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Funabiki, H., Kumada, K., and Yanagida, M. (1996). EMBO J. *15*, 6617–6628.

Holloway, S.L., Glotzer, M., King, R.W., and Murray, A.W. (1993). Cell *73*, 1393–1402.

Jager, H., Herzig, A., Lehner, C.F., and Heidmann, S. (2001). Genes Dev. *15*, 2572–2584.

Jallepalli, P.V., Waizenegger, I.C., Bunz, F., Langer, S., Speicher, M.R., Peters, J., Kinzler, K.W., Vogelstein, B., and Lengauer, C. (2001). Cell *105*, 445–457.

Mei, J., Huang, X., and Zhang, P. (2001). Curr. Biol. *15*, 1197–1201.

Nasmyth, K., Peters, J.M., and Uhlmann, F. (2000). Science *288*, 1379–1385.

Parry, D.H., and O'Farrell, P.H. (2001). Curr. Biol. *11*, 671–683.

Stemmann, S., Zou, H., Gerber, S.A., Gygi, S.P., and Kirschner, M.W. (2001). Cell *107*, 715–726.

Uhlmann, F., Lottspeich, F., and Nasmyth, K. (1999). Nature *400*, 37–42.

Yanagida, M. (2000). Genes Cells *5*, 1–8.

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The mitotic spindle is a bipolar assembly of microtubules (MTs) that performs multiple functions during cell division. Most notably, kinetochore MTs capture and segregate to daughter cells the duplicated parental chromosomes. The spindle also performs a less obvious but well-documented role in the assembly or activation of a cortical microfilament-based contractile ring that divides the parent cell during cytokinesis. More recent studies have demonstrated that the central region of the mitotic spindle is required for the completion of cytokinesis. The central spindle, or midzone, consists of bundles of antiparallel, overlapping MTs that form during anaphase and persist after division as the midbody. In this issue of *D* *.* *.C* ., Glotzer and colleagues focus on two key components of the central spindle, a mitotic kinesin-like protein (MKLP) and a Rho-GTPase activating protein (RhoGAP) (Mishima et al., 2002). They use a compelling combination of biochemical and genetic approaches to identify and dissect a tetrameric complex composed of these two proteins. This complex likely mediates central spindle assembly by crosslinking antiparallel, nonkinetochore MTs. The stage is now set for a mechanistic understanding of the role that the central spindle plays in the final act of cytokinesis.

Genetic studies in the nematode *C* [illegible] *.* - have identified two distinct classes of proteins contrast, disrupting a central spindle component prevents formation of the midzone and results in regression of the cleavage furrow after extensive ingression. Chromosome segregation appears normal in many central spindle mutants, suggesting that the midzone is required specifically for cytokinesis. Two central spindle cytokinesis genes, called *-4* and *-4*, encode an MKLP and a RhoGAP that are interdependent for localization to the central spindle (Jantsch-Plunger et al., 2000; Powers et al., 1998; Raich et al., 1998). A vertebrate relative of ZEN-4, called MKLP-1, can crosslink antiparallel MTs in vitro (Nislow et al., 1992), suggesting that this kinesin subfamily is directly involved in central spindle assembly.

Mishima et al. now show that ZEN-4 and CYK-4 form a tetrameric complex that they call centralspindlin. Intriguingly, the N terminus of CYK-4 binds to the neck linker and coiled-coil region of ZEN-4. Because the neck linker is important for force production in other kinesins, CYK-4 may regulate the motor activity of ZEN-4. Alternatively, CYK-4 may influence the orientation of the ZEN-4 neck and head regions to facilitate crosslinking of antiparallel MTs.

Genetic data strongly support the functional significance of the CYK-4/ZEN-4 interaction. An allele that introduces a single amino acid substitution in the N-terminal region of CYK-4 eliminates binding to ZEN-4, disrupts central spindle assembly, and results in a late cytokinesis defect. Morever, Mishima et al. found seven extragenic suppressors of this allele that introduce substitutions in the CYK-4 binding region of ZEN-4 and rescue central spindle assembly and cytokinesis. Bringing the analysis full circle, one such suppressor mutation restores binding of ZEN-4 to the mutant form of CYK-4 in vitro. Finally, the authors show that CYK-4 and ZEN-4 can each self-associate. Based on the associative properties of the two proteins, and on their stoichiometry in purified native complexes, the authors conclude that two copies of each protein interact to form a tetrameric

