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The NoCut Pathway Links Completion of Cytokinesis to Spindle Midzone Function to Prevent Chromosome Breakage

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SUMMARY

During anaphase, spindle elongation pulls sister chromatids apart until each pair is fully separated. In turn, cytokinesis cleaves the cell between the separated chromosomes. What ensures that cytokinesis proceeds only after that all chromosome arms are pulled out of the cleavage plane was unknown. Here, we show that a signaling pathway, which we call NoCut, delays the completion of cytokinesis in cells with spindle-midzone defects. NoCut depends on the Aurora kinase Ipl1 and the anillin-related proteins Boi1 and Boi2, which localize to the site of cleavage in an Ipl1-dependent manner and act as abscission inhibitors. Inactivation of NoCut leads to premature abscission and chromosome breakage by the cytokinetic machinery and is lethal in cells with spindle-elongation defects. We propose that NoCut monitors clearance of chromatin from the midzone to ensure that cytokinesis completes only after all chromosomes have migrated to the poles.

INTRODUCTION

Mitosis is a highly coordinated process in which the two copies of each chromosome (sister chromatids) are segregated away from each other to opposite poles of the cell. Subsequently, the cell is cleaved between the two newly formed nuclei, leading to two independent cells. To ensure that each daughter cell inherits a single and complete copy of the genome, chromosome segregation and cell division are tightly coordinated. The fidelity of these events is ensured by complex surveillance mechanisms that detect and correct errors (for review, see Hartwell and Weinert [1989]).

The spindle checkpoint, together with kinetochore proteins and the kinase Aurora B (Ipl1 in yeast), ensures that chromosome segregation begins only after each pair of sister chromatids achieves bipolar attachment to the mitotic spindle (Musacchio and Hardwick, 2002). Chromosome segregation is then fulfilled by elongation of the anaphase spindle. Elongation is supported by the spindle midzone, which bundles antiparallel nonkinetochore spindle microtubules (Glotzer, 2005; McCollum, 2004). Numerous molecules localize to the spindle midzone. The microtubule-bundling protein PRC1 (Ase1 in budding yeast) is required for spindle stability. In addition, a number of yeast kinetochore components such as Ndc10, Ndc80, and Slk19 and the chromosomal passenger proteins Aurora B, INCENP, and survivin (Ipl1, Sli15, and Bir1 in budding yeast) also localize to the midzone (Adams et al., 2001; Bouck and Bloom, 2005; Buvelot et al., 2003; Glotzer, 2005; Sullivan et al., 2001). When the spindle becomes longer than twice the longest chromosome arm, chromosome segregation is achieved.

Cytokinesis, the final step of cell division, physically dissociates the two daughter cells from each other (Glotzer, 2005; Guertin et al., 2002). In animal cells, cytokinesis starts with contraction of the equatorial actomyosin ring, leading to membrane furrowing. As furrow ingression completes, the cells remain linked by a cytoplasmic bridge containing the remnant of the spindle midzone, the midbody. The actomyosin ring then disassembles while resolution of the plasma membrane, called abscission, completes cytokinesis.

In animal cells, spindle-midzone defects generally cause the furrow to regress, leading to the formation of binucleated cells (Glotzer, 2005; Guertin et al., 2002; McCollum, 2004). Cases of furrow regression have also been described in cells with incomplete DNA segregation (Meraldi et al., 2004a; Mullins and Biesele, 1977). However, it is not clear how and why defects in DNA segregation lead to cytokinesis failure.

Like in animal cells, yeast cytokinesis is a multistep process that also starts with the assembly and contraction of

an actomyosin ring. Contraction depends on activation of the mitotic exit network (MEN) and Cdk1 inactivation (for review, see Tolliday et al. [2001]). After contraction, the ring disassembles and the narrow cytoplasmic bridge left between the two daughter cells is resolved by abscission (Dobbelaere and Barral, 2004). Three independent molecular pathways fulfill these events. Type II myosin, Myo1, ensures actomyosin ring contraction. Hof1, homologous to *Schizosaccharomyces pombe* Cdc15p, defines a second pathway required for proper actin organization and septation. The third and least understood pathway depends on the protein Cyk3 and acts downstream of actomyosin ring contraction in septation and perhaps abscission. Inactivation of either of the *MYO1*, *HOF1*, or *CYK3* genes affects cytokinesis only mildly, whereas simultaneous disruption of any two of them abolishes cytokinesis and is lethal (Korinek et al., 2000; Tolliday et al., 2001).

Here, we investigate how cytokinesis is coordinated with anaphase. We show that similar to animal cells, yeast spindle-midzone defects prevent the completion of cytokinesis. Abscission inhibition depended on NoCut, a signaling pathway that involved the Aurora kinase, Ipl1, and the anillin-related proteins Boi1 and Boi2. Inactivation of this pathway causes chromosome breakage due to precocious abscission. Thus, our results provide a model for how the timing of cytokinesis is coordinated with chromosome segregation.

RESULTS

Ase1 Is Required for Timely Cytokinesis

In animal cells, spindle-midzone defects lead to cytokinesis failure. We therefore investigated whether the midzone is also required for cytokinesis in budding yeast. The microtubule binding protein Ase1, the homolog of human PRC1, is the only yeast protein known to localize exclusively to the spindle midzone. Yeast cells lacking Ase1 are viable and form highly unstable spindles that break down prematurely during anaphase (Schuyler et al., 2003). Tetrad analysis demonstrated that cells lacking Myo1 and Hof1 depend on Ase1 for survival, indicating that Ase1 is required for cytokinesis and suggesting that it may function in the Cyk3 pathway (data not shown). In support of this conclusion, the *ase1*Δ *cyk3*Δ double mutant was fully viable. These data indicate that Ase1 or the spindle midzone participates in yeast cytokinesis.

To characterize the role of Ase1 in cytokinesis, we visualized the plasma membrane at the bud neck of dividing *ase1*Δ cells using Ras2-GFP as a marker (Whistler and Rine, 1997). Cells that had initiated anaphase were identified by the fact that they had one spindle pole body (SPB) in the mother cell and the other in the bud. SPBs were visualized using Spc42-CFP as a marker. In ana-/telophase cells, the plasma membrane at the bud neck was in one of three states (Figure 1A): continuous ("open" neck, in which the furrow has not ingressed), contracted (the furrow had ingressed), or resolved into two (indicating that abscission was completed but mother cells had not yet separated from their buds). To determine which stage of cytokinesis was affected in *ase1*Δ mutants, we quantified the frequency of these different classes in asynchronous wild-type and *ase1*Δ cultures (Figure 1B). In wild-type, 45% of the ana- and telophase cells had not yet undergone contraction, while in 52% of the cells the membrane was clearly resolved into two. Only about 3% of the cells showed a "pinched" neck, in which the membrane had ingressed. The small fraction of cells with pinched bud necks reflects the short interval between furrow ingression and membrane resolution in wild-type cells. This category of cells was specifically and strongly increased (8-fold) in the *ase1*Δ population (Figure 1B). Thus, furrow ingression progressed properly but membrane resolution was delayed in the *ase1*Δ cells. These data are consistent with Ase1 acting in cytokinesis posterior to actomyosin ring contraction, most likely in abscission.

To further analyze the role of Ase1, we created a conditional *ASE1* shut-off allele. The Ase1 promoter was replaced by a weak version of the regulatable *GAL* promoter (GALS; Janke et al., 2004), which allowed Ase1 synthesis to be turned on to appropriate levels in galactose and turned off in glucose media. Accordingly, *GALS:HA-ASE1* cells did not contain detectable levels of HA-Ase1 protein when grown in glucose medium (Figure 1C). In these cells, the spindle failed to elongate and broke down prematurely compared to wild-type cells (see Figure 6B). Thus, the *GALS:HA-ASE1* allele allowed efficient depletion of Ase1 within a single cell cycle, consistent with Ase1 being unstable in G_1 (Juang et al., 1997). Similar to the *ase1*Δ mutant cells, the *GALS:HA-ASE1* cells delayed abscission upon shifting to the repressing medium (Figure 1B). In addition, a high fraction of *GALS:HA-ASE1* cells formed chains that failed to separate, where primary septa failed to form properly, as indicated by chitin staining (Figure 1E). These cells were not merely kept together by cell wall remnants but rather by a continuous plasma membrane, since digestion of the cell wall using zymolyase failed to dissociate the chains (Figure 1D). Together, these data indicate that Ase1 function is required for efficient resolution of the plasma membrane after furrow ingression and hence for proper abscission.

