

Formin' the Connection between Microtubules and the Cell Cortex

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IN higher eukaryotes, the position of cytokinesis is determined by the position of the mitotic spindle (Rapaport, 1990). Generally, the spindle sits in the middle of the cell and cytokinesis produces two equivalent cells. However, many developmental processes require specific positioning of the cleavage plane and, hence, the mitotic spindle. For example, control of spindle position can be used to asymmetrically distribute cell fate determinants between the two daughter cells, to form polar bodies during oogenesis, and for tissue morphogenesis (Stearns, 1997). Positioning of the spindle appears to be mediated through the attachment of astral microtubules to filamentous actin at the cell cortex (Lutz et al., 1988; Hyman, 1989; Waddle et al., 1994).

In budding yeast, the site of cell division is specified at the start of the cell cycle by the location of the bud site. The mitotic spindle must then be positioned in the neck between mother and bud to achieve segregation of chromosomes between the mother and daughter cells. Positioning the mitotic spindle in yeast involves three processes (Fig. 1, Table I). Before mitotic spindle formation, a kinesin, Kip3p, is required for movement of the nucleus to the mother-bud neck (DeZwaan et al., 1997). After spindle formation, Kip3p is needed to orient the pre-anaphase spindle along the mother bud axis. Dynein functions later in mitosis to move the anaphase spindle into the neck (Stearns, 1997). How Kip3p and dynein function in these movements is not known. They may pull on microtubules in the traditional sense of a motor, or they may regulate microtubule length via effects on dynamic instability at microtubule ends. Astral microtubules are highly dynamic and occasionally span the distance from the spindle pole body to the cell cortex (Shaw et al., 1997). Therefore, spindle movements are hypothesized to depend on transient, short-lived interactions between astral microtubules and the cell cortex. Two papers in this issue of the Journal define components involved in microtubule-cortex interactions during the early spindle movements that require Kip3p (Lee et al., 1999; Miller et al., 1999).

Lee and colleagues and Miller and colleagues found that Bni1p, a formin, and Bud6p participate in movement of the nucleus to the bud neck and in orienting the pre-

anaphase spindle (Lee et al., 1999; Miller et al., 1999). Formins control actin-dependent processes in many systems (Frazier and Field, 1997). The new results showing that Bni1p and actin are necessary for spindle positioning suggest that microtubules interact with actin at the cell cortex. The localization of Kar9p, another protein involved in Kip3p-dependent movements, depends on actin, Bni1p and Bud6p (Miller et al., 1999).

Bni1p, a Formin, Functions in Kip3p-dependent Spindle Positioning and Movement

Both groups found that *bni1* mutants had defects in spindle positioning, using different approaches. The nucleus did not migrate to the neck efficiently, and the spindle was not aligned along the mother-bud axis, based in part on movies of live cells (Lee et al., 1999). The spindle then moved into the neck (Lee et al., 1999; Miller et al., 1999).

These phenotypes are similar to ones observed previously in *kip3* and *kar9* mutants (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Miller and Rose, 1998; Miller et al., 1998). Previous genetic analyses suggested that Kip3p and Kar9p act together to position the spindle before the action of dynein (Miller et al., 1998). Genetic analyses in the new reports (Lee et al., 1999; Miller et al., 1999) indicate that Bni1p functions in the same process as Kip3p and Kar9p (Fig. 1). Bud6p, a protein that physically interacts with Bni1p, has a similar but less important role, based on milder phenotypes (Lee et al., 1999; Miller et al., 1999) and a weaker genetic interaction with dynein (Miller et al., 1999).

Interestingly, *bni1* mutants do display movements of the pre-anaphase spindle, including exaggerated transits back-and-forth through the neck. Therefore, alternative mechanisms for movement may exist, and Bni1p may act as a governor to focus or restrict the action of these other mechanisms.

Bni1p Participates in Kar9p Localization

Bni1p forms a cap at the incipient bud site and remains at the bud tip, suggesting that Bni1p interacts with microtubules to pull the spindle toward the bud. Kar9p is present as a spot at the bud tip, presumably overlapping the cap of Bni1p. In *kar9* null mutants, astral microtubules do not orient into the bud, and, consequently, Kip3p-dependent spindle movements are impaired (Miller et al., 1998; Fig.

