myosin Myp2p to the contractile ring, in spite of many other mutations causing delays in the appearance of these proteins (Fankhauser et al., 1995; Bezanilla et al., 1997; Mulvihill and Hyams, 2003; Coffman et al., 2009; Sladewski et al., 2009; Roberts-Galbraith, 2010). We found that contractile rings form with precisely normal...
timing in \textit{blt1}Δ cells, confirming previous qualitative observations (Moseley \textit{et al.}, 2009; Ye \textit{et al.}, 2012; Guzman-Vendrell \textit{et al.}, 2013; Jourdain \textit{et al.}, 2013). Furthermore, rings in \textit{blt1}Δ cells constricted at a normal rate in spite of a 10-min delay in the onset of constriction. This shows that the cytokinesis defect in \textit{blt1}Δ cells is confined to the events that initiate constriction.

\textbf{Blt1p recruits Mob1p and Sid2p to the contractile ring and promotes ring constriction}

Our mutant screen revealed strong genetic interactions between \textit{blt1}Δ and mutations in genes (\textit{cdc25} and \textit{mid1}+) that function in interphase or at the G2/M transition (Paoletti and Chang, 2000; Martin and Berthelot-Grosjean, 2009; Moseley \textit{et al.}, 2009), but the most extensive interactions were with genes for SIN components that lead to phosphorylation of Sid2p (Sparks \textit{et al.}, 1999; Hou \textit{et al.}, 2004). The Sid2p/Mob1p complex carries the SIN signal from the SPBs to the contractile ring (Sparks \textit{et al.}, 1999; Chen \textit{et al.}, 2008). Mutations of SIN pathway genes compromise maturation of the contractile ring, which can delay the onset of constriction (Hachet and Simanis, 2008).

Our measurements indicate that Blt1p is required for the timely recruitment and retention of Sid2p and Mob1p at the division site (Figure 4B). The kinase complex may interact directly with Blt1p, but the large Blt1p tetramer may also function as a scaffold and contribute to the architecture of nodes and the ability of other receptors to bind Sid2p/Mob1p. Previous work implicated microtubules in localizing Mob1p and Sid2p to the division plane (Sparks \textit{et al.}, 1999), but the mechanisms retaining these proteins at the contractile ring were unknown. In both wild-type and \textit{blt1}Δ cells, contractile rings begin to constrict when \sim 1000 molecules of Sid2p/Mob1p accumulate in the ring. Contractile rings in cells without Blt1p or depleted of Mob1p or Sid2p recruit Sid2p/Mob1p complexes more slowly and accumulate 1000 complexes \sim 10 min later than wild-type cells. Mutations that reduced Sid2p/Mob1p complex activation also extend the time required to recruit Sid2p/Mob complexes, possibly accounting for the growth defects in \textit{blt1}Δ mutants with a SIN mutation (Table 2 and Supplemental Figure S4).

Blt1p must cooperate with other proteins to recruit and retain Sid2p and Mob1p in contractile rings, since both proteins appear in rings in reduced numbers in \textit{blt1}Δ cells. Our results indicate that putative Rho-GEF Gef2p is unlikely to be this second receptor, and our genetic screen (Table 2) did not provide other candidates. In fact, Gef2p might antagonize Blt1p in regulating SIN activation, given that the \textit{gef2}Δ mutation partially suppresses growth defects of the mutations \textit{sid2-250} and the \textit{sid2p/Mob1p} receptor \textit{cdc11-136} (Ye \textit{et al.}, 2002).

We propose that the onset of contractile ring constriction depends on a threshold level of Sid2p/Mob1p in the ring to phosphorylate the Cdc14-like phosphatase Clp1p. This hypothesis offers an explanation for why contractile rings in \textit{blt1}Δ cells with decreased Sid2p also have half the normal Clp1p. Phosphorylation of Clp1p promotes its retention in the cytoplasm and its concentration in the contractile ring, so with defects in SIN signaling, Clp1p is targeted back to the nucleus, resulting in cytokinesis defects (Trautmann \textit{et al.}, 2001; Mishra \textit{et al.}, 2004; Chen \textit{et al.}, 2008).

