It's a kar9ochore to capture microtubules

Kerry Bloom

Microtubule orientation to cortical spatial cues is essential for the fidelity of asymmetric cellular processes. A cortical microtubule-capture site, composed of Bim1p and Kar9p, has now been identified in yeast. Bim1p is the yeast homologue of EB1, a binding partner of the adenomatous polyposis coli (APC), indicating that important features of this complex may be highly conserved.

The mitotic spindle is a highly efficient mechanism for segregating duplicated chromosomes. The mechanical linkage between chromosomes and the spindle is provided by the kinetochore, a highly specialized microtubule-capture site. After chromosome segregation and spindle disassembly, the bipolar microtubule array undergoes a conformational change, forming a highly elaborate structure that lacks the simple elegance of the spindle, but which is nonetheless essential for cell function {**AU: this sentence ok?**}. This dynamic microtubule structure recognizes positional information and sites of polarized growth and is orientated towards specific cortical sites, allowing it to direct the specific localization of bodies such as secretory vesicles and cellular organelles {**AU: OK?**}. In budding yeast, nuclear movement and the orientation of the mitotic spindle must be tightly regulated to ensure proper cell division, requiring a cytoplasmic microtubule-capture mechanism that dynamically couples microtubules to the cell cortex. Recent studies of nuclear movement and spindle orientation have demonstrated a direct interaction between Kar9p, a protein that is localized to the daughter-cell cortex, and the microtubule-binding protein Bim1, shedding new light on the molecular mechanism of cortical microtubule capture[1,2] {**AU: is it ok to remove 'p' suffixes from protein names, as it is not** *Nature* **style to mark yeast proteins as such**}.

The interphase microtubule array in yeast is one of the simplest in nature (Fig. 1a). Three to four astral microtubules emanate from a single spindle-pole body (the yeast equivalent of a centrosome). The spindle-pole body is embedded in the nuclear envelope, which, unlike in most cells, does not break down during the cell cycle. The site of cell division, also known as the incipient bud site {**AU: OK?**}, is chosen before the previous cycle is completed, by an elaborate selection strategy that predisposes haploid cells to clustering (axial division, favouring mating between neighbouring cells) and diploid cells to spreading (bipolar division, the closest approximation to foraging for a sessile being). In contrast to mammalian cells, in which the spindle position determines the cleavage plane, the budding yeast nucleus must migrate through the division plane that corresponds to the neck of a budded cell in order for genetic material to be deposited into both mother and bud.

Whereas nuclei have been observed to make precise, directed turns towards specific cortical sites in a number of systems, the yeast nucleus is simply moved around the cell interior, in the opposite to that of microtubule growth {**AU: OK?**}. These nuclear rotations through the cell are driven by microtubule growth and/or sliding against the cortex[3,4] (Fig. 1b). Thus, this random movement exploits the dynamic instability of microtubules to probe the cell cortex for the growing bud, thereby contributing to the fidelity of nuclear migration. When a microtubule encounters the incipient bud site or the bud tip, its plus end is 'captured' {**AU: OK?**} and a transition to pulling ensues, ensuring that the nucleus migrates towards the bud neck (Fig. 1c).