Mutations in Kinetochore Proteins that Affect the Spindle Midzone Also Prevent Normal Cytokinesis

The cytokinesis defect observed in cells lacking Ase1 suggested that the spindle midzone is involved in the control of abscission. Alternatively, cytokinesis might have been repressed until completion of chromosome segregation, which is delayed in these cells (Figure 7A). To distinguish between these possibilities, we tested whether mutations that impair chromosome segregation also impair cell cleavage. Kinetochore components fall into two classes relative to chromosome separation and spindle function. Ndc10, an inner kinetochore component, also localizes to the spindle midzone during anaphase and is required for both kinetochore assembly and midzone stability (Bouck and Bloom, 2005; Buvelot et al., 2003; see Figures S1A and S1B in the Supplemental Data available with this

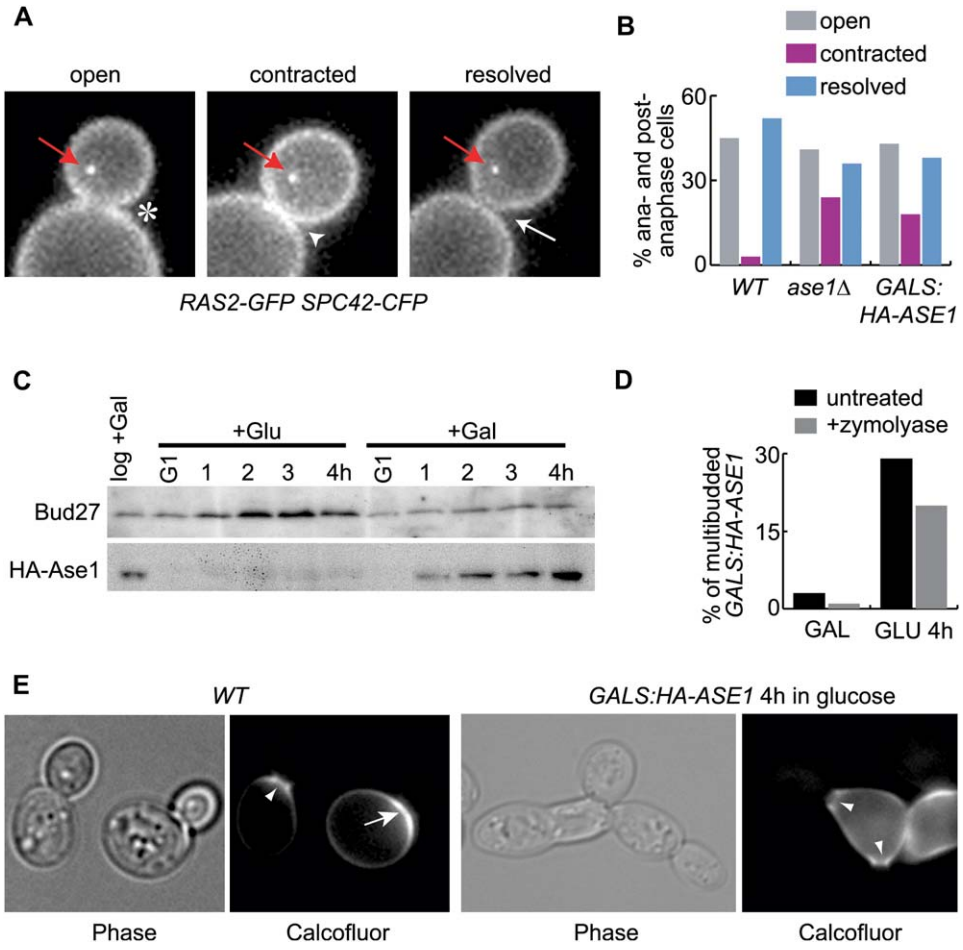

Figure 1. Ase1-Defective Cells Have a Cytokinetic Delay

(A) Visualization of the plasma membrane, labeled with Ras2-GFP, during cytokinesis (projections of nonconfocal planes along the z axis). Segregation of one SPB into the bud (red arrow) indicates that the cells have initiated anaphase. White symbols mark open bud necks (asterisk) and necks with contracted (arrowhead) or resolved membranes (arrow).

(B) Fraction of anaphase and postanaphase cells in the cytokinesis stages defined in (A). Resolution of the plasma membrane is delayed in the *ase1*Δ and *GAL:HA-ASE1* strains.

(C) Western blots of cell extracts from *GALS:HA-ASE1* cells probed with anti-HA and anti-Bud27 antibodies (loading control). Ase1 fails to accumulate in cells grown in glucose medium. The carbon source (Glu = glucose; Gal = galactose) and the time (hr) after release from G_1 are indicated.

(D) Fraction of *GALS:HA-ASE1* bibudded cells grown under inducing and noninducing conditions (4 hr), prior to and after zymolyase treatment.

(E) Morphology of wild-type and *GALS:HA-ASE1* cells grown for 4 hr in glucose medium. Staining of the cell wall with calcofluor white is shown. Arrowheads mark open bud necks while arrows point at closed septa.

article online). Using the midzone marker Slk19, we found that the central kinetochore component Ndc80 falls in the same category (Figure 2A). In contrast, the central kinetochore proteins Nnf1 and Mtw1 were required only for chromosome attachment to the spindle and not for midzone formation (Figures 2A and 2B). Progression of the *ndc80-1*, *nnf1-17*, and *mtw1-1* mutants through anaphase was obtained by deleting the *MAD2* gene. Analysis of bud formation indicated that all strains entered and exited mitosis with similar kinetics as wild-type (Figure S1C and data not shown).

Inactivation of kinetochore function in *nnf1-17 mad2*Δ and *mtw1-1 mad2*Δ double mutants resulted in little or no DNA being pulled into the bud upon anaphase and led to an increased number of cells that rebudded without separating from their first bud (Figure 2B). However, these mutants were not defective in cytokinesis because the bibudded cells were efficiently resolved by zymolyase treatment. Thus, the *nnf1-17 mad2*Δ and *mtw1-1 mad2*Δ double mutants completed cytokinesis but were defective in the subsequent degradation of the primary septum. In contrast, the *ndc10-1* single and *ndc80*Δ *mad2*Δ double mutants formed chains of cells that could not be separated by zymolyase treatment and were therefore defective in cytokinesis (Figure 2B). Thus, chromosome segregation was not required for proper completion of cytokinesis,

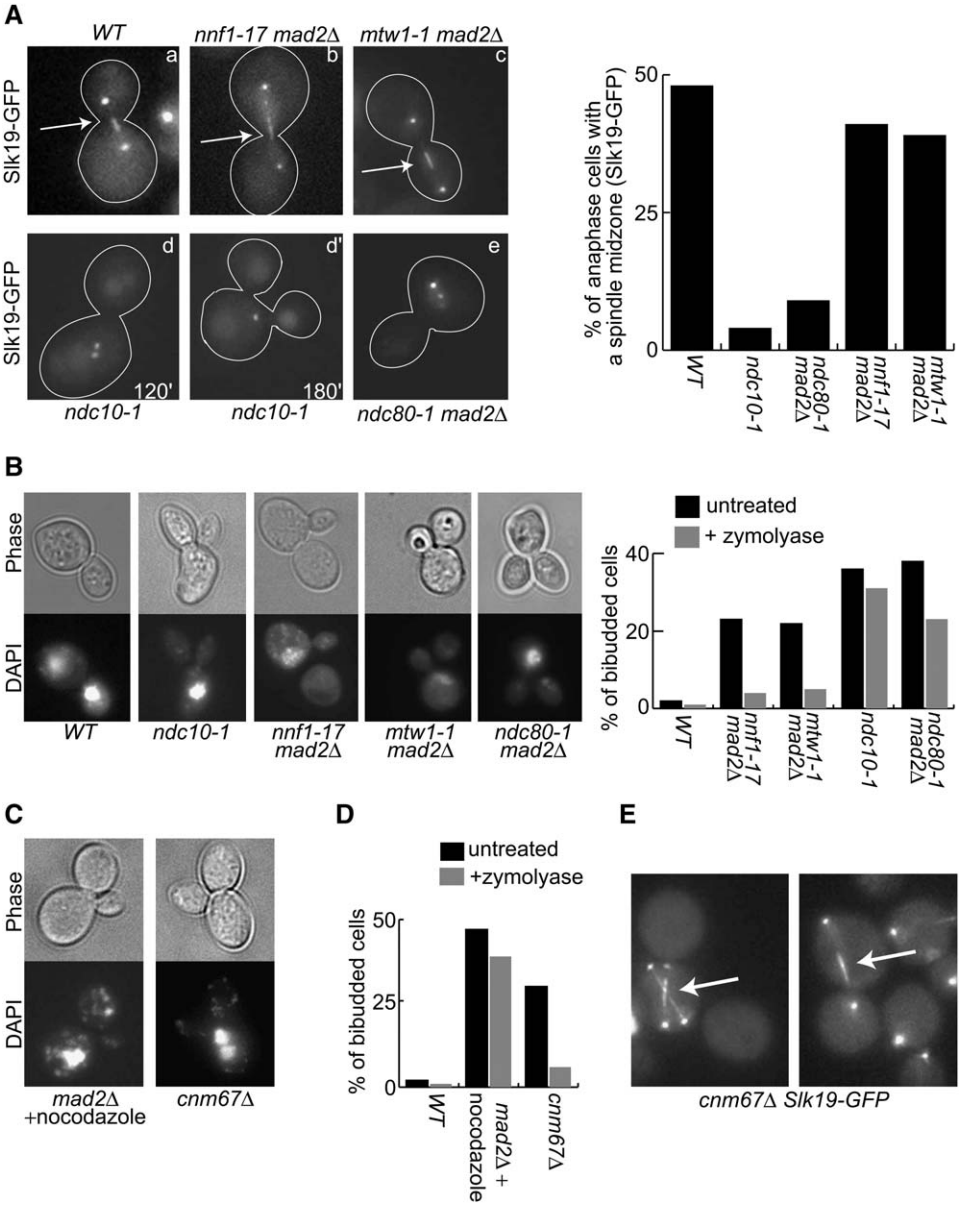

Figure 2. Role of Kinetochore Components in Spindle Midzone Assembly and Cytokinesis

(A) Localization of Slk19-GFP in kinetochore mutants undergoing anaphase. Cells (n > 100) were arrested in G_1 and released at the restrictive temperature. The fraction of cells with spindle midzones is shown, as indicated by Slk19 localization in wild-type and mutant strains. Arrows indicate the spindle midzones.

(B) Phase micrographs and DAPI staining (to visualize DNA) of kinetochore mutants allowed to progress through mitosis. Cells were synchronized as in (A) and examined 240 min after the temperature shift. The graph shows the fraction of bibudded cells before and after digestion of the cell wall with zymolyase.

(C) Morphology and DNA distribution (DAPI staining) of bibudded *cnm67*Δ and nocodazole-treated *mad2*Δ cells.

(D) Fraction of bibudded *cnm67*Δ and nocodazole-treated *mad2*Δ cells before and after zymolyase treatment.

