A Mechanism for Coupling Exit from Mitosis to Partitioning of the Nucleus

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Summary
Exit from mitosis must not occur prior to partitioning of chromosomes between daughter cells. We find that the GTP binding protein Tem1, a regulator of mitotic exit, is present on the spindle pole body that migrates into the bud during S phase and mitosis. Tem1’s exchange factor, Lte1, localizes to the bud. Thus, Tem1 and Lte1 are present in the same cellular compartment (the bud) only after the nucleus enters the bud during nuclear division. We also find that the presence of Tem1 and Lte1 in the bud is required for mitotic exit. Our results suggest that the spatial segregation of Tem1 and Lte1 ensures that exit from mitosis only occurs after the genetic material is partitioned between mother and daughter cell.

Introduction
In budding yeast, entry into mitosis and mitotic spindle formation rely on the mitotic cyclin-dependent kinases (Cdk1 complexes containing Cbl1 to Cbl5). After formation of the mitotic spindle in the mother cell, the nucleus migrates to the neck between the mother and the daughter cell where it awaits the onset of anaphase. Entry into anaphase is controlled by the ubiquitin-protein ligase APC/C (Anaphase Promoting Complex/Cyclosome; reviewed in King et al., 1996). The APC/C initiates anaphase by degrading the anaphase inhibitor Pds1 (Cohen-Fix et al., 1996). Proteolysis of Pds1 liberates Esp1, allowing it to initiate cleavage of protein complexes (cohesins) that hold sister chromatids together (Guccci et al., 1997; Michaelis et al., 1997; Ciosk et al., 1998; Uhlmann et al., 1999). When the bonds between sister chromatids have been dissolved, nuclear division commences with the nucleus extending into daughter cell. After nuclear division is completed, mitotic kinases are inactivated, which triggers exit from mitosis and cytokinesis (reviewed in Morgan, 1999).

The highly conserved phosphatase Cdc14 plays a pivotal role in promoting inactivation of mitotic kinases and thus exit from mitosis (Visintin et al., 1998; Jaspersen et al., 1999). Cdc14 dephosphorylates the APC/C specificity factor Cdh1/Hct1 thereby stimulating APC-dependent degradation of mitotic cyclins. By dephosphorylating the CDK inhibitor Sic1 and its transcription factor Swi5, Cdc14 induces stabilization of Sic1 and Sic1 transcription, respectively.

Cdc14 activity is regulated by Cfi1/Net1 (Shou et al., 1999; Straight et al., 1999; Visintin et al., 1999). Cfi1/Net1 sequesters and inhibits Cdc14 in the nucleolus during G1, S phase, and early mitosis. During nuclear division, Cdc14 is released from Cfi1/Net1 in the nucleolus, allowing it to reach its targets in the nucleus (Sic1 and Cdh1) and cytoplasm (Swi5). The release of Cdc14 from the nucleolus is regulated by the mitotic exit pathway. This pathway includes the Ras-like GTP binding protein Tem1, the prototypical exchange factor Lte1, the two-component GTPase-activating enzyme (GAP) Bub2-Byr4, the protein kinases Cdc5, Cdc15, Dbf2, and Dbf20, and the Dbf2-associated protein Mob1 (reviewed in Morgan, 1999). Genetic interactions among these genes indicate that they function in a common pathway and that Tem1 and Lte1 act together at or near the top of this signaling cascade (Shirayama et al., 1994a; Jaspersen et al., 1998; Morgan, 1999). Pds1 and Esp1 are also required for release of Cdc14 from the nucleolus and inactivation of mitotic kinases (Celinson-Fix and Kosholand, 1999; Shirayama et al., 1999; Tinker-Kulberg and Morgan, 1999). How these proteins control the exit pathway is not known. Release of Cdc14 from the nucleolus is not sufficient for it to trigger inactivation of mitotic kinases. Cbl5-Cdc28 kinase, a potent antagonist of Cdc14, also needs to be inactivated for Cdc14 to efficiently dephosphorylate its targets. This is achieved by the APC, which degrades Cbl5 during anaphase (Shirayama et al., 1999).