But how are the plus ends of astral microtubules captured by the daughter-cell cortex? As reported recently by Lee *et al.*[1] and Korinek *et al.*[2], the key molecules involved in this process are Bim1p and Kar9p. Bim1p, a member of the EB1 family of conserved proteins, binds to microtubules *in vivo*[5] and apparently concentrates at their plus ends[6]. EB1 interacts with the APC tumour suppressor, which in turn has been shown to bind to microtubules and accumulate at regions of the cell periphery that are active in cell migration (reviewed in ref. 7). Bim1p is also found along the mitotic spindle, where it contributes to the fidelity of chromosome segregation — mutations in Mal3, the BIM1 homologue in *Schizosaccharomyces pombe*, have been shown to cause improper segregation of chromosomes[8] {**AU: OK?**}. Bim1p and its homologues are therefore key intermediates for microtubule interactions at chromosomes and sites of cell growth, migration and polarity establishment. Kar9p was first identified in a screen for karyogamy mutants and has a role in nuclear migration[9]. Kar9p tagged with green fluorescent protein (Kar9p–GFP) localizes to regions of cortical cell growth, as well as along cytoplasmic microtubules[1,2,10]. Lee *et al.* and Korinek *et al.* have now shown that cytoplasmic Bim1p recruits Kar9p to microtubules, thereby establishing the microtubule–cortex connection[1,2]. Data from two-hybrid analyses and co-immunoprecipitation studies indicate that Kar9p and Bim1p physically interact, and that Bim1p may be sufficient to recruit Kar9p to microtubules, as suggested by *in vitro* microtubule assembly[1]. The relative molecular mass of the Kar9p–Bim1p complex was estimated at 250,000 (M_r250K; ref. 1), indicating either that the complex contains several Kar9p or Bim1p subunits or that it possesses further components. Kar9p interacts with itself, as well as with Stu2p, another microtubule binding protein (R. K. Miller, personal communication). Clearly, the ability of Bim1p to recruit accessory proteins to the microtubule cytoskeleton has opened new avenues in understanding the function of the EB1 family {**AU: OK?**}.

Is Kar9p–Bim1p the cortical kinetochore? Evidence is mounting that nuclear migration towards the cortical 'kar9ochore' results from shortening of astral microtubules{**AU: OK?**}. Microtubules shorten by losing subunits either at their spindle poles (minus end) or at cortical sites (plus end). Imaging of fluorescent speckles in the lattice of dynamic astral microtubules indicates that, in yeast, they may lose or gain tubulin subunits from their plus ends[11]. The assembly and disassembly of microtubule plus ends is coupled to pushing and pulling of the nucleus at the tips of mating cells[12]. It is therefore conceivable that Kar9p–Bim1p complexes anchored in the corteces of vegetative cells may promote plus-end microtubule disassembly while maintaining contact with the shortening end, thereby effecting nuclear movement.

An intriguing question is how the Kar9p– Bim1p complex is actually attached to the cell cortex. As Bim1p is a microtubule-binding protein, it is unlikely to be directly anchored to the cortex. Kar9p is not a transmembrane protein and does not interact directly with cortical actin. However, localization of Kar9p to the tips of budded cells is dependent on several actin-interacting proteins (Bud6p and Bni1p) and an intact actin cytoskeleton[10]. In the absence of Bud6p or Bni1p, Kar9p is released from the bud tip, resulting in its redistribution to astral microtubules. Bni1p is a member of the FH protein family (the members of which contain proline-rich homology domains) and has an important function in cytokinesis and establishment of cell polarity[13]. The actin-binding protein

Bud6p/Aip3p is required for accurate bud-site selection as well as polarity establishment[14], making it difficult to assign a specific role to either Bud6p or Bni1p in nuclear migration. Actin itself is known to have an important role in nuclear migration, but as nuclear migration becomes actin-independent in later stages of the cell cycle, it has been difficult to asses its precise function[15].

