

Coupling spindle position with mitotic exit in budding yeast: The multifaceted role of the small GTPase Tem1

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The budding yeast *S. cerevisiae* divides asymmetrically and is an excellent model system for asymmetric cell division. As for other asymmetrically dividing cells, proper spindle positioning along the mother-daughter polarity axis is crucial for balanced chromosome segregation. Thus, a surveillance mechanism named Spindle Position Checkpoint (SPOC) inhibits mitotic exit and cytokinesis until the mitotic spindle is properly oriented, thereby preventing the generation of cells with aberrant ploidies. The small GTPase Tem1 is required to trigger a Hippo-like protein kinase cascade, named Mitotic Exit Network (MEN), that is essential for mitotic exit and cytokinesis but also contributes to correct spindle alignment in metaphase. Importantly, Tem1 is the target of the SPOC, which relies on the activity of the GTPase-activating complex (GAP) Bub2-Bfa1 to keep Tem1 in the GDP-bound inactive form. Tem1 forms a hetero-trimeric complex with Bub2-Bfa1 at spindle poles (SPBs) that accumulates asymmetrically on the bud-directed spindle pole during mitosis when the spindle is properly positioned. In contrast, the complex remains symmetrically localized on both poles of misaligned spindles. We have recently shown that Tem1 residence at SPBs depends on its nucleotide state and, importantly, asymmetry of the Bub2-Bfa1-Tem1 complex does not promote mitotic exit but rather controls spindle positioning.

Spindle Positioning in Budding Yeast

In asymmetrically dividing cells, proper spindle positioning is crucial to ensure the

unequal fate of daughter cells. In stem cells, derangement of the mechanisms controlling asymmetric cell division, including spindle positioning, affects the developmental fate of daughter cells and can promote tumorigenesis. (reviewed in ref.¹).

The budding yeast *Saccharomyces cerevisiae* divides asymmetrically and has long been used as model system to study the mechanisms underlying asymmetric cell division. Because accurate spindle positioning is critical for asymmetric cell division, 2 redundant pathways are responsible for correct spindle positioning in *S. cerevisiae*: a pathway dependent on the microtubule-binding protein Kar9 and another pathway requiring the minus end-directed motor dynein. The Kar9 pathway is supposed to act mainly in metaphase and to mediate the sliding of astral microtubule ends along actin cables.² Conversely, the dynein-dependent pathway is thought to act predominantly in anaphase.³ However, its ability to compensate for the lack of the Kar9 pathway indicates that is likely already active in metaphase.⁴ Consistent with the notion that the 2 spindle positioning mechanisms are largely redundant, *kar9* or dynein single mutants display only mild spindle mispositioning, while double mutants are lethal.⁵ One critical feature of Kar9 is its asymmetric localization to the astral microtubules emanating only from the bud-oriented SPB.^{2,6-8} The asymmetry of Kar9 ensures that only one pole of the mitotic spindle is oriented toward the bud. Interestingly, also dynein is localized asymmetrically at spindle poles, with a strong bias for the bud-directed SPB.⁹ Asymmetry of Kar9 and dynein, however, seems to be controlled by different mechanisms.^{7,9,10}

Keywords: asymmetric cell division, Kar9, mitotic exit network, spindle positioning, spindle position checkpoint, spindle pole bodies, Tem1

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The Tem1 GTPase

The budding yeast *TEM1* gene was identified through a genetic screen in 1994 as encoding for a novel GTP-binding protein.¹¹ The deletion of *TEM1* was lethal, and the *tem1* temperature-sensitive mutants arrested at telophase, indicating that *TEM1* is required to exit from mitosis. Later on, Tem1 was shown to be indeed a Rab-like GTPase.¹² The counterpart of Tem1 in *S. pombe*, named Spg1, is also a GTPase that is dispensable for mitotic exit and solely required for cytokinesis.¹³ In spite of the essential roles played by these GTPases in yeasts, no mammalian counterpart has been identified so far.

