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SPOTLIGHT

A back-up source of microtubules for the midbody during cytokinesis

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During cytokinesis, microtubules become compacted into a dense midbody prior to abscission. Using genetic perturbations and imaging of *C. elegans* zygotes, Hirsch et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202011085) uncover an unexpected source of microtubules that can populate the midbody when central spindle microtubules are missing.

Microtubules of the mitotic spindle control successive stages of cell division. They first align and segregate the duplicated sets of chromosomes to opposite poles of the spindle during mitosis. Spindle microtubules then specify where the contractile ring assembles to drive the furrowing stage of cytokinesis. Finally, microtubules form the midbody within the intercellular bridge that links sister cells prior to abscission. Midbodies are dense, relatively stable structures that adopt a characteristic architecture with a central Flemming body and flanking midbody arms (1). Midbodies contain proteins from both the spindle and the contractile ring, which become organized in different subregions of the midbody as it matures, thins, and prepares for abscission (1, 2). Although the maturation process remains poorly understood, the source of the microtubules that form the central scaffold of the midbody is clear. Or so we thought.

Initiation of cytokinesis involves two distinct subpopulations of microtubules (Fig. 1): astral microtubules and central spindle microtubules (3). Astral microtubules are dynamic microtubules that emanate from the spindle poles and are thought to cause relaxation of the polar cortex. Conversely, the central spindle constitutes a more stable array of antiparallel microtubules that appear in the spindle midzone during anaphase. The minus ends of central spindle microtubules are oriented toward the separating chromosomes, while their plus-ends interdigitate and overlap at the cell equator. Key proteins concentrate at this plus-end overlap zone, where they bundle these microtubules and drive contractile ring assembly. It is toward this site that the contractile ring closes on its way to forming the intercellular bridge (Fig. 1). Because the contractile ring closes around the central spindle, it has long been assumed that the midbody must require the gathering and compaction of these central spindle microtubules. However, a new study by Canman and colleagues using the one-cell *Caenorhabditis elegans* embryo suggests that this widely held view is not the full story (4). Using genetic manipulations and quantitative live-cell microscopy, they present evidence that midbodies can in fact still form without any preceding central spindle, using astral microtubules as their source.

Hirsch et al. build on prior work showing that central spindle assembly in *C. elegans* zygotes requires the translocation of the centromere protein F homologues holocentric chromosome-binding proteins 1/2 (HCP-1/2) and the downstream cytoplasmic linker-associated protein family protein CLS-2 from kinetochores to the midzone (5). The researchers sought to better understand this central spindle assembly pathway by further defining the individual requirements for HCP-1 and HCP-2 and uncovering potential redundancies between them. First, they carefully measured the spindle midzone levels of GFP reporters of various central spindle proteins in live embryos deficient in HCP-1, HCP-2, or both. These reporters included GFP::β-tubulin, the chromosomal passenger complex component, AIR-2^{AuroraB}::GFP, and the microtubule bundling protein SPD-1^{PRC1}::sfGFP. These analyses clearly showed that HCP-1 is the primary contributor to central spindle assembly, but that HCP-2 also contributes in a partially redundant manner.

However, what caught the authors' attention was the observation that, even when HCP-1 and HCP-2 were co-depleted and there was no detectable evidence of central spindle formation, the cells still went on to form midbodies and undergo successful cytokinesis. They measured the rates of contractile ring closure, which were normal, and found that midbody components such as cytokinesis defective 4 (CYK-4) and zygotic epidermal enclosure defective 4 (ZEN-4) of the centralspindlin complex, AIR-2, and SPD-1 suddenly appeared at the close of furrowing, despite having been absent from the spindle midzone.

