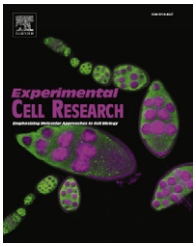
EXPERIMENTAL CELL RESEARCH 318 (2012) 1400–1406

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/yexcr



Review Article

Astral microtubule asymmetry provides directional cues for spindle positioning in budding yeast

*Steven M. Markus, Katelyn A. Kalutkiewicz, Wei-Lih Lee**

Biology Department, University of Massachusetts Amherst, 221 Morrill South, 611 North Pleasant Street, Amherst, MA 01003, USA

ARTICLE INFORMATION

Article Chronology:
Received 1 January 2012
Revised version received 6 April 2012
Accepted 6 April 2012
Available online 19 April 2012

Keywords:
Spindle orientation
Kar9 pathway
Dynein
Spindle position checkpoint

ABSTRACT

Cortical force generators play a central role in the orientation and positioning of the mitotic spindle. In higher eukaryotes, asymmetrically localized cortical polarity determinants recruit or activate such force generators, which, through interactions with astral microtubules, position the mitotic spindle at the future site of cytokinesis. Recent studies in budding yeast have shown that, rather than the cell cortex, the astral microtubules themselves may provide polarity cues that are needed for asymmetric pulling on the mitotic spindle. Such asymmetry has been shown to be required for proper spindle positioning, and consequently faithful and accurate chromosome segregation. In this review, we highlight results that have shed light on spindle orientation in this classical model of asymmetric cell division, and review findings that may shed light on similar processes in higher eukaryotes.

Contents

Introduction

Unlike mitosis in higher eukaryotes, the site of cell division in budding yeast occurs at a predetermined site. Upon commitment to cell division (at the G1/S boundary), cells initiate processes that result in DNA replication, duplication of the spindle pole bodies (SPBs; equivalent to centrosomes in animal cells), and the formation of a daughter cell (also referred to as the bud). As a result, the duplicated genetic material must be physically translocated from the interior of the mother cell to the daughter cell compartment in a reliable and tightly regulated manner. Gross errors in this process result in the complete exclusion of

* *Corresponding author.* Fax: +1 4135452944.
E-mail address: wlee@bio.umass.edu (W.-L. Lee).

0014-4827/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2012.04.006

chromosomes from the daughter cell, and an accompanying increase in genetic material within the mother cell (from 1 N to 2 N). This latter event (i.e., polyploidy) can lead to an increased demand on the DNA replication and chromosome maintenance machinery, causing changes in gene dosage, which can alter protein stoichiometry, and can have severe consequences on cell fitness [1].

In higher eukaryotes, the position of the mitotic spindle often dictates the position of the cleavage furrow and thus determines whether a division occurs symmetrically or asymmetrically. Thus, the proper regulation of spindle dynamics – orientation, rotation, and movement – at the moments leading up to and following chromosome separation (i.e., anaphase) is a crucial process during mitosis that is important for generating cell-type diversity in organisms across evolution.

Variations on a common theme

A common theme underlying the mechanisms that govern regulated spindle positioning in various organisms is that they generally require asymmetric localization of protein complexes that interact directly with the cytoskeletal machinery powering spindle movements. In many cases, the protein complexes that are involved in the process are targeted to the cell cortex where they recruit or activate force generators, which then act on the mitotic spindle through astral microtubules emanating from the spindle poles, resulting in asymmetric cortical pulling forces. For instance, in dividing *Drosophila* neural stem cells, asymmetric localization of the Gα/Pins/Mud complex to the apical cortex (apical–basal polarity pre-established by the apical Par complex, Par-3/Par-6/aPKC, at late interphase) provides a docking site for attracting astral microtubules nucleated from the apical spindle pole [2]. Since Mud interacts with microtubules and the minus end-directed microtubule motor dynein, it is thought that cortical Mud either recruits dynein as the force generator or tethers the captured astral microtubules as a means to achieve alignment of the mitotic spindle along the apical–basal axis. Similar to *Drosophila* neuroblasts, in the one-cell *Caenorhabditis elegans* zygotes, the activity of the homologous complex (GOA-1 and GPA-1, GPR-1/2, LIN-5) is also asymmetrically restricted to a specific cortical domain (but, in the *C. elegans* case, the posterior cell cortex) [3]. Here, differential enrichment of GPR-1/2 at the posterior cortical domain has been suggested to promote cortical anchoring of dynein for posterior-directed anaphase pulling force, or to facilitate microtubule plus end depolymerization-mediated force generation via a dynein-dependent end-on interaction with astral microtubules [4].

