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Cytokinesis: Closing in on the Central Spindle

The central spindle is important for the completion of cytokinesis. Genetic and biochemical approaches have identified a tetrameric complex, made up of a mitotic kinesin-like protein and a Rho-GTPase activating protein, that mediates central spindle assembly.

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 *Developmental Cell*, 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 *Caenorhabditis elegans* have identified two distinct classes of proteins required for cytokinesis: a contractile ring group and a central spindle group. Reducing or eliminating the function of a contractile ring component results in an early cytokinesis defect: no membrane ingression occurs, and mitosis produces a single binucleate cell. In

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 *zen-4* and *cyk-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. Moreover, 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 complex. This complex promotes the bundling of MTs in vitro—while ZEN-4 alone does not. Thus, centralspindlin likely mediates the assembly of the central spindle. MKLP-1 and MgcRacGAP, human orthologs of ZEN-4 and CYK-4, form a similar complex. Depleting MgcRacGAP in a human cell line by RNA interference disrupts

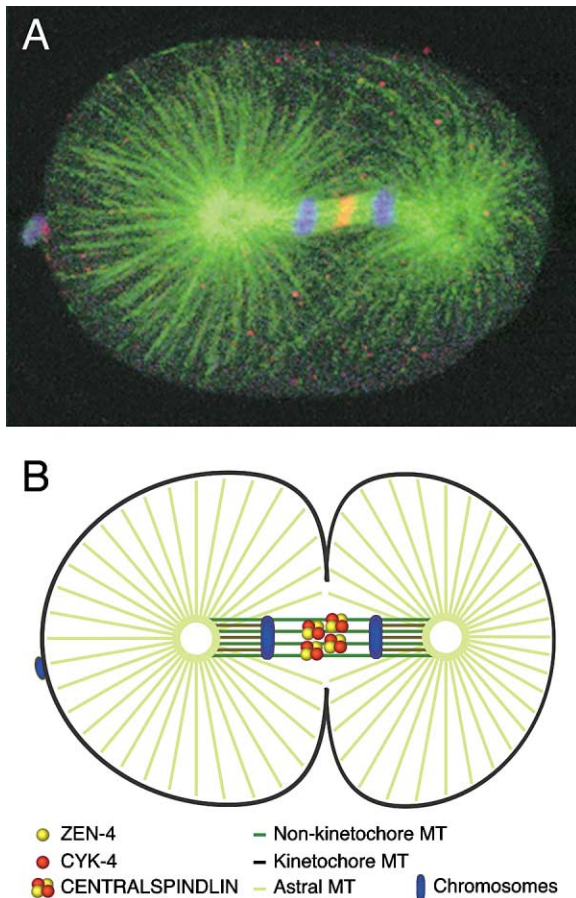


Figure 1. The First Mitotic Spindle in the *Caenorhabditis elegans* Embryo

(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 molecular mechanisms underlying this process, several questions remain. Importantly, it is not known if either CYK-4 or ZEN-4 is directly involved in the completion of cytokinesis. The participation of a RhoGAP in centralspindlin function suggests that regulation of Rho-family small GTPases might be essential for the completion of cytokinesis. In *C. elegans* embryos, one of three small G proteins, RhoA, is known to be required for furrow ingression early in cytokinesis. Because GAP activity antagonizes Rho function, perhaps CYK-4 promotes disassembly or deactivation of the contractile ring as it contacts the spindle midzone late in cytokinesis. Alternatively, the central spindle may provide a scaffold for anchoring the contractile ring during the completion of cytokinesis. Perhaps MKLPs stabilize cleavage furrows directly by tethering the contractile ring to the central spindle or

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. elegans*, 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. elegans* ZEN-4, the *Drosophila* MKLP *Pavarotti* 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 *Drosophila* cells is a large disc that extends nearly to the cell cortex, while the central spindle during the first embryonic mitosis in *C. elegans* 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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Membrane-Associated MMP Regulators: Novel Cell Adhesion Tumor Suppressor Proteins?

Matrix metalloproteinases are enzymes that regulate tissue behavior by interactions with extracellular matrix proteins. RECK, a membrane-anchored inhibitor of MMPs was recently characterized for its role in development, tissue homeostasis, and for tumor angiogenesis.