During the past 2 decades, several studies shed light on the role of Tem1 as a molecular switch for activation of the Mitotic Exit Network (MEN), an essential kinase cascade that promotes mitotic exit and cytokinesis and is organized similarly to the Septation Initiation Network (SIN) in fission yeast and the Hippo pathway in metazoans (reviewed in ref.¹⁴). The MEN effector of Tem1 is the Ste20-like kinase Cdc15,¹⁵⁻¹⁸ which in turn promotes the activation of the downstream Mob1-Dbf2 kinase complex (LATS-NDR in the Hippo pathway).^{19,20} that ultimately leads to the release and activation of Cdc14,²¹⁻²³ the main CDK-counteracting phosphatase that triggers mitotic exit through dephosphorylation of mitotic CDK substrates.²⁴ One notable feature of the MEN is that most of its components, including the Tem1 GTPase, reside at spindle pole bodies (SPBs), i.e. the yeast microtubule-organizing centers (Fig. 1A-B). Thus, not only Tem1 is necessary for cell cycle progression, but is also ideally localized to get positional feedback from the spindle poles. Not surprisingly, Tem1 is the target of the Spindle Position Checkpoint (SPOC), a surveillance mechanism that blocks mitotic exit when the spindle is not properly oriented, in order to prevent the formation of aneuploid and polyploid cells after cytokinesis (reviewed in. ref.^{25,26}, see below).

Regulation of the Tem1 GTPase

As long as the spindle is not properly positioned at the bud neck and oriented

along the mother-bud polarity axis, the SPOC prevents Tem1 activation (reviewed in ref.^{25,26}), thereby preventing premature exit from mitosis or aberrant mitosis. The key actor in this control is the two-component GTPase-activating protein (GAP) Bub2-Bfa1 that inactivates Tem1 by stimulating GTP hydrolysis.^{12,27} (Fig. 1B). The GAP activity of the Bub2-Bfa1 complex resides on Bub2, which carries a TBC domain (Tre-2, Bub2 and Cdc16; reviewed in ref.²⁸), whereas Bfa1 mediates Bub2 interaction with Tem1 and acts as guanine-nucleotide dissociation inhibitor (GDI), stabilizing Tem1 in its GDP- or GTP-bound state.^{12,27,29} Interestingly, Bfa1 can inhibit Tem1 and mitotic exit also in a Bub2-independent manner, likely by competing with Cdc15 for Tem1 interaction³⁰ and possibly by locking Tem1 in the GDP-inactive state.²⁹

Although activation of most GTPases requires nucleotide exchange factors (GEFs) that catalyze the release of GDP and promote its replacement by GTP, the identity of the GEF(s) for Tem1, if any, remains elusive. The ability of recombinant Tem1 to efficiently load guanine nucleotides *in vitro*.^{12,27} raises the possibility that Tem1 does not need a GEF. The putative GEF Lte1, which activates Tem1 *in vivo* and has long been thought to catalyze Tem1 binding to GTP, does not seem in fact to bear GEF activity for Tem1 *in vitro*.²⁹

The activity of the GAP complex Bub2-Bfa1 is itself finely regulated by the partition of the cell in 2 distinct regulatory compartments: the mother- and the bud-compartment, which respectively inhibit and activate Tem1 and the downstream MEN cascade.³¹ (Fig. 1A-B). These regulatory compartments are defined by the activity of 2 asymmetrically distributed proteins, i.e. Kin4 and Lte1, that localize specifically in the mother cell and in the bud, respectively.³²⁻³⁶ In the mother compartment, the Kin4 protein kinase activates the Bub2-Bfa1 GAP complex by direct phosphorylation of Bfa1, thereby preventing its inhibitory phosphorylation by the Polo kinase Cdc5.^{33,35,37} Moreover, it creates a docking site on Bfa1 for the 14-3-3 protein Bmh1, which in turn weakens the association of Bfa1 with the

SPB and accelerates the turnover of the GAP complex at SPBs.³⁸ In the bud compartment, Lte1 prevents Kin4 spreading into the mother cell, thus allowing the Cdc5 kinase to inactivate the GAP through Bfa1 phosphorylation.³⁹⁻⁴¹ Therefore, when both SPBs remain in the mother cell following spindle mispositioning (Fig. 1B), Tem1 and the MEN are kept inactive, while translocation of one SPB into the bud promotes Tem1 activation and allows mitotic exit.⁴² SPB-localized Tem1 thus acts as a sensor that relays the positional information from the spindle and translates it into a biochemical signal to license or prevent mitotic exit.