Prior studies have shown that midbodies formed at this first zygotic division undergo abscission during the following division, when they are released and internalized (6). Hirsch et al. therefore tracked their central

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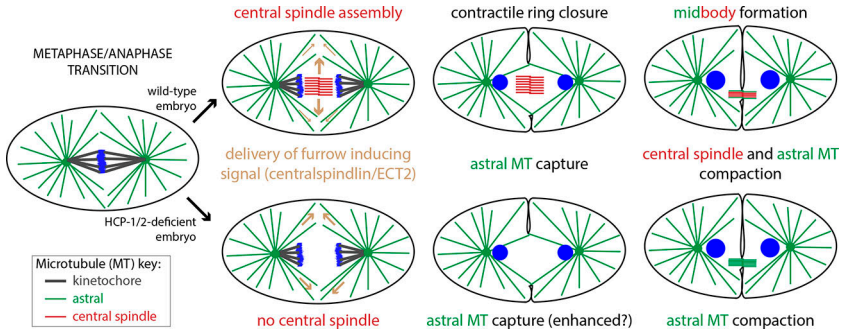

Figure 1. **Schematic depicting the contributions of different populations of microtubules to midbody formation.** Upper cartoons depict a wild-type *C. elegans* zygote, while the lower cartoons depict the midbody assembly pathway uncovered by Hirsch et al. (4) upon loss of the HCP-1/2–dependent central spindle.

spindle–independent midbodies through the next cell cycle and found that they were released and internalized with comparable kinetics to controls, confirming that they were functionally able to promote abscission. Finally, the researchers carefully examined GFP::β-tubulin in HCP-1 and HCP-2 doubly deficient embryos to determine the origin of microtubules that populated these central spindle–independent midbodies. This revealed that astral microtubules were being gathered by the incoming contractile ring and bundled together into the nascent midbodies. Thus, midbodies do not obligatorily derive from the central spindle, as has always been assumed, but can also form from astral microtubules (Fig. 1).

The work raises several interesting questions. If central spindle microtubules are dispensable for midbody formation and astral microtubules are sufficient, then is the reverse also true? At first it seems unlikely that astral microtubules would be required, given that specific perturbations of astral microtubules in different systems do not reportedly affect midbody formation (7, 8), but this may warrant closer scrutiny. In any case, it would be interesting to compare the ultrastructure of astral microtubule–derived midbodies to control midbodies by electron microscopy in worm embryos.

This work is significant not only for understanding midbody assembly, but also for understanding the preceding furrowing stage, where astral microtubules and central spindle microtubules are often considered to play quite different roles. Astral microtubules are thought to relax the polar cortex through poorly defined inhibitory signals (3). Central spindle microtubules deliver the stimulatory, furrow-inducing signal, which includes the centralspindlin complex (ZEN-4/MKLP1 and CYK-4/MgcRacGAP) (3). Centralspindlin not only bundles and stabilizes the central spindle, but it also localizes the RhoGEF ECT2 to the equator and promotes its activation at the plasma membrane to drive RhoA-dependent contractile ring assembly and closure (9). Experiments in echinoderm embryos have also shown that astral microtubules can adopt central spindle–like attributes, becoming bundled at their plus-ends and accumulating centralspindlin and ECT2, in both the presence or absence of a central spindle (10). That study and this latest one by Hirsch et al. (4) suggest that equatorially directed astral microtubules and central spindle microtubules may not be so different from one another. Both subpopulations can deliver ECT2 to drive furrowing, and both subpopulations can form a midbody after furrowing (Fig. 1). It is unclear whether central spindle disruption enhanced astral microtubule capture and bundling by the contractile ring, but this is a possibility if the two subpopulations of microtubules compete for the same factors.

Hirsch et al.'s central finding is also unexpected because the central spindle does appear to be essential for midbody formation and successful cytokinesis in fly and mammalian cells (11, 12). This discrepancy may reflect genetic differences between systems and/or differences in cell size or geometry. For example, in flies and humans, the augmin complex is important for both central spindle and midbody assembly (12, 13), but no orthologous genes are present in the worm genome. Alternatively, the larger *C. elegans* zygotes with their more prominent asters may have a greater capacity to gather enough astral microtubules to form a midbody than smaller cells. In this regard, it may be interesting to determine whether the smaller cells of later *C. elegans* divisions can also form functional midbodies from astral microtubules alone. Either way, the work serves as an important reminder that one needs to exercise caution when making what seem like well-supported assumptions. Fundamental processes such as cytokinesis are likely so robust because of the back-up mechanisms that have evolved to ensure the fidelity of their function.

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