Stromal-epithelial interactions play a critical role during organ development and maintenance of tissue homeostasis. Numerous studies both *in vivo* and *ex vivo* have documented that dynamic interactions between the cells and the extracellular matrix microenvironment (ECM; the proteinaceous component of the tissue stroma) dictate developmental processes such as branching morphogenesis during glandular development and normal angiogenesis and facilitate tissue specific differentiation. At present, it is known that strictly regulated cycles of ECM synthesis, deposition, and degradation are necessary in order to mediate these processes. Dynamically modulated ECM turnover events are essential to regulate cell behavior on multiple levels by modifying the physical scaffold upon which cells can migrate, by transmitting biochemical and biophysical cues to the cells to initiate cell proliferation, survival, and tissue-specific gene expression, and by functioning as a reservoir for sequestering and releasing soluble factors, such as morphogens and growth factors, into the tissue microenvironment. In addition, the multicellular composition of the tissue, cell shape, and tissue organization dictate how the cell responds to these cues. Perturbed ECM secretion and turnover and loss of proper cellular responsiveness to ECM cues are associated with pathological states such as malignancy (Radisky et al., 2001). Precisely how these processes are regulated or dysregulated at the molecular level remains ill defined and is currently an area of intense investigation.

Matrix metalloproteinases (MMPs) are secreted enzymes that process and degrade ECM and have been shown to function as important mediators of ECM-regulated tissue behavior (Vu and Werb, 2000). The activity of MMPs are known to be regulated by secreted tissue inhibitors of metalloproteinases (TIMPs). However, regulatory factors such as TIMPs that are secreted distal to the target cell and subsequent to MMP processing fail to adequately explain how normal cells are able to directionally and tightly regulate ECM turnover to facilitate normal controlled processes such as branching morphogenesis. A paper by Oh and colleagues in the December 14 issue of *Cell* summarizes a series of experiments that define a new type of ECM MMP regulatory

molecule that can function as a novel tumor suppressor (Oh et al., 2001).

RECK is a membrane-anchored glycoprotein that has now been shown to repress MMP-9, MMP-2, and MTI-MMP synthesis and secretion and to influence ECM integrity. The physiological relevance of RECK expression and function to tissue homeostasis is elegantly illustrated in the recent article and also in earlier work by the authors (Oh et al., 2001; Takahashi et al., 1998). In the recent work, they demonstrate that perturbed control of secretion and activation of MMPs such as MMP-9, MMP-2, and MTI-MMP and dysregulated ECM turnover in the absence of RECK expression is sufficient to facilitate the execution of pathological processes such as tumor invasion and metastasis and malignancy-associated angiogenesis (Oh et al., 2001). The significance of identifying a cell-associated MMP modifier that is involved in regulating processing and secretion of MMPs emphasizes the existence of master upstream modulators of ECM homeostasis that also has implications as a regulator of normal morphogenic developmental programs such as glandular morphogenesis. In this regard, RECK, which is a membrane-associated upstream MMP regulator, can be distinguished from the vast majority of previously described secreted TIMPs, that function downstream to inhibit MMP activity. Whether RECK is a unique regulator of ECM turnover or if it constitutes the first candidate molecule of a new class of regulators awaits further study.

Intriguingly, RECK is a GPI-linked protein. GPI-linked proteins, such as fyn, by virtue of their propensity to associate with hydrophobic lipid microdomains, characteristically sequester into cholesterol-rich lipid rafts. Lipid rafts have been implicated as membrane regulatory domains that participate in diverse cellular processes, including endocytosis, secretion, and cell signaling (Brown and London, 1998). This raises the interesting possibility that RECK might also participate in lipid raft-mediated processes. Given the dynamic and reciprocal link that exists between the ECM, ECM receptors, ECM turnover, and tissue architecture, it is perhaps not surprising that RECK, presumably acting as an MMP regulator, also appears to participate in ECM assembly and secretion.

Analogous to conventional lipid raft components, RECK itself is likely subject to spatial regulation as occurs following ligand-induced receptor clustering of lipid raft components, or is induced in concert with partitioning of lipid moieties to the apical membrane domain in association with acquisition of tissue polarity. If true, then the relevance of the dynamic polarity switches that have been documented to occur during branching morphogenesis or the sustained loss of polarity as occurs during tumor progression to RECK function merits further investigation. Indeed, whether RECK dynamically shuttles between basal and apical membrane domains during branching morphogenesis would suggest that