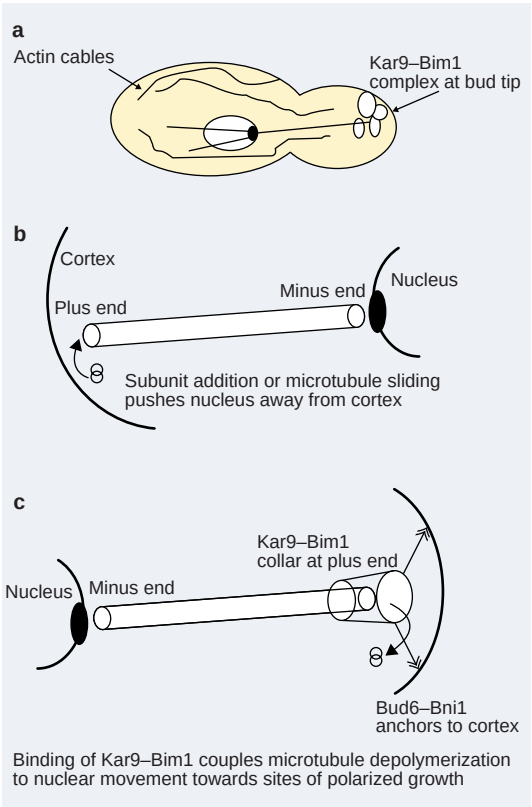
Bud6 and Bni1, however, have alternative functions that may help to elucidate their roles in the cortical anchoring of Kar9p. BNI1 has recently been identified as a She mutant (she5) that is defective in the transport of ASH1 mRNA[16]. Ash1 mRNA is deposited to the tip of budded cells where translation of the Ash1p transcriptional repressor silences HO expression in the bud. Ash1 mRNA is transported along the actin cytoskeleton by type-V myosin (Myo4p). She5/Bni1 and AIP3/BUD6 (which has a slight She defect) are not defective in mRNA transport, but in anchoring of Ash1 mRNA to bud tips[17] {**AU: please indicate where genes are referred to and where proteins in the above passage. OK to mark genes in italics?**}. Thus, Bud6p and Bni1p comprise a cortical scaffold for mRNA localization at sites of polarized cell growth. Bud6p is transported by the type-V myosin motor Myo2p for delivery to the bud tip[18], whereas the Bni1p -related protein Cdc12p in *S. pombe* is transported by microtubules and actin filaments[19]. The actin cytoskeleton is therefore essential for delivering Bud6p and Bni1p to sites of polarized growth where a cortical scaffold is constructed for binding of Ash1 mRNA as well as the kar9ochore. Once the cortical scaffold is established, the actin cytoskeleton is no longer required for nuclear migration and is redistributed to the neck for subsequent cell cycles.

Despite the intriguing link between Bim1p and Kar9p, many questions remain. How, for example, are microtubules guided towards the bud site? Kar9p has also been shown to facilitate microtubule penetration into the bud[9], indicating that the kar9ochore may be assembled in the mother cell and may function before the bud is formed. This model is particularly attractive when considering the process of microtubule search and capture. Rather than randomly encountering the incipient bud site or the bud, microtubules may be guided to sites of polarized growth through linkages with the actin cytoskeleton. Several proteins besides Bud6p and Bni1p may contribute to early encounters between microtubules and the actin cytoskeleton. Coronin, an actin-associated protein, binds directly to both actin filaments and microtubules *in vitro*, through separate domains[20], and crn mutants display microtubule defects. Mutations in the CLIP-170 homologue Bik1 cause defects in nuclear migration, as well as impairment of its function in nuclear fusion during mating. Early encounters with actin-microtubule 'linkers' would facilitate the search-and-capture mechanism and contribute to the fidelity of nuclear migration.

The force that pulls the nucleus towards the bud must be generated either by microtubule-based motors or by depolymerization of microtubules. It is not clear, however, how the kar9ochore couples force generation to microtubule capture. The kinesin Kip3p, which has been implicated in the same genetic 'pathway' as Kar9p, may itself be part of the kar9ochore. However, Kip3p is not known to interact with Kar9p, Stu2p or Bim1p. Bim1p directly influences the dynamic properties of microtubules (dynamicity[4,6]), and its ability to bind to microtubule plus ends may be sufficient to couple nuclear movement to microtubule shortening. Further studies of the modes of action of Kip3p and/or Bim1p will distinguish these mechanisms.

A deeper knowledge of nuclear movement in yeast is likely to increase our understanding of organelle stability and inheritance {**AU: OK?**}. Our attention to errors in genetic and genomic stability has had an enormous impact on understanding the aetiology of the cancerous state. The discovery of the Bim1p microtubule-binding protein in the nucleus and cytoplasm highlights the issue of organelle segregation. The problem of nuclear migration in yeast is similar to those encountered by asymmetrically dividing cells in orienting their mitotic spindles. *Drosophila* APC2 (which may interact with EB1/Bim1p) accumulates at both poles of the spindle in asymmetrically dividing neural stem cells[7]. Errors in spindle orientation and/or nuclear migration do not lead to cell death in yeast, as cytokinesis is delayed until accurate segregation is attained[21]. Loss of Bim1p abrogates this checkpoint[22] and leads to the production of anucleate daughters. Thus, nuclear migration, cytoarchitecture and, more broadly, organelle distribution are subject to control by cellular checkpoints. The identification of Bim1p at the plus ends of microtubules in yeast has not only revealed mechanisms of spindle positioning but has also provided insights into the links between cellular polarity and accurate distribution of organelles such as the nucleus. Clearly, the loss of polarity determinants leads to errors in cellular architecture, and it will be important to examine these in propagating cells.□