The components of the mitotic exit pathway are highly conserved among eukaryotes but it is not known how they function to promote Cdc14 release. We report here that Tem1 is synthesized and localized to the spindle pole body that migrates into the bud concomitant with mitotic spindle formation. Tem1’s exchange factor is present throughout the cell during G1, but localizes to the bud as soon as it forms. Owing to the changes of Tem1 protein levels during the cell cycle and the localization patterns of Tem1 and Lte1, the two proteins are only present in the same cellular compartment after the nucleus enters the bud during nuclear division. Indeed, we find that Tem1 and Lte1 need to be in the same compartment to initiate mitotic exit. We also show that Tem1’s GAP Bub2 contributes to restraining mitotic exit when the nucleus is not partitioned between mother and daughter cell. Our results suggest that one function of the mitotic exit pathway is to ensure that exit from mitosis occurs only after the genetic material has been partitioned between mother and daughter cell, thereby guaranteeing maintenance of ploidy.

Results
Tem1 and Lte1 Protein Levels during the Cell Cycle
Epistasis analyses indicate that TEM1 and LTE1 function at or near the top of the mitotic exit pathway. Overexpression of CDC15 and CDC14 rescues the mitotic exit defect of temperature-sensitive (ts) tem1-3 mutants. In contrast, overexpression of TEM1 does not rescue the
Addition of calf intestinal phosphatase (CIP) to Lte1-HA immunoprecipitates led to the presence of only a fast migrating form of Lte1, indicating that phosphorylation was responsible for the change in Lte1's electrophoretic mobility (Figure 1E).

Tem1 Is Present on One Spindle Pole Body during Mitosis

Next, we analyzed the subcellular localization of Tem1 in exponentially growing cells by indirect in situ immunofluorescence using an anti-Myc antibody. Consistent with our Western blot analysis little or no Tem1 staining was detectable in G1 cells or in cells that had not yet formed a mitotic spindle (Figure 2B). In 75% of cells containing a mitotic spindle Tem1 was present as a single dot at one end of spindles (Figure 2B). In the remaining 25% of mitotic cells, a strong Tem1 signal was observed at one end of the mitotic spindle and a faint one at the other end (data not shown).

The subcellular localization of Tem1 was similar to that of components of spindle pole bodies (SPBs). Indeed Tem1 colocalized, although not in its entirety, with the γ-tubulin Tub4, which localizes to the inner and outer plaques of the SPB (Figure 2C; Rout and Kilmartin, 1990; Sobel and Snyder, 1995; Knop et al., 1997). To determine more precisely where on the SPB Tem1 was localized, we examined Tem1 localization in nud1-44 mutants, where the outer plaque of the SPB dissociates from the rest of the organelle (Adams and Kilmartin, 1999). Although similar amounts of Tem1 protein were present in nud1-44 as in wild-type cells (data not shown), only 10% of nud1-44 cells showed localization of Tem1 at the end of mitotic spindles. In 40% of cells, Tem1 was present as a “dot” in the cytoplasm (for examples see Figure 2D). Tem1 was undetectable by indirect immunofluorescence in 49% of mitotic cells. Our results suggest that Tem1 is present on the outer plaque of one SPB or a structure associated with it during late S phase, G2, and mitosis.

Next we determined whether the localization of Tem1 was controlled by components of the mitotic exit pathway. Tem1 was present on one spindle pole body in cdc15-2, cdc5-1, dbf2-2 and cfi1::URA3 mutants (Figures 3A and 3B and data not shown), showing that components of the mitotic exit pathway are not required for Tem1 localization. We also analyzed whether microtubules were important for Tem1 localization. Interestingly, in cells treated with the microtubule-depolymerizing drug nocodazole, although Tem1 was present at high levels as judged by Western blot analysis, the protein was only weakly present at the SPB (data not shown). Thus, either microtubules are important for localizing Tem1 to the SPB or Tem1 is masked by other proteins that assemble onto the SPB in response to spindle damage or proteins present on this organelle that are modified in response to microtubule depolymerization.

Tem1 Is Present at the Spindle Pole Body that Migrates into the Bud

Because Tem1 was present on predominantly one SPB, we asked if the Tem1-bearing SPB migrated into the bud or remained in the mother cell. To address this
that Tem1 localized to the SPB destined to migrate into the bud.