In a somewhat similar scenario, in budding yeast, differential localization of proteins to mother and bud cortex establishes distinct cortical domains along which the position of the mitotic spindle is monitored by checkpoint proteins (discussed further below). However, it is not clear whether an asymmetrically targeted receptor complex, such as Gα/Pins/Mud, recruits force generators, such as dynein, to a specific domain at the yeast cell cortex (e.g., the bud cortex). Instead, insights from new data in yeast suggest that the astral microtubules might play an active role in ensuring that the force generator is asymmetrically delivered to the cortical receptor, which, in the case of the dynein pathway, is uniformly distributed at the cell cortex (discussed further below). As in numerous organisms, astral microtubules and dynein play a major role in positioning the yeast spindle along the polarized cell axis. However, budding yeast deficient in dynein are viable and can in fact compensate for the loss of dynein by utilizing a seemingly independent pathway to properly orient their spindle (i.e., the Kar9 pathway), and consequently segregate their genetic material equally between the mother and daughter cell. Below we review current paradigms for these pathways and briefly discuss how spindle orientation information is transmitted to cell cycle progression by the spindle position checkpoint (referred to as SPC or SPOC), which prevents premature mitotic exit from occurring.

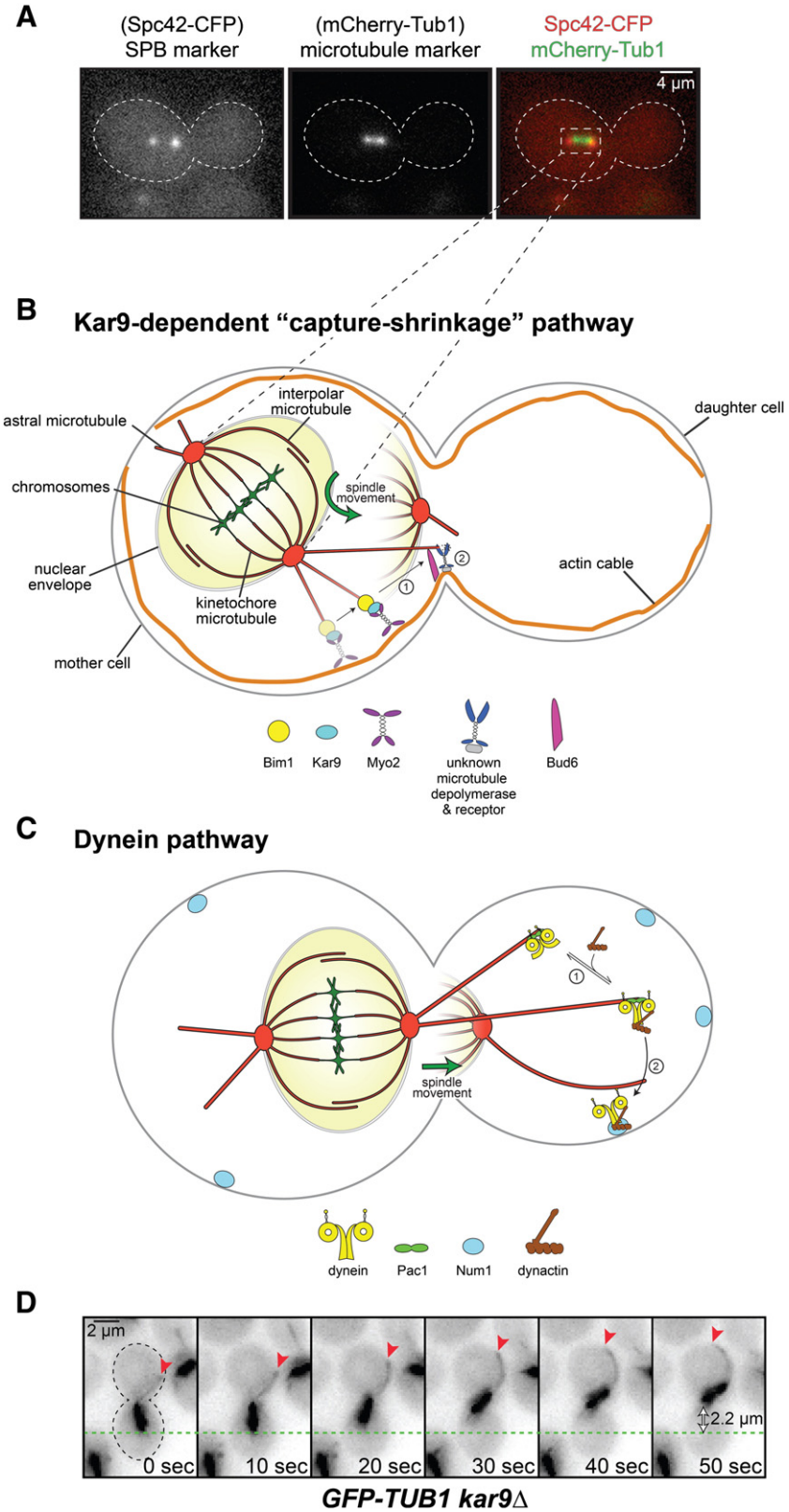
The 'early' Kar9 pathway

Initial studies on spindle orientation in budding yeast revealed that the mechanism responsible for orienting the spindle early in the cell cycle is quite distinct from that occurring later in the cell cycle. In particular, elegant F-actin perturbation experiments showed that the early mechanism requires actin cables [5], whereas the later mechanism does not [5,6]. During the early stages of the cell cycle (i.e., in small- and medium-budded cells), the mother cell contains a cortical basket of actin cables converging on the bud tip or the bud neck, from where the actin cables are nucleated. Perturbing F-actin (using latrunculin-A) at these early stages significantly decreased the proportion of cells displaying astral microtubules extending into the bud, leading to a preanaphase spindle misorientation phenotype in the majority of the treated cells [5]. This observation provided the initial clues that, early on in the cell cycle, actin cables play an important role in either guiding astral microtubules into the bud, anchoring them within the bud, or both.

The early actin-dependent mechanism, now known as the Kar9 pathway, uses a class-V myosin, Myo2, to guide the plus ends of astral microtubules along cortical actin cables toward the bud neck or apex of the bud cell cortex (Fig. 1B) [7–9]. Myo2 is recruited to microtubule plus ends through an interaction with Kar9, the yeast homologue of the mammalian adenomatous polypopsis coli (APC) protein. Kar9, in turn, binds Bim1, the yeast homologue of mammalian EB1, which interacts directly with the plus end of astral microtubules. As Myo2 motors along the actin cables, it transports Kar9, Bim1, and the associated microtubule plus end (as cargo) toward the bud neck or the bud cell tip [10]. In this manner, Kar9 can be viewed as an adaptor that promotes interactions between astral microtubules and the cortical actin cables, leading to frequent penetration or maintenance of the microtubules in the bud.