At the division plane, Clp1p dephosphorylates Cdc15p, contributing to formation and constriction of the contractile ring (Clifford \textit{et al.}, 2008; Roberts-Galbraith \textit{et al.}, 2010). Low numbers of Cdc15p in contractile rings of \textit{sid2} mutants (Hachet and Simanis, 2008) are presumably due to a failure to stabilize Clp1p at the division plane and a corresponding decrease in Cdc15p dephosphorylation. We found normal numbers of Cdc15p molecules in contractile rings of \textit{blt1}Δ mutants, showing that half the number of Clp1p at the division site provides enough enzyme activity to retain Cdc15p at the contractile ring, although not enough activity to avoid a delay in the onset of ring constriction.

\textbf{Blt1p contributes to primary septum formation and division plane stability}

The absence of Blt1p delays recruitment of Bgs1p to the cleavage furrow and the onset of primary septum synthesis, since these processes depend on output from SIN (Le Goff \textit{et al.}, 1999; Liu \textit{et al.}, 1999; Cortes \textit{et al.}, 2002; Jin \textit{et al.}, 2006) and cell wall synthesis contributes to ingestion of the cleavage furrow (Liu \textit{et al.}, 1999; Proctor \textit{et al.}, 2012). In both wild-type and \textit{blt1}Δ cells, contractile ring constriction and primary septum synthesis begin at the point where \sim 8000 molecules of Bgs1p accumulate at the division plane, but reaching this number of Bgs1p molecules takes 10 min longer without Blt1p. During this delay in the initiation of primary septum synthesis and ring constriction, contractile rings may slide away from the cell equator and displace the division plane, as observed under other conditions that disrupt assembly of the primary septum (Liu \textit{et al.}, 1999; Pardo and Nurse, 2003; Huang \textit{et al.}, 2008). Although the direct link between the Mob1p/Sid2p and Bgs1p has not been identified, it is possible that the Clp1p phosphatase may also contribute to primary septum synthesis, as Clp1p overexpression can promote septum assembly and completion of cytokinesis in cells with disrupted contractile rings (Mishra \textit{et al.}, 2004).

\textbf{MATERIALS AND METHODS}

\textbf{Strain construction and growth methods}

Supplemental Table S1 lists the fission yeast strains used in this study. Strains were constructed by tagging genes at their endogenous loci using PCR-based gene targeting protocols (Bahler \textit{et al.}, 1998) so fusion proteins were expressed under the control of their native promoters. Cells for microscopic studies were grown in exponential phase at 25°C in YE5S (yeast extract with amino acid supplements) liquid medium in 50-ml flasks to a density between 0.2 and 0.4 OD595. Cells with genes under control of the nmt promoter were grown in exponential phase at 25°C for 24 h before transitioning to EMM5S (Edinburgh minimal medium with amino acid supplements) liquid medium with 15 μM thiamine to deplete protein expression for 10 h before imaging.

\textbf{Microscopy}

Cells were centrifuged at 500 \times g for 1 min and washed three times with EMM5S. Cells were plated on a thin pad of 25% gelatin (Wu \textit{et al.}, 2008; Arasada and Pollard, 2011). Fluorescence images of live cells were acquired between 23 and 25°C with an Olympus IX-71 inverted microscope with a 63\times numerical aperture 1.4 Plan Apo lens, Andor Technology (Belfast, Northern Ireland) CSU-X1 confocal spinning disc confocal system, and Andor Technology iXON-EM-CCD camera. Unless otherwise noted, Z-stacks of 16 slices at 0.5-μm intervals were acquired at 2-min intervals with an exposure time of 100 ms. For each condition, data were collected from multiple cells in multiple experiments.

\textbf{Image analysis}

Image analysis was conducted with ImageJ software and macros (National Institutes of Health, Bethesda, MD). Cells expressed the fluorescently tagged protein of interest along with the spindle pole body protein Sad1p-CFP or Sad1p-RFP to place events on a time scale in which time zero is defined as separation of the spindle pole...
bodies (Wu et al., 2003). We defined cell cycle events as follows: transition of interphase nodes to cytokinesis nodes was the time when fluorescently tagged Cdc15p and Myo2p appeared in punctae at the middle of cells in maximum intensity projection images; formation of a complete contractile ring was when nodes condensed into a continuous ring in maximum intensity projection images and three-dimensional reconstructions; the onset of contractile ring constriction and septum deposition was when the diameter of the ring began to change; and completion of ring constriction or septum deposition was when the ring diameter stopped changing.

