Dispatches

Asymmetric Division: A Kinesin for Spindle Positioning

The meiotic spindles of animal eggs move to extremely asymmetric positions, close to the cell cortex. A recent paper has identified a motor complex that may move the meiotic spindle toward the cortex in *Caenorhabditis elegans* eggs.

Erin K. McCarthy and Bob Goldstein

Some of the most extreme cases of asymmetric cell division are the meiotic divisions of maturing oocytes. Each meiotic division results in the partitioning of chromosomes between the oocyte and a polar body. These two cells must differ in size drastically to provide the maturing oocyte with a substantial amount of cytoplasm to support development. How oocytes position meiotic spindles is largely an open question. Additionally, it is not well understood how an oocyte regulates meiotic events in the same cytoplasm that will later sustain mitotic events, as these events may rely on very different mechanisms.

One of the hurdles in understanding meiotic divisions is the surprising variety of strategies that appear to be used in different systems. Oocytes of the worm *Chaetopterus* have spindles that, when pulled away from the cortex, will return to the original cortical site [1]. Such experiments have suggested that there is a site in the cortex that can pull on astral microtubules of the meiotic spindle. Astral microtubules function in similar movements during meiosis in certain other systems, such as fission yeast [2,3].

In many other systems, including the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila* and mice [4–6], meiotic spindles lack centrosomes and astral microtubules. Even in these anastral systems, studies indicate that a diversity of mechanisms are used. For example, meiotic spindle positioning in mice depends on an actin-based mechanism, while *C. elegans* meiotic spindles can move normally even when actin filaments are depolymerized [7,8].

C. elegans meiotic spindles provide us with a fascinating model in which to study how a spindle with minimal tools can position itself near the cortex. If there are no astral microtubules that can be used to pull the spindle to the cortex, and actin filaments do not play an active role, what mechanisms remain? A recent paper by Yang *et al.* [9] has provided some initial clues. These authors have identified players required to translocate the *C. elegans* meiotic spindle to the cortex. From this, we can begin to build models for how a meiotic spindle can be positioned without the use of astral microtubules or actin filaments.

Yang *et al.* [9] speculated that kinesin motors might function to translocate the meiotic spindle to the cortex and began an RNA interference (RNAi) screen of the *C. elegans* kinesin homologs, using live imaging to monitor meiotic spindle translocation inside living worms. During both meiosis I and II in wild-type oocytes, the spindle is generally translocated to the cortex with its long axis parallel to the cortex, followed by spindle rotation and spindle shortening at the cortex (Figure 1).

Yang *et al*. [9] found that, in oocytes depleted of the kinesin-I homolog UNC-116, meiotic spindles remain stationary when wild-type spindles would normally translocate, and polar bodies often fail to form. RNAi downregulation of two kinesin light-chain homologs, KLC-1 and KLC-2, produced a similar result.

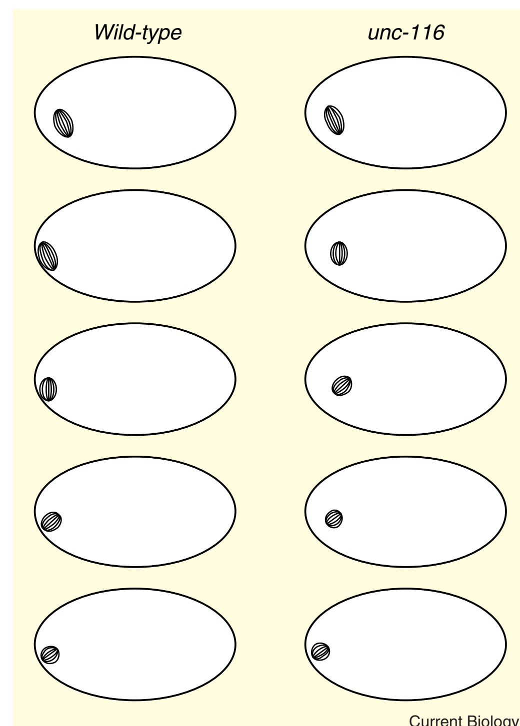

Figure 1. *C. elegans* meiosis.