Intriguingly, Kar9's role in spindle orientation requires its asymmetric localization to the astral microtubules that grow out of the daughter bound spindle pole (dSPB) but not the mother bound spindle pole (mSPB) (representing a variation of the common theme described above). Kar9 asymmetry requires the function of Clb4 [11], an early mitotic cyclin that preferentially localizes to the mSPB, although this localization pattern appears to depend on its level of expression [10]. There, the active Clb4/Cdc28 (Cdk1 homologue) complex phosphorylates Kar9 and inhibits its association with the pole destined for the mother cell [11]. Conceivably, Clb4/Cdc28 forms a local gradient of kinase activity that precludes Kar9 recruitment onto the microtubules growing out of the mSPB, as phosphorylation by Cdc28 also decreases the ability of Kar9 to interact with Bim1 [11], which could in turn inhibit Kar9's function as an adaptor molecule. Time-

lapse imaging of microtubule behavior during the Kar9 pathway revealed that, upon emergence from the dSPB, short Kar9-loaded microtubules rapidly orient toward the bud (within 30 s) as they elongate and 'sweep' along the cortex (presumably along cortical actin cables) [11]. Interestingly, microtubules emanating from the mSPB, which lack Kar9, do not reorient toward the bud, suggesting that a random search mechanism is not sufficient to target the microtubules into the bud. These phenomena support the notion that astral microtubules (rather than the cell cortex) carry with them the cues for positioning the spindle along a predetermined cell axis, as asymmetric loading of Kar9 onto the microtubules does not appear to depend on cortical polarity.



When overexpressed, GFP-Kar9 has been seen to localize as cortical dots independently of microtubules [8,9,12], suggesting that a yet uncharacterized cortical interaction/capture site exists for the Kar9 pathway. At the capture site (Fig. 1B), an attachment-coupled microtubule depolymerization (or 'capture–shrinkage') event results in the orientation of the spindle along the mother-bud axis, and the translocation of the spindle toward the bud neck, as observed [13]. These capture–shrinkage events are thought to be mediated by a microtubule depolymerase, the identity of which is still unknown, although evidence points to Kip3 (a kinesin-8) and Kar3 (a kinesin-14), both of which exhibit plus end-specific microtubule depolymerase activity [14,15]. The motor may induce microtubule depolymerization while keeping the microtubule attached to either Bud6 [16,17], or a yet unknown cortical receptor protein. Based on in vitro force measurements, such depolymerization-driven events could produce at least 30–65 pN of force per microtubule [18]. Thus, after microtubule guidance toward the capture site, a capture–shrinkage mechanism could conceivably generate enough pulling force to move the spindle close to the bud neck and align it on the mother-bud axis.

The dynein pathway

In contrast to the early Kar9-mediated pathway for spindle positioning, the dynein pathway, which operates later in the cell cycle, employs cortically-anchored dynein motors that walk along the microtubule lattice to power spindle movement into the mother-bud neck (Fig. 1C). Because the motor is anchored, this movement generates pulling forces that originate from the cell cortex. Since the minus end of the astral microtubule is stably attached to the SPB, the astral microtubule that is being pulled by dynein slides along the bud cortex, dragging the spindle through the neck (Fig. 1D), thereby aligning the spindle along the polarized cell axis.

