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A different angle on cell division

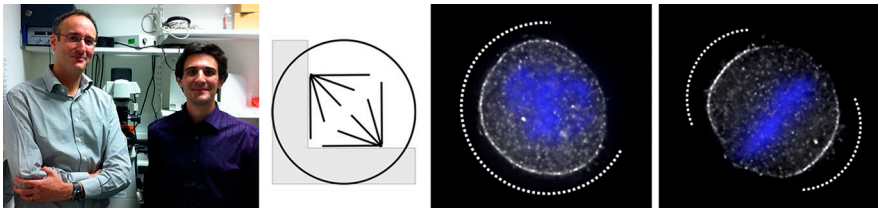
Study shows how actin-binding proteins help tilt the mitotic spindle.

Machicoane et al. reveal how three actin-organizing proteins cooperate to determine the axis of cell division (1). The proteins help establish the unequal distribution of complexes that are essential for orienting the mitotic spindle.

The F-actin mesh at the cell cortex does more than shape and support the cell. Recent studies have revealed that it also helps set the orientation of the mitotic spindle (2). Researchers are still trying to work out how. Machicoane et al. uncovered a mechanism by connecting two seemingly separate teams of molecules. One team involves the so-called force generator complexes, such as the one containing Gαi, LGN, and NuMA. They anchor dynein motors—which orient the mitotic spindle by pulling on spindle microtubules—to the cell cortex (3). The other set of molecules includes three related proteins, the ERM proteins ezrin, radixin, and moesin, that bind to F-actin and fasten it to the cell membrane (4). Blocking the *Drosophila* version of moesin, the only ERM protein the insects produce, induces the spindle to rock back and forth, suggesting that ERM proteins help align the structure (5).

To explore this possibility further, Machicoane et al. first determined what kinases phosphorylate and switch on the ERM trio in mitosis. Although the identity of these activators has been controversial, recent results implicated members of the SLK family. During early metaphase in human cells, the researchers found, SLK colocalizes with activated ERM proteins at the cortex and is therefore in the right place at the right time to turn on the ERM proteins. Indeed, SLK phosphorylated ERM proteins in vitro, and knocking down the kinase prevented the activation of ERM proteins and hindered their localization to the cortex of mitotic cells.

Focal Point



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(Left to right) Arnaud Echard, Mickael Machicoane, and colleagues (not pictured) probed how the actin-binding proteins ezrin, radixin, and moesin affect the orientation of the mitotic spindle. They found that the three proteins exert their influence on two members of a force-generating complex, LGN and NuMA. The researchers grew cells on coverslips coated with L-shaped fibronectin micropatterns (left) that turn the spindle to a 45° angle. In these cells, LGN (white, dotted lines) localizes throughout most of the cell cortex in prometaphase (center) but is limited to the cortex opposite the spindle poles by metaphase (right).

To uncover the effects of ERM proteins on spindle orientation, the researchers grew human cells on coverslips coated with L-shaped swatches, or micropatterns, of fibronectin. The technique is useful, says senior author Arnaud Echard, because "it forces the cells to orient in a particular direction." When a cell nestles in the angle of the "L," its spindle tilts at a 45° angle. Spindles are often far from this angle when prometaphase begins, but they typically rotate into the right orientation within 15 minutes. Machicoane et al. discovered that, in cells lacking SLK, spindles start out with the same wide range of angles but rarely reach the correct alignment. Removing individual ERM proteins had no effect on spindle orientation, but depleting all three proteins sent the spindles off-kilter, indicating that the proteins' functions in determining spindle orientation are redundant.

> "LGN and NuMA are polarized, and that's what drives the spindle orientation in a particular direction."

By studying the developing neocortex of mouse embryos, Machicoane et al. uncovered evidence that ERM proteins behave similarly in vivo. In this tissue, the spindles of neuronal progenitors align parallel with the apical ventricular surface. However, far fewer spindles rotated into the right position after the researchers depleted SLK.

But how do ERM proteins control spindle orientation? Using the micropattern technique, the team found that the force-generating proteins show different distributions on the cortex when the spindles rotate. NuMA and LGN cluster on the portions of the cortex facing the sticky part of the micropattern. Gαi, in contrast, is almost evenly spread around the cortex. Removing SLK or all three ERM proteins from the cells had no effect on the distribution of Gαi, but NuMA and LGN were no longer arranged asymmetrically on the cortex.

That discovery ties the evidence together. "In prometaphase, Gαi is uniform on the cortex," explains Echard, "but LGN and NuMA are polarized, and that's what drives the spindle orientation in a particular direction." The ERM proteins, which are turned on by SLK, work in parallel with Gαi to establish the uneven distribution of LGN and NuMA. The authors now want to determine how activated ERM proteins influence the localization of NuMA and LGN on the cortex.

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