**Tem1 Is Associated with Cdc15 in Mitosis but Not during G1**

In *S. pombe* the Cdc15 and Tem1 homologs cdc7p and spg1p are found to be associated (Schmidt et al., 1997). Cdc15 also coimmunoprecipitated with Tem1 in exponential growing cultures, cells arrested in metaphase and telophase (Figure 3D) when Tem1 is found on the spindle pole body. During G1 Tem1 protein levels are low and no Tem1 protein is detected on the SPB (Figures 1A and 2B). We were nevertheless able to detect immunoprecipitated Tem1, but Cdc15 was not found in these immunoprecipitates. The absence of Cdc15 in Tem1 immunoprecipitates obtained from G1 cells was not due to low levels of Tem1 obtained in the immunoprecipitation. The amount of Tem1 immunoprecipitated from G1 cells was 3- to 4-fold lower than the amount obtained from exponentially growing cultures, yet Cdc15 could not be detected in Tem1 immunoprecipitates even in long exposures (Figure 3D). Similar amounts of Cdc15 protein were present in various arrests (Figure 3D), excluding the possibility that the lack of a Cdc15-Tem1 interaction during G1 was due to low levels of Cdc15 protein in this cell cycle stage (Figure 3D). These results suggest a correlation between the ability of Tem1 to associate with Cdc15 and the presence of Tem1 on the SPB. During mitosis Tem1 is localized on the SPB and associates with Cdc15. During G1 Tem1 is not found on the SPB, and an association between Tem1 and Cdc15 is not detected.

**Lte1 Is Localized to the Bud**

Next we determined the subcellular localization of Lte1-Ha using an anti-Ha antibody. This analysis revealed that Lte1-Ha was present throughout the cell during early G1 (Figure 4B). As soon as the bud emerged, Lte1 was found only in the bud. The asymmetric localization of Lte1 persisted until the completion of nuclear division. Due to the small size of the Tem1 signal and space limitations, individual cells representing different cell cycle stages rather than fields of cells are shown.

**Exit from Mitosis Is Inhibited when Nuclear Division Occurs in the Mother Cell**

Our localization data indicate that Tem1 and Lte1 are spatially segregated until the onset of nuclear division, but in the same compartment after the nucleus translocates into the bud during nuclear division, and continue to remain so until cells exit mitosis. If the presence of Tem1 and Lte1 in the same compartment were required for mitotic exit to occur, mutants in which the nucleus does not translocate into the bud during nuclear division should not release Cdc14 from the nucleolus and exit mitosis. Cells lacking cytoplasmic dynein (DYN1/DHC1) are defective in positioning the nucleus correctly during nuclear division (Saunders et al., 1995; Yeh et al., 1995; Carminati and Stearns, 1997; Shaw et al., 1997).
As a result of this defect, a small portion (~10%) of dyn1/dhc1Δ cells undergo nuclear division in the mother cell. Most of such cells eventually correct the defect, and half of the already divided nucleus is moved into the bud.

If entry of the Tem1-bearing spindle pole body into the bud was important for exit from mitosis, dyn1/dhc1Δ cells in which nuclear division had occurred in the mother cell (henceforth dyn1/dhc1Δ binucleate mother cell) should not release Cdc14 from the nucleolus and exit from mitosis should be inhibited. The appearance of binucleate mother cells is a transient phenomenon in dyn1/dhc1Δ mutants as most binucleate mother cells eventually move half of the divided nucleus into the bud. We, therefore, manipulated cells to undergo a synchronous cell cycle to analyze Cdc14 localization and cell cycle position. Wild-type and dyn1/dhc1Δ cells were arrested in early S phase with hydroxyurea at 30°C and then released into the cell cycle at 16°C (the nuclear positioning defect of dyn1/dhc1Δ mutants is more pronounced at lower temperatures; Yeh et al., 1995). After release from the hydroxyurea block, α-factor pheromone was added to cultures to prevent reentry into the next cell cycle. We then analyzed the localization of Cdc14 when dyn1/dhc1Δ cells where in telophase and distinguished between two classes of dyn1/dhc1Δ cells; (1) dyn1/dhc1Δ cells, in which partitioning of the nucleus between mother and daughter cells was not perturbed (henceforth dyn1/dhc1Δ normal telophase cells) and (2) dyn1/dhc1Δ cells where nuclear division occurred in the mother cell (dyn1/dhc1Δ binucleate mother cells). Time points between 4 and 6.5 hr after release from the hydroxyurea arrest were chosen for this analysis when cells with telophase spindles were present in cultures (Figure 5B).