Recent studies have begun to reveal the intricate series of events that result in the targeting of dynein to the bud cell cortex in a spatially and temporally restricted manner. As it turns out, localization of dynein to the bud cortex depends on its initial loading onto astral microtubule plus ends [19]. As with Kar9 (see above), the loading of dynein onto astral microtubules appears to occur preferentially on those emanating from the dSPB [20] (representing another variation of the common theme described above). Although some of the dynein heavy chain (Dyn1) molecules that are present at the microtubule plus end are transported from the minus end in a kinesin-mediated manner [19,21], most of them are recruited directly from the cytoplasm in complex with Pac1, the yeast homologue of mammalian LIS1 [22]. After assembly in the cytoplasm, the dynein–Pac1 complex subsequently interacts with Bik1, the yeast homologue of mammalian CLIP-170, which may bind directly to the plus end through its NH_2-terminal CAP-Gly domain [23]. It is unclear how dynein, a minus end-directed motor, stays associated with the plus end of a microtubule. However, recent studies have demonstrated that Pac1, which is required for dynein plus end-targeting, can reduce the velocity of dynein motility in vitro [24], as described for LIS1 [25,26]. Thus, Pac1 may function by keeping dynein in an 'off' state at the plus end. Plus end-associated dynein exploits the dynamic instability of the microtubule to search for cortical capture sites containing Num1, onto which dynein offloads and becomes anchored [24]. Interestingly, although Num1 assembles cortical patches that are distributed along the bud and mother cell compartments [27,28], dynein offloading is strongly biased toward the bud cortex [24]. Whether this is due to the observed asymmetric recruitment of dynein to astral microtubules oriented toward the bud cell (i.e., from the dSPB), as described above, or some other phenomenon is unknown.

What prevents dynein from being recruited directly from the cytoplasm to cortical Num1 receptor sites? Localization studies of truncated Dyn1 constructs suggested that dynein offloading is negatively regulated by an intramolecular 'masking' of its NH_2-terminal tail (cortical association) domain by its COOH-terminal motor head [19]. At the plus end, dynein recruits dynactin – the dynein processivity factor and cargo adaptor molecule [29,30] – and this event is thought to trigger unmasking of the dynein tail domain, allowing it to interact with cortical Num1 upon a

Fig. 1 – Spindle dynamics in budding yeast. (A) The metaphase spindle as observed in cells expressing Spc42-CFP (spindle pole marker; red) and GFP-Tub1 (microtubule marker; green). All 16 chromosomes and kinetochores associated with each pole are situated within a single diffraction-limited spot. Note the bi-lobed appearance of the spindle, with all 16 kinetochore microtubules from each pole situated within the brighter spots, and a dimmer region (of approximately 800 nm) corresponding to the spindle midzone where overlapping interpolar microtubules are found (reviewed in [49]). Astral microtubules are not apparent due to the short exposure time used in the acquisition of this image (see panel D for longer exposures). (B) The 'early' Kar9 pathway. Astral microtubules emanating from the dSPB are guided toward the bud neck by Myo2-Kar9-Bim1, as depicted, along actin cables that are polarized toward the bud. Upon encountering the neck, the microtubule is captured either in a Bud6-dependent manner or subsequently depolymerized by a yet unknown microtubule depolymerase. This depolymerization-driven force orients the spindle along the mother-bud axis, and also moves the spindle close to the neck. (C) The 'late' pathway, which is mediated by dynein and its accessory proteins (many of which are omitted for simplicity), positions the spindle within the neck between the mother and daughter cell compartments. Dynein is offloaded from the plus ends of dynamic microtubules to Num1 receptor sites at the cell cortex. A conformational change within the dynein heavy chain ('unmasking'; see text), which may be triggered by dynactin (step 1), appears to regulate offloading to the cell cortex (step 2). Cortically anchored dynein–dynactin pulls along astral microtubules, resulting in the movement of the nucleus and the associated spindle into the neck. (D) Example time-lapse images of GFP-labeled microtubules undergoing dynein-mediated pulling forces in a Kar9-deficient cell. Red arrowhead indicates the astral microtubule plus end, which can be seen moving along the cell cortex toward the bud cell apex. Dashed green line indicates the starting point for the distal spindle pole (i.e., at $t = 0$ s). Double-headed arrow indicates approximate spindle displacement caused by dynein pulling forces. Note that the velocity of in vivo dynein-mediated spindle movements is ~41 nm/s (under load) [22], compared to 70–85nm/s for the velocity of single molecules of dynein walking in vitro (no load) [24,50].

productive contact with the bud cortex (Fig. 1C). In support of the unmasking mechanism, time-lapse microscopy showed that a constitutively 'unmasked', but motility-competent dynein mutant offloads directly to the bud cortex from astral microtubule plus ends [24]. Whether the proposed conformational change occurs in vivo, and how it is regulated require further study. As has been determined for the Kar9 pathway, these recent findings support the emerging notion that astral microtubules, rather than the cell cortex, carry with them the cues and machinery (e.g., dynein) for orienting the mitotic spindle along a predetermined cell axis.