Regulation of Tem1 Localization

Besides on its nucleotide state, Tem1 activity also depends on its localization. Indeed, Tem1 loading onto the SPBs is an essential step to trigger the mitotic exit cascade.^{43,44} A *tem1* mutant that fails to localize at SPBs is deficient in mitotic exit,¹⁵ while artificially tethering Tem1 to SPBs is sufficient to drive out of mitosis cells experiencing spindle misalignment.⁴⁴ The downstream MEN kinases are also recruited to SPBs once Tem1 gets activated. Localization at spindle poles of MEN components, including the inhibitory GAP Bub2-Bfa1 and the kinase Kin4, is critical for MEN activity and regulation and is mainly accomplished at the cytoplasmic face of the SPBs through association to the SPB scaffold Nud1.^{15,35,43,44} Consistently, *nud1* temperature-sensitive mutants fail to exit mitosis and arrest in telophase.⁴³ Several observations indicate that Tem1 activation is necessary but not sufficient to promote mitotic exit during the unperturbed cell cycle. Indeed, covalent tethering of Tem1 to SPBs promotes its activation and increases Cdc15 levels at spindle poles, but does not promote premature mitotic exit.⁴⁴ Similarly, fusion of Cdc15 to the SPBs leads to premature activation of the Dbf2 kinase, but not premature mitotic exit.¹⁷ Inhibition of Cdc15 and Dbf2-Mob1 by cyclinB-CDKs is likely responsible for the inability of active Tem1 to trigger mitotic exit in metaphase and provides a safety mechanism to ensure the correct

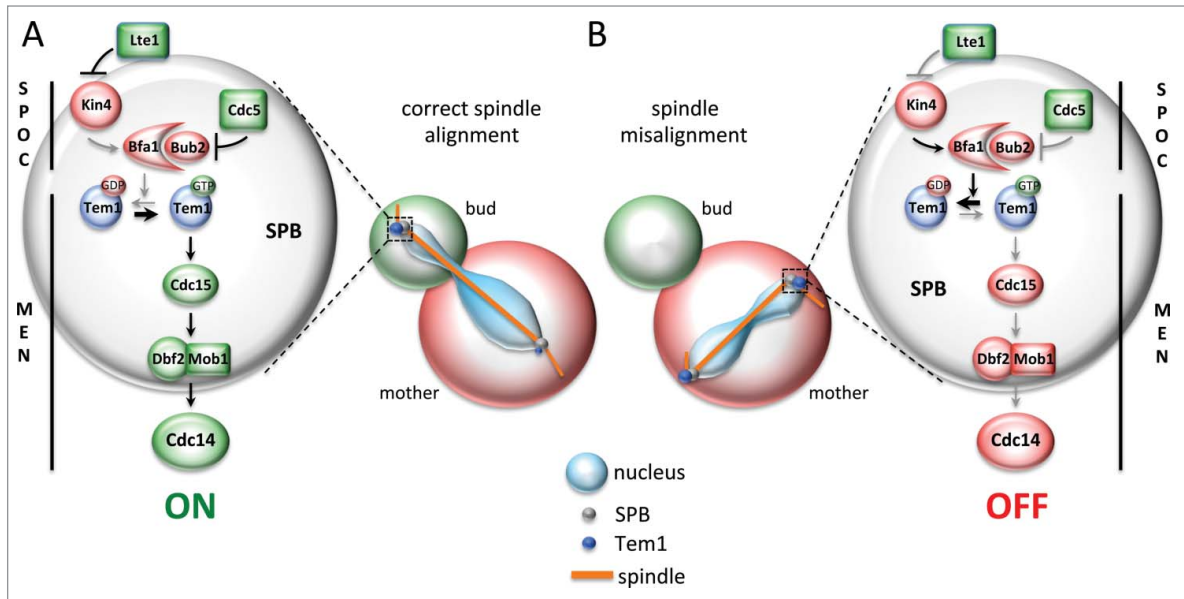


Figure 1. Asymmetry of the Tem1 GTPase at SPBs is broken upon spindle misalignment. **(A)** When cells align properly their mitotic spindles along the mother-to-bud polarity axis the Tem1 GTPase localizes preferentially to the bud-directed SPB and triggers the Mitotic Exit Network (MEN) kinase cascade (Cdc15, Dbf2-Mob1) that leads to the activation of Cdc14 phosphatase and mitotic exit. Two separated compartments for inhibition and activation of the MEN are defined in the mother cell and in the bud by Kin4 and Lte1, respectively, and depicted in red and green. **(B)** Upon spindle misalignment Tem1 localizes symmetrically on both SPBs and the Spindle Position Checkpoint (SPOC) inhibits Tem1 through the GTPase-Activating Protein (GAP) complex Bub2-Bfa1, thereby restraining the MEN until the spindle repositions correctly. The GAP is in turn kept active by the kinase Kin4, which counteracts the inhibitory phosphorylation of the GAP by the Polo kinase Cdc5.

