Current Biology, Vol. 13, R430–R432, May 27, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00362-2

Microtubule Cytoskeleton: Navigating the Intracellular Landscape

Kerry Bloom

Recent studies have significantly advanced our understanding of how a dividing cell asymmetrically positions the mitotic spindle — a key process in metazoan development — while maintaining a dynamic spindle state that can respond and reorient when necessary.

The intracellular cytoskeleton in embryos and single-cell eukaryotes is extremely dynamic and constantly remodeled during growth and differentiation. Microtubules and actin filaments comprise the filamentous cytoskeletal network. We are starting to understand how microtubules and actin filaments collaborate to 'read' intracellular cues. Microtubules are nucleated from microtubule organizing centers, called centrosomes or spindle pole bodies. Proteins bound to dynamic microtubule plus ends mediate interactions with polarized actin filaments or asymmetrically distributed cell polarity cues in the cell cortex [1,2]. Actin is required for polarized growth and directed membrane secretion. In budding yeast, the direction of bud growth dictates the axis of mitotic spindle alignment and microtubules, guided by actin cables, align the spindle apparatus.

In embryos of the nematode *Caenorhabditis elegans*, polarity is established through asymmetric segregation of several Par proteins, including Par-2 and Par-3, which specify the anterior–posterior axis of the embryo. After the first division, Par-2 and Par-3 are asymmetrically localized in one daughter cell (P1), while Par-3 is present throughout the cortex of the second daughter cell (AB). The spindle rotates 90° to align along the anterior–posterior axis in P1 cells with asymmetrically localized Par-2 and Par-3. As in the case of spindle orientation in budding yeast, actin is involved in spindle positioning by directing Par-2 and Par-3 localization [3]. Understanding the mechanism of asymmetric protein accumulation that facilitates mitotic spindle orientation will provide new insights into basic processes that underlie development.

In budding yeast, Kar9p links microtubules to the actin cytoskeleton. Kar9p binds microtubules via the yeast EB1 homolog, Bim1p [4–6]. EB1/Bim1p is highly conserved and functions at microtubule plus ends [4]. Mammalian EB1 interacts with the adenomatous polyposis coli protein (APC) to guide microtubule plus ends to specific cortical sites. Kar9p interacts with actin through the type V myosin Myo2p, thereby linking the microtubules to polarized actin [1,2]. The old and newly duplicated spindle poles are distinct in budding yeast: the old pole is oriented toward the bud [7], and microtubules emanating from this pole lead the mitotic spindle into the bud [8]. One of the outstanding problems in the field has been to understand the genetic control of spindle pole 'fate' and how Kar9p directs the old pole toward the bud.

Two recent papers [9,10] report new insights into the mechanistic basis of spindle pole differentiation. Protein binding and release from the spindle pole is a highly regulated event. The cell-division kinase Cdc28p and cyclin are central to spindle pole differentiation. Cdc28p–Clb5p is required for proper spindle assembly and orientation in yeast [11]. Maekawa *et al.* [10] screened for proteins that interact with Cdc28p and Clb5p, and found Kar9p, among others. Kar9p has fifteen consensus sites for phosphorylation by Cdc28p and is phosphorylated in a cell-cycle-dependent fashion. Kar9p binds spindle poles, microtubule plus ends and the neck of budded cells in G1/S phase of the cell cycle. Interestingly, Kar9p is lost from the tip of microtubule plus ends in cells with reduced Cdc28p–Clb5p activity, and spreads along the entire cytoplasmic microtubule.

Cdc28p–Clb5p thus confers tight spatial control of Kar9p. Microtubules grow prematurely to the tip of the bud in *cdc28 clb5* mutants, resulting in net migration of the entire spindle into the bud [10,11]. How, then, does Clb5p promote microtubule tip binding by Kar9p? Phosphorylated Kar9p is transported by the microtubule-based motor protein Kip2p from spindle poles to microtubule plus-ends [10]. But the question remained as to how Kar9p binds to the old pole but not to the new pole which is destined to remain in the mother.

Liakopoulos *et al.* [9] found that Kar9p phosphorylation is significantly reduced in *clb4* mutants and, importantly, Kar9p localized symmetrically to both spindle poles in these cells. The regulation of Kar9p by phosphorylation is thus required for its spatial distribution to one and only one spindle pole. Furthermore, the phosphorylated form of Kar9p has reduced affinity for Bim1p. Clb4p was found to bind to the mother pole, thereby restricting 'active' Kar9p to the old daughter bound pole. Kar9p loading to the daughter pole promotes microtubule penetration into the bud by binding to Myo2p and, subsequently, to actin cables.