How is spindle position communicated to cell cycle progression?

Following correct orientation of the spindle by the concerted efforts of the Kar9 and dynein pathways, anaphase ensues and the spindle rapidly disassembles prior to the onset of cytokinesis. In order for cells to prevent mitotic exit from occurring prior to the correct positioning of the spindle, a cell cycle checkpoint mechanism is in place that presumably monitors spindle orientation and communicates this information to the mitotic exit network (MEN). The spindle position checkpoint (SPC; also see review by Caydasi and Pereira in this issue [31]) acts by inhibiting the MEN, the latter of which is comprised of a signal transduction cascade that ultimately initiates spindle disassembly and cytokinesis. When the spindle is misaligned, the SPC works to delay mitotic exit by inhibiting the MEN. The primary switch of the MEN is a small Ras-like GTPase, Tem1, which appears to be activated by Lte1, a component originally thought to function as the guanine nucleotide exchange factor (GEF) that localizes specifically along the bud cell cortex [32,33]. However, the mechanism by which Lte1 activates Tem1 does not appear to be direct, since it has been shown to lack GEF activity toward Tem1 [34]. Prior to spindle elongation, Tem1 localizes to the dSPB. Upon entry of the dSPB into the daughter cell, Tem1 encounters Lte1, where the former is presumably triggered to activate Cdc15, a protein kinase that directly promotes mitotic exit. Thus, the SPC may assess spindle position by using the SPB as a sensor that responds to spatial cues situated within the mother and daughter cells: the MEN 'activating' zone is located in the bud as a result of Lte1 localization; conversely, a MEN 'inhibiting' zone in the mother is established by the presence of Kin4, a kinase that maintains the GTPase-activating protein complex Bfa1/Bub2 in an active state [35–37]. Interestingly, recent evidence suggests that Kin4 is negatively regulated by Lte1, which in turn initiates mitotic exit (also see [31]). Active Bfa1/Bub2 inhibits Tem1, and consequently precludes initiation of the MEN [32,38,39]. Therefore, the SPB containing Tem1 must *not only* enter the bud to signal mitotic exit, *but also* escape inhibition by Kin4 in the mother.

Kin4 is therefore a key player in regulating the SPC, in part by establishing a zone of MEN inhibition within the mother cell. Kin4 has the unusual property of localizing exclusively to the mother cell cortex: while many proteins localize to the daughter cell, very few exhibit selectivity for the mother. Although the means by which Kin4 exhibits this selectivity is unknown, association of Kin4 with the mother cell cortex is likely a prerequisite for its subsequent targeting to the mSPB [35], which is required for an intact SPC. Ectopically targeting Kin4 to both SPBs is sufficient to delay mitotic exit [37], indicating the importance of selective SPB-targeting for this kinase. Kin4 activity is directly regulated by phosphorylation: it is in a hypophosphorylated state during the stages of the cell cycle when its activity is needed to inhibit the MEN, and it is rapidly phosphorylated following mitotic exit [40,41]. Recent studies have found that the protein phosphatase 2A (PP2A) is important to maintain Kin4 in its hypophosphorylated state, and that dephosphorylation by PP2A is required for Kin4 to associate with the SPB [41]. PP2A is therefore a major factor in the selective localization of Kin4 to the mother cortex, and is thus a key player in SPC maintenance.

In addition to the SPB-mediated checkpoint mechanism, several lines of evidence indicate that interactions between astral MTs and the bud neck play an important role in the SPC. Situated at the bud neck is a network of septin filaments, GTPases that have been proposed to form a ring-shaped diffusion barrier between the mother and daughter cell compartments [42,43]. Mutations that disrupt septin ring formation at the neck result in a defective SPC, possibly due to the mislocalization of Lte1 to the mother cell [44]. A recent study noted that cells with a mispositioned anaphase spindle exhibit at least one astral MT penetrating the neck that persists for long periods of time. Laser ablation of these MTs, but not the SPB or spindle MTs, was often followed by spindle disassembly and cytokinesis [45]. This was only observed in cells in which the astral MT crossing the neck was cut, suggesting that an interaction between astral MTs and the bud neck is important for the maintenance of the SPC. Subsequent screening of bud-neck localized proteins revealed that Elm1, a protein kinase functioning in various pathways, is needed for SPC activity [46]. Elm1 was shown to be important for phosphorylation of Kin4 at threonine 209 [46,47], an event that is required for the kinase activity, but not the localization or expression of Kin4 [37]. The function of Elm1 in the SPC depends on its localization to the bud neck, since an Elm1 truncation that is unable to associate with the bud neck is deficient in the SPC. Fusing a heterologous bud neck-association domain to this Elm1 mutant, or overexpressing it, was sufficient to rescue the SPC deficiency, suggesting that an intact SPC requires a threshold of Elm1 activity at the neck.

