Search, capture and signal: games microtubules and centrosomes play

Scott C. Schuyler and David Pellman

Department of Pediatric Oncology, The Dana-Farber Cancer Institute and Pediatric Hematology, The Children's Hospital, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA
(e-mail: schuyler@fas.harvard.edu; david_pellman@dfci.harvard.edu)

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Summary

Accurate distribution of the chromosomes in dividing cells requires coupling of cellular polarity cues with both the orientation of the mitotic spindle and cell cycle progression. Work in budding yeast has demonstrated that cytoplasmic dynein and the kinesin Kip3p define redundant pathways that ensure proper spindle orientation. Furthermore, it has been shown that the Kip3p pathway components Kar9p and Bim1p (Yeb1p) form a complex that provides a molecular link between cortical polarity cues and spindle microtubules. Recently, other studies indicated that the cortical localization of Kar9p depends upon actin cables and Myo2p, a type V myosin. In addition, a *BUB2*-dependent cell cycle checkpoint has been described that inhibits the mitotic exit network and cytokinesis until proper centrosome position is achieved. Combined, these studies provide molecular insight into how cells link cellular polarity, spindle position and cell cycle progression.

Key words: Mitosis, Spindle orientation, Centrosome, Spindle pole body, Microtubule, Kinesin, Dynein, Actin, Myosin, Polarity, Cell cycle checkpoint

Introduction

Positioning of the mitotic spindle within dividing cells lies at the crossroads of many interesting areas of cell biology. The process involves communication of polarity signals to the cytoskeleton, interactions between different cytoskeletal systems and coordination of cytoskeletal rearrangements with cell cycle progression. In all dividing cells, spindle position and cytokinesis must be coordinated to ensure the accurate distribution of the genome (Rappaport, 1996; Wheatley, 1999). Interactions between microtubules and the cell cortex that mediate spindle positioning generate pulling forces on the spindle poles that are important for anaphase (Aist and Berns, 1981; Aist et al., 1991; Saunders et al., 1995). Additionally, accurate positioning of the spindle is important for asymmetric cell divisions that control cell fate determination during development (Rhyu and Knoblich, 1995; Chant, 1999). In asymmetrically dividing cells, cortical interactions at specific sites are dictated by the axis of polarity (Hyman and White, 1987; Hyman, 1989; Nishikata et al., 1999; Jan and Jan, 2000). Thus, physical interactions between microtubules and defined cortical sites enable the spindle to 'sense' the polarity of the cell and move into alignment with it. Microtubule orientation toward specific membrane sites is not only important in dividing cells but is also crucial for the navigation of neural growth cones, the polarization of T cells towards antigens and the establishment of apical-basal polarity of epithelial cells (Baas, 1999; Drubin and Nelson, 1996).

Physical interactions between microtubules and capture sites on the cortex, or the chromosome (the kinetochore), are thought to arise by a 'search and capture' mechanism driven by the dynamic instability of microtubules (Kirschner and Mitchison, 1986). Dynamic instability enables microtubules to 'search' the intracellular space for binding sites (Desai and Mitchison, 1997; Holy and Leibler, 1994). Chance encounters bring them into contact with capture sites, allowing the formation of semi-stable attachments (Mitchison and Kirschner, 1985). 'Targeting' of microtubule polymerization towards capture sites might also promote attachment (Kaverina et al., 1998, Wadsworth, 1999; Brunner and Nurse, 2000). Once semi-stable attachments are made, movement can then be generated by shortening or lengthening of the attached microtubules or by ATP-dependent mechanochemical action (Inoue and Salmon, 1995; Vallee and Gee, 1998; Hildebrandt and Hoyt, 2000; Goldstein and Philip, 1999). Additionally, dynamic instability together with spatially defined sites of nucleation and capture might allow self-organization of structures such as the spindle (Kellogg et al., 1994; Hyman and Karsenti, 1998; Kirschner et al., 2000; Sharp et al., 2000; Heald, 2000).

The molecular basis for microtubule capture at the cortex and other sites has remained elusive. Until recently, the positioning of the spindle in some way was known to involve actin, microtubules and microtubule-based motors, but specific molecular interactions remained unidentified. A flurry of papers on the spindle-positioning mechanism in the budding yeast *Saccharomyces cerevisiae* has now brought potential mechanisms into sharper focus. These results have implications beyond the understanding of spindle alignment in yeast. A number of the proteins required for spindle positioning are conserved. Further, some of the proteins involved in cortical-microtubule capture might well play a role in capture at other sites, such as the kinetochore. Here we focus on the most recent progress in spindle positioning in budding yeast and discuss the implications for other systems.