(E) Localization of Slk19-GFP in *cnm67*Δ cells, showing that these cells fail to orient their spindles but assemble normal midzones (arrows).

and instead the cytokinesis failure correlated with midzone defects.

Accordingly, microtubules were found to be required for cytokinesis. When synchronized in G_1 with α factor and released into fresh medium containing nocodazole, *mad2*Δ cells progressed through the cell cycle, exited mitosis, and entered a new cycle, indicated by the formation of a second bud (Li and Murray, 1991; Figure 2C). These bibudded cells were not resolved by zymolyase (Figure 2D), indicating that they failed to complete cytokinesis. Our

analysis of the kinetochore mutants *nnf1-17* and *mtw1-1* indicated that kinetochore microtubule interactions were not involved in this process. To test whether cytoplasmic microtubules were involved, we analyzed the division of the *cnm67*Δ cells. Cnm67 is a component of the outer plaque of the SPB that anchors cytoplasmic microtubules to the SPB. Cells lacking Cnm67 fail to properly position their spindle but exit mitosis on schedule, due to defective organization of the SPB and the spindle orientation checkpoint (Hoepfner et al., 2000). We found that *cnm67*Δ cells completed cytokinesis properly (Figures 2C–2E). In these cells, Slk19-GFP localized to the spindle midzone normally (Figure 2E). Thus, neither microtubule-kinetochore attachment nor cytoplasmic microtubules are required for cytokinesis. We concluded that the microtubules required for cytokinesis corresponded to those of the midzone. It is important to note that cytokinetic defects did not correlate with a failure in mitotic exit, since *ndc10-1* and nocodazole-treated *mad2*Δ cells went on to rereplicate DNA, degrade Pds1 and Clb2, and accumulate Sic1 with kinetics similar to wild-type (Fraschini et al., 2001; Figure 3C and data not shown). It is also noteworthy that *cnm67*Δ cells misposition the spindle midzone relative to the bud neck but complete cytokinesis normally. Therefore, the midzone position is irrelevant for yeast cytokinesis.

Cells with Damaged Spindles Are Defective in Abscission

Because Ase1-depleted cells delay abscission, we tested whether *ndc10-1*, *ndc80-1 mad2*Δ, and nocodazole-treated *mad2*Δ cells also suffer of abscission defects. Calcofluor staining showed that these cells failed to complete septation (Figure 3A). Moreover, time-lapse microscopy showed that the actomyosin ring contracted and disassembled with wild-type kinetics in *ndc10-1* and nocodazole-treated *mad2*Δ cells (Figure 3B). Thus, in these cells, cytokinesis was impaired after actomyosin ring contraction.

To investigate whether abscission failed, we monitored the plasma membrane at the bud neck. Cells containing Ras2-GFP were imaged in three dimensions by spinning disk confocal microscopy. In *cnm67*Δ and untreated *mad2*Δ mutant cells, the plasma membrane was continuous through the bud neck of mitotic cells. In postmitotic cells, two plasma membranes were observed that were clearly separated by the unlabeled septum (Figure 3D), indicating that these cells completed abscission. In contrast, the plasma membrane of the postmitotic *mad2*Δ cells treated with nocodazole had ingressed but was not resolved, indicating that abscission failed (Figure 3D). Consistent with this, *ndc10-1* and nocodazole-treated *mad2*Δ cells displayed disorganized septin rings, a phenotype frequently observed in abscission mutants (Dobbelaere and Barral, 2004; Dobbelaere et al., 2003; Figure 3E). By comparison, abscission-competent *cnm67*Δ cells showed septin rings indistinguishable from wild-type. Taken together, these results indicated that yeast cells with spindle-midzone defects display septin disorganization and delay or fail abscission.

The Aurora Kinase Ipl1 Inhibits Abscission in Cells with Spindle-Midzone Defects

Two scenarios can account for the cytokinesis defect of midzone-defective cells. The spindle midzone might provide a positive signal necessary for abscission. Alternatively, active inhibition of abscission might delay cytokinesis as a secondary response to midzone defects. In the latter case, cells that are defective in inhibition should complete abscission even in the absence of a midzone. The yeast Aurora kinase Ipl1 and its associated protein Sli15/INCENP localize to the spindle midzone during anaphase and might be required for midzone stability (Pereira and Schiebel, 2003). However, *ipl1-321* mutants do not exhibit a cytokinesis defect (Figure 4A; Biggins and Murray, 2001; Buvelot et al., 2003). We therefore tested whether the cytokinesis defect in midzone mutants requires Ipl1 kinase activity and asked whether the *ipl1-321* and *sli15-1* mutations rescued cytokinesis in midzone-defective cells. As shown in Figure 4A, inactivation of Ipl1 or Sli15 did not prevent the formation of bibudded cells. However, all bibudded cells formed a septum at postmitotic necks (Figure 4B) and were efficiently separated by zymolyase (Figure 4A). Spindle-midzone defects were not rescued in these cells (Figure 4C), indicating that inactivation of Ipl1 and Sli15 directly restored abscission. Thus, the spindle midzone is not required per se for cytokinesis. Instead, midzone defects cause Aurora/Ipl1- and INCENP/Sli15-dependent inhibition of abscission.

Ipl1-Dependent Inhibition of Abscission Requires the Bud Neck Proteins Boi1 and Boi2

To elucidate how Ipl1 inhibits abscission, we investigated whether it translocates from the closed nucleus to the bud neck in response to midzone defects. In *ndc10-1* cells, Ipl1-3GFP localized along the broken spindles (Figure 4D). In nocodazole-treated *mad2*Δ cells, no spindle assembled and Ipl1 localized to nuclear dots (data not shown). Ipl1 was never observed at the bud neck. Thus, we postulated that Ipl1 inhibits abscission through proteins that shuttle between the nucleus and the bud neck.

Boi1 and Boi2 are highly related proteins involved in polarized growth. They are structurally related to anillins, a family of cytokinesis proteins characterized by the presence of a pleckstrin-homology (PH) domain at their C terminus and by the ability to shuttle between the nucleus and the cell cortex. Accordingly, Boi1 and Boi2 carry a PH domain at their C termini, localize to the bud cortex during bud emergence and to the bud neck during mitosis (Hallett et al., 2002). Furthermore, they are occasionally observed in the nucleus of G_1 cells (E. Bailly, personal communication; Figure 5C). Finally, *boi1*Δ *boi2*Δ double-mutant cells separate normally (Figure 4A), indicating that they might not act positively in cytokinesis. Thus, we asked whether they acted as cytokinetic inhibitors.

Therefore, we constructed the *ndc10-1 boi1*Δ *boi2*Δ triple mutant and analyzed its phenotype. Four hours after α factor release, 26% of the *boi1*Δ *boi2*Δ *ndc10-1* triple mutant cells were bibudded (versus 36% in *ndc10-1*). Most of

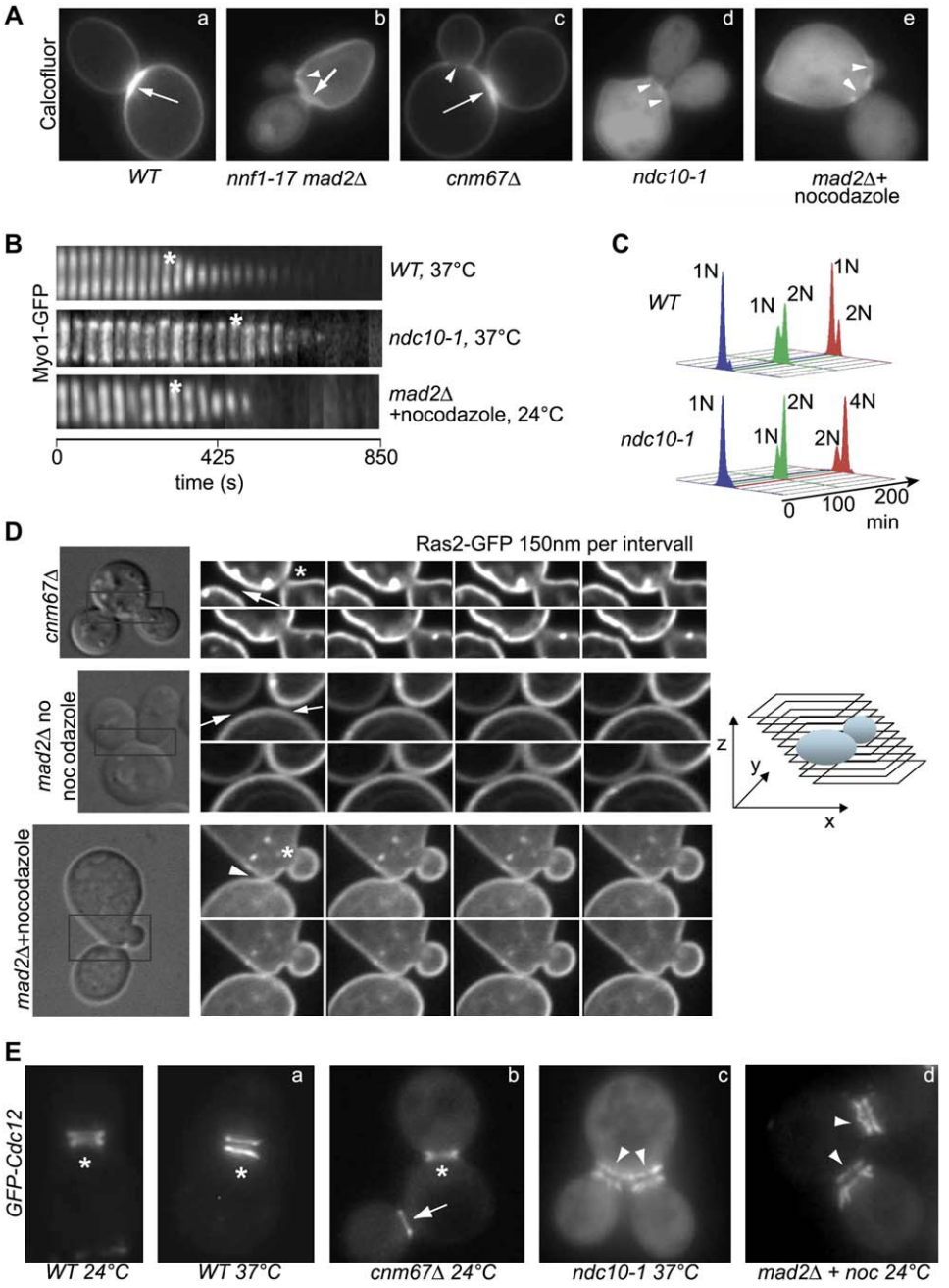

Figure 3. Abscission Is Impaired in Cells with Midzone Defects

(A) Cells were stained with calcofluor white to visualize septa. Arrows indicate closed septa while arrowheads indicate open bud necks.