Although it is well established that a transfer of information takes place between a misoriented spindle and cell cycle progression (i.e., SPC and MEN), it is not clear if a reciprocal exchange is taking place; namely, is spindle position information conveyed to dynein or Kar9 pathway components? When a spindle is misoriented, it would seem prudent for a cell to respond by triggering Kar9 or dynein pathway activity. In fact, given the nature of a misoriented spindle in budding yeast (i.e., contained entirely within the mother cell), it would seem most appropriate for dynein activity to be selectively upregulated in the bud. Asymmetric pulling forces generated in the bud-ward direction would result in the translocation of the spindle into the daughter cell. It is unclear if such a scenario exists, or how such uneven forces could be generated. Although dynein offloading appears to occur preferentially at the bud cortex (see above), preexisting dynein motors associated with the mother cortex may hinder bud-directed movement of the mitotic spindle. Regulators of dynein activity may be well suited to this task. For instance, selective association of dynein (i.e., at the bud cortex) with its activator dynactin, the latter of which is important for high force generation in vivo [29] and processivity in vitro [30], may prompt asymmetric pulling forces. Previous studies have shown that She1

can regulate the association between dynein and dynactin at the cell cortex [22]. Alternatively, other factors that communicate with the cell cycle progression machinery may serve to affect dynein pathway activity. It is interesting to note that deletion of Kin4 [40] or disruption of the Aurora B mitotic kinase Ipl1 [48] results in a modest but significant spindle misorientation phenotype. In fact, cells with misoriented anaphase spindles exhibit higher Kin4 activity compared to cells with correctly oriented anaphase spindles [40]. Thus, Kin4 or Ipl1 kinase activity may be upstream regulators that mediate crosstalk between spindle position (i.e., SPC) and dynein or Kar9-mediated spindle orientation activity.

Summary

Spindle orientation is a critical mitotic process that governs asymmetric cell divisions in a variety of model organisms. Studies from higher eukaryotes have suggested that proper spindle orientation is achieved through asymmetrically situated cortical cues, which in turn recruit dynein to appropriate sites of action. However, emerging evidence from yeast indicates that the astral microtubules, and not the cell cortex, play an important role in carrying the positional cues for spindle orientation, raising the question of whether a similar mechanism exists in higher organisms. Interestingly, an intact SPC also relies on feedback from astral microtubules, in addition to spindle pole bodies, both of which communicate spindle position information to the cell cycle progression machinery. An interesting question that should be the focus of future studies is whether any communication takes place from the SPC to the mechanisms that mediate spindle positioning (i.e., dynein and Kar9), which would, in theory, allow the cell to recruit or activate appropriate force generators in the event of a misoriented spindle, and thus achieve proper orientation prior to mitotic exit.

Acknowledgments

We thank G. Pereira for prepublication communications. This work was supported by a UMass Commonwealth College Undergraduate Research Assistantship to K. K. and an NIH grant (1R01GM076094) to W. Lee.