Spindle orientation in budding yeast

In budding yeast the polarity of the cell is established by the

signaling molecules that control the budding pattern (Palmieri and Haarer, 1998; Chant, 1999; Pruyne and Bretscher, 2000a; Pruyne and Bretscher, 2000b). To distribute replicated chromosomes between the mother and daughter cells, the spindle must be aligned with the pre-existing axis of division. This is achieved by interactions between the 3-4 astral microtubules emanating from the centrosomes or spindle pole bodies (SPBs) and the cell cortex. The in vivo dynamics of cytoplasmic microtubules in budding (Carminati and Stearns, 1997; Shaw et al., 1997; Shaw et al., 1998; Bloom et al., 1999; Tirnauer et al., 1999; Maddox et al., 2000; Adames and Cooper, 2000) and fission yeast (Drummond and Cross, 2000) have been described in detail. When new daughter cells are born and before polarization of the cytoskeleton towards the site of the future bud, the SPB, and therefore the nucleus, appears to be pushed by polymerizing microtubules hitting the cell cortex (Shaw et al., 1997). This is a well-established mechanism for moving centrosomes and nuclei around in other cell types (Reinsch and Gonczy, 1998). Close to the time of bud emergence, the duplicated SPBs separate, and microtubules from the daughter-bound pole enter the bud (Byers and Goetsch, 1975; Vallen et al., 1992). Microtubules that have entered the bud are pulled and swing the spindle into alignment (Carminati and Stearns, 1997; Shaw et al., 1997). Pulling forces can be generated either by end-on interactions between microtubules and the cortex that are coupled to microtubule depolymerization or by sliding of the side of the astral microtubules along the cortical surface (Carminati and Stearns, 1997; Shaw et al., 1997; Maddox et al., 2000; Adames and Cooper, 2000). During anaphase, the spindle elongates across the entire distance between the mother and daughter cell, driving the SPBs towards opposite sides of the cell cortex.

The dynein/dynactin-dependent mechanism for spindle orientation

Genetic studies suggest that at least two partially overlapping mechanisms position spindles in budding yeast. The first is mediated by the microtubule motor dynein and its regulator the dynactin complex (Stearns, 1997; Heil-Chapdelaine et al., 1999; Hildebrandt and Hoyt, 2000). Cells that lack dynein (Eshel et al., 1993; Li et al., 1993; Yeh et al., 1995; Dick et al., 1996; Carminati and Stearns, 1997) or dynactin (Clark and Meyer, 1994; Muhua et al., 1994; McMillan and Tatchell, 1994; Kahana et al., 1998) assemble a normal bipolar spindle and position it appropriately near the neck between the mother and daughter cell. However, they undergo a defective anaphase in which the spindle is not inserted properly across the neck and chromosomes are divided entirely within the mother, which thus leaves a temporarily empty bud. Remarkably, loss of dynein and/or dynactin is not lethal. Rather than dividing into one cell that contains two copies of the chromosomes and another that has none, most of the cells lacking dynein and/or dynactin delay the cell cycle until the problem can be fixed (Yeh et al., 1995; Muhua et al., 1998). The cell cycle delay is mediated by a recently elucidated checkpoint mechanism (see below), (Bardin et al., 2000; Pereira et al., 2000; Bloecher et al., 2000).

The fact that dynein and dynactin play a critical role in nuclear movement and positioning of the budding yeast spindle is consistent with the requirement of these proteins for nuclear movement and spindle position in other organisms, such as *Schizosaccharomyces pombe* (Yamashita et al., 1997; Yamamoto et al., 1999; Hiraoka et al., 2000), *Aspergillus nidulans* (Xiang et al., 1994), *Neurospora crassa* (Plamann et al., 1994; Tinsley et al., 1996), *Nectria haematococca* (Inoue et al., 1998), *Dictyostelium discoideum* (Koonce et al., 1999), *Caenorhabditis elegans* (Skop and White, 1998; Gonczy et al., 1999), *Drosophila melanogaster* (McGrail and Hays, 1997; Deng and Lin, 1997; Robinson et al., 1999; Swan et al., 1999) and mammals (Vaisberg et al., 1993; O'Connell and Wang, 2000).

