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Making more microtubules by severing: a common theme of noncentrosomal microtubule arrays?

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Two related enzymes, katanin and spastin, use the energy from ATP hydrolysis to sever microtubules. Two new studies (one in this issue; see McNally et al., p. 881) show that microtubule severing by katanin provides a means for increasing microtubule density in meiotic spindles. Interestingly, loss of spastin leads to a sparser microtubule array in axons and synaptic boutons. Together, these studies hint at a wider role for microtubule-severing enzymes in the formation and organization of noncentrosomal microtubule arrays by generating new seeds for microtubule growth.

Although considerable in girth (25 nm) and held together by multiple tubulin–tubulin interactions, microtubules have been observed to “snap” in half in various cell types (Waterman-Storer and Salmon, 1997; Kalil et al., 2000). The first evidence that microtubule breakage reflects the action of an enzyme rather than just mechanical strain came from experiments with *Xenopus laevis* egg mitotic extracts (Vale, 1991). Fittingly, this microtubule-severing enzyme (which consists of a catalytic subunit, p60, belonging to the AAA ATPase family and a regulatory subunit, p80) was named katanin, after the Japanese word for sword, *katana* (McNally and Vale, 1993). Katanin is found in a variety of organisms ranging from *Chlamydomonas reinhardtii* to humans, but its biological roles remain poorly understood. However, two recent studies significantly advance our understanding of katanin-mediated microtubule severing. In this issue, McNally et al. (p. 881) show that microtubule severing by katanin controls the length and microtubule distribution in the *Caenorhabditis elegans* meiotic spindle and contributes to spindle shortening during anaphase. These findings are complemented by a recent electron microscopic study by Srayko et al. (2006) showing that katanin-mediated severing generates many short microtubules around meiotic chromatin, which helps increase the microtubule polymer mass of the *C. elegans* meiotic spindle.

The *C. elegans* female meiotic spindle has several unusual features that make it particularly well suited to investigate

katanin's function. In the absence of MEI-1 or MEI-2 (the names for the *C. elegans* katanin p60 and p80 subunits, respectively), microtubules form around meiotic chromatin but fail to assemble a bipolar spindle (Mains et al., 1990; Clandinin and Mains, 1993). This essential function for katanin in spindle formation has not been observed in any other system thus far. A plausible explanation for katanin's importance in the female *C. elegans* meiotic spindle may reside in the way that this spindle forms. Animal cell mitosis is dominated by a centrosome-based pathway for microtubule nucleation and bipolar spindle formation. In contrast, oocytes do not contain centrosomes, and spindle formation occurs by chromatin-based microtubule nucleation followed by the reorganization of microtubules into a bipolar spindle by motor proteins. *C. elegans* meiotic spindles also are unusual in that they shorten during chromosome segregation, rather than elongate, as occurs in most mitotic spindles.

McNally et al. (2006) examined spindle size and dynamics in a *C. elegans* mutant with reduced levels of MEI-2 (because katanin-null mutations cannot assemble a bipolar meiotic spindle). This hypomorph most likely has impaired microtubule severing, as the authors show that the catalytic subunit MEI-1 cannot sever microtubules in vitro without its partner subunit MEI-2. In *mei-2* mutants, the meiotic spindle is longer, indicating that severing controls spindle length. Examining the dynamics of these spindles by time-lapse microscopy, McNally et al. (2006) define the role of katanin in two phases of spindle shortening. The first phase is not accompanied by microtubule disassembly, is katanin independent, and is likely driven by motor-dependent sliding of overlapping microtubules. In contrast, the second spindle-shortening phase, which occurs during anaphase chromosome segregation, is accompanied by a precipitous decrease in microtubule density at the poles and a shift in microtubule density from the poles to the chromosomes. This second shortening phase, including the redistribution of microtubule density, was severely inhibited in the hypomorphic katanin mutant. From this data, the authors conclude that severing by katanin at the poles results in microtubule disassembly and spindle shortening. Microtubule fragments, either generated at the poles and transported to the spindle midzone or generated locally near the midzone, may account for the increased microtubule density at the center of the spindle (Fig. 1).

The live-cell imaging by McNally et al. (2006) is nicely complemented by higher resolution snapshots of the *C. elegans* meiotic spindle by Srayko et al. (2006). Using tomographic

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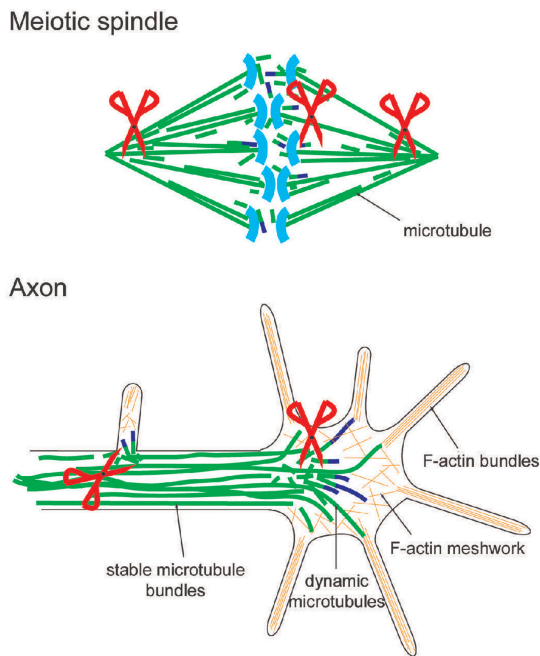