The spindle pole is thus the regulatory center for spindle positioning, and Kar9p delivery to specific microtubule plus ends from one pole guides these structures to their destination (Figure 1). These data reflect a spatial feature inherent in differential regulatory cascades. Cdc28p–Clb4p prevents Kar9p from binding to the new pole in the mother cell, while a different Cdk–cyclin, perhaps Cdc28p–Clb5p, promotes transport of Kar9p from the old pole to cytoplasmic microtubule plus ends destined for the bud.

In addition to this programmed genetic control of its positioning, the spindle is a dynamic structure that can reposition or realign within cells experiencing a variety of mutational or external perturbations [12]. Even in the

Department of Biology, 623 Fordham Hall CB#3280, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3280, USA.

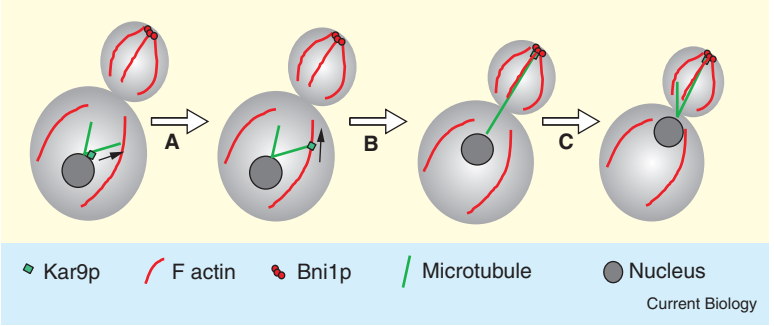

Figure 1. Bim1p–Kar9p orient microtubule plus ends via the actin cytoskeleton.

Kar9 binds to the daughter spindle pole and is transported to microtubule plus-ends (A). At the plus-ends, Kar9p via Myo2p and actin directs microtubules to the bud (B). Once in the bud, microtubule shortening from the plus end and/or sliding powers nuclear migration (C).

highly orchestrated yeast cell cycle, old and new poles [13] as well as Kar9p can 'switch' from their predetermined fate if spindle positioning cues are abrogated [10]. What is the nature of extrinsic mechanisms that preside over these situations? An elegant new study by Tsou *et al.* [14] reveals that cell shape provides important cues for spindle positioning. These authors geometrically perturbed cell shape in *C. elegans* embryos by ablating the AB cell after the first division, causing the adjacent P1 cell to become more spherical. In otherwise wild-type cells, nuclear rotation and spindle positioning are not perturbed. Mechanisms of spindle alignment thus resist changes in cell shape. In *par-3* mutants, however, nuclear rotations are severely diminished in these 'pseudo-spherical' cells: Par-3 thus abrogates geometric constraints that might interfere with the genetic program governing nuclear movement.

How does geometry regulate spindle positioning? Tsou *et al.* [14] hypothesize that the angle of interaction of microtubules with the cortex regulates force. Direct microtubule interactions (90°) with the cortex provide less force, while the more oblique the microtubule–cortical interaction, the greater the force. In a cell-free system, it is clear that 'walls' influence microtubule dynamics [15]. In these systems, more force is exerted when microtubules hit the wall perpendicularly, whereas less force is exerted in the oblique interactions [15]. But *in vivo*, cellular boundaries — the cortex — are populated by microtubule-based motors [16]. In particular, dynein and dynactin accumulate at microtubule plus ends in yeast, where they function to slide microtubules along the cortex, resulting in nuclear migration toward the bud [17,18] (Figure 2). The dynamic properties of microtubule interactions at the cortex are influenced by the geometry of the interaction at the boundary as well as motors, microtubule-associated proteins (MAPs) and proteins such as Kar9p. The next step will be to measure the force generated at these critical junctures.

Labbe *et al.* [19] have developed a powerful imaging strategy to address microtubule stability at boundaries. The technique, coined cortical imaging of microtubule stability (CIMS), focuses at the cortex of embryos containing GFP-labeled microtubules. In this way, a microtubule end appears as a dot at the cell surface. The residence time of the fluorescent dot at the surface is a measure of the stability of the microtubule–cortex interaction. While the technique does not directly measure the force at individual ends, it reveals a 15% difference in microtubule stability between the anterior and posterior cortices, which is dependent upon Par-3. The microtubule ends are more dynamic in the posterior pole, where pulling forces promote spindle displacement [20].

One possibility is that more dynamic microtubules reflect increased opportunity for interactions with microtubule-based motor proteins responsible for generating force. Alternatively, according to the geometry-based hypothesis, end on interactions may generate less force, and decreased stability may represent a mechanism to promote catastrophes of 'less productive' interactions. Imaging of the microtubule-based motor proteins together with the microtubule plus ends will resolve this issue.

References

1. Yin, H., Pruyne, D., Huffaker, T.C., and Bretscher A (2000). Myosin V orientates the mitotic spindle in yeast. Nature *406*, 1013-1015.
2. Beach. D.L., Thibodeaux, J., Maddox, P., Yeh, E., and Bloom, K. (2000). The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. Curr. Biol. *10*, 1497-1506.