Kerry Bloom is in the Department of Biology, 623 Fordham Hall, University of North Carolina, Chapel Hill, North Carolina 27599, USA.
e-gmail: kerry_bloom@unc.edu



Binding of Kar9–Bim1 couples microtubule depolymerization to nuclear movement towards sites of polarized growth

Figure 1 **Microtubule dynamics and nuclear movement in yeast. a, Three to four astral microtubules are nucleated by the spindle-pole body (grey oval) embedded in the nuclear envelope. Actin cables extend from the mother to the bud. Kar9p–Bim1p localizes to the tip of the budded cell. b, Mechanism of nuclear movement in G1-phase cells. Sliding of microtubules along the cortex and addition of tubulin subunits provide the motive force for nuclear migration. c, Mechanism of nuclear movement towards sites of polarized growth. Kar9p interacts with Bim1p (and Stu2p; see text), forming a microtubule-capture site at the cell cortex. Nuclear movement towards the bud is accompanied by microtubule-plus-end depolymerization. The capture complex is thought to dynamically couple microtubule shortening with directed nuclear movement.**

1. Lee, L. *et al. Science* **287,** 2260–2262 (2000).
2. Korinek, W. S., Copeland, M. J., Chaudhuri, A. & Chant, J. *Science* **287,** 2257–2259 (2000).
3. Shaw, S. L., Yeh, E., Maddox, P., Salmon, E. D. & Bloom, K. *J. Cell Biol.* **139,** 985–994 (1997).
4. Adames, N. R. & Cooper, J. A. *J. Cell Biol.* **149,** 1–13 (2000).

5. Schwartz, K., Richards, K. & Botstein, D. *Mol. Biol. Cell* **8,** 2677–2691 (1997).
6. Tirnauer, J. S., O"Toole, E. O., Berrueta, L., Bierer, B. E. & Pellman, D. *J. Cell Biol.* **145,** 993–1007 (1999).
7. McCartney, B. M. & Peifer, M. *Nature Cell Biol.* **2,** E58–E60 (2000).
8. Beinhauer, J. D., Hagan, I. M., Hegemann, J. & Fleig, U. *J. Cell Biol.* **139,** 717–728 (1997).
9. Miller, R. K. & Rose, M. D. *J. Cell Biol.* **140,** 377–390 (1998).
10. Miller, R., Matheos, D. & Rose, M. *J. Cell Biol.* **144,** 963–975 (1999).
11. Maddox, P., Bloom, K. & Salmon, E. D. *Nature Cell Biol.* **2,** 36–41 (2000).
12. Maddox, P. *et al. J. Cell Biol.* **144,** 977–987 (1999).
13. Evangelista, M. *et al. Science* **276,** 118–122 (1997).
14. Amberg, D. C., Zahner, J. E., Mulholland, J. W., Pringle, J. R. & Botstein, D. *Mol. Biol. Cell* **8,** 729–753 (1997).
15. Theesfeld, C. L., Irazoqui, J. E., Bloom , K. & Lew, D. J. *J. Cell Biol.* **146,** 1019–1032 (1999).
16. Bobola, N., Jansen, R-P., Shin, T. H. & Nasmyth, K. *Cell* **84,** 699–709 (1996).
17. Beach, D. L., Salmon, E. D. & Bloom, K. *Curr. Biol.* **9,** 569–578 (1999).
18. Jin, H. & Amberg, D. C. *Mol. Biol. Cell* **11,** 647–661 (2000).
19. Chang, F. *Curr. Biol.* **9,** 849–852 (1999).
20. Goode, B. L. *et al. J. Cell Biol.* **144,** 83–98 (1999).
21. Yeh, E., Skibbens, R. V., Cheng, J. W., Salmon, E. D. & Bloom, K. *J. Cell Biol.* **130,** 687–700 (1995).
22. Muhua, L., Adames, N. R., Murphy, M. D., Shields, C. R. & Cooper, J. A. *Nature* **393,** 487–491 (1998).