In wild-type *C. elegans* oocytes, the meiotic spindle translocates to the cortex prior to spindle rotation and shortening (left). In oocytes lacking UNC-116 (right), the meiotic spindle does not translocate to the cortex until after spindle rotation and shortening begin.

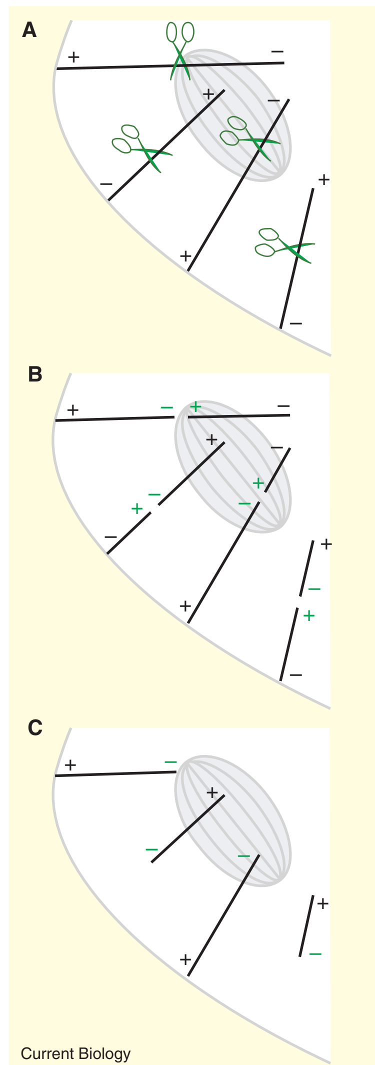

Figure 2. Microtubule severing for spindle translocation.

(A) The *C. elegans* katanin homolog MEI-1 (represented by green scissors), may function in severing cytoplasmic microtubules near the meiotic spindle. (B) Severing generates new plus and minus ends (marked in green). (C) It is plausible that the newly created plus and minus ends may behave differently. In the scenario drawn, newly created plus ends undergo catastrophe, and new minus ends are stable. This would leave only plus ends contacting the oocyte's cortex.

Although the spindle did not move at the correct time in these backgrounds, it did move to the cortex later, at the time when wild-type meiotic spindles would normally undergo spindle rotation and shortening, suggesting that a partially redundant mechanism exists for spindle positioning.

As more than 5000 *C. elegans* protein–protein interactions have been identified by two-hybrid screens and by other methods [10], checking for interaction partners has become a routine step for *C. elegans* researchers who develop an interest in new proteins. Yang *et al*. [9] showed that both of the kinesin light chains, KLC-1 and KLC-2, can bind a protein that, by RNAi experiments, is also required for spindle translocation. This protein, which they call KCA-1, for kinesin cargo adaptor, appears to be a novel and nematode-specific kinesin cargo protein. KCA-1 can also bind a heterochromatin protein [10], suggesting a possible direct link between the kinesin motor complex and the meiotic chromatin.

How might kinesin-I function to move the meiotic spindle? Yang *et al*. [9] have proposed a model in which KCA-1 serves as a cargo adaptor to bridge the meiotic chromosomes and UNC-116. They propose that UNC-116 walks along cytoplasmic microtubules toward the cortex, carrying along KCA-1 and the spindle. Although KCA-1 has been shown also to bind a heterochromatin protein, whether the heterochromatin protein is required for spindle translocation has not been reported. One alternative to this model is that kinesin-I might act more indirectly, for example to set up a microtubule architecture required for spindle movement, or to carry other motors to the spindle or the cortex.

Earlier studies by Yang and colleagues [8] demonstrated a role for another protein in this process. A putative katanin-like microtubule severing protein, MEI-1, also functions in translocation of the meiotic spindle to the cortex. The microtubule severing activity of MEI-1 keeps microtubules short during meiosis. Later, during mitosis, when the mitotic spindle must be much larger, MEI-1 is degraded [11–13]. MEI-1 protein is enriched at spindles in *C. elegans* oocytes [12], and oocytes depleted of MEI-1 have defects in spindle translocation, such as delayed movement to the cortex [8]. From these findings, it has been hypothesized that MEI-1 functions to keep meiotic spindles both small and close to the cortex [8].