Important questions remain, however. Because of the lack of localization data in budding yeast, the site of action of dynein remains unclear. The bud cortex is likely to be one important site, because a recent study found that the sliding of microtubules along the bud cortex is mediated by dynein (Adames and Cooper, 2000). Dynein might also act at the mother-bud neck and/or at the cortex of the mother cell. Another critical issue is the molecular role of the dynactin complex: does it target dynein to the appropriate site or, rather, regulate its activity? The studies on budding yeast dynein and dynactin have two interesting implications. First, the fact that dynein and dynactin are not essential suggests that an alternative spindle-positioning mechanism exists. Second, the cell must 'know' that the spindle is mispositioned and stall cell cycle progression until the problem is fixed (see below). There has been significant recent progress on both of these questions.

The Kip3p-dependent pathway of spindle orientation

A host of proteins have recently presented themselves as alternatives to dynein-dynactin for positioning the spindle. We refer to this group as the 'Kip3p' pathway after the first member to be shown to overlap functionally with dynein (DeZwaan et al., 1997; Cottingham and Hoyt, 1997; Miller et al., 1998). The Kip3p group includes the kinesin motor Kip3p, the yeast formin Bni1p, the cortical and microtubule-associated protein Kar9p and the microtubule-binding protein Bim1p (Lee et al., 1999; Fujiwara et al., 1999; Miller and Rose, 1998; Miller et al., 1998; Tirnauer et al., 1999). Cells lacking any of these proteins have a variety of common features: they exhibit defective initial alignment of the metaphase spindle towards the bud; they are inviable if they also lack dynein or dynactin; and in general they fail to show additive defects in conjunction with null mutants of other Kip3p pathway proteins. Importantly, several of the Kip3p pathway proteins are localized to the bud cortex and are functionally linked to actin (Pruyne and Bretscher, 2000a; Pruyne and Bretscher, 2000b), which is required for spindle positioning in many systems (Fuchs and Yang, 1999; Goode et al., 2000). Although the genetic studies suggest these proteins are part of a pathway, it is not yet clear whether all of the members of the Kip3p pathway in fact participate in a single biochemical function.

Of the Kip3p group proteins, we now know the most about the function of Kar9p and Bim1p. Kar9p is required for nuclear fusion during mating of budding yeast (Kurihara et al., 1994; Rose, 1996) and for the related processes of spindle and nuclear orientation towards the bud and the mating projection (Miller and Rose, 1998; Miller et al., 1999). It has a fascinating pattern of localization: it is often found as a dot at the ends of microtubules extending into the bud and also in discrete foci along the length of microtubules directed into the bud (Miller and Rose, 1998; Lee et al., 2000). In the absence of

microtubules, Kar9p is seen as a highly mobile dot on the bud cortex (Beach et al., 2000; J. Liu and D. Pellman, unpublished results). Additionally, in the absence of polymerized actin, Kar9p cortical localization is lost (Miller et al., 1999). Together, these findings suggest that Kar9p shuttles between microtubules and actin, which makes it a prime candidate for the agent that captures microtubules at the bud cortex. Note that the full extent of Kar9p function is not yet clear, because Kar9p is also found at the SPB and at the septum (Lee et al., 2000; Miller et al., 2000).

The Kar9p/Bim1p link between microtubules and the cell cortex

It turns out that Kar9p does not bind microtubules directly but rather is linked to microtubules by the microtubule-binding protein Bim1p (also known as Yeb1p) (Lee et al., 2000; Korinek et al., 2000; Miller et al., 2000). Bim1p is one of the most highly conserved components of the microtubule cytoskeleton. Its human homologue EB1, was identified as a binding partner for the adenomatous polyposis coli (APC) tumor suppressor protein (Su et al., 1995; Tirnauer and Bierer, 2000). Loss of *mal3*$^{+}$, the *S. pombe* homolog of *BIM1*, can be complemented by human EB1, which demonstrates true functional conservation (Beinhauer et al., 1997). Budding yeast Bim1p was identified because of its interaction with tubulin and, notably, cells lacking Bim1p have similar phenotypes to those lacking Kar9p: they exhibit defective karyogamy and also a strikingly similar pattern of genetic interactions with other mutations that affect microtubules (Schwartz et al., 1997; Tirnauer et al., 1999; Lee et al., 2000; Korinek et al., 2000; Miller et al., 2000; Adames and Cooper, 2000). Bim1p was also identified in a screen for checkpoint mutants that abolish the cell cycle delay in a dynactin mutant (see below), (Muhua et al., 1998). Bim1p and Mal3p are found on both astral and spindle (nuclear in yeast) microtubules (Schwartz et al., 1997; Beinhauer et al., 1997), and at native levels it is highly concentrated near microtubule plus ends (Tirnauer et al., 1999) – as is human EB1 (Berrueta et al., 1998; Morrison et al., 1998; Mimori-Kiyosue et al., 2000a).