The numbers of GFP or YFP molecules in the cleavage furrow region were acquired by measuring the fluorescence intensity in sum images of a rectangular area enclosing the nodes or ring. The measurements were corrected for camera noise, uneven illumination, diffuse cytoplasmic fluorescence, and acquisition photobleaching (Hoffman et al., 2001; Wu and Pollard, 2005). The number of molecules per pixel was calculated from a calibration curve based on the fluorescence intensity of seven mGFP or mYFP tagged proteins: capping protein (Acp2p), α-actinin (An1p), actin-related protein 2 (Arp2p), actin-related protein 3 (Arp3p), Arp complex protein C5 (ArpC5p), fimbrin (Fim1p), and type II myosin (Myo2p; Wu and Pollard, 2005; Wu et al., 2008).

We used a log-rank test for Kaplan–Meier curves (Klein and Moeschberger, 2003; Rich et al., 2010) implemented with software from MedCalc for Windows 12.7.8 (MedCalc Software, Ostend, Belgium) to compare whole outcomes curves and calculate a p value for a pair of curves being different. Statistical significance for molecule quantification measurements was calculated using the t test.

Cellular viability and growth assays

*S. pombe* cells were grown in YE5S liquid medium at 25°C for 24 h, diluted 1:100, and grown an additional 12 h at 25°C to maintain a population in exponential growth phase at a density between 0.2 and 0.4 OD595. Equal numbers of cells were serially diluted 10-fold four times, and 5-μl aliquots were spotted onto YE5S agar plates for growth at 25, 30, or 36°C for 36–72 h. Plates were placed on a backlight box (Hall Productions, San Luis Obispo, CA), and a digital camera (Kodak DC290; Rochester, NY) was used to acquire images by transmitted light. Images were loaded into ImageJ, and colony growth was measured by densitometry using the NIH Image Gel Analyzer plug-in. Percentage growth for each condition is shown relative to wild-type growth in Table 2.

Expression and purification of Blt1p

The *S. pombe* DNA sequence encoding full-length Blt1p amino acid residues 1–700 was cloned into the BamHI site of pQE80L vector (Qiagen, Valencia, CA), which added a hexahistidine tag to the C-terminus. Clones were transformed into ArcticExpress-competent cells (Qiagen) equilibrated with bacterial lysis buffer and washed with 50 ml of Ni-NTA wash buffer (50 mM sodium phosphate, 300 mM NaCl, 20 mM imidazole, pH 8.0). Proteins were eluted with 15 ml of Ni-NTA elution buffer (50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, pH 8.0) and dialyzed overnight at 4°C into MonoQ buffer A (20 mM Tris, pH 8.0, 50 mM NaCl, 1 mM diithiothreitol [DTT]).

Step 2: Anion exchange chromatography. The dialyzed sample was loaded onto an 8-ml column of Mono Q 5/50GL (GE Healthcare) in MonoQ buffer A, and unbound proteins were washed out using two column volumes of MonoQ buffer A. Fractions of 0.5 ml were collected during elution with a linear salt gradient from 0 to 100% MonoQ buffer B (20 mM Tris, pH 8.0, 500 mM NaCl, 1 mM DTT) over 40 column volumes at 0.5 ml/min. The Blt1p-6xHis peak eluted at 38–48% MonoQ buffer B, corresponding to 215 mM NaCl.

Step 3: Size exclusion chromatography. Fractions 30–38 from step 2 containing Blt1p were pooled and concentrated to <1 ml with an Amicon Ultra 3000 MWCO centrifugal filter (Millipore, Billerica, MA) and purified on a 120-ml column of Superdex 200 (GE Healthcare Bio-Sciences, Piscataway, NJ) in gel filtration buffer (20 mM Tris, pH 8.0, 20 mM NaCl, 0.5 mM EDTA, 1 mM DTT). Fractions of 1.3 ml were collected over one column volume at 0.3 ml/min. Blt1p-6xHis eluted in fractions 42–48, which were pooled and concentrated to <500 μl with an Amicon Ultra 3000 MWCO centrifugal filter (Millipore). The final purified Blt1p retained the hexahistidine tag.