REFERENCES

[1] E.M. Torres, B.R. Williams, A. Amon, Aneuploidy: cells losing their balance, Genetics 179 (2008) 737–746.
[2] J.A. Knoblich, Mechanisms of asymmetric stem cell division, Cell 132 (2008) 583–597.
[3] X. Morin, Y. Bellaiche, Mitotic spindle orientation in asymmetric and symmetric cell divisions during animal development, Dev. Cell 21 (2011) 102–119.
[4] E.M. Gusnowski, M. Srayko, Visualization of dynein-dependent microtubule gliding at the cell cortex: implications for spindle positioning, J. Cell Biol. 194 (2011) 377–386.
[5] C.L. Theesfeld, J.E. Irazoqui, K. Bloom, D.J. Lew, The role of actin in spindle orientation changes during the *Saccharomyces cerevisiae* cell cycle, J. Cell Biol. 146 (1999) 1019–1032.
[6] R.A. Heil-Chapdelaine, N.K. Tran, J.A. Cooper, Dynein-dependent movements of the mitotic spindle in *Saccharomyces cerevisiae* do not require filamentous actin, Mol. Biol. Cell 11 (2000) 863–872.
[7] H. Yin, D. Pruyne, T.C. Huffaker, A. Bretscher, Myosin V orientates the mitotic spindle in yeast, Nature 406 (2000) 1013–1015.
[8] W.S. Korinek, M.J. Copeland, A. Chaudhuri, J. Chant, Molecular linkage underlying microtubule orientation toward cortical sites in yeast, Science 287 (2000) 2257–2259.
[9] L. Lee, J.S. Tirnauer, J. Li, S.C. Schuyler, J.Y. Liu, D. Pellman, Positioning of the mitotic spindle by a cortical-microtubule capture mechanism, Science 287 (2000) 2260–2262.
[10] H. Maekawa, E. Schiebel, Cdk1-Clb4 controls the interaction of astral microtubule plus ends with subdomains of the daughter cell cortex, Genes Dev. 18 (2004) 1709–1724.
[11] D. Liakopoulos, J. Kusch, S. Grava, J. Vogel, Y. Barral, Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment, Cell 112 (2003) 561–574.
[12] R.K. Miller, M.D. Rose, Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast, J. Cell Biol. 140 (1998) 377–390.
[13] N.R. Adames, J.A. Cooper, Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*, J. Cell Biol. 149 (2000) 863–874.
[14] M.L. Gupta Jr., P. Carvalho, D.M. Roof, D. Pellman, Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle, Nat. Cell Biol. 8 (2006) 913–923.
[15] P.S. Maddox, J.K. Stemple, L. Satterwhite, E.D. Salmon, K. Bloom, The minus end-directed motor Kar3 is required for coupling dynamic microtubule plus ends to the cortical shmoo tip in budding yeast, Curr. Biol. 13 (2003) 1423–1428.
[16] S.M. Huisman, O.A. Bales, M. Bertrand, M.F. Smeets, S.I. Reed, M. Segal, Differential contribution of Bud6p and Kar9p to microtubule capture and spindle orientation in *S. cerevisiae*, J. Cell Biol. 167 (2004) 231–244.
[17] M. Segal, K. Bloom, S.I. Reed, Bud6 directs sequential microtubule interactions with the bud tip and bud neck during spindle morphogenesis in *Saccharomyces cerevisiae*, Mol. Biol. Cell 11 (2000) 3689–3702.
[18] E.L. Grishchuk, M.I. Molodtsov, F.I. Ataullakhanov, J.R. McIntosh, Force production by disassembling microtubules, Nature 438 (2005) 384–388.
[19] S.M. Markus, J.J. Punch, W.L. Lee, Motor- and tail-dependent targeting of dynein to microtubule plus ends and the cell cortex, Curr. Biol. 19 (2009) 196–205.
[20] S. Grava, F. Schaerer, M. Faty, P. Philippsen, Y. Barral, Asymmetric recruitment of dynein to spindle poles and microtubules promotes proper spindle orientation in yeast, Dev. Cell 10 (2006) 425–439.
[21] P. Carvalho, M.L. Gupta Jr., M.A. Hoyt, D. Pellman, Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-170) regulates microtubule stability and dynein activation, Dev. Cell 6 (2004) 815–829.
[22] S.M. Markus, K.M. Plevock, B.J. St Germain, J.J. Punch, C.W. Meaden, W.L. Lee, Quantitative analysis of Pac1/LIS1-mediated dynein targeting: implications for regulation of dynein activity in budding yeast, Cytoskeleton (Hoboken) 68 (2011) 157–174.
[23] H. Lin, P. de Carvalho, D. Kho, C.Y. Tai, P. Pierre, G.R. Fink, D. Pellman, Polyploids require Bik1 for kinetochore–microtubule attachment, J. Cell Biol. 155 (2001) 1173–1184.
[24] S.M. Markus, W.L. Lee, Regulated offloading of cytoplasmic dynein from microtubule plus ends to the cortex, Dev. Cell 20 (2011) 639–651.
[25] M. Yamada, S. Toba, Y. Yoshida, K. Haratani, D. Mori, Y. Yano, Y. Mimori-Kiyosue, T. Nakamura, K. Itoh, S. Fushiki, M. Setou, A. Wynshaw-Boris, T. Torisawa, Y.Y. Toyoshima, S. Hirotsune, LIS1 and NDEL1 coordinate the plus-end-directed transport of cytoplasmic dynein, EMBO J. 27 (2008) 2471–2483.