In yeast, cytoplasmic microtubules are most dynamic during G1 phase, when they are searching for cortical binding sites in the new bud site (Carminati and Stearns, 1997; Tirnauer et al., 1999; Adames and Cooper, 2000). Live-cell imaging of cells lacking Bim1p revealed that Bim1p is required for the dynamicity of microtubules during G1 phase but has little or no effect on microtubule dynamics during mitosis (Tirnauer et al., 1999; Adames and Cooper, 2000). This suggested that Bim1p is required for the search for cortical binding sites. Bim1p is also directly involved in the cortical capture process, binding Kar9p: Bim1p and Kar9p physically interact and were found in an ~250-kDa complex in yeast extracts (Lee et al., 2000; Korinek et al., 2000; Miller et al., 2000). Furthermore, Bim1p is required for microtubules to interact with cortical Kar9p in vivo, and therefore *bim1Δ* cells fail to orient microtubules towards the bud or towards the shmoo in mating cells (Tirnauer et al., 1999; Lee et al., 2000; Korinek et al., 2000; Adames and Cooper, 2000). A direct role for Bim1p in linking microtubules with Kar9p is supported by the finding that, although Kar9p has little or no microtubule binding on its own, pure Bim1p can recruit Kar9p onto microtubules (Lee et al., 2000; Korinek et al., 2000). Finally, the idea that the interaction between Bim1p and Kar9p is important for cortical microtubule capture is strengthened by live-cell microscopy showing that cells lacking either Bim1p or Kar9p have decreased pulling of astral microtubules towards the bud cortex (Adames and Cooper, 2000; Yeh et al., 2000). Together, these studies suggest a simple mechanism for the initial step in the capture of microtubules at the cortex of the bud. Bim1p promotes microtubule dynamicity during G1 phase, which enhances the searching by microtubules for cortical binding sites. A physical interaction between Bim1p and Kar9p then tethers the microtubule to the bud cortex.

Although these studies give us a sketch of the capture process, interesting biochemical details are yet to come. First, how does Bim1p or other EB1-family proteins preferentially interact with microtubule plus ends? Human EB1 localizes along growing microtubule plus ends in a manner very similar to that used by the other plus-end-associated linker family, the CLIP-170-like proteins (Mimori-Kiyosue et al., 2000a; Perez et al., 1999; Brunner and Nurse, 2000). In fact, it appears that, in budding yeast, polymerizing microtubules in vivo are always capped by Bim1p and/or the CLIP-170 family member Bik1p (Berlin et al., 1990; Tirnauer et al., 1999; D. Kho and D. Pellman, unpublished results). The simplest presumption is that EB1-family proteins and CLIP-170 either bind to a unique structure at the growing plus ends or to a specific nucleotide-bound state of tubulin (Tirnauer and Bierer, 2000). Cryoelectron microscopy or atomic force microscopy may provide insight into the structure of Bim1p complexed to microtubule ends. Another interesting line of experiments will be to examine how Bim1p or the Bim1p-Kar9p complex affects microtubule dynamics in vitro. Measurements of microtubule dynamics in vivo suggest that Bim1p promotes plus-end dynamicity (Tirnauer et al., 1999; Adames and Cooper, 2000). Rather than promoting dynamicity, the Bim1p-Kar9p complex might either stabilize microtubule ends or promote their depolymerization. The idea that the Bim1p-Kar9p complex depolymerizes plus ends is appealing because, if the complex remains tethered to the cortex during depolymerization, a pulling force towards the cortex must be generated. The kinesin Kip3p might also play a role in producing a bud-directed pulling force, potentially by promoting microtubule depolymerization. We have not been able to detect an interaction between Bim1p and Kip3p by co-immmunoprecipitation, but it remains possible that Kip3p is transiently recruited to the complex (L. Lee and D. Pellman, unpublished results). Clearly, more biochemical work needs to be done, and observations from other model systems should be utilized. For example, the localization of a cortical kinesin-like protein to the centrosome-attracting body (CAB) in ascidians makes this system an attractive one for studying how microtubules interact with the cell cortex (Nishikata et al., 1999; Iseto and Nishida, 1999).