(B) Kymographs showing actomyosin-ring contraction (Myo1-GFP) in cells of the indicated genotype. Asterisks mark the start of actomyosin-ring contraction. The time in seconds is indicated in the x axis.

(C) *ndc10-1* cells accumulate 4N DNA content after 200 min at the restrictive temperature. Aliquots from synchronized cultures were stained with propidium iodide, and their DNA content was analyzed by FACS. DNA content is indicated.

(D) The status of the plasma membrane was examined using Ras2-GFP as a marker. Cells were inspected 180 min after release from G_1 block. Images were acquired at 150 nm intervals along the z axis using a spinning disk confocal microscope. Arrowheads indicate unresolved plasma membrane bridges, arrows point at resolved plasma membranes, and asterisks mark open-bud necks.

(E) Analysis of septin-ring morphology, using the septin Cdc12-GFP as a reporter. Normal double rings are indicated with an asterisk. Arrowheads point to abnormal discontinuous rings. An arrow points at a single ring lying on the bud side of a *cnm67*Δ mutant.

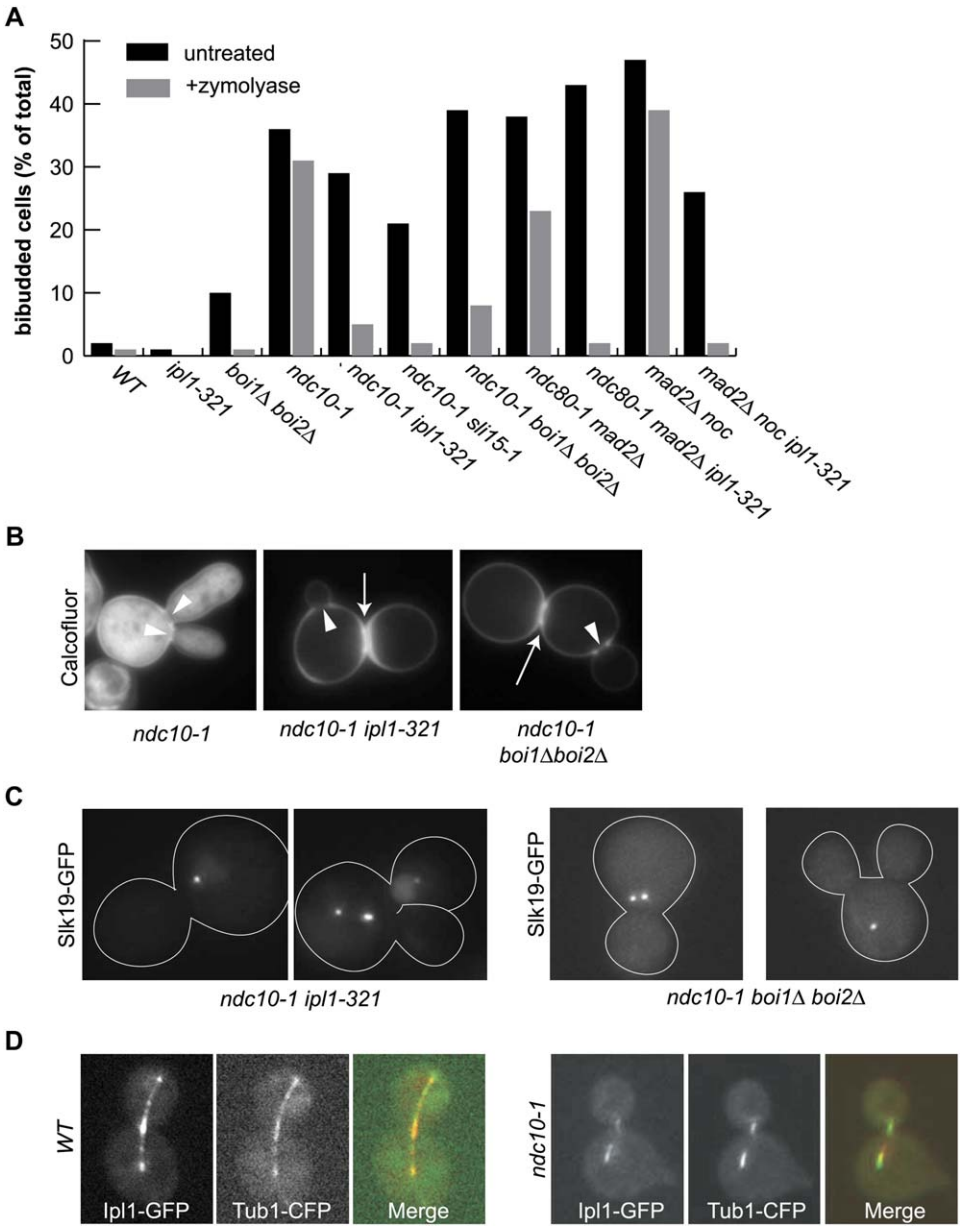

Figure 4. Aurora/Ipl1, INCENP/Sli15, and the Anillin-like Boi1 and Boi2 Proteins Inhibit Abscission in Cells with Midzone Defects

(A) Fraction of bibudded cells before and after zymolyase treatment. Cells were synchronized with α factor and analyzed 240 min after release from G_1 block at 37°C.

(B) Calcofluor staining of bibudded cells 240 min after release as in Figure 4A. Arrows indicate completed septa while arrowheads mark open-bud necks.

(C) Formation of the central spindle, as assayed by localization of Slk19-GFP as a reporter, is not restored in *ndc10-1 ipl1-321* and *ndc10-1 boi1Δ boi2Δ* cells. Cells were treated as in Figure 2A.

(D) Localization of Ipl1-3GFP (red) and Tub1-CFP (green) during anaphase of wild-type and *ndc10-1* cells grown at the restrictive temperature.

these cells formed proper septa (Figure 4B) and were resolved to unbudded and single-budded cells by zymolyase treatment (Figure 4A). Furthermore, as in the *ndc10-1 ipl1-321* double mutants, midzone defects were not suppressed in these cells (Figure 4C). Thus, Boi1 and Boi2 functioned in the inhibition of abscission in midzone defective cells.

We next carried out a detailed analysis of Boi1 and Boi2 localization. Coexpression of Boi1-GFP and CFP-Tub1 revealed that Boi1 translocated from the nucleus, where it was detected in a fraction of G_1 cells, to the cortex of the emerging bud in G_2 and to the bud neck of anaphase cells (Figures 5A, arrows, and 5B). After spindle breakdown, low

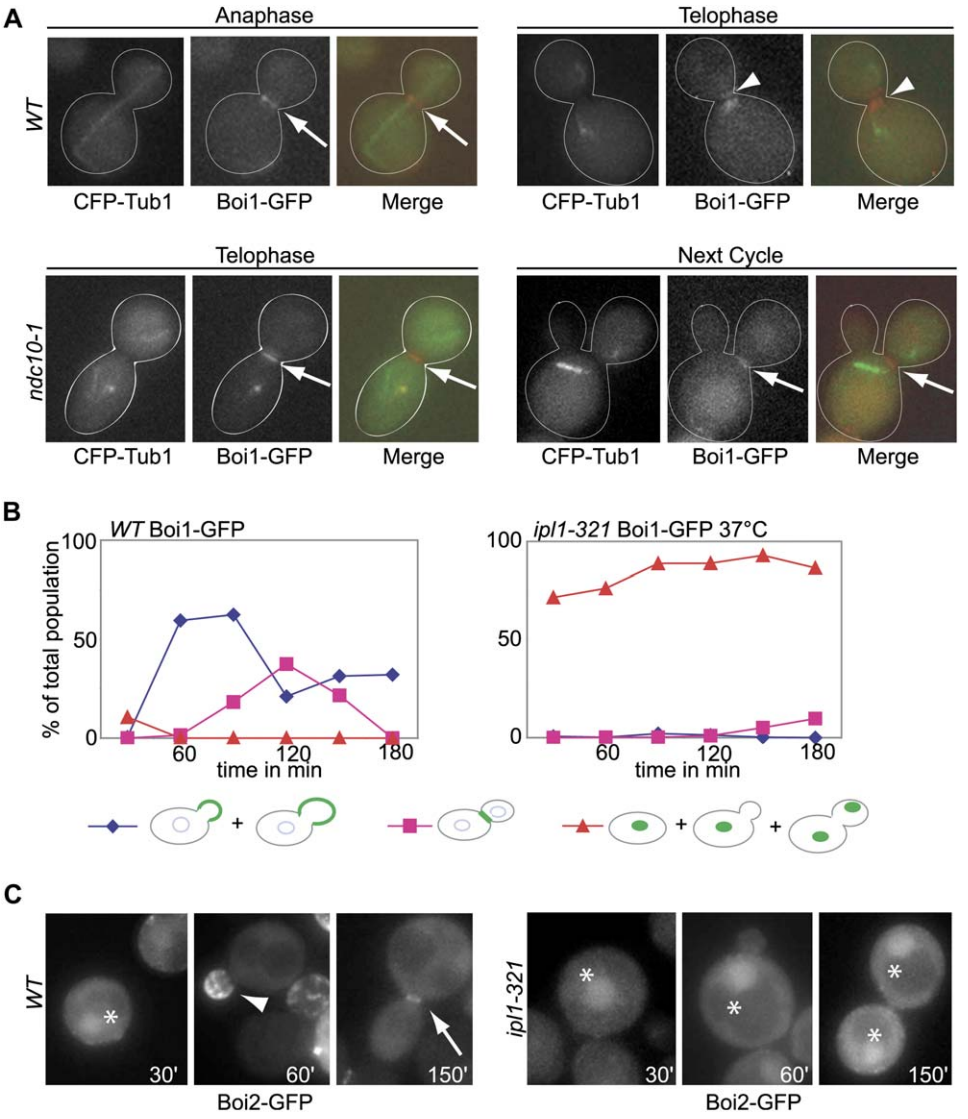

Figure 5. Boi1 and Boi2 Localization Depends on Ipl1 Function

(A) Localization of endogenously expressed Boi1-GFP in wild-type and *ndc10-1* cells during anaphase and telophase. CFP-Tub1 was used to monitor the status of the spindle. Arrows mark bud-neck localization of Boi1-GFP.