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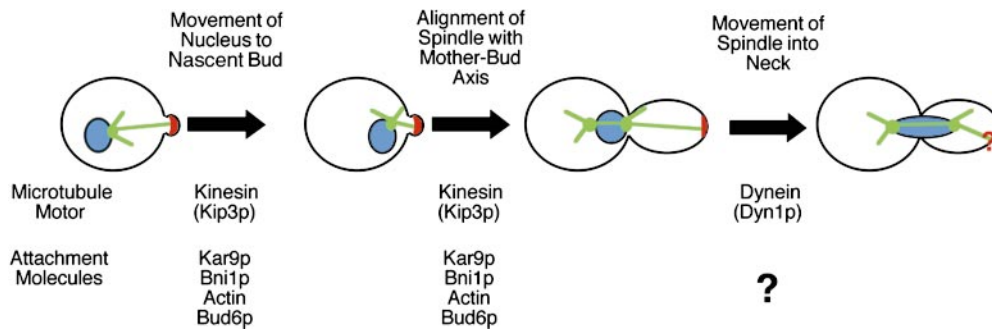


Figure 1. Steps in mitotic spindle position and movement. First, the nucleus (blue) moves to nascent bud, which requires Kip3p and presumably the attachment of astral microtubules (green) to a site in the cortex of the bud (red). The attachment is transient, not permanent. Kar9p, Bni1p, filamentous actin, and Bud6p function in the movement, presumably

because they are necessary for the attachment. These proteins also function in the second step, aligning the pre-anaphase spindle along the mother-bud axis. Third, concurrent with anaphase initiation, the spindle is pulled into the neck. This movement requires dynein and a presumed cortical attachment site that in some respects appears different from the one used in steps 1 and 2.

1). In the current work, Kar9p was mislocalized in cells lacking Bni1p, Bud6p, or filamentous actin (Miller et al., 1999). Mislocalization of Kar9p correlated with defects in astral microtubule orientation and spindle positioning.

These results suggest that Bni1p and Bud6p localize Kar9p to the cortex, and that the mislocalization of Kar9p in *bni1* and *bud6* mutants is responsible for the nuclear positioning and pre-anaphase spindle orientation defects in these mutants. However, microtubules were appropriately oriented in the *bni1* mutant, and the spindle positioning defects in *kar9* mutants were more severe than the defects in *bni1* mutants. Therefore, some Kar9p function appears to be retained despite its mislocalization in *bni1* mutants.

A Role for Filamentous Actin

Studies with a conditional actin mutant have implicated actin in pre-anaphase spindle orientation (Palmer et al., 1992). To examine the role of filamentous actin more directly,

both groups used the actin-depolymerizing drug latrunculin A. Spindle orientation was lost with latrunculin treatment, as seen in *kip3* mutants (Lee et al., 1999). Kar9p localization also was lost in latrunculin (Miller et al., 1999). These results confirm that actin is necessary for Kip3p-dependent spindle movements.

What element of the actin cytoskeleton provides this function? Cortical actin patches have been widely assumed to be the attachment site for microtubules because the patches cluster at the bud tip. However, clustering of actin patches may not be necessary for pre-anaphase spindle orientation. An actin cytoskeleton mutant with largely delocalized patches, *sla1ΔSH3#3*, showed normal spindle orientation and positioning (Lee et al., 1999). Also, Kar9p localized normally in a *sla1* null mutant (Miller et al., 1999). In a similar analysis, the bipolar pattern of bud site selection in diploid yeast depended on actin but not patches (Yang et al., 1997). Furthermore, several proteins are involved in both bipolar bud site selection and spindle

Table I. Summary of Results on the Function of Proteins Involved in Connections between Microtubules and the Cell Cortex

Protein	Location during mitosis	Synthetic lethal with dynein	Loss of function causes a defect in these processes					
			Astral microtubule orientation into the bud	Kar9p localization	Movement of pre-anaphase spindle to neck	Pre-anaphase spindle orientation along mother-bud axis	Movement of anaphase spindle into neck	Microtubule orientation and nuclear movement in shmoos
Bni1p	Cap at bud tip ¹	Yes ^{2,3}	No* ²	Yes ²	Yes ³	Yes ³	No ³	Yes ²
Bud6p	Cap at bud tip ⁴	Mild ²	Not tested	Yes ²	Not tested	Yes ³	Not tested	Yes ²
Actin	Cortical patches, cytoplasmic cables	Not tested	Not tested	Yes ²	Not tested	Yes ³	No ³	Not tested
Kar9p	Spot at bud tip ⁶	Yes ⁶	Yes ⁶	NA	Yes ²	Not tested	Yes ^{2,6}	Yes ⁶
Kip3p	Microtubules, astral and spindle ^{7,8}	Yes ⁷⁻⁹	Yes ⁸	No ²	Yes ⁷	Yes ⁷	No ⁷	Not tested ⁸
Dynein	Uncertain	NA	No ⁸	No ²	No ¹⁰	Yes ⁷	Yes ¹⁰	No ⁶