Kar9p localization at the cortex depends upon actin and myosin

The studies discussed above suggest that Bim1p connects microtubules to Kar9p, but how is Kar9p connected to actin and the cell cortex? This is another area in which there has been significant progress. Studies using mutants of actin or actin-associated proteins and actin-depolymerizing drugs reveal that, in many cell types, positioning of the spindle requires actin

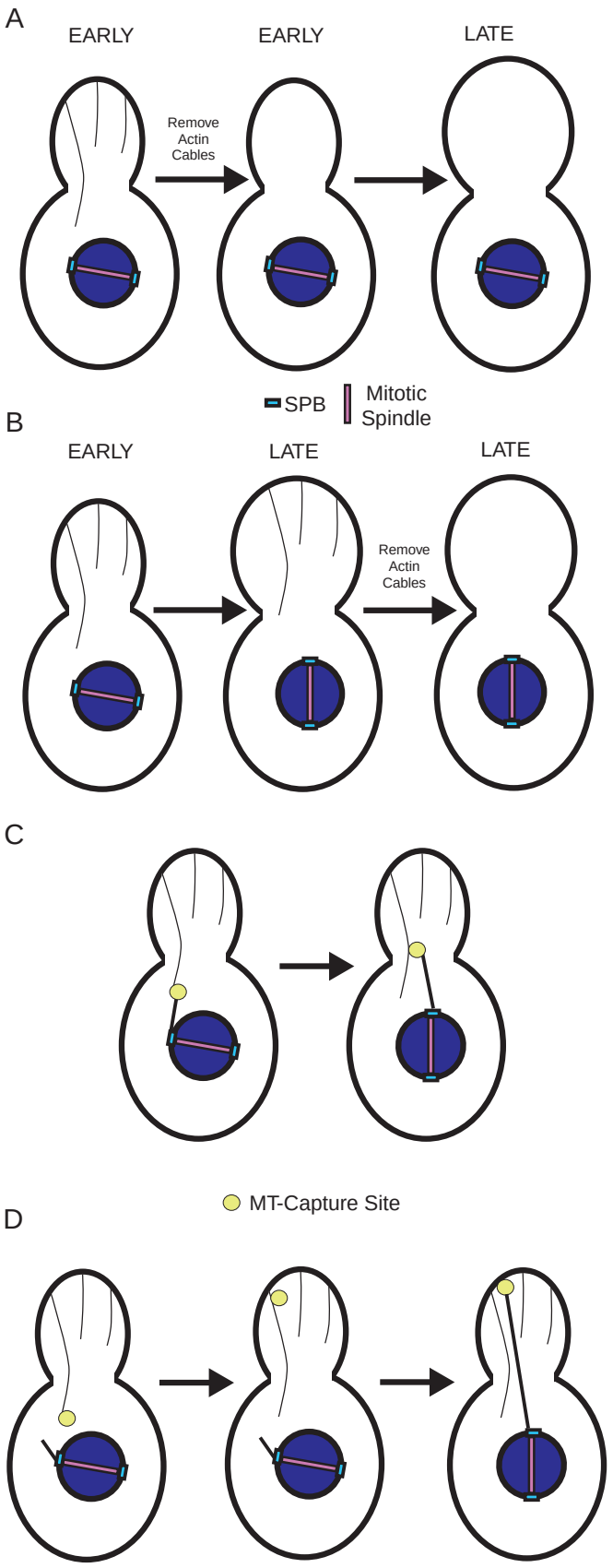

Fig. 1. Actin cables are required early in the cell cycle, but not late, for proper spindle orientation. Spindle orientation is marked by the position of the spindle pole bodies (SPBs) relative to the mother-bud axis. (A) Cells in which actin cables (thin lines) are disrupted early in the cell cycle do not orient spindles. (B) Spindle orientation is maintained if actin cables are disrupted late in the cell cycle. (C) Actin cables might directly promote spindle orientation by coupling a microtubule (MT) capture site to actin cables. (D) Actin cables might be required to transport a microtubule-capture site to the cell cortex.

(Palmer et al., 1992; Fuchs and Yang, 1999; Goode et al., 2000). Kar9p localization to the bud cortex requires actin and other proteins functionally linked to actin (Miller et al., 1999). There is mounting evidence that actin cables are a critical actin structure for aligning the spindle. One study found that a mutation that alters the organization of the actin patches does not affect spindle orientation (Lee et al., 1999). Other studies found that disruption of actin cables, early in the cell cycle but not late (Fig. 1), dramatically disrupts microtubule orientation (Kopecka and Gabriel, 1998; McMillan et al., 1998; Theesfeld et al., 1999). The implication is that actin cables themselves are directly linked to microtubules (Fig. 1C) or that some protein(s) critical for spindle positioning are transported to the bud via actin cables (Fig. 1D).