(B) Time course of Boi1-GFP localization in wild-type and *ipl1-321* cells progressing through the cell cycle.

(C) Localization of plasmid-borne Boi2-GFP in wild-type and *ipl1-321* cells. Arrows (at bud necks) and arrowheads (at the bud surface) indicate cortical Boi2-GFP while asterisks indicate nuclear localization.

levels of Boi1-GFP were briefly present on both sides of the neck (Figure 5A, arrowheads), and faded away prior to cell separation. Similar results were obtained for Boi2-GFP expressed from a multicopy plasmid, in which case the accumulation of Boi2-GFP to G_1 nuclei was more obvious (Figure 5C). In contrast to wild-type cells, most *ndc10-1* cells that failed abscission maintained Boi1 at the bud neck even after the emergence of a new bud (Figure 5A). Thus, the localization of Boi1 and Boi2 was consistent with a role in the inhibition of abscission.

Remarkably, in the *ipl1-321* mutant shifted to the restrictive temperature, Boi1-GFP failed to localize to the cortex of growing buds and to the bud neck of anaphase cells. Instead, it remained nuclear for the whole cell cycle (Figure 5B). Similar results were obtained for plasmid borne Boi2-GFP (Figure 5C). Thus, Ipl1 activity was required for the proper localization of Boi1 and Boi2, establishing that Boi1 and Boi2 act downstream of Ipl1. Boi1 and Boi2 contain several Ipl1 consensus phosphorylation sites. However, simultaneous mutation of all sites in both Boi1 and Boi2 did not alter their localization (data not shown). Thus, some other Ipl1 substrate might function in the control of Boi1 and Boi2 localization. Alternatively, additional Ipl1 sites might exist on these proteins.

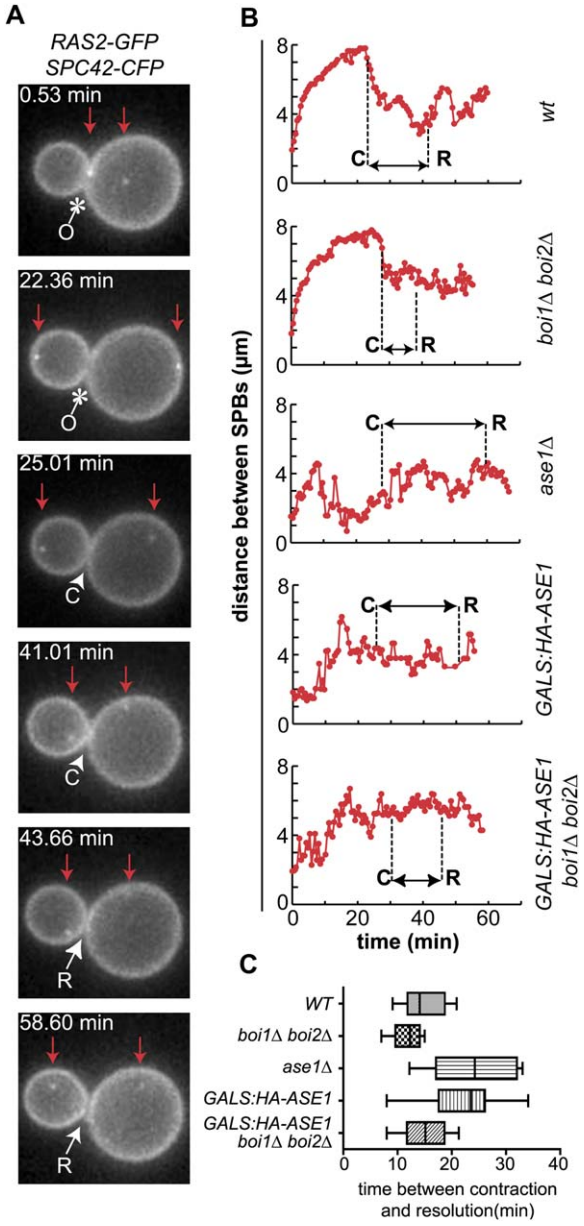

Figure 6. Boi1 and Boi2 Prevent Premature Abscission in Wild-Type and Delay Abscission in Ase1-Depleted Cells

(A) Selected frames (projections of three focal planes spaced 0.3 μm) from a movie of a wild-type cell during mitosis and cytokinesis. The signals of Ras2-GFP and Spc42-CFP were acquired simultaneously in the CFP channel to visualize the plasma membrane and the SPBs, respectively. Time 0 indicates anaphase onset. Red arrows indicate the position of the SPBs. Symbols and letters indicate whether membranes at the bud neck are open (O, asterisks), contracted (C, arrowheads), or resolved (R, arrows).

(B) Quantification of the distance between SPBs during mitosis and cytokinesis in representative cells of the indicated strains expressing Ras2-GFP and Spc42-CFP. The timing of membrane contraction (C) and resolution (R) is indicated. The upper graph corresponds to the wild-type cell shown in (A).

(C) "Box-and-whiskers" graph summarizing the time elapsed between membrane contraction and separation for all cells examined of the indicated strains. Boxes extend from the 25 to the 75 percentile, with a line at the median; whiskers extend to the most extreme values.

Boi1 and Boi2 Prevent Premature Abscission in Wild-Type Cells

To test whether Boi1/Boi2 are required to regulate abscission in all cells or only in cells with midzone defects, we analyzed the progression of cytokinesis in wild-type and *boi1*Δ *boi2*Δ double mutant cells using time-lapse microscopy. As in Figure 1A, we monitored mitotic progression and abscission using the reporters Spc42-CFP and Ras2-GFP. In all movies, the bud neck membrane contracted shortly after spindle breakdown, visualized by the sudden decrease in SPB-to-SPB distance (Figure 6A; t = 25 min; upper graph in Figure 6B; wild-type = 1.6 ± 1.2 min after spindle breakdown; *boi1*Δ *boi2*Δ = 1.3 ± 1.1 min; n = 13 and 9, respectively). In wild-type cells, the membrane remained "pinched" for an average of 15 min (±1.05 min; n = 13) before being resolved by abscission (Figure 6A; t = 43 min). Remarkably, the interval between membrane contraction and resolution was significantly reduced in the *boi1*Δ *boi2*Δ double-mutant cells (11.9 ± 0.9 min; n = 9; $p < 0.05$). Anaphase proceeded normally in these cells (Figure 6B). Thus, abscission was advanced in the *boi1*Δ *boi2*Δ double mutant as compared to wild-type, demonstrating that Boi1 and Boi2 control the timing of abscission in wild-type cells.

Boi1 and Boi2 Delay Abscission in Ase1-Depleted Cells

Consistent with our original observation (Figure 1B), time-lapse analysis showed that membrane resolution in *ase1*Δ mutant cells occurs approximately 24 min after contraction, 9 min later than wild-type (Figures 6B and 6C; mean *ase1*Δ = 24.2 ± 2.3 min; n = 10; $p < 0.005$). Similar results were obtained with the *GALS:HA-ASE1* cells grown in glucose (22.2 ± 2.3 min; n = 11; $p < 0.05$). This delay depended on Boi1 and Boi2, since the *boi1*Δ *boi2*Δ *GALS:HA-ASE1* triple mutant completed cytokinesis with wild-type kinetics (15.2 ± 1.3 min; n = 10). In these cells, spindle dynamics were identical to that of the *GALS:HA-ASE1* and *ase1*Δ cells, indicating that the midzone defect was not suppressed (Figure 6B). The timing of cytokinesis could not be determined for the *boi1*Δ *boi2*Δ *ase1*Δ triple-mutant cells because this strain was not viable. Micromanipulation of *boi1*Δ *boi2*Δ *ase1*Δ spores indicated that they germinated properly and underwent a few rounds of division, completing cytokinesis and cell separation before arrest (data not shown). Together, these data establish that Boi1 and Boi2 delay abscission and are required for cell viability in response to spindle-midzone defects.