In the wild-type strain, Cdc14 was found to be released throughout the nucleus and cytoplasm in 98% of cells in telophase (mean value of all time points analyzed) and to be sequestered in the nucleolus in 2% of cells (Figure 5D). Cdc14 was also present throughout the cell during telophase in 99.6% of dyn1/dhc1Δ normal telophase cells (Figure 5D). In contrast, in 77% of dyn1/dhc1Δ binucleate mother cells Cdc14 was sequestered in the nucleolus during telophase (Figure 5E; for an example see Figure 5A). The fraction of binucleate mother cells with telophase spindles was also high (82.5%), suggesting that these cells failed to or were severely delayed in exit from mitosis (Figure 5C). We noted that
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Figure 4. Subcellular Localization of Lte1-Ha during the Cell Cycle
(A) Exponentially growing wild-type cells (K699) without an LTE1-HA fusion (no tag).
(B) Lte1-Ha and microtubules of strain A1949 were visualized using anti-Ha antibodies (α-Ha) and anti-tubulin antibodies (α-tubulin), respectively. DAPI was used to stain DNA. Due to space limitations, individual cells representing different cell cycle stages rather than fields of cells are shown.
Abbreviations: early S, early S phase; late S, late S phase; G2/Meta, G2/metaphase; Ana, anaphase; Telo, telophase.

(C) Lte1-Ha staining of cells expressing LTE1-HA from the endogenous (left panel, A1949) or the GAL1-10 promoter (A2067; right panel).

a small proportion of dyn1/dhc1Δ binucleate mother cells, after a long delay, exited mitosis and formed a mother cell with two nuclei and a daughter cell without a nucleus (Figure 5G). Low levels of Lte1 in the mother cell, another exchange factor, or slow Tem1-intrinsic exchange activity could be responsible for this slow mitotic exit. Our results are consistent with the idea that entry of the Tem1-bearing SPB into the bud is required for exit from mitosis.

Overexpression of LTE1 Induces Cdc14 Release and Exit from Mitosis in dyn1/dhc1Δ Binucleate Mother Cells
To determine whether the presence of Tem1 and Lte1 in the same compartment was indeed required for release of Cdc14 from the nucleolus and mitotic exit, we analyzed the consequences on Cdc14 localization and mitotic exit in dyn1/dhc1Δ binucleate mother cells when Lte1 was forced into the mother cell. In cells overexpressing LTE1 from the galactose inducible GAL1-10 promoter, the majority of Lte1 protein localized to the bud, but some protein was also present in mother cells (Figure 4C). High levels of Lte1 had little effect on cell cycle progression (data not shown), but a small proportion of cells (5%-10%) showed premature release of Cdc14 from the nucleolus at 16°C (Figure 5F).

Overexpression of LTE1 had a dramatic effect on Cdc14 localization in dyn1/dhc1Δ binucleate mother cells (Figure 5E). Only 16.5% of dyn1/dhc1Δ binucleate mother cells (mean value of all time points analyzed) had Cdc14 sequestered in the nucleolus. Overexpression of LTE1 also caused a decrease in the percentage of dyn1/dhc1Δ binucleate mother cells with telophase spindles (Figure 5C) and an increase in multinucleate and anucleate cells (but not binucleate mother cells) after longer incubation times (Figure 5G). These findings indicate that overexpression of LTE1 induced exit from mitosis in these cells. We conclude that the presence of Tem1 and Lte1 in the same compartment is required for release of Cdc14 from the nucleolus and exit from mitosis.

Nuclear Position Correlates with the Ability of esp1-1 Cells to Exit from Mitosis
To further test the hypothesis that entry of the Tem1-bearing SPB into the bud was important for exit from mitosis, we analyzed Cdc14 localization and exit from
mitosis in esp1-1 mutants. Cells impaired for ESP1 function progress through mitosis normally until metaphase. In more than 90% of esp1-1 cells, the undivided nucleus migrates into the bud (Jansen et al., 1996; Figure 6A). Cells then disassemble the metaphase spindle, exit mitosis without having undergone nuclear division and enter a new cell cycle, form a bud and replicate their DNA (McGrew et al., 1992; Ciosk et al., 1998). If entry of the Tem1-bearing SPB into the bud were important for the initiation of mitotic exit, esp1-1 cells should exit mitosis only when the nucleus is present in the bud but not when it remains in the mother cell. esp1-1 mutants were incubated with α-factor pheromone until they formed a mating projection to distinguish mother cells from buds, followed by synchronous release into the cell cycle. Metaphase spindle formed in the mother cell 90 min after release and, concomitantly, the nucleus translocated into the bud in the majority of cells (Figure 6A). Cdc14 was released from the nucleolus 15 min after translocation of the nucleus into the bud (Figure 6A), which is consistent with the idea that the Tem1-bearing SPB has to enter the bud for Cdc14 to be released from the nucleolus.