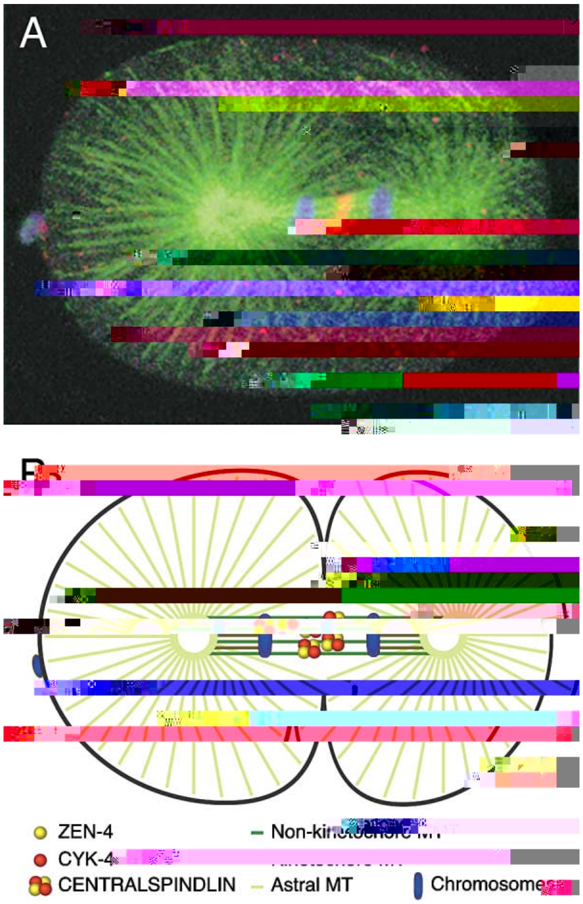

Figure 1. The First Mitotic Spindle in the *C* . Embryo

(A) An embryo in anaphase of the first cell cycle, stained to show microtubules (green), DNA (blue), and the mitotic kinesin-like protein ZEN-4, which accumulates at the central spindle.

(B) A schematic of the first mitotic spindle, showing three populations of microtubules. ZEN-4 and CYK-4 associate in a complex, called centralspindlin, which bundles antiparallel, nonkinetochore microtubules to form the central spindle.

central spindle assembly and cytokinesis, suggesting that this complex has been functionally conserved.

Although the results of Mishima et al. move the field toward an understanding of the mnstitutecc342.8()-270.6(tfe)-32TD[(to-629.4(Econkin)-2ggesting2Aor2stigesting2lro -0.0471 TD[(to

midbody. Consistent with this possibility, ZEN-4 is required to stabilize furrows well after the apparent completion of cytokinesis (Severson et al., 2000). Finally, it is also possible that the central spindle mediates the delivery of new membrane vesicles to the cleavage furrow to promote the final membrane fusion events that presumably ultimately partition daughter cells (Bowerman and Severson, 1999).

The general significance of the late step in cytokinesis identified in *C.* ., and the extent to which the central spindle and the contractile ring function independently during cytokinesis in other cell types, remain to be determined. In contrast to *C.* . ZEN-4, the *D* . MKLP *P* is required not only for central spindle assembly but also for assembly of the contractile ring early in cytokinesis (Adams et al., 1998). Intriguingly, the central spindle in some *D* . cells is a large disc that extends nearly to the cell cortex, while the central spindle during the first embryonic mitosis in *C.* . is only a few microns in diameter, embedded within a cell that is roughly 20 microns wide. Perhaps centralspindlin acts late in cytokinesis in all animal cells and also early in cytokinesis in cell types where the large central spindle is essential for specification of the cleavage plane. It will be interesting to learn whether the cytokinesis defect observed after depletion of centralspindlin in human cells results in an early or late cytokinesis defect, an issue not addressed by Mishima et al.

Finally, other factors may regulate centralspindlin, since CYK-4 and ZEN-4 likely associate throughout the cell cycle (Mishima et al., 2002). Both Polo kinase and an Aurora B-type kinase can bind to MKLPs, and both proteins accumulate at the central spindle and are required for midzone assembly and cytokinesis (Severson et al., 2000; Adams et al., 1998; Carmena et al., 1998; Lee et al., 1995). Moreover, Mishima et al. have shown that some MKLP-1 migrates through a gel with reduced mobility, suggesting that MKLP-1 may be phosphorylated at some point in the cell cycle. Undoubtedly, future research will lead to rapid progress in understanding the regulation of centralspindlin and its role in the completion of cytokinesis.

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