order of cell cycle events.⁴⁵ Indeed, cyclinB degradation at the onset of anaphase is a requisite that must be satisfied for MEN to get fully active.

Tem1, together with its GAP Bub2-Bfa1, is present on both SPBs in metaphase, when it accumulates preferentially on the SPB that is most proximal to the bud.^{27,46-48} (Fig. 2A). Its localization becomes even more asymmetric in anaphase, when it keeps concentrating on the bud-directed SPB (Fig. 1A). Conversely, if the spindle fails to elongate properly along the cell polarity axis, the Bub2-Bfa1-Tem1 complex is symmetrically retained on both SPBs and shows high turnover, exchanging with its cytoplasmic pool.^{42,46,48} (Fig. 1B). Surprisingly, chimeric proteins obtained by fusing Bfa1 or Bub2 to both SPBs cause unscheduled mitotic exit upon spindle misalignment,^{46,49} similar to constitutive recruitment of Tem1 to SPBs. These data led to the idea that the Bub2-Bfa1 complex, while acting as inhibitory GAP on Tem1, also stabilizes Tem1 at SPBs where it becomes active.^{44,49} Since the Bub2-Bfa1 complex is completely dispensable for

mitotic exit, in its absence Tem1 is likely recruited to SPBs through another receptor, that has been proposed to be the SPB

scaffold Nud1.⁴⁴ Consistently, in the absence of Bub2-Bfa1 Tem1 levels at SPBs are drastically reduced throughout

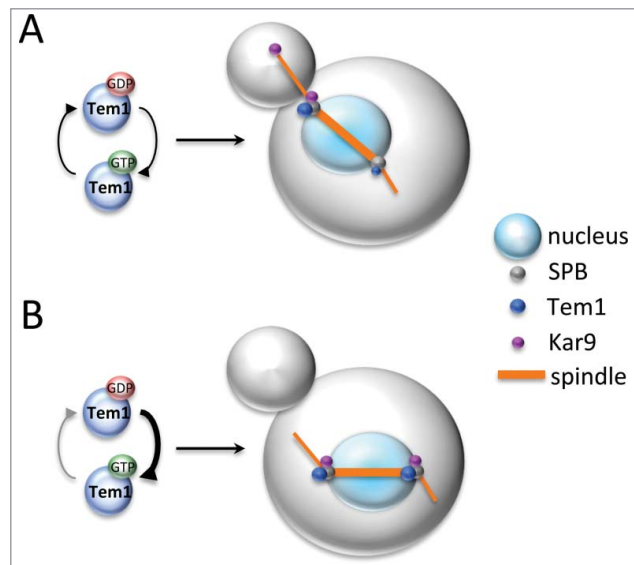


Figure 2. Tem1 asymmetry contributes to asymmetric distribution of Kar9 and proper spindle positioning. **(A)** Partial asymmetry of Tem1 at SPBs is already established in metaphase and contributes to the asymmetry of Kar9 on the bud-directed SPB and the tip of the astral microtubules that nucleate from it. Kar9 asymmetry is in turn necessary for proper spindle positioning in metaphase. **(B)** Constitutive activation or SPB tethering of Tem1 breaks its asymmetry at SPBs leading to a more symmetric distribution of Kar9 and spindle position defects. See text for further details.

most of the cell cycle, to then sharply rise in telophase.⁴⁸ The molecular basis for SPB-driven Tem1 activation remains elusive. A recent proposal envisions that an as-yet unidentified GEF for Tem1 resides at SPBs.⁵⁰