Next we analyzed the localization of Cdc14 in esp1-1 metaphase cells (time points between 105 and 225 min after release from the α-factor arrest were chosen for this analysis) and distinguished between two classes of cells; (1) esp1-1 cells, in which the nucleus resided in the mother cells, and (2) esp1-1 cells where the nucleus was in the bud or bud neck. As observed in the dyn1/dhc1Δ mutant analysis (Figure 5), there was a remarkable correlation between nuclear position and Cdc14 localization. Cdc14 was sequestered in the nucleolus during metaphase in cells, in which the nucleus was in the mother cell (Figure 6D). In contrast, Cdc14 was released from the nucleolus in a high proportion of esp1-1 cells where the metaphase nucleus was found in the bud (Figure 6C). Furthermore, more than 90% of cells where the nucleus was found in the bud formed a new bud, indicating that cells had exited
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Figure 6. Mitotic Exit in esp1-1 Mutants

esp1-1 (A2277) and esp1-1, GAL-LTE1 (A2278) cells were arrested in G1 using 20 μg/ml α-factor at 23°C in YEP medium containing raffinose and galactose (YEPRaf-Gal). When cells had formed a mating projection (after 3.25 hr) cells were released at 37°C into YEPRaf-Gal medium.

(A) Percentage of (1) esp1-1 cells in metaphase (open triangles), (2) esp1-1 cells with Cdc14 released from the nucleolus (closed triangles), and (3) esp1-1 cells with the nucleus located in the bud or bud neck (small squares).

(B) Percentage of esp1-1, GAL-LTE1 cells (1) in metaphase (open triangles), (2) with Cdc14 released from the nucleolus (closed triangles), and (3) the nucleus located in the bud or bud neck (small squares).

(C) Percentage of Cdc14 released from the nucleolus in esp1-1 (open squares) and esp1-1, GAL-LTE1 (closed squares) cells with metaphase nuclei in the bud or bud neck.

(D) Percentage of Cdc14 released from the nucleolus in esp1-1 (open circles) and esp1-1, GAL-LTE1 (closed circles) cells with metaphase nuclei in the mother cell.

(E) Percentage of rebudded cells with nuclei in the bud or bud neck in esp1-1 (open squares) and esp1-1, GAL-LTE1 (closed squares) mutants.

(F) Percentage of rebudded cells with nuclei in the mother cells in esp1-1 (open circles) and esp1-1, GAL-LTE1 (closed circles) mutants.

The cartoons of cells within the graphs indicate the cell type scored.

(G) The photographs show one esp1-1 cell where the nucleus remained in the mother cell and rebudding did not occur (right cell) and one cell where the nucleus translocated into the bud, which subsequently formed a new bud. M marks the mother cell, which has a mating projection; D marks the bud (spherical).

mitosis and entered a new cell cycle (Figure 6E, for an example see Figure 6G). In contrast, rebudding was severely inhibited in cells where the nucleus remained in the mother cell (Figure 6F). Overexpression of LTE1 led, although with a delay, to release of Cdc14 from the nucleolus in mother cells (Figure 6D) and allowed these mother cells to exit mitosis as judged by the formation of a new bud (Figure 6F). As observed in the dyn1/dhc1Δ mutant analysis (Figure 5B), overexpression of LTE1 in wild-type cells delayed progression through mitosis (Figure 6B), suggesting that GAL-LTE1 causes, albeit minor, cell cycle defects. Our results indicate that entry of the Tem1-bearing SPB into the bud, where Lte1 resides, is required for release of Cdc14 from the nucleolus and exit from mitosis.

BUB2 Is Required to Prevent Mitotic Exit when Nuclear Division Occurs in the Mother Cell

To determine whether Tem1's GAP, Bub2-Byr4 participates in inhibiting exit from mitosis when nuclear division occurred in the mother cell, we analyzed Cdc14 localization and cell cycle position in dyn1/dhc1Δ and dyn1/dhc1Δ, bub2::HIS3 cells. Cells were arrested in early S phase with hydroxyurea at 30°C and then released into the cell cycle at 16°C in the presence of α-factor. A portion of dyn1/dhc1Δ cells was delayed in disassembly of the mitotic spindle (Figures 5B and 7A), which was partially abolished by deleting BUB2 (Figure 7A). The analysis of Cdc14 localization and the status of the mitotic spindle in dyn1/dhc1Δ and dyn1/dhc1Δ, bub2::HIS3 normal telophase and binucleate mother cells is shown in Figures 7B-7D. Cdc14 was sequestered in the nucleolus in a large proportion of dyn1/dhc1Δ binucleate mother cells (Figure 7D) and the fraction of telophase spindles was high in these cells (Figure 7B). Deletion of BUB2 led to a decrease in binucleate mother cells with Cdc14 sequestered in the nucleolus and telophase spindles (Figures 7B and 7D) and an increase in multinucleate and anucleate cells after longer incubation times (Figure 7E). Deletion of BUB2 had a less dramatic effect than overexpressing LTE1 on dyn1/dhc1Δ binucleate mother cells (compare Figures 5 and 7).
Figure 7. Analysis of Mitotic Exit in dyn1/dhc1 and dyn1/dhc1, bub2::HIS3 Mutants