[26] R.J. McKenney, M. Vershinin, A. Kunwar, R.B. Vallee, S.P. Gross, LIS1 and NudE induce a persistent dynein force-producing state, Cell 141 (2010) 304–314.
[27] X. Tang, J.J. Punch, W.L. Lee, A CAAX motif can compensate for the PH domain of Num1 for cortical dynein attachment, Cell Cycle 8 (2009) 3182–3190.
[28] R.A. Heil-Chapdelaine, J.R. Oberle, J.A. Cooper, The cortical protein Num1p is essential for dynein-dependent interactions of microtubules with the cortex, J. Cell Biol. 151 (2000) 1337–1344.
[29] J.K. Moore, D. Sept, J.A. Cooper, Neurodegeneration mutations in dynactin impair dynein-dependent nuclear migration, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5147–5152.
[30] J.R. Kardon, S.L. Reck-Peterson, R.D. Vale, Regulation of the processivity and intracellular localization of *Saccharomyces cerevisiae* dynein by dynactin, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 5669–5674.
[31] A.K. Caydasi, G. Pereira, SPOC alert — when chromosomes get the wrong direction, Exp. Cell Res. (current issue) (2012).
[32] A.J. Bardin, R. Visintin, A. Amon, A mechanism for coupling exit from mitosis to partitioning of the nucleus, Cell 102 (2000) 21–31.
[33] J.N. Molk, S.C. Schuyler, J.Y. Liu, J.G. Evans, E.D. Salmon, D. Pellman, K. Bloom, The differential roles of budding yeast Tem1p, Cdc15p, and Bub2p protein dynamics in mitotic exit, Mol. Biol. Cell 15 (2004) 1519–1532.
[34] M. Geymonat, A. Spanos, G. de Bettignies, S.G. Sedgwick, Lte1 contributes to Bfa1 localization rather than stimulating nucleotide exchange by Tem1, J. Cell Biol. 187 (2009) 497–511.
[35] L.Y. Chan, A. Amon, Spindle position is coordinated with cell-cycle progression through establishment of mitotic exit-activating and -inhibitory zones, Mol. Cell 39 (2010) 444–454.
[36] A.K. Caydasi, G. Pereira, Spindle alignment regulates the dynamic association of checkpoint proteins with yeast spindle pole bodies, Dev. Cell 16 (2009) 146–156.
[37] H. Maekawa, C. Priest, J. Lechner, G. Pereira, E. Schiebel, The yeast centrosome translates the positional information of the anaphase spindle into a cell cycle signal, J. Cell Biol. 179 (2007) 423–436.
[38] M. Geymonat, A. Spanos, S.J. Smith, E. Wheatley, K. Rittinger, L.H. Johnston, S.G. Sedgwick, Control of mitotic exit in budding yeast. In vitro regulation of Tem1 GTPase by Bub2 and Bfa1, J. Biol. Chem. 277 (2002) 28439–28445.
[39] G. Pereira, T. Hofken, J. Grindlay, C. Manson, E. Schiebel, The Bub2p spindle checkpoint links nuclear migration with mitotic exit, Mol. Cell 6 (2000) 1–10.
[40] K.E. D'Aquino, F. Monje-Casas, J. Paulson, V. Reiser, G.M. Charles, L. Lai, K.M. Shokat, A. Amon, The protein kinase Kin4 inhibits exit from mitosis in response to spindle position defects, Mol. Cell 19 (2005) 223–234.
[41] L.Y. Chan, A. Amon, The protein phosphatase 2A functions in the spindle position checkpoint by regulating the checkpoint kinase Kin4, Genes Dev. 23 (2009) 1639–1649.
[42] P.A. Takizawa, J.L. DeRisi, J.E. Wilhelm, R.D. Vale, Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier, Science 290 (2000) 341–344.
[43] Y. Barral, V. Mermall, M.S. Mooseker, M. Snyder, Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast, Mol. Cell 5 (2000) 841–851.
[44] G.A. Castillon, N.R. Adames, C.H. Rosello, H.S. Seidel, M.S. Longtine, J.A. Cooper, R.A. Heil-Chapdelaine, Septins have a dual role in controlling mitotic exit in budding yeast, Curr. Biol. 13 (2003) 654–658.
[45] J.K. Moore, V. Magidson, A. Khodjakov, J.A. Cooper, The spindle position checkpoint requires positional feedback from cytoplasmic microtubules, Curr. Biol. 19 (2009) 2026–2030.
[46] J.K. Moore, P. Chudalayandi, R.A. Heil-Chapdelaine, J.A. Cooper, The spindle position checkpoint is coordinated by the Elm1 kinase, J. Cell Biol. 191 (2010) 493–503.
[47] A.K. Caydasi, B. Kurtulmus, M.I. Orrico, A. Hofmann, B. Ibrahim, G. Pereira, Elm1 kinase activates the spindle position checkpoint kinase Kin4, J. Cell Biol. 190 (2010) 975–989.
[48] S. Buvelot, S.Y. Tatsutani, D. Vermaak, S. Biggins, The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly, J. Cell Biol. 160 (2003) 329–339.
[49] D.C. Bouck, A.P. Joglekar, K.S. Bloom, Design features of a mitotic spindle: balancing tension and compression at a single microtubule kinetochore interface in budding yeast, Annu. Rev. Genet. 42 (2008) 335–359.
[50] S.L. Reck-Peterson, A. Yildiz, A.P. Carter, A. Gennerich, N. Zhang, R.D. Vale, Single-molecule analysis of dynein processivity and stepping behavior, Cell 126 (2006) 335–348.