Figure 1. **Model of microtubule severing in the meiotic spindle (top) and axon (bottom).** Potential sites of action of microtubule-severing enzymes are indicated by scissors. Microtubules growing from severed ends are shown in dark blue.

electron microscopic reconstructions to trace individual microtubules, the authors show that wild-type spindles are composed of a large number of short, overlapping microtubules distributed along the spindle axis. The authors also observed lateral defects in the walls of a subset of microtubules, bite marks that might have been left by a microtubule-severing enzyme. In contrast, their tomographic reconstructions of *mei-1*-null spindles revealed far fewer (>80% less) and longer microtubules around chromosomes when compared with wild-type spindles, as well as a lack of short microtubules throughout the entire spindle. These observations indicate that katanin-mediated severing increases microtubule number and suggest that severed microtubules serve as templates for tubulin addition to increase microtubule density in the spindle. Consistent with the latter idea, McNally et al. (2006) find that a reduction in the activities of both katanin and γ -tubulin (involved in chromatin-based microtubule nucleation) produces a dramatic loss in microtubules, suggesting that multiple mechanisms contribute to the generation of microtubule density in the meiotic spindle.

Cooperation between γ -tubulin and katanin in the building of noncentrosomal microtubule arrays has been proposed in plants (Wasteneys, 2002). To create a cortical array of microtubules, plant γ -tubulin complexes bind to the sides of existing microtubules and then nucleate new microtubules, a mechanism similar to that of Arp2/3-mediated nucleation of actin filaments. Severing of these new microtubule branches by katanin would free the γ -tubulin complex to nucleate a new microtubule. In the absence of functional katanin, the *Arabidopsis thaliana* cortical microtubule array is sparse and disorganized (Wasteneys, 2002), reminiscent of the meiotic spindle in *C. elegans* katanin mutants.

The neuron represents another cell type in which microtubule severing could be used for regulating the density of non-centrosomal microtubules. How microtubules are generated to fill the long processes of neurons is still controversial. One idea is that microtubules are continuously nucleated at the centrosome in the cell body, severed from their centrosomal connection by katanin, and transported by molecular motors into the axon (Ahmad et al., 1999). However, relying solely on a microtubule nucleation center in the cell body (which can be 1 m away from the nerve terminal) would likely limit a neuron's ability to rapidly remodel after injury or during development. Microtubule nucleation from tubulin monomers or small oligomers might generate new microtubules in such situations (Keating and Borisy, 1999), although firm evidence for such a mechanism is lacking. Alternatively, local severing might increase microtubule polymer numbers and mass by creating seeds for new microtubule growth (Fig. 1), as described for the *C. elegans* meiotic spindle. In fact, an accumulation of short microtubules in growth cones and newly developing axonal branches has been observed (Kalil et al., 2000).

Templated nucleation from severed microtubules in axons has not been demonstrated but is a tempting hypothesis to explain the phenotype of neurons with defective spastin, a microtubule-severing protein related to katanin (Evans et al., 2005; Roll-Mecak and Vale, 2005). Spastin was originally identified as one of the most commonly mutated genes in hereditary spastic paraplegia (Hazan et al., 1999), a human neurodegenerative disease characterized by lower extremity weakness. Aspects of the human disease are mimicked by spastin loss of function in lower organisms, thereby allowing dissection of the underlying mechanism. Spastin localizes to growth cones, synaptic boutons, and axonal branches (Errico et al., 2004; Sherwood et al., 2004; Trotta et al., 2004). *Drosophila* spastin-null mutants have sparse and disorganized microtubule arrays at synaptic boutons (Sherwood et al., 2004), whereas spastin-knockdown zebrafish show a disorganized and sparse axonal microtubule array and impaired axonal outgrowth (Wood et al., 2006). However, it has not been directly demonstrated that spastin-mediated microtubule severing occurs in boutons or growth cones, and the small size of these structures poses challenges for such measurements. However, a connection between microtubule severing and the hereditary spastic paraplegic disease phenotype is at least indirectly suggested by the finding that many spastin disease mutants have impaired microtubule severing in vitro (Roll-Mecak and Vale, 2005).

In summary, studies in *C. elegans* meiotic spindles, plant cortical microtubule arrays, and neuronal axons/synaptic terminals suggest that microtubule severing provides an important mechanism for regulating microtubule numbers and lengths. These systems share an absence of a proximal centrosome-based microtubule nucleation site, hence their greater reliance on alternate mechanisms for generating microtubules. However, many questions remain. To increase microtubule polymer mass, severed microtubule must be stabilized and allowed to grow, but how is stabilization accomplished in these different cell types? Is microtubule severing operating constitutively, or can it be up- or down-regulated in response to signals?

Are microtubule-severing enzymes playing similar roles in cells with centrosomal microtubule arrays, as is suggested by additional studies on mitotic cells by McNally et al. (2006), but less obvious because of the dominant role of centrosomes? Can severing also be used to reduce or destroy a microtubule network (perhaps suggested by the role of katanin in the deflagellation of *C. reinhardtii* flagella; Lohret et al., 1998)? Two decades after the identification of the first microtubule-severing enzyme, the biology and future directions for this interesting class of enzymes are at last coming into focus.

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