An exciting new development is that Kar9p appears to be transported to the bud cortex along actin cables by the type V myosin Myo2p (Yin et al., 2000). Myo2p is required for Kar9p localization to the bud cortex, and Kar9p binds to the Myo2p tail. An elegant genetic analysis supports the idea that this binding transports Kar9p to the bud cortex: some but not all of a series of mutations in the Myo2p tail block spindle orientation, and these mutations also block binding to Kar9p. A requirement for Myo2p in Kar9p transport to the bud has also been directly observed by live-cell microscopy (Beach et al., 2000). These findings strongly suggest that Myo2p transports Kar9p to the bud cortex and raise the possibility that Myo2p both maintains Kar9p at the bud cortex and controls its movement.

The Myo2p-dependent transport of Kar9p suggests two models for how actin cables could promote spindle positioning. One hypothesis, suggested by Yin et al., is that Myo2p-dependent transport creates a gradient of Kar9p along actin cables that orients microtubules even before they interact with the cortex (Fig. 2A) (Yin et al., 2000). In this model, during spindle orientation, actin and microtubules are physically connected by the Kar9p-Bim1p complex. Although this model is appealing, one finding is difficult to reconcile with this idea: Kar9p appears to move to the cortex >50-fold faster than the rate of nuclear migration or the rate of microtubule polymerization (Beach et al., 2000). Therefore the bulk Kar9p-GFP that has been observed moving to the cortex is unlikely to be stably bound to microtubules. Additionally, the Bim1p-Kar9p complex identified in native extracts, although larger than predicted for a Bim1p-Kar9p heterodimer, is not large enough to contain stoichiometric amounts of Myo2p (Lee et al., 2000). However, it remains possible that a subpopulation of Kar9p exists in an actin-Myo2p-Kar9p-Bim1p-microtubule complex.

An alternative idea is that Kar9p is rapidly transported to the cortex and perhaps maintained there by Myo2p. This would allow Bim1p-bound microtubule ends to ‘pick up’ Kar9p

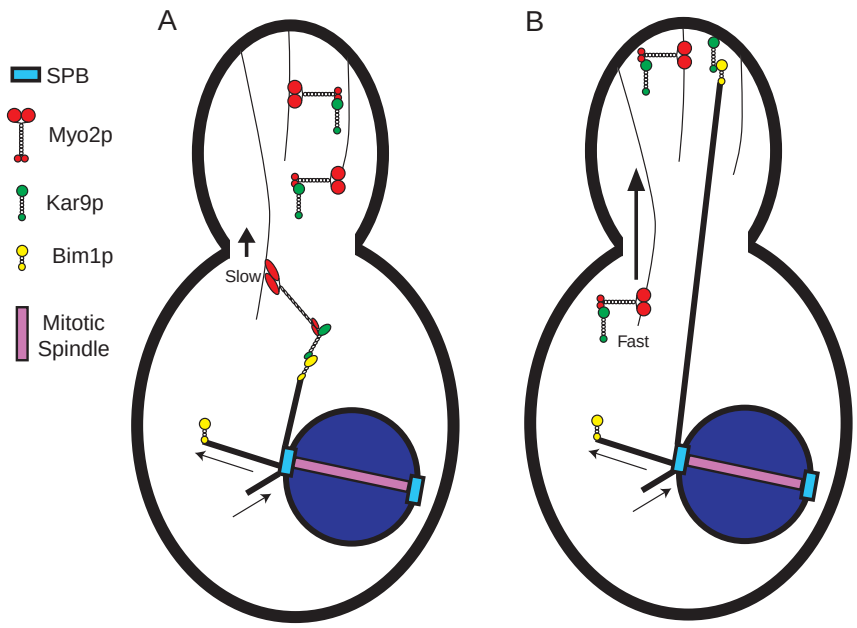

Fig. 2. Two models for the role of Myo2p in spindle orientation. Early in the cell cycle a small bud has formed, and the spindle pole bodies (SPBs) have duplicated. For simplicity, only daughter SPB microtubules are shown. (A) A Myo2p-Kar9p-Bim1p complex actively promotes proper nuclear orientation. Myo2p-Kar9p complexes along actin cables (thin lines) create a gradient of Bim1p-capture sites. Upon capture, the new trimeric complex would slowly orient the SPB towards the bud. For clarity, many other known Myo2p complexes are not shown. (B) Myo2p rapidly transports Kar9p to the cortex, where it interacts with other cortical factors. Myo2p may be required to maintain Kar9p localization at the cortex. Once Kar9p interacts with Bim1p, a smaller Kar9p-Bim1p complex is formed that connects the microtubule to other cortical polarity factors.

by sweeping along the cortex. The Kar9p-Bim1p-bound microtubule ends would then form a semi-stable attachment to a cortical actin structure (Fig. 2B). There are several experiments that would help distinguish between these models. First, the idea that a Kar9p gradient orients microtubules would be supported by the identification of a tri-molecular complex containing Myo2p, Kar9p and Bim1p. Second, it will be important to characterize Kar9p levels during the cell cycle and to determine whether any of the physical interactions with Kar9p are temporally regulated.