Wild-type (open circles; A1411), dyn1/dhc1Δ (closed circles; A2077), bub2::HIS3 (open squares; A1901) and dyn1/dhc1Δ, bub2::HIS3 (closed squares; A2270) cells were arrested in early S phase using 5 mg/ml hydroxyurea at 30°C in YEPD medium. When arrest was complete (after 2.25 hr) cells were washed and released into YEPD medium containing α-factor (5 μg/ml) at 16°C.

(A) Total amount of cells in telophase.
(B) Percentage of telophase spindles in dyn1/dhc1Δ and dyn1/dhc1Δ, bub2::HIS3 binucleate mother cells.
(C) Percentage of Cdc14 sequestered in the nucleolus in telophase wild-type, dyn1/dhc1Δ, bub2::HIS3 and dyn1/dhc1Δ, bub2::HIS3 normal telophase cells.
(D) Percentage of Cdc14 sequestered in the nucleolus in telophase dyn1/dhc1Δ and dyn1/dhc1Δ, bub2::HIS3 binucleate mother cells. The cartoons of cells within the graphs indicate the cell type scored.
(E) Percentage of anucleate (black bars) and multinucleate (gray bars) cells in wild-type, bub2::HIS3, dyn1/dhc1Δ and dyn1/dhc1Δ, bub2::HIS3 cells grown at 16°C for 24 hr.

dyn1/dhc1Δ, bub2::HIS3 cells contain almost as many anucleate and multinucleate cells as dyn1/dhc1Δ, GAL-LTE1 cells, despite the fact that the effects of deleting BUB2 on Cdc14 release from the nucleolus and spindle disassembly were not as dramatic as that caused by overexpression of LTE1. This is because glucose was used as a carbon source instead of raffinose and galactose in this experiment, which allowed for more cell divisions to occur.

These findings suggest that BUB2 participates in ensuring that exit from mitosis only occurs after the nucleus has translocated into the bud. However, deletion of BUB2 has less dramatic consequences than forcing LTE1 into the mother cell.

Discussion

The mitotic exit pathway is highly conserved among eukaryotes (Morgan, 1999), yet its role in promoting exit from mitosis has been unclear. Our studies on Tem1 and Lte1 shed light on this question. We find that Tem1 and Lte1 need to be in the same compartment in order to induce mitotic exit and that this occurs as the nucleus migrates into the bud during nuclear division. In wild-type cells under optimal growth conditions entry of the nucleus into the bud coincides with the onset of nuclear division. As exit from mitosis requires Tem1 and Lte1 to be in the same compartment, the spatial segregation of Tem1 and Lte1 until the onset of nuclear division ensures that exit from mitosis does not occur prior to nuclear division. Under some conditions (e.g., cold) nuclear division can occur entirely in the mother cell. Even under these aberrant conditions the mode of Tem1 and Lte1 localization ensures that the nucleus is partitioned between mother and daughter cell prior to exit from mitosis. Thus, the mitotic exit pathway guarantees that the outcome of cell division is productive and two cells, each with a nucleus, are generated.

The Localization of Tem1

Two lines of evidence suggest that the presence of Tem1 on the SPB is critical for the protein to promote exit from mitosis. First, unlike most other mutants that disrupt SPB function, which arrest in G2 (Geissler et al., 1996; Knop et al., 1997; Theesfeld et al., 1999), nud1-44 mutants arrest in telophase (Adams and Kilmartin, 1999), indicating that they fail to exit from mitosis. Although other explanations are possible, the nud1-44 phenotype may be a consequence of a failure to localize Tem1 on the SPB. Second, Tem1 interacts with Cdc15 only in cell cycle stages when Tem1 is present on the SPB, suggesting that SPB localization of Tem1 is required for the protein to associate with Cdc15.