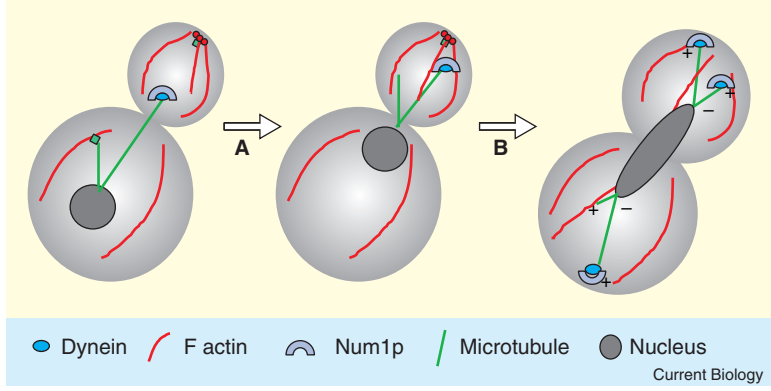

Figure 2. Cytoplasmic dynein binds microtubule plus-ends and promotes nuclear migration through the bud.

Dynein is bound by cortically anchored Num1p. Dynein promotes microtubule sliding along the cortex (A), or microtubule plus end shortening (B).

3. Severson, A.F., Bowerman, B. (2003). Myosin and the PAR proteins polarize microfilament-dependent forces that shape and position mitotic spindles in *C. elegans*. J. Cell Biol. *161*, 21-26.
4. Miller, R.K., Cheng, S.C., and Rose, M.D. (2000). Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. Mol. Biol. Cell *11*, 2949-2959.
5. Lee, L., Tirnauer, J.S., Li, J., Schuyler, S.C., Liu, J.Y., and Pellman, D. (2000). Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. Science *287*, 2260-2262.
6. Korinek, W.S., Copeland, M.J., Chaudhuri, A., and Chant, J. (2000). Molecular linkage underlying microtubule orientation toward cortical sites in yeast. Science *287*, 2257-2259.
7. Pereira, G., Tanaka, T.U., Nasmyth, K., and Schiebel, E. (2001). Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. EMBO J. *20*, 6359-6370.
8. Yeh, E., Skibbens, R.V., Cheng, J.W., Salmon, E.D., and Bloom, K. (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. J. Cell Biol. *130*, 687-700.
9. Liakopoulos, D., Kusch, J., Grava, S., Vogel, J., and Barral, Y. (2003). Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. Cell *112*, 561-574.
10. Maekawa, H., Usui, T., Knop, M., Schiebel, E. (2003). Yeast Cdk1 translocates to the plus end of cytoplasmic microtubules to regulate bud cortex interactions. EMBO J. *22*, 438-449.
11. Segal, M., Clarke, D.J., Maddox, P., Salmon, E.D., Bloom, K., Reed, S.I. (2000). Coordinated spindle assembly and orientation requires Clb5p-dependent kinase in budding yeast. J. Cell Biol. *148*, 441-452.
12. Schultz, N., and Onfelt, A. (2001). Spindle positioning in fibroblasts supports an astral microtubule length dependent force generation at the basal membrane. Cell Motil. Cytoskel. *50*, 69-88.
13. Yeh, E., Yang, C., Chin, E., Maddox, P., Salmon, E.D., Lew, D.J., and Bloom, K. (2000). Dynamic positioning of mitotic spindles in yeast: role of microtubule motors and cortical determinants. Mol. Biol. Cell *11*, 3949-3961.
14. Tsou, M.F., Ku, W., Hayashi, A., and Rose, L.S. (2003). PAR-dependent and geometry-dependent mechanisms of spindle positioning. J. Cell Biol. *160*, 845-855.
15. Dogterom, M., and Yurke, B. (1997). Measurement of the force-velocity relation for growing microtubules. Science *278*, 856-860.
16. Adames, N.R., and Cooper, J.A. (2000). Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*. J. Cell Biol. *149*, 863-874.
17. Lee, W.L., Oberle, J.R., and Cooper, J.A. (2003). The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. J. Cell Biol. *160*, 355-364.
18. Sheeman, B., Carvalho, P., Sagot, I., Geiser, J., Kho, D., Hoyt, M.A., and Pellman, D. (2003). Determinants of S. cerevisiae dynein localization and activation. Implications for the mechanism of spindle positioning. Curr. Biol. *13*, 364-372.
19. Labbe, J.-C., Maddox, P., Salmon, E.D., and Goldstein, B. (2003). PAR proteins regulate microtubule dynamics at the cell cortex in *C. elegans*. Curr. Biol. *13*, 29th April issue.
20. Grill, S.W., Gonczy, P., Stelzer, E.H., and Hyman, A.A. (2001). Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. Nature *409*, 630-633.