Analytical gel filtration

We calibrated a 24-ml column of Superdex 200 (GE Healthcare Bio-Sciences) with five purified proteins in gel filtration buffer at 0.5 ml/min: thyroglobulin, ferritin, catalase, aldolase, and serum albumin. The peak elution volume for each protein was used to calculate a partition coefficient using the formula

\[
p = \frac{\text{elution volume} - \text{void volume}}{\text{column volume} - \text{void volume}}
\]

where the total column volume was 24.4 ml and the void volume was 8.3 ml. An inverse complement error function was calculated for each protein using the Wolfram Functions Site InverseFrc program (Wolfram Research, Champaign, IL), and these values were plotted against the Stokes radius for each protein standard to obtain a formula for calculating the Stokes radius of Blt1p from its InverseFrc partition coefficient (Akers, 1967).

Sedimentation velocity analytical ultracentrifugation

Sedimentation velocity analytical ultracentrifugation was carried out at 20°C using a Beckman XL-1 analytical ultracentrifuge. Samples of 400 μl of purified Blt1p or gel filtration buffer were loaded in two-channel centerpieces fitted with quartz windows in a four-hole rotor. Samples were centrifuged at 42,000 rpm and monitored by scanning absorbance at 280 nm along the radial length of the cell every 5 min. SEDFIT and SEDNTERP software were used to analyze the sedimentation profiles and calculate molecular weights, diffusion coefficients, density and viscosity of the buffer, and partial specific volume of the protein.

Binding experiments

*S. pombe* strains expressing Cdc15p-mEGFP, Mob1p-mEGFP, or Sid2p-mEGFP were grown in YE5S liquid medium at 25°C for 24 h, diluted 1:100 into 100 ml of YE5S, and grown an additional 12 h at 25°C to maintain a population in exponential growth phase at a density between 0.2 and 0.4 OD595. Cultures were centrifuged at 1000 × g for 15 min at 25°C, and cells were washed three times in...
S. pombe lysis buffer (20 mM phosphate buffer, 10 mM NaCl, 1% Triton X-100, 0.2 mM DTT, 1 mM phenylmethylsulfonyl fluoride, pH 7.4, with two Roche protease inhibitor tablets per 10-ml buffer). Cells were lysed with a bead beater (Wu and Pollard, 2005) and clarified by centrifugation at 13,000 × g for 15 min at 4°C. Lysates were incubated 1:1 with 150 μl of Ni-NTA agarose resin (Qiagen) in S. pombe lysis buffer for 30 min at 4°C on a rotary wheel. Samples were centrifuged at 1000 × g for 5 min, and supernatants were removed.

A 1:1 solution of Ni-NTA agarose resin (Qiagen) in S. pombe lysis buffer (200-μl total volume) was incubated with or without 10 μM Bt1p-hexahistidine for 30 min at 4°C on a rotary wheel. Ni-NTA agarose was washed three times with 200 μl of S. pombe lysis buffer to remove unbound Bt1p-hexahistidine. Samples of 150 μl of the precleared lysates from cells expressing Cdc15p-mEGFP, Mob1p-mEGFP, or Sid2p-mEGFP were added to the 50 μl of Ni-NTA resin and incubated at 4°C for 2 h on a rotary wheel. Samples were centrifuged at 1000 × g for 5 min, the 150-μl supernatants were removed, and 50 μl of SDS protein sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA, and 0.02% bromophenol blue) was added to each sample (200-μl total volume). Pelleted beads were washed three times with 150 μl of S. pombe lysis buffer and resuspended in 50 μl of protein sample buffer. Supernatant and pellet fractions were boiled for 5 min, loaded on a 4−20% SDS-polyacrylamide gel, and run at 120 V for 1.5 h.

**Immunoblotting**

Electrophoresis was used to transfer proteins from SDS–polyacrylamide gels to nitrocellulose membranes (GE Healthcare Bio-Sciences). GFP fusion proteins from S. pombe lysates were detected by immunoblotting (Wu and Pollard, 2005) using a 1:4000 dilution of ab290 anti-GFP primary antibody (Abcam, Cambridge, England) and 1:15,000 dilution of horseradish peroxidase–conjugated anti-rabbit secondary antibody (4050-05; Southern Bio Tech, Birmingham, AL). Membranes were incubated with ECL reagent (GE Healthcare Bio-Sciences), exposed to x-ray film (GE Healthcare Bio-Sciences), and developed.