Failure to Delay Abscission Causes Chromosome Breakage by the Cytokinetic Machinery

Together, our data indicate that spindle-midzone defects lead to Ipl1- and Boi1/Boi2-dependent inhibition of abscission. We next wondered about the biological function of

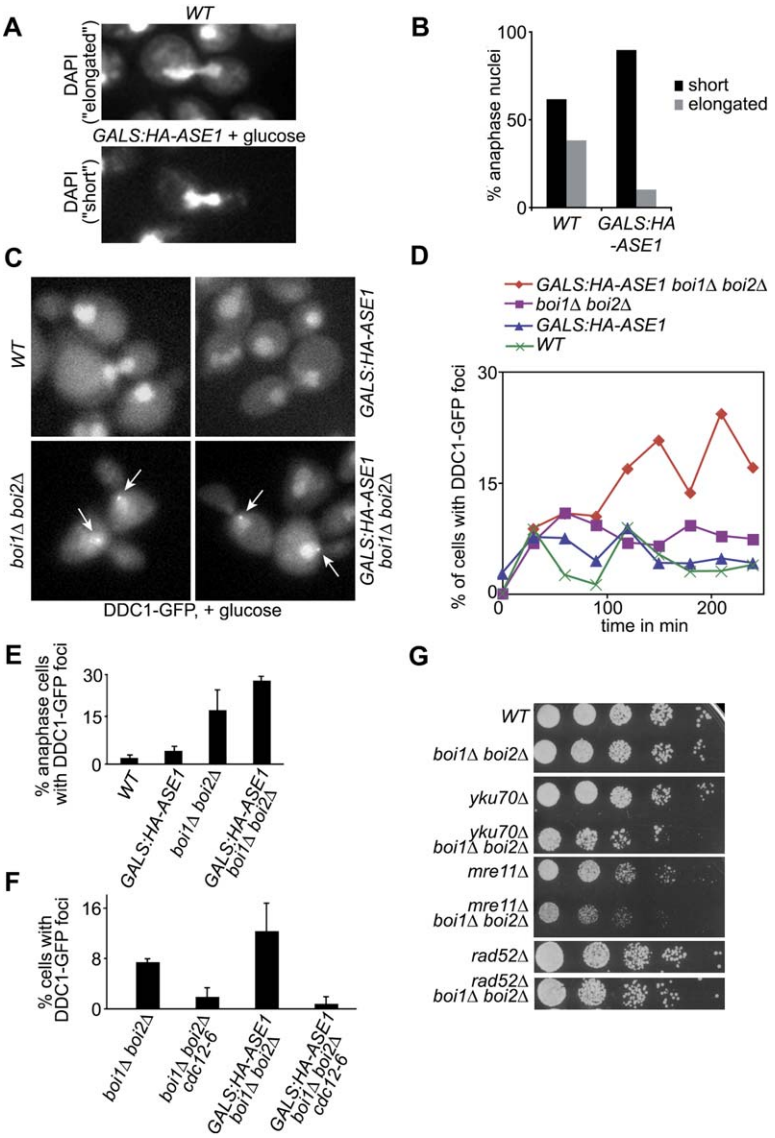

Figure 7. Boi1 and Boi2 Protect Chromosomes from Breakage by the Cytokinesis Machinery

(A and B) DAPI staining (A) and quantification of DNA separation (B) of wild-type and Ase1-depleted cells undergoing anaphase. Cells of the indicated strains were grown for 4 hr in glucose medium, and the shape of anaphase nuclei was scored. ("short": unsegregated chromosomes; "elongated": chromosomes stretched through the bud neck).

(C) Ddc1-GFP localization to double-strand break foci (arrows). Note that in *boi1*Δ *boi2*Δ and *GALS:HA-ASE1 boi1*Δ *boi2*Δ cells these foci frequently localize to the vicinity of the bud neck.

(D) Time course of Ddc1-GFP focus formation in cells released from G_1 into glucose medium.

(E) Fraction of anaphase cells with Ddc1-GFP foci, treated as in (D). In this and the next panel, data corresponding to three independent experiments are represented as mean ± SD.

(F) Ddc1-GFP focus formation in asynchronous cultures after 4 hr in glucose media at 37°C.

(G) Genetic interactions between *boi1*Δ *boi2*Δ mutations and mutations in nonhomologous end joining and homologous recombination genes. Cells were grown to log phase in liquid media. 5-fold dilutions were plated and inspected after 3 days.

this inhibition. Central spindle collapse during anaphase delays the clearance of the cleavage plane from chromatin. Indeed, upon shift to the repressive condition, chromosome segregation was strongly impaired in *GALS:HA-ASE1* cells, as assayed by DAPI staining (Figures 7A and 7B). Therefore, we rationalized that inhibition of abscission might prevent the chromosome arms still lagging in the cleavage plane from being broken by the cytokinetic machinery.

To test this possibility, we monitored the effect of Boi1 and Boi2 inactivation on chromosome integrity, using Ddc1-GFP as a reporter of DNA damage. Ddc1 is involved in the recognition and repair of double-strand breaks (DSBs), and Ddc1-GFP foci accumulate at sites of DNA damage (Melo et al., 2001). When wild-type and *GALS:HA-ASE1* cells were released from α factor arrest into repressing conditions, a small fraction of cells with Ddc1-GFP foci appeared in both strains at early time points, likely corresponding to S phase (Figure 7D). In both strains, the frequency of cells with Ddc1-GFP foci then dropped to about 4% as the cells progressed through mitosis and into the next G_1. Thus, Ase1 depletion did not increase the frequency of double-strand breaks compared to wild-type cells, despite the frequent presence of chromatin in the cleavage plane. In contrast, cells lacking Boi1 and Boi2 showed Ddc1-GFP foci at a higher frequency of up to 8%. The frequency of these foci increased further (up to 27%) in the Ase1-depleted, *boi1*Δ *boi2*Δ cells. The increased frequency of Ddc1 foci was most obvious at later times in the experiment, when cells went through mitosis (Figure 7D). In the *boi1*Δ *boi2*Δ double- and *boi1*Δ *boi2*Δ *GALS:HA-ASE1* triple-mutant cells, most Ddc1-GFP dots were found in anaphase cells (see Figure 7E) and frequently localized to the bud neck (Figure 7C). No such

correlation was observed in *BOI*$^{+}$ cells. Thus, Boi1 and Boi2 have DNA-protecting activity in a late stage of the cell cycle.

To test whether DNA damage was caused by premature cytokinesis in *boi1*Δ *boi2*Δ cells, we tested whether the frequency of Ddc1-GFP foci was suppressed when we prevented cytokinesis using the *cdc12-6* septin mutation. Asynchronous cultures were shifted from galactose to glucose at the restrictive temperature for *cdc12-6*, and Ddc1-GFP foci were scored after four hours. Consistent with cytokinesis causing DNA damage, the frequency of Ddc1 foci was reduced to wild-type levels in the *boi1*Δ *boi2*Δ *cdc12-6* triple and *GALS:HA-ASE1 boi1*Δ *boi2*Δ *cdc12-6* quadruple mutants (Figure 7F).

Double-strand breaks can be repaired by two alternative mechanisms. Homologous recombination (HR) uses the sequence of the homologous chromosome to repair the break, whereas nonhomologous end joining (NHEJ) joins the ends of broken chromosomes irrespective of sequence homology (Krogh and Symington, 2004). We reasoned that if haploid *boi1*Δ *boi2*Δ double-mutant cells experience an increased incidence of chromosome breaks during abscission, these cells should strongly rely on NHEJ for survival. Indeed, HR would not be able to process such breaks, since the damage would occur after segregation of homologous chromatids to opposite daughter cells. In agreement with this, *boi1*Δ *boi2*Δ cells carrying mutations in the NHEJ genes *YKU70* and *MRE11* showed impaired growth compared to the parental strains (Figure 7G). Disruption of the HR gene *RAD52* had no such effect. Thus, the *boi1*Δ *boi2*Δ cells experienced an increased incidence of chromosome breaks specifically after sister-chromatid disjunction. Altogether, our results establish that Boi1 and Boi2 function in a pathway that protects chromosomes from being broken by premature cytokinesis.

DISCUSSION

In this study, we show that spindle-midzone defects lead to inhibition of abscission in yeast. Abscission inhibition depends on Ipl1/Aurora and the anillin-related proteins Boi1 and Boi2. Failure to inhibit abscission leads to an increased frequency of chromosome breakage during cell division and loss of viability, indicating that cells possess a mechanism to delay cytokinesis when chromosome segregation is impaired.

Cells with Spindle-Midzone Defects Fail to Complete Abscission

Here, we report that spindle-midzone defects impair cytokinesis in yeast. Failure of cytokinesis was not due to defects in mitotic exit and/or cell cycle progression, since cytokinesis-defective cells started a new round of budding and DNA replication on schedule. In addition, mutants that affected aspects of spindle function other than the spindle midzone (like *nnf1-1 mad2*Δ, *mtw1-1 mad2*Δ, and *cnm67*Δ) often caused cell separation defects but did not impair cytokinesis. Cell separation failed in these mutants probably because DNA did not segregate into the bud, preventing the bud-specific transcription factor Ace2 from initiating primary septum degradation (Colman-Lerner et al., 2001; Racki et al., 2000). In contrast, mutations that affected the spindle midzone, such as *ase1*Δ, *ndc10-1*, and *ndc80-1*, delayed or completely abrogated cytokinesis. Cell wall digestion assays, as well as imaging of the cell wall, plasma membrane, and actomyosin-ring dynamics, indicated that abscission failed. Accordingly, genetic data placed Ase1 in the Cyk3 pathway, which is likely to act in abscission. Although some of the mutants that affected the midzone and cytokinesis also affected microtubule-kinetochore interactions or cytoplasmic microtubules, these defects alone did not cause failure of cytokinesis. Thus, we conclude that similar to animal cells, the spindle midzone is required for proper completion of cell cleavage in budding yeast.

How Direct Is the Role of the Spindle Midzone during Abscission?