A Bub2p-dependent spindle-position checkpoint

A final interesting question is how the cell can sense when the spindle position is abnormal and hence respond by delaying cell cycle progression. Time-lapse microscopy experiments first demonstrated that cells lacking dynein (*DYN1*) delay mitotic exit when the spindle is mispositioned (Yeh et al., 1995). Because *dyn1Δ* cells delay the cell cycle in late anaphase, this delay could be mediated by a checkpoint distinct from the well-characterized mitotic checkpoint that monitors bivalent attachment of kinetochores (Yeh et al., 1995; Hoyt, 2000). This idea of a spindle-position-sensing checkpoint is further supported by the report of mutations that can bypass the delay in cells lacking dynactin function (Muhua et al., 1998). Intriguingly, the primary mutant characterized from this screen was in *BIM1* (called *YEB1* in this work). The cytoskeletal function of EB1-family proteins is now well established (Tirnauer and Bierer, 2000). What remains unknown is whether EB1 proteins have additional signaling functions.

Although how Bim1p influences the spindle-position checkpoint is not known, there has been significant progress on other aspects of this signaling mechanism. The main new finding is that the cell cycle delay in dynein- or dynactin-deficient cells requires a known mitotic checkpoint protein, Bub2p (Bardin et al., 2000; Pereira et al., 2000; Bloecher et al., 2000). Bub2p was known to be an outlier in the mitotic checkpoint pathway, acting in parallel to the proteins that sense the bivalent attachment of kinetochores to spindles (Li, 1999; Fraschini et al., 1999; Alexandru et al., 1999; Fesquet et al., 1999; Gardner and Burke, 2000). Further supporting a distinct role for Bub2p, these experiments suggest that Bub2p might regulate mitotic exit rather than the metaphase-anaphase transition, which other mitotic checkpoint proteins target.

The recent work suggests that Bub2p monitors the position of the centrosome rather than the kinetochore and directly influences the signaling pathway known as the mitotic exit network (MEN). In budding yeast the MEN promotes mitotic exit by inhibiting the function and expression of mitotic cyclins (Hoyt, 2000). Like its homologue in *S. pombe*, Bub2p is asymmetrically localized on the centrosome: during mitosis it is preferentially associated with the daughter-bound centrosome (Cerutti and Simanis, 1999; Pereira et al., 2000; L. Lee and D. Pellman, unpublished results). A combination of genetic data, homology and analogy to biochemical work in *S. pombe* (Balasubramanian et al., 2000) suggests that Bub2p is a component of a GTPase-activating protein (GAP) that inactivates the Rab-family GTPase Tem1p (Schmidt et al., 1997; Furge et al., 1998). Tem1p is thought to be activated by a GTPase-exchange factor (GEF), Lte1p, that is also part of the MEN (Shirayama et al., 1994a; Shirayama et al., 1994b). The recent work suggests that spatial separation of Tem1p and Lte1p is a key mechanism for controlling the timing of mitotic exit. Like Bub2p, Tem1p is preferentially associated with the daughter-bound centrosome (Bardin et al., 2000; Pereira et al., 2000). By contrast, Lte1p is confined to the daughter cell (Bardin et al., 2000; Pereira et al., 2000).

Together, the localization and genetic analyses suggest an appealing model for how the cell senses abnormal spindle position (Fig. 3). In a normal cell cycle, Bub2p bound to Tem1p would restrain mitotic exit until the daughter-bound centrosome comes into contact with Lte1p in the bud. This would effectively couple mitotic exit with nuclear segregation. In cells in which the spindle is misaligned, the pole that should have entered the bud remains in the mother. Mitotic exit is then inhibited because Tem1p is exposed to the inhibitory influence