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**REFERENCES**

Akers GK (1967). A new calibration procedure for gel filtration columns. J Biol Chem 242, 3237–3238.

Almonacid M, Celton-Morizur S, Jakubowski JL, Dingli F, Loew D, Mayeux A, Chen JS, Gould KL, Clifford DM, Paoletti A (2011). Multicellular control of cell-cycle commitment by Plo1 regulation of myosin II recruitment in S. pombe. Mol Biol Cell 22, 5195–5210.

Akers GK (1967). A new calibration procedure for gel filtration columns. J Biol Chem 242, 3237–3238.

Baker KE, Badoglou A, Fairlamb J, Gething MJ, Hinchcliffe MJ, Keates AK, McCollum D (2000). The SIN kinase Sid2 regulates cytokinesis. Mol Biol Cell 11, 1959–1999.

Bezanilla M, Forsburg SL, Pollard TD (1997). Identification of a second myosin-II in Schizosaccharomyces pombe: Myp2p is conditionally required for cytokinesis. Mol Biol Cell 8, 693–705.

Coffman VC, Niles A, Lui J, Wu JQ (2009). Roles of formin nodes and myosin motor activity in Mid1p-dependent contractile-ring assembly during fission yeast cytokinesis. Mol Biol Cell 20, 5195–5210.

Cortes JC, Ishiguro J, Duran A, Ribas JC (2002). Localization of the (1, 3)-beta-D-glucan synthase catalytic subunit homologue Bgs1p/Cps1p from fission yeast suggests that it is involved in septation, polarized growth, mating, spore wall formation, and spore germination. J Cell Sci 115, 4081–4096.

Cuelle N, Salimova E, Estaban V, Blanco M, Moreno S, Bueno A, Simanis V (2001). Fli1, a fission yeast orthologue of the S. cerevisiae CDC14 gene, is not required for cyclin degradation or rum1p stabilisation at the end of mitosis. J Cell Sci 114, 2649–2664.

Dingli F, Mathé A, Mory K, Sokolowska C, Mayeux A (1999). Blt1 and Mob1 provide overlapping membrane anchors to position the division plane in fission yeast. Mol Biol Cell 13, 388–408.

Erickson HP (2009). Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol Proced Online 11, 32–51.

Estaban V, Blanco M, Cuelle N, Simanis V, Moreno S, Bueno A (2004). A role for the Cdc14-family phosphatase Fis1p at the end of the cell cycle in controlling the rapid degradation of the mitotic inducer Cdc25p in fission yeast. J Cell Sci 117, 2461–2468.

Fankhauser C, Reidman A, Cerutti L, Utzig S, Hofmann K, Simanis V (1995). The S. pombe cdc15 gene is a key element in the reorganization of Fa-actin at mitosis. Cell 82, 435–444.

Guzman-Vendrell M, Baldissard S, Almonacid M, Mayeux A, Paoletti A, Moseley JB (2013). Blt1 and Mob1 provide overlapping membrane anchors to position the division plane in fission yeast. Mol Biol Cell 33, 418–428.

Hachet O, Simanis V (2008). Mid1p/anillin and the septation initiation network orchestrate contractile ring assembly for cytokinesis. Genes Dev 22, 3205–3216.

Hoffman DB, Pearson CG, Yen TJ, Howell BJ, Salmon ED (2001). Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at Ptk1 kinetochores. Mol Biol Cell 12, 1995–2009.

Hou MC, Guerchin DA, McCollum D (2004). Initiation of cytokinesis is controlled through multiple modes of regulation of the Sid2p-Mob1p kinase complex. Mol Cell Biol 24, 3262–3276.

Hou MC, Salek J, McCollum D (2000). Mob1p interacts with the Sid2p kinase and is required for cytokinesis in fission yeast. Curr Biol 10, 619–622.

Huang T, Tan H, Balasubramanian MK (2008). Assembly of normal actomyosin rings in the absence of Mid1p and cortical nodes in fission yeast. J Cell Biol 183, 979–988.