How spindle-midzone defects interfere with cytokinesis in animal cells has remained elusive. It has been assumed that the spindle midzone provides spatial information and/or structural support for the assembly of the abscission machinery, i.e., that it is an intrinsic player of cytokinesis. Alternatively, the cell might respond to midzone defects by preventing abscission, similar to a checkpoint response. In the latter model, the midzone does not play a role in cytokinesis. Rather, a sensing mechanism would detect midzone defects or their consequence(s) and respond by inhibiting abscission. Inactivation of this machinery should therefore render abscission independent of midzone integrity. Strikingly, we show here that midzone-defective cells lacking Aurora kinase activity or the anillin-related molecules Boi1 and Boi2 restore cytokinesis without suppressing the midzone defects. Thus, our results indicate that Ipl1 and the Boi proteins are part of an inhibitory pathway, which we call NoCut, that represses abscission in cells with midzone defects. Ipl1 localizes exclusively to the nucleus and most likely acts upstream in NoCut. In turn, Boi1 and Boi2 translocate from the nucleus to the cortex in response to Ipl1/Sli15 activity. There, they repress abscission, possibly directly or indirectly by recruiting or activating other inhibitor(s). Thus, the spindle midzone is not intrinsically required for abscission in yeast.

The NoCut Pathway Prevents Chromosome Damage during Cytokinesis

The spindle assembly and orientation checkpoints ensure the faithful segregation of chromosomes between mother and daughter cells (Bardin and Amon, 2001; Musacchio and Hardwick, 2002). What is the role of yet another surveillance mechanism during mitosis? Visualization of DNA-damage sites using Ddc1-GFP indicated that *boi1*Δ *boi2*Δ cells suffer increased levels of DNA damage during anaphase. The frequency of DNA damage further increased when the *boi1*Δ *boi2*Δ cells failed to elongate their

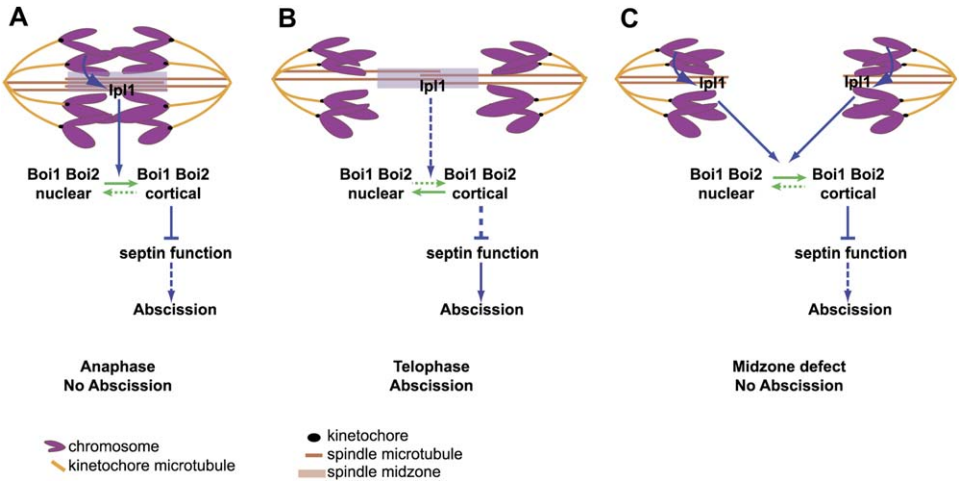

Figure 8. Model of the NoCut Pathway

(A) During early anaphase, Ipl1 at the central spindle is activated by surrounding chromatin. Active Ipl1 causes Boi1 and Boi2 to translocate to the cortex, where they inhibit septin function. Abscission is repressed.

(B) Upon segregation of the chromosomes away from the central spindle, Ipl1 is no longer kept active by chromatin. Boi1 and Boi2 leave the bud neck. Abscission can take place.

(C) In cells with midzone defects, Ipl1 stays close to chromatin even after chromosome segregation. Boi1 and Boi2 are not removed from the bud neck. Abscission remains inhibited.

spindle properly. Preventing cytokinesis in the *boi1*Δ *boi2*Δ *cdc12-6* and *GALS:ASE1 boi1*Δ *boi2*Δ *cdc12-6* cells was sufficient to prevent this damage. Thus, a major conclusion from our study is that the cytokinesis machinery can cause DNA damage if not properly regulated. A main function of NoCut therefore appears to be the protection of chromosomes while they are still engaged in the cleavage plane. Our finding that an *ase1*Δ *boi1*Δ *boi2*Δ triple mutant is inviable underscores the importance of this control.

Remarkably, our data also show that NoCut delays abscission during normal mitosis in addition to midzone-defective cells. The increased frequency of DNA damage in the *boi1*Δ *boi2*Δ double mutants indicates that, even when spindle elongation is normal, some chromatin lingers at the bud neck in a significant fraction of telophase cells. Thus, clearance of chromatin from the cleavage plane is error prone and requires the existence of a surveillance mechanism. Shielding genetic material from the cytokinesis machinery is probably a common theme in cell division because a NoCut-like system also exists in bacteria (Wu and Errington, 2004), although the molecular mechanisms differ. In mammalian cells, merotelically attached chromatids, which are pulled toward both poles of the spindle and therefore remain in the cleavage plane, cause an abscission delay until they segregate to one side of the midzone (Cimini et al., 2002). Similarly, studies in animal cells have established that DNA bridges due to incomplete separation of sister chromatids also cause abscission to fail and the cleavage furrow to regress (Meraldi et al., 2004a; Mullins and Biesele, 1977). Furrow regression does not occur in midzone-defective budding yeast cells, probably due to cell wall deposition during actomyosin-ring contraction. Thus, NoCut-like mechanisms might exist in most cell types.

In summary, NoCut appears to be a previously unidentified cell cycle checkpoint: it represses a cell cycle event, cytokinesis, in response to defects in a previous step, anaphase. This repression prevents a catastrophic division and helps the cell to remain viable. However, unlike classical checkpoints, NoCut does not lead to cell cycle arrest.

A Model for the NoCut Pathway

It is not clear what primary signal is being monitored by NoCut. Similarly, it is unknown how NoCut inactivation allows abscission. Because midzone defects cause abscission inhibition, it is possible that NoCut directly monitors midzone integrity. Three observations argue against this idea: first, Boi1 and Boi2 localize to the bud neck in anaphase, suggesting that NoCut is already active at this stage, although the midzone is intact. Second, Boi1/Boi2 control the timing of abscission in all cells, not only in those with damaged spindles. Third, in *ase1*Δ, NoCut delays abscission but does not prevent it. The first two observations indicate that NoCut is already active during anaphase in all cells. Thus, an attractive, although speculative, model is that the spindle midzone is required for NoCut inactivation upon chromosome separation. We propose that NoCut monitors the clearance of chromatin from the cleavage plane and that the spindle midzone functions as a sensor in this process (Figure 8). For example, the proximity of chromatin might activate Ipl1, perhaps through a mechanism similar to the activation of Aurora-A by Ran-GTP (Gruss and Vernos, 2004). Ipl1 activity would then remain high as long as chromatin surrounds the midzone, and Boi1 and Boi2 would stay at the bud neck and hold abscission in check (Figure 8A). During spindle elongation, the spindle midzone would sequester Ipl1 away from

chromatin, allowing its inactivation and permitting abscission to be relieved from Boi1- and Boi2-dependent repression (Figure 8B). On the other hand, in midzone-defective cells, Ipl1, which remains around chromatin, maintains its inhibition activity (Figure 8C), explaining the abscission defect of midzone mutants. In the kinetochore mutants *mtw1* and *nnf1*, chromosomes are successfully segregated from the midzone, in that they remain around the spindle pole in the mother cell. This would explain why defects in chromosome separation do not lead to a cytokinesis defect in these cells. Thus, our observations are consistent with the incomplete clearance of chromatin from the spindle midzone triggering NoCut.

The "cut" phenotype has been most clearly described in *S. pombe* (Yanagida, 1998). In this organism, a number of mutations lead to completion of cytokinesis in the absence of chromosome segregation and hence to cutting of the nucleus that is still lying on the cleavage plane. Although this could indicate that the coordination between chromosome segregation and abscission is not conserved, other interpretations could reconcile the different findings. For example, the *S. pombe* cut mutants could in fact be components of both anaphase initiation and the fission yeast NoCut pathway. Alternatively, since NoCut causes a delay and not a complete block in cytokinesis, DNA cuts could still occur in the absence of chromosome segregation after a prolonged time; in fact, "adaptation" is known to occur with other surveillance mechanisms, like the spindle and DNA replication checkpoints.

In higher eukaryotes, Aurora-B proteins are required for furrowing and cytokinesis (Adams et al., 2001; Meraldi et al., 2004b) and might therefore not function in NoCut. However, a role for Aurora-B in abscission inhibition cannot be excluded at this point, since such a role would have been masked by its earlier role in furrow ingression. In this perspective, it is worth noting that overexpression of Aurora-A causes abscission failure in animal cells (Marumoto et al., 2005). It will be interesting to investigate whether this phenomenon is related to Aurora-A acting in a mammalian NoCut pathway.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Growth Conditions

Strains are derivatives of W303 or S288C. All strains were grown in rich medium (YPD) at room temperature, unless indicated otherwise. Gene deletion, promoter replacement, and tagging was performed using a single-step PCR-based approach as described (Janke et al., 2004; Longtine et al., 1998). The *ndc10-1*, *nnf1-17*, *mtw-1*, *ndc80-1*, *ipl1-321*, *sli15-1*, and *cdc12-6* mutations have been described (Barral et al., 2000; Biggins et al., 1999; Euskirchen, 2002; Goh and Kilmartin, 1993; Goshima and Yanagida, 2000; Kim et al., 1999; Wigge and Kilmartin, 2001). The *cnm67*Δ (Hoepfner et al., 2000) and *boi1*Δ *boi2*Δ and Boi2-GFP strains were kind gifts from P. Philippsen (Biozentrum, Basel) and Eric Bailly (CNRS, Marseille). The RAS2-GFP construct has been described (Whistler and Rine, 1997).