*Astral microtubules from both spindle pole bodies occasionally enter the bud in *bni1* mutants (K. Bloom et al., personal communication).

¹Tested in fixed populations of asynchronous cells.

²Bilateral karyogamy assays of *kip3* mutants were normal, suggesting that microtubule orientation and nuclear migration may be normal as well (Miller et al., 1998).

³Evangelista et al., 1997

⁴Miller et al., 1999

⁵Lee et al., 1999

⁶Amberg et al., 1997

⁷This paper and unpublished results

⁸Miller and Rose, 1998

⁹DeZwaan et al., 1997

¹⁰Miller et al., 1998

¹¹Cottingham and Hoyt, 1997

¹²Yeh et al., 1995

orientation. Thus, both processes may involve some as yet undefined form of filamentous actin; alternatively, a small amount of actin patch clustering may be sufficient.

Nuclear Positioning during Mating

The nucleus moves during mating, and some of the molecular mechanisms are shared with the Kip3-dependent movements of the nucleus and spindle in dividing cells. During mating, haploid cells undergo polarized cell growth toward each other, forming a projection that makes cells resemble shmoos. Nuclei migrate into projections via astral microtubules that interact with the cortex at projection tips. Upon cell fusion, astral microtubules from each nucleus contact each other, permitting the nuclei to move together and fuse.

In shmoos, *bni1* and *bud6* mutations impaired Kar9p localization, astral microtubule orientation and nuclear movement into the projection. The extent of Kar9p mislocalization correlated with the severity of the defects in microtubule orientation and nuclear movement. However, the phenotypes in shmoos were more severe than those in dividing cells. Therefore, mating may provide a simpler model for cortical capture of astral microtubules.

Conclusions

These papers provide important new information about how microtubules interact with the cell cortex in yeast. Astral microtubules are presumed to connect the mitotic spindle to the cell cortex and thereby dictate the position and movement of the spindle. This work should represent another case where discoveries in yeast influence research on related processes in other systems.

Bni1p, actin and Kar9p are all necessary for the early phases of spindle positioning and orientation, which depend on astral microtubules and Kip3p, a kinesin. Bni1p and actin function together to localize Kar9p.

Future Directions

In yeast, much remains to be learned about how these proteins interact with each other and how they function to mediate the attachment between microtubules and the cortex. Additional proteins will surely be identified as necessary for the attachment, and biochemical studies will be needed to define the activities.

The mechanism of force production to move the spindle is unknown. The kinesin Kip3p is presumably involved, but whether Kip3 functions as a microtubule motor or causes microtubule shortening by destabilizing ends is an important open question.

Whether this microtubule/cortex attachment mechanism operates outside of yeast is also unknown. Formins, such as Bni1p, are found in many different organisms. Formins appear to influence the actin cytoskeleton but have not yet been implicated in interactions between actin and microtubules or been shown to have primary effects on microtubules. Kar9p has no obvious homologues in the sequence databases. Studies of formins and associated proteins, including perhaps functional equivalents of Kar9p, in other systems will be important.

In addition, little is known about how microtubules at-

tach to the cell cortex during the dynein-mediated movement of the spindle into the neck in yeast. Dynein-dependent spindle movements are known to occur in organisms other than yeast (Morris et al., 1995; Skop and White, 1998).

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Note Added in Proof: A submitted manuscript by K. Bloom and colleagues (Yang, C., P. Maddox, E. Chin, E. Yeh, E.D. Salmon, D.J. Lew, and K. Bloom, manuscript submitted for publication). Cytoplasmic dynein, Bni1p and Bud6p are required for mitotic spindle orientation and nuclear migration in yeast) describes results consistent with those discussed here.

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