Remarkably, in most cells Tem1 is found on only the SPB that migrates into the bud. How this asymmetry is generated is not known. As Tem1 protein levels fluctuate during the cell cycle, it is tempting to speculate that degradation of Tem1 during exit from mitosis and G1 clears Tem1 from the SPB. If Tem1 were then only capable of loading onto the newly assembled SPB, asymmetry would be generated. Whether the absence of Tem1 on the spindle pole that remains in the mother cell is important for the regulation of exit from mitosis is also
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unclear. 25% of mitotic spindles show, albeit weakly, Tem1 localization on the spindle pole body that remains in the mother cell. In S. pombe, the Tem1 homolog spg1p is present on both SPBs (Schmidt et al., 1997), but its two-component GAP cdc16p-byr4p localizes to only one SPB where it keeps spg1p inactive (Cerutti and Simanis, 1999). Why cells choose to keep Tem1/spg1p activity restricted to one spindle pole body is a mystery. Our analyses showed that Bub2, which localizes to both spindles (Fraschini et al., 1999; Li, 1999), also participates in restraining exit from mitosis when nuclear division occurred in the mother cell. Whether Bub2-Byr4 activity is regulated or whether its GAP activity is constitutive and antagonized by Lte1 once Tem1 enters the bud is unknown. However, it is clear that Bub2-Byr4 is critical to prevent mitotic exit when cell cycle progression is halted in response to spindle damage (Hoyt et al., 1991; Alexandru et al., 1999; Fesquet et al., 1999; Fraschini et al., 1999; Li, 1999). Indeed, we found that Cdc14 was released from the nucleolus in a small portion (1%) of metaphase cells (A. B., unpublished observations). We speculate that Bub2-Byr4 functions as a safeguard to prevent low levels of Lte1 in the mother cell, another exchange factor, or slow Tem1-intrinsic exchange activity to activate the mitotic exit pathway when onset of anaphase is inhibited due to mitotic spindle defects.

The Role of Bub2 in Restraining Mitotic Exit

Our analyses showed that Bub2, which localizes to both SPBs (Fraschini et al., 1999; Li, 1999), also participates in restraining exit from mitosis when nuclear division occurred in the mother cell. Whether Bub2-Byr4 activity is regulated or whether its GAP activity is constitutive and antagonized by Lte1 once Tem1 enters the bud is unknown. However, it is clear that Bub2-Byr4 is critical to prevent mitotic exit when cell cycle progression is halted in response to spindle damage (Hoyt et al., 1991; Alexandru et al., 1999; Fesquet et al., 1999; Fraschini et al., 1999; Li, 1999). Indeed, we found that Cdc14 was released from the nucleolus in a small portion (1%) of metaphase cells (A. B., unpublished observations). We speculate that Bub2-Byr4 functions as a safeguard to prevent low levels of Lte1 in the mother cell, another exchange factor, or slow Tem1-intrinsic exchange activity to activate the mitotic exit pathway when onset of anaphase is inhibited due to mitotic spindle defects.

Importance of the Spatial Segregation of Lte1 and Tem1

Genetic evidence suggests that LTE1 and TEM1 function in a common pathway and that LTE1 functions as the exchange factor for TEM1. Deletion of TEM1 is lethal causing cells to arrest in telophase (Shirayama et al., 1994b). Deletion of LTE1 also causes arrest in telophase at low temperatures (Shirayama et al., 1994a) and a transient delay in telophase at 25°C (20 min; A. B., unpublished observations). Furthermore, overexpression of TEM1 suppresses the cold-sensitive lethality of cells deleted for LTE1 (Shirayama et al., 1994b). However, unlike TEM1, LTE1 is only essential for viability at low temperatures. Whether other exchange factors exist that substitute for LTE1 at higher temperatures or whether Tem1 contains an intrinsic exchange activity, is at present unclear.

Our analysis of dyn/dhc1Δ and esp1-1 mutants indicates that the presence of Tem1 and Lte1 in the bud is required for release of Cdc14 from the nucleolus and exit from mitosis. In dyn1/dhc1Δ cells where the nucleus was partitioned between mother and daughter cell and esp1-1 mutants where the nucleus was in the bud or in the bud neck, Cdc14 was released from the nucleolus and exit from mitosis occurred. In contrast, in dyn1/dhc1Δ binucleate mother cells and esp1-1 mutants in which the nucleus resided in the mother cell, Cdc14 was sequestered in the nucleolus and exit from mitosis was inhibited. Overexpression of LTE1 allowed a large proportion, but not all, of such dyn1/dhc1Δ or esp1-1 cells to release Cdc14 from the nucleolus and exit from mitosis. We can envision two reasons or a combination thereof for this incomplete rescue. Lte1 may not be as active in the mother cell due to insufficient amount of protein accumulating or due to lack of phosphorylation that occurs only upon its the translocation of Lte1 into the bud. Alternatively, activation of other components of the mitotic exit pathway may be necessary to efficiently trigger exit from mitosis. This idea is consistent with the following two observations. (1) Overexpression of LTE1 had little effect on cell cycle progression in wild-type cells and (2) Cdc14 release from the nucleolus did not immediately occur after the nucleus translocated into the bud in esp1-1 mutants, but was delayed by 15 min.