Given the roles of both MEI-1 and the UNC-116 complex, it is interesting to speculate how these proteins may function together in translocating the meiotic spindle to the correct location at the cortex. As kinesin-I is typically a plus-end-directed motor, the model proposed by Yang *et al.* [9] of kinesin-I-dependent translocation would require that many microtubules near the meiotic spindle are oriented with their plus ends at the cortex, something that has not yet been examined.

One interesting possibility is that the microtubule severing activity of MEI-1 may produce a directional bias in microtubule orientation that a plus-end motor could exploit for spindle translocation — a bias in which most microtubules near the spindle have their plus ends at the cell cortex. Depending on the balance of plus end- and minus end-stabilizing proteins near microtubules, it is conceivable that severed microtubules could undergo catastrophe at newly created plus ends and might be stable at newly created minus ends. This would leave intact primarily the microtubules with their plus ends near the cortex (Figure 2), a bias that could result in a plus end directed motor moving toward the cell cortex.

Although little is yet known about the molecular mechanisms of meiotic spindle positioning, it is clear that various systems employ strikingly different mechanisms. By using a genetically tractable organism in which these events also can be well visualized, Yang and colleagues [8,9] have created a new model for how a spindle can be positioned. Whether similar strategies are used in other systems to move mitotic or meiotic spindles will be an interesting question for future work.

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Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3280, USA.
E-mail: emccarthy@unc.edu; bobg@unc.edu

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Guard Cells: Transcription Factors Regulate Stomatal Movements

Recent work shows that transcription factors are necessary for stomatal movements in plants. Different members of the plant-specific R2R3-MYB transcription factor family are required for mediating stomatal opening in response to light and stomatal closure in response to darkness.

Julie Gray

Stomatal pores on the surface of plants allow gaseous exchange across the cuticle of leaves and stems. The apertures of stomatal pores are controlled by a pair of guard cells which regulate the uptake of CO_2 from the atmosphere and the loss of water vapor from the plant. To act as effective regulators of gas exchange, guard cells process information from simultaneous, often conflicting, signals, such as light intensity, atmospheric CO_2 concentration and various plant hormones, including the drought response hormone abscisic acid (ABA) [1].

Environmental signals, such as reductions in light intensity or water availability, bring about reductions in stomatal gas exchange by promoting stomatal closure and inhibiting stomatal opening. These are two distinct turgor-driven processes which involve the co-ordinated activation and inhibition of ion channels present on the membranes of the guard cells. Recently there have been major advances in our understanding of the cellular events that underlie guard cell signaling. In addition to ion channels, many signaling components have been identified that are involved in the control of stomatal aperture, including second messengers, protein kinases, protein phosphatases and phospholipases [2–5].

Although, until recently, the role of transcription factors in regulating stomatal apertures had not been directly investigated, there was some evidence indicating that changes in gene expression patterns were involved in controlling stomatal movements. For example, the application of transcriptional inhibitors inhibits stomatal opening under some conditions [6], and RNA processing has been implicated in ABA-induced stomatal closure [7,8]. A guard cell expressed transcription factor has been reported [9], and the ectopic expression of ABI3 — a transcription factor involved in ABA-regulated seed dormancy — has effects on ABA signaling in guard cells [10]. Furthermore, it is clear that changes in gene expression are associated with stomatal movements. A decade ago, ABA-induced changes in guard cell gene expression were reported by Taylor *et al.* [11], and since then many other detailed reports have followed [12–14]. But it has not been established whether such changes are required during changes in stomatal aperture.

Two papers published very recently in *Current Biology* [15,16], demonstrate the involvement of two R2R3-MYB transcription factors in the regulation of stomatal apertures, implicating gene expression as an addition level of control in the proposed intracellular guard cell signaling network that controls stomatal aperture [1].

Plant genomes encode a comparatively large number of putative transcription factors. But even in the case of the most intensively studied of the model species, *Arabidopsis thaliana*, the function of only ~5% of these transcription factors has been determined by detailed phenotypic analysis of the corresponding mutants [17]. The MYB family is one of the largest groups of plant transcription factors, of which the major