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Previews

Microtubule Length Control, a Team Sport?

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Kinesin-8 family members function in microtubule length control and exhibit highly processive plus-end directed motility in conjunction with microtubule dissassembly activity. In a recent issue of *Cell*, Varga and colleagues describe how these two activities may be used to simultaneously measure and adjust the length of cellular microtubules.

Mechanisms that regulate microtubule length are thought to play crucial roles in a number of cellular processes including nuclear positioning, the establishment and maintenance of mitotic spindles, the regulation of mitotic chromosome alignment, process extension, and directed cell motility. However, the molecular control of microtubule length in cells is not well understood. Members of the kinesin-8 family of motors have been implicated in the direct regulation of microtubule length in cells (Gupta et al., 2006; Stumpff et al., 2008; Varga et al., 2006). A recent paper from Varga et al. (2009) now indicates that the budding yeast kinesin-8, Kip3p, uses a surprising cooperative mechanism to monitor microtubule lengths and shorten them "as needed."

Kip3p uses the energy of ATP to "walk" toward the plus-ends of microtubules for unusually long distances without releasing from the polymer. This ensures that a motor that lands anywhere on the microtubule lattice, even on long cellular microtubules, is likely to eventually reach the plus end, leading to an accumulation of motors there. Surprisingly, once a sufficient number of Kip3p molecules reach the plus-end of the polymer, microtubule disassembly commences at a rate that is proportional to the concentration of Kip3p molecules. Due to the motor's inherently high processivity, the depolymerization rate, by extension, is proportional to the length of the microtubule (Varga et al., 2006). This microtubule length-dependent activity was proposed as a mechanism by which the kinetic properties of the motor could be used to measure and "trim" microtubules to a length that is appropriate to the needs of the cell.

Regulation of microtubule length is consistent with many of the described cellular activities of Kip3p (Figure 1). The molecule is found associated with both astral (cytoplasmic) and spindle (nuclear) microtubules in the budding yeast *Saccharomyces cerevisiae*. It is required to transport the pre-anaphase nucleus to the daughter bud site, a function dependent on the adjustment of astral microtubule length. Loss of Kip3p leads to excessively long and bent anaphase spindles, suggesting that length-dependent microtubule depolymerization activity is utilized at the plus ends of spindle midzone microtubules to limit the length of anaphase spindles (Hildebrandt and Hoyt, 2000). The mechanism by which Kip3p "knows" how to disassemble a microtubule, but only so far, to set spindle position and length was puzzling. An impressive study by Varga and colleagues (Varga et al., 2009) using TIRF microscopic assays in conjunction with purified GFP-Kip3p now show that once a motor reaches the end of the microtubule, it will linger there until another motor arrives to knock it off along with one to two tubulin dimers, thereby reducing the filament length. Thus, one motor molecule (likely consisting of a dimer of two motor domains) removes only one or two tubulin dimers before recycling rather slowly to rebind to the microtubule. By comparison to kinesin-13 microtubule depolymerases, this makes Kip3p an extraordinarily weak microtubule depolymerizer. High processivity combined with exceptionally weak, cooperative removal of terminal tubulin dimers are features that could enable Kip3p to establish a gradient of motors at the microtubule tip that serves as a readout of microtubule length. If the processive motors translocated to the end of the microtubule and then halted benignly, the gradient would disappear over time as the lattice became saturated with motors, thereby abolishing the positional information along the microtubule.

An important question that remains is what, structurally, the kinesin-8 family of motors does at the end of the microtubule. Kinesin-13s stabilize a curled structure at the end of the microtubule protofilament in the ATP-bound state (Moores et al., 2002). In contrast, electron microscopic studies of kinesin-8 family molecules (klp5/6 from the fission yeast *S. pombe*) liberally decorating the microtubule lattice and protofilament extensions showed no evidence of protofilament curling (Grissom et al., 2009). Mutational analysis of kinesin-13 motors demonstrates that bending and detachment of a tubulin dimer from the microtubule and the subsequent release of this dimer from the motor is tightly coupled to the ATP hydrolysis cycle (Wagenbach et al., 2008). However, it is unknown whether loss of the terminal tubulin dimer is coupled to any part of the ATP hydrolysis cycle of the tubulin-associated Kip3p motor. Kip3p's tubulin removal mechanism may differ significantly from the extraordinarily efficient ATP-coupled bending employed by kinesin-13 family members. Its comparatively weak tubulin removal activity begs the question: does this activity completely explain the mechanism used by Kip3p to modulate microtubule polymer length in cells?