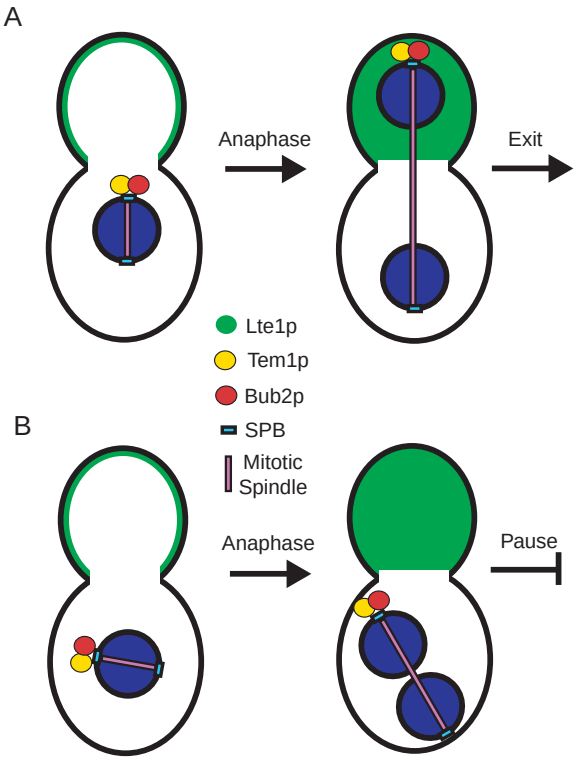

Fig. 3. A Bub2p-dependent mitotic checkpoint monitors the position of the daughter spindle pole body (SPB). Tem1p and Bub2p are localized specifically at the daugter SPB. Lte1p is confined to the daughter cell. (A) In wild-type cells, spindle elongation brings Tem1p into contact with Lte1p and promotes the exit from mitosis. (B) In cells that have a spindle-orientation defect, anaphase occurs in the mother. The cell cycle is paused because Tem1p is inhibited by Bub2p at the daughter SPB.

of Bub2p and cannot come into contact with its activator Lte1p. Future work to determine how the asymmetric localization of Tem1p and Bub2p at the daughter SPB and Lte1p in the daughter cell is established and maintained will be important for our understanding of how the cell couples spindle elongation with mitotic exit and cell cycle progression. It will also be interesting to explore the similarities and differences in the functions of conserved components of the mitotic exit network in other organisms (Balasubramanian et al., 2000; Guertin et al., 2000).

Future directions

Will the insights from budding yeast be transferable to other cell types? Although the answer is not yet available, the outlook is promising. The recent work on the Kip3p pathway raises the possibility that EB1-family proteins have a general function to link microtubules to other cellular structures, such as the cell cortex or the chromosomes. There are at least six EB1 family members in the human genome, one of which binds to dynactin directly (Berrueta et al., 1999; Tirnauer and Bierer, 2000). Furthermore, the adenomatous polyposis coli tumor suppressor protein (APC) localizes to asymmetrically distributed spots near the membrane of migrating epithelial cells and near the tips of microtubules (Mimori-Kiyosue et al., 2000b; Askham et al., 2000). This microtubule-end localization of APC depends at least in part on the EB1-binding domain. This creates an appealing parallel between the interactions of Kar9p-Bim1p and APC-EB1. The APC-EB1 interaction may be important to orient microtubules towards the leading edge of migrating epithelial cells. Additionally, Bim1p and EB1 both localize to spindle microtubules and probably to kinetochore microtubules. It is therefore possible that these proteins have a role in capture of microtubules at kinetochores, which is essential for proper chromosome segregation. Chromosomal instability is a hallmark of colon cancer. >85% of colon cancers have mutations in APC that delete the C-terminal domain that binds EB1 (Kinzler and Vogelstein, 1996). Thus, the APC-EB1 interaction might have a direct role in the chromosomal instability of colon cancers.

The checkpoint mechanism that senses kinetochore attachment is highly conserved between yeast and humans. Some of these human proteins are also implicated in the chromosomal instability of colon cancers (Cahill et al., 1998). The conserved checkpoint function of the mammalian proteins has been driven home by recent knockout experiments in the mouse (Dobles et al., 2000; Kalitsis et al., 2000). Will the Bub2p checkpoint that monitors centrosome position also be conserved? Here, much less is known, but it is intriguing that potential human orthologues of Bub2p have been described (Richardson and Zon, 1995; White et al., 2000). Significant morphological and temporal differences in the relationship of spindle and centrosome position to cytokinesis exist between yeast and animal cells. However, it has been found time and again that in different cell types similar molecular mechanisms mediate similar processes even when these processes appear morphologically distinct. The Bub2p spindle-position checkpoint provides a new frontier to test the generality of basic cell cycle control mechanisms.

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