We have recently shown a close link between Tem1 activity and the establishment of its asymmetry at spindle poles.⁴⁹ The hyperactive *TEM1-Q79L* mutant allele, where a leucine replaces the catalytic glutamine in the G domain, is completely refractory to stimulation of GTP hydrolysis by Bub2-Bfa1 both *in vitro* and *in vivo*, without affecting its interaction with the GAP. The mutant protein shows a markedly increased symmetry at anaphase spindle poles and also impairs the asymmetry of Bub2 and Bfa1.⁴⁹ Likewise, mutations abolishing Bub2-Bfa1 GAP activity perturb the asymmetry of the Tem1-Bub2-Bfa1 complex, indicating that GTP hydrolysis promotes asymmetry of both Tem1 and its GAP at SPBs.^{27,49}

Tem1 in the Control of Spindle Positioning

Although the most prominent MEN function is closely coupled with the final stages of mitosis, recent observations showed that MEN components also have important functions earlier on during the cell cycle. These include a role in orientation of the mitotic spindle in metaphase and in establishing Kar9 asymmetry.⁵¹ Indeed, MEN downregulation by inactivation of Nud1, Tem1, Cdc15 or Dbf2, prevents the Kar9 protein to properly accumulate on the aster microtubules of the bud-directed SPB in early mitosis and allows it to bind also the second spindle pole. This leads to spindle misorientation due to the ability of both asters to pull along actin cable in the bud. At the heart of this mechanism there is the Dbf2-dependent phosphorylation of Kar9 that stabilizes its asymmetric binding to the bud-directed SPB. Consistently, the downstream Cdc14 phosphatase is not involved in Kar9 distribution.⁵¹ Localization of Tem1 and its downstream kinases at SPBs is likely critical for the control of Kar9 asymmetry. Indeed, the lack of the Bub2-Bfa1 complex, which prevents

Tem1 recruitment to SPBs in early mitosis, compromises Kar9 asymmetry and spindle orientation in a similar way to MEN inactivation.⁴⁹

Our recent data indicate that Tem1 asymmetry plays a critical role in directing Kar9 asymmetry. Indeed, chimeric proteins that stabilize Tem1 at both SPBs or expression of the constitutively active Tem1-Q79L GTPase that remains more symmetric at SPBs both impair Kar9 asymmetry and spindle positioning.⁴⁹ (Fig. 2B). Thus, at a first glance inactivation or hyperactivation of Tem1 similarly impact on Kar9 asymmetry. One notable difference, however, is that while Tem1 inactivation causes only spindle orientation defects (where orientation is defined by the angle between the metaphase spindle and the bud neck),⁵¹ Tem1 hyperactivation causes in addition spindle mispositioning (where position is defined by the distance between the proximal SPB and the bud neck).⁴⁹ The molecular bases of this difference remain to be established. One possibility to be explored further is that the absolute amounts and/or turnover of Kar9 at SPBs differ in the 2 conditions.

Conclusions

One crucial question that remains unsolved is how Tem1 activity toward mitotic exit is restrained until telophase while being already operational in metaphase toward spindle positioning. One possible scenario is that only the fraction of Tem1 at SPBs gets activated in metaphase, while a more global activation of Tem1 in the cytoplasm is required for mitotic exit.⁵² A differential requirement of different pools of Tem1 in processes that must occur in different cell cycle phases would intrinsically ensure the proper coordination between spindle positioning and mitotic exit.

Another important issue that remains to be addressed is what triggers Tem1 activation at SPBs. As mentioned above, it is possible that an unidentified GEF resides at SPBs. Alternatively, Tem1 might not need a GEF; rather, at SPBs it could be simply refractory to inhibition by its GAP. It is worth noticing that the Polo kinase Cdc5, which downregulates the

GAP activity of the Bub2-Bfa1 complex,^{41,53} is found at both SPBs during metaphase⁵⁴ and is therefore likely part of this regulatory mechanism. Moreover, Tem1 itself is subject to post-translational modifications, such as phosphorylation and ubiquitylation,⁵⁵ that could contribute to its regulation.

The control of Tem1 and the MEN is a beautiful example of the strategies that eukaryotic cells have evolved to couple spindle positioning and cell cycle progression. Surveillance mechanisms analogous to the SPOC have been identified in asymmetrically dividing stem cells,⁵⁶ highlighting their importance in ensuring the right fate of a cell lineage and preserving the proper ploidy of cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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