Jin QW, Zhou M, Bimbo A, Balasubramanian MK, McCollum D (2006). A role for the septation initiation network in septum assembly revealed by genetic analysis of sid2-250 suppressors. Genes Dev 20, 2101–2112.

Jourdain I, Brzezinska EA, Toda T (2013). Fission yeast Nod1 is a component of cortical nodes involved in cell size control and division site placement. J Cell Biol 199, 2015–2026.

Klein JP, Moeschberger ML (2003). Survival Analysis: Techniques for Censored and Truncated Data, 2nd ed., New York: Springer.

Krap A, Simanis V (2008). An overview of the fission yeast septation initiation network (SIN). Biochem Soc Trans 36, 411–415.

Laporte D, Coffman VC, Lee JU, Wu JQ (2011). Assembly and architecture of precursor nodes during fission yeast cytokinesis. J Cell Biol 192, 1005–1021.

Le Goff X, Motegi F, Salimova E, Mabuchi I, Simanis V (2000). The S. pombe ncl1 gene encodes a putative myosin regulatory light chain that binds the type II myosins Myo3p and Myo2p. J Cell Sci 113, 4158–4163.

Le Goff X, Woollard A, Simanis V (1999). Analysis of the cps1 gene provides evidence for a septation checkpoint in Schizosaccharomyces pombe. Mol Gen Genome 262, 163–172.

Liu J, Wang H, Balasubramanian MK (2000). A checkpoint that monitors cytokinesis in Schizosaccharomyces pombe. J Cell Sci 113, 1223–1230.
Roberts-Galbraith RH, Ohi MD, Ballif BA, Chen JS, McLeod I, McDonald WH, Gygi SP, Yates JR3rd, Gould KL (2010). Dephosphorylation of F-BAR protein Cdc15 modulates its conformation and stimulates its scaffolding activity at the cell division site. Mol Cell 39, 86–99.

Saha S, Pollard TD (2012). Anillin-related protein Mid1p coordinates the assembly of the cytokinetic contractile ring in yeast. Mol Biol Cell 23, 3982–3992.

Salanova E, Sohmann M, Fournier N, Simanis V (2000). The S. pombe orthologue of the S. cerevisiae mob1 gene is essential and functions in signaling the onset of septum formation. J Cell Sci 113, 1695–1704.

Sladewski TE, Previs MJ, Lord M (2009). Regulation of fission yeast myosin-II function and contractile ring dynamics by regulatory light chain and heavy chain phosphorylation. Mol Biol Cell 20, 3941–3952.

Sparks CA, Morphew M, McCollum D (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. J Cell Biol 146, 777–790.

Trautmann S, Wolfe BA, Jorgensen P, Tyers M, Gould KL, McCollum D (2001). Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. Curr Biol 11, 931–940.

Vavylonis D, Wu JQ, Hao S, O’Shaughnessy B, Pollard RD (2008). Assembly mechanism of the contractile ring for cytokinesis by fission yeast. Science 319, 97–100.

Wolfe BA, Gould KL (2004). Fission yeast Clp1p phosphatase affects G(2)/M transition and mitotic exit through Cdc25p inactivation. EMBO J 23, 919–929.

Wu JQ, Kuhn JR, Kovar DR, Pollard TD (2003). Spatial and temporal pathway for assembly and constriction of the contractile ring in fission yeast cytokinesis. Dev Cell 5, 723–734.

Wu JQ, McCormick CD, Pollard TD (2008). Chapter 9: counting proteins in living cells by quantitative fluorescence microscopy with internal standards. Methods Cell Biol 89, 253–273.

Wu JQ, Proctor SA, Minc N, Boudaoud A, Chang F (2012). Contributions of turgor pressure, the contractile ring, and septum assembly to forces in cytokinesis in fission yeast. Curr Biol 22, 1601–1608.

Rich JT, Neely JG, Paniello RC, Voelker CC, Nussenbaum B, Wang EW (2010). A practical guide to understanding Kaplan-Meier curves. Otolaryngol Head Neck Surg 143, 331–336.

Ye Y, Lee UJ, Runge KW, Wu JQ (2012). Roles of putative Rho-GEF Gel2 in division-site positioning and contractile-ring function in fission yeast cytokinesis. Mol Biol Cell 23, 1181–1195.