Cell Synchronization, Staining Procedures, and Nocodazole and Zymolyase Treatment

Cells arrested with α factor (10 μg/ml, 2 hr at 22°C) were washed twice in YPD and released at the restrictive temperature (for temperature-sensitive mutants) or at 22°C. For DAPI staining, cells were fixed for 30 min in 70% ethanol, washed in PBS, and resuspended in PBS containing 1 μg/ml DAPI. Nocodazole was used at a concentration of 50 μg/ml. For septum digestion, cells were fixed with 3.7% formaldehyde for 30 min, washed twice with PBS, and resuspended in 1M sorbitol. Cell separation was evaluated by light microscopy after 30 min zymolyase digestion (2 mg/ml) at 22°C. Calcofluor (Sigma) was used at 0.01 mg/ml. FACS measurements were carried out as described (Lim et al., 1996).

Western Blotting

Samples for extract preparation were collected at the indicated times after release in fresh media containing glucose or galactose. Extracts were prepared using alkaline lysis and TCA precipitation as described (Janke et al., 2004). Total protein concentration was measured by a colorimetric assay (Bio-Rad), and equal amounts were loaded on SDS gels. Upon transfer to PVDF membranes, the relative amount of Ase1 was estimated by immunoblotting using α-HA antibodies (Sigma) detected with an ECL kit (Amersham).

Microscopy

Acquisition of Z stacks for visualization of the plasma membrane at the bud neck was carried out on a Zeiss 200 M spinning disk confocal microscope (Figure 3) or on an Olympus BX50 fluorescence microscope equipped with a piezo motor (Figures 1 and 6) essentially as described (Kusch et al., 2002). Unpaired t tests were used to assess the statistical significance of the data in Figure 6 (Prism software, GraphPad).

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.cell.com/cgi/content/full/125/1/85/DC1/.

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REFERENCES

Adams, R.R., Carmena, M., and Earnshaw, W.C. (2001). Chromosomal passengers and the (aurora) ABCs of mitosis. Trends Cell Biol. *11*, 49–54.

Bardin, A.J., and Amon, A. (2001). Men and sin: what's the difference? Nat. Rev. Mol. Cell Biol. *2*, 815–826.

Barral, Y., Mermall, V., Mooseker, M.S., and Snyder, M. (2000). Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Mol. Cell *5*, 841–851.

Biggins, S., and Murray, A.W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev. *15*, 3118–3129.

Biggins, S., Severin, F.F., Bhalla, N., Sassoon, I., Hyman, A.A., and Murray, A.W. (1999). The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. Genes Dev. *13*, 532–544.

Bouck, D.C., and Bloom, K.S. (2005). The kinetochore protein Ndc10p is required for spindle stability and cytokinesis in yeast. Proc. Natl. Acad. Sci. USA *102*, 5408–5413.

Buvelot, S., Tatsutani, S.Y., Vermaak, D., and Biggins, S. (2003). The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. J. Cell Biol. *160*, 329–339.

Cimini, D., Fioravanti, D., Salmon, E.D., and Degrassi, F. (2002). Merotelic kinetochore orientation versus chromosome mono-orientation in the origin of lagging chromosomes in human primary cells. J. Cell Sci. *115*, 507–515.

Colman-Lerner, A., Chin, T.E., and Brent, R. (2001). Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. Cell *107*, 739–750.

Dobbelaere, J., and Barral, Y. (2004). Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. Science *305*, 393–396.

Dobbelaere, J., Gentry, M.S., Hallberg, R.L., and Barral, Y. (2003). Phosphorylation-dependent regulation of septin dynamics during the cell cycle. Dev. Cell *4*, 345–357.

Euskirchen, G.M. (2002). Nnf1p, Dsn1p, Mtw1p, and Nsl1p: a new group of proteins important for chromosome segregation in Saccharomyces cerevisiae. Eukaryot. Cell *1*, 229–240.

Fraschini, R., Beretta, A., Lucchini, G., and Piatti, S. (2001). Role of the kinetochore protein Ndc10 in mitotic checkpoint activation in Saccharomyces cerevisiae. Mol. Genet. Genomics *266*, 115–125.

Glotzer, M. (2005). The molecular requirements for cytokinesis. Science *307*, 1735–1739.

Goh, P.Y., and Kilmartin, J.V. (1993). NDC10: a gene involved in chromosome segregation in Saccharomyces cerevisiae. J. Cell Biol. *121*, 503–512.

Goshima, G., and Yanagida, M. (2000). Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. Cell *100*, 619–633.

Gruss, O.J., and Vernos, I. (2004). The mechanism of spindle assembly: functions of Ran and its target TPX2. J. Cell Biol. *166*, 949–955.

Guertin, D.A., Trautmann, S., and McCollum, D. (2002). Cytokinesis in eukaryotes. Microbiol. Mol. Biol. Rev. *66*, 155–178.

Hallett, M.A., Lo, H.S., and Bender, A. (2002). Probing the importance and potential roles of the binding of the PH-domain protein Boi1 to acidic phospholipids. BMC Cell Biol. *3*, 16.

Hartwell, L.H., and Weinert, T.A. (1989). Checkpoints: controls that ensure the order of cell cycle events. Science *246*, 629–634.

Hoepfner, D., Brachat, A., and Philippsen, P. (2000). Time-lapse video microscopy analysis reveals astral microtubule detachment in the yeast spindle pole mutant cnm67. Mol. Biol. Cell *11*, 1197–1211.

Janke, C., Magiera, M.M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., Moreno-Borchart, A., Doenges, G., Schwob, E., Schiebel, E., and Knop, M. (2004). A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. Yeast *21*, 947–962.

Juang, Y.L., Huang, J., Peters, J.M., McLaughlin, M.E., Tai, C.Y., and Pellman, D. (1997). APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. Science *275*, 1311–1314.

Kim, J.H., Kang, J.S., and Chan, C.S. (1999). Sli15 associates with the ipl1 protein kinase to promote proper chromosome segregation in Saccharomyces cerevisiae. J. Cell Biol. *145*, 1381–1394.

Korinek, W.S., Bi, E., Epp, J.A., Wang, L., Ho, J., and Chant, J. (2000). Cyk3, a novel SH3-domain protein, affects cytokinesis in yeast. Curr. Biol. *10*, 947–950.

Krogh, B.O., and Symington, L.S. (2004). Recombination proteins in yeast. Annu. Rev. Genet. *38*, 233–271.

Kusch, J., Meyer, A., Snyder, M.P., and Barral, Y. (2002). Microtubule capture by the cleavage apparatus is required for proper spindle positioning in yeast. Genes Dev. *16*, 1627–1639.

Li, R., and Murray, A.W. (1991). Feedback control of mitosis in budding yeast. Cell *66*, 519–531.

Lim, H.H., Goh, P.Y., and Surana, U. (1996). Spindle pole body separation in Saccharomyces cerevisiae requires dephosphorylation of the tyrosine 19 residue of Cdc28. Mol. Cell. Biol. *16*, 6385–6397.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953–961.

Marumoto, T., Zhang, D., and Saya, H. (2005). Aurora-A—a guardian of poles. Nat. Rev. Cancer *5*, 42–50.

McCollum, D. (2004). Cytokinesis: The central spindle takes center stage. Curr. Biol. *14*, R953–R955.

Melo, J.A., Cohen, J., and Toczyski, D.P. (2001). Two checkpoint complexes are independently recruited to sites of DNA damage in vivo. Genes Dev. *15*, 2809–2821.

Meraldi, P., Draviam, V.M., and Sorger, P.K. (2004a). Timing and checkpoints in the regulation of mitotic progression. Dev. Cell *7*, 45–60.

Meraldi, P., Honda, R., and Nigg, E.A. (2004b). Aurora kinases link chromosome segregation and cell division to cancer susceptibility. Curr. Opin. Genet. Dev. *14*, 29–36.

Mullins, J.M., and Biesele, J.J. (1977). Terminal phase of cytokinesis in D-98s cells. J. Cell Biol. *73*, 672–684.

Musacchio, A., and Hardwick, K.G. (2002). The spindle checkpoint: structural insights into dynamic signalling. Nat. Rev. Mol. Cell Biol. *3*, 731–741.

Pereira, G., and Schiebel, E. (2003). Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. Science *302*, 2120–2124.

Racki, W.J., Becam, A.M., Nasr, F., and Herbert, C.J. (2000). Cbk1p, a protein similar to the human myotonic dystrophy kinase, is essential for normal morphogenesis in Saccharomyces cerevisiae. EMBO J. *19*, 4524–4532.

Schuyler, S.C., Liu, J.Y., and Pellman, D. (2003). The molecular function of Ase1p: evidence for a MAP-dependent midzone-specific spindle matrix. Microtubule-associated proteins. J. Cell Biol. *160*, 517–528.

Sullivan, M., Lehane, C., and Uhlmann, F. (2001). Orchestrating anaphase and mitotic exit: separase cleavage and localization of Slk19. Nat. Cell Biol. *3*, 771–777.

Tolliday, N., Bouquin, N., and Li, R. (2001). Assembly and regulation of the cytokinetic apparatus in budding yeast. Curr. Opin. Microbiol. *4*, 690–695.

Whistler, J.L., and Rine, J. (1997). Ras2 and Ras1 protein phosphorylation in Saccharomyces cerevisiae. J. Biol. Chem. *272*, 18790–18800.

Wigge, P.A., and Kilmartin, J.V. (2001). The Ndc80p complex from Saccharomyces cerevisiae contains conserved centromere components and has a function in chromosome segregation. J. Cell Biol. *152*, 349–360.

Wu, L.J., and Errington, J. (2004). Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. Cell *117*, 915–925.

Yanagida, M. (1998). Fission yeast cut mutations revisited: control of anaphase. Trends Cell Biol. *8*, 144–149.