It is important to note that restriction of Lte1 to the bud does not only ensure that mitotic exit is induced after the nucleus is partitioned between mother and daughter cell. Cells lacking the actin motor MYO2 fail to form a bud and the mother cell grows unusually large (Johnston et al., 1991; Govindan et al., 1995). In such cells release of Cdc14 from the nucleolus is delayed. The delay in Cdc14 release is, to a large extent, abolished by overexpression of LTE1 (A. B., unpublished observations). Thus, restricting Lte1 also ensures that sufficient amounts of Lte1 are present to activate Tem1 once the nucleus enters the bud during nuclear division.

Signals for Exit from Mitosis

Inhibition of cytokinesis was previously observed when nuclear division occurred in the mother cell and shown to be due to activation of the spindle-positioning checkpoint (Yeh et al., 1995; Muhua et al., 1998). Our results show that this is likely to be due to inhibition of Cdc14 release from the nucleolus and that this is in large part due to Lte1 and Tem1 not being in the same compartment. Mutants have been identified that abolish the delay in mitotic exit when the mitotic spindle is mispositioned (Muhua et al., 1998). It will be interesting to determine whether these mutants affect Tem1, Lte1, or Bub2 localization.

Improper nuclear position is probably not the only event that prevents exit from mitosis. Defects in mitotic spindle formation inhibit mitotic exit through Bub2-Byr4 (Alexandru et al., 1999; Fesquet et al., 1999; Fraschini et al., 1999; Li, 1999). Two regulators of sister-chromatid separation, Pds1 and Esp1, also inhibit exit from mitosis and Cdc14 release from the nucleolus (Cohen-Fix and Koshland, 1999; Shirayama et al., 1999; Tinker-Kulberg and Morgan, 1999). Whether these proteins regulate Tem1 activation or other aspects of the mitotic exit pathway is, at present, not known. Thus, it appears that the mitotic exit pathway is responsive to a variety of signals—such as onset of nuclear division, correct positioning of the nucleus, the activity of proteins that control sister-chromatid separation, and the integrity of the mitotic spindle—and only triggers mitotic exit when all these processes have occurred properly.

A Model for How Exit from Mitosis Is Coupled to Partitioning of the Nucleus between Mother and Daughter Cell

Our results provide a mechanism for ensuring that exit from mitosis does not occur prior to partitioning of the
genetic material between mother and daughter cell. During G1 Lte1 is localized uniformly throughout the cell. During this cell cycle stage, Tem1 protein levels are very low and it fails to associate with the mitotic exit pathway component Cdc15, suggesting that Tem1 is not capable of activating the mitotic exit pathway during G1. Perhaps, the presence of Bub2-Byr4 on the SPB acts as a safeguard to prevent premature activation of Tem1. During early S phase, when the bud forms, Lte1 is recruited to the bud. Tem1 protein accumulates during late S phase when the mitotic spindle forms and, after mitotic spindle formation, is loaded onto the bud destined SPB. As nuclear division begins, the nucleus moves to the bud neck and is pulled into the bud as chromosome segregation commences. This leads to the presence of Tem1 and Lte1 in the same compartment, allowing for its activation. How activation of Tem1 at the spindle pole body leads to release of Cdc14 in the nucleolus is an important question that remains to be addressed. As cells exit mitosis, Tem1 is cleared from the SPB, perhaps by protein degradation, preventing further stimulation of the mitotic exit pathway and allowing for the rapid return of Cdc14 into the nucleolus.

Our results point toward a novel mechanism for regulating the activity of signal transduction pathways: the coupling of activation of a signal transduction pathway to the physical movement of an organelle. In the case of the mitotic exit pathway, this mechanism establishes a dependency of mitotic exit on partitioning of the genetic material between mother and daughter cell.

Experimental Procedures

Plasmid and Strains


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References


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