To begin to answer this question, one must revisit the effects that kinesin-8 motors have on "live," dynamic microtubules. In *S. cerevesiae*, loss of Kip3p reduces both the catastrophe frequency and the rescue frequency of dynamic microtubules (Gupta et al., 2006). The catastrophe frequency is the frequency with which a microtubule will transition to depolymerization (one would expect a microtubule depolymerizer to increase this frequency). The rescue frequency is the frequency with which the microtubule

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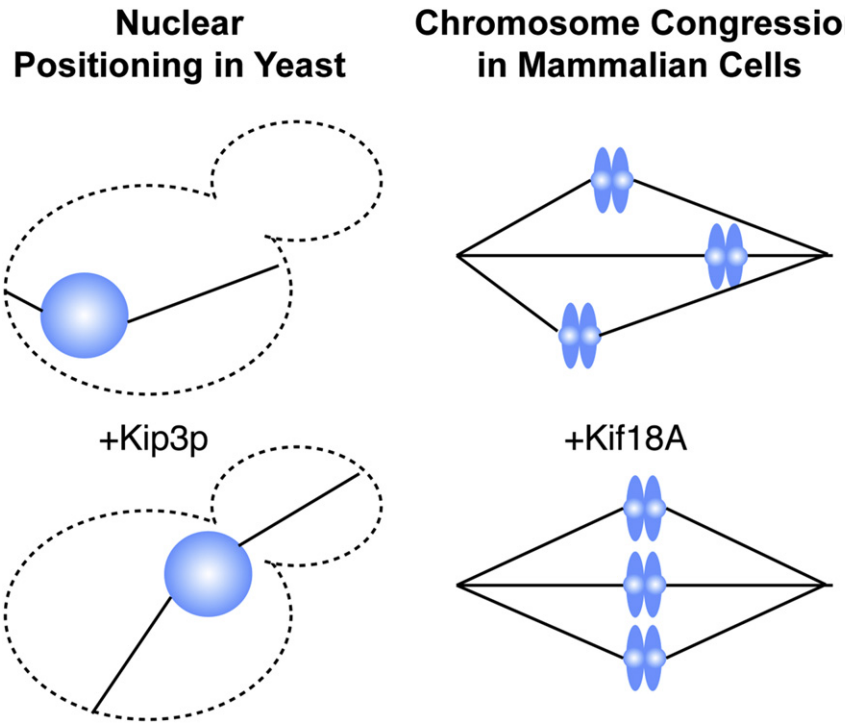

Figure 1. Kinesin-8 Motor Functions
Kinesin-8 motors such as Kip3p in budding yeast and Kif18A in mammalian cells are implicated in processes that lead to the equalization of microtubule length. Both pre-anaphase nuclear positioning in budding yeast and chromosome congression in mammalian cells tend to equalize cytoplasmic or kinetochore microtubule (solid black lines) lengths, respectively. Nuclei and chromosomes are shown in blue.

will transition to polymerization. This is not an activity one would expect from a microtubule depolymerizer. In addition to promoting both catastrophes and rescues of microtubules, Kip3p also slows the disassembly rate of live microtubules in *S. cerevesiae*. These activities contrast with the kinesin-13 depolymerase, MCAK, which promotes catastrophes and reduces rescues of dynamic, unstabilized microtubules (Newton et al., 2004). By simultaneously increasing both rescues and catastrophes over time, Kip3p has the unexpected potential to limit microtubules to a median steady-state length. This activity is compatible with its known role in promoting the transport of the pre-anaphase nucleus to the bud site (Hildebrandt and Hoyt, 2000)). In this case, long astral microtubules extending toward the bud will shorten and short astral microtubules in the mother cell will lengthen in order to center the nucleus near the bud neck.

Similar to the role of Kip3p in nuclear positioning, the mammalian kinesin-8 motor Kif18A, which is restricted to kinetochore fibers during mitosis, decreases the speed of chromosome movement and increases the frequency with which chromosome movement switches directions (Stumpff et al., 2008). Within the context of the kinetochore fiber, these activities are analogous to the measured changes in microtubule dynamics described for Kip3p. Addition of increasing amounts of Kif18A motor to the kinetochore fibers strongly restricts the movement of chromosomes to the spindle midline. This, in effect, normalizes the length of all the kinetochore fiber bundles to the same median length, in turn promoting the congregation of chromosomes at the metaphase plate (Figure 1). In contrast, a potent kinetochore-coupled depolymerase, such as the kinesin-13 MCAK, would be expected to increase the speed of chromosome movement (Wordeman et al., 2007) and increase the dynamics of live microtubules (Newton et al., 2004), as published studies have indicated. The fact that Kif18A seems to have the opposite effect is puzzling but would be reconcilable if kinesin-8 motors function to increase both catastrophes and rescues at microtubule ends. Presently, all signs point toward kinesin-13 and kinesin-8 motors as having opposite, even antagonistic roles at the kinetochore in mammalian cells, a counterintuitive conclusion if both motors are depolymerizers. The Varga et al. (2009) study illustrates beautifully the likely source and mechanism of the catastrophe-promoting activity of kinesin-8 motors and how this could contribute to length regulation. However, published work strongly suggests that a key component of kinesin-8 activity—the rescue-promoting activity—that is distinct from other depolymerizers like kinesin-13 motors remains to be explored. Further understanding of both of these activities will be required to fully explain the role of kinesin-8 motors in living cells.

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