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CENP-E hangs on at dynamic microtubule ends

Melissa K. Gardner

During mitosis, kinetochores attach to microtubule plus ends, thus allowing dynamic microtubules to properly segregate chromosomes. How this type of 'end-on' attachment between microtubule plus ends and kinetochores is formed and maintained is unclear. CENP-E, a kinesin-7 family member, is now shown to have a role in associating kinetochores with dynamic microtubule plus ends.

During mitosis in vertebrate cells, multiple dynamic microtubules make attachments to large multi-protein complexes termed kinetochores. These are the essential linkers between the dynamic microtubules that align and ultimately segregate duplicated chromosomes, and the chromosomes themselves[1]. To achieve the correct alignment and segregation of duplicated chromosomes during mitosis, it is important that the kinetochore complex forms 'end-on' attachments with the dynamic plus ends of kinetochore microtubules[2]. This architecture allows the microtubule plus ends to align chromosomes at the centre of the mitotic spindle during metaphase, achieving chromosome congression, and then to mechanically segregate the chromosomes during anaphase through microtubule plus-end depolymerization and sliding[3]. How the cell is able to make and maintain an end-on configuration between microtubule plus ends and kinetochores is a major question in mitosis, especially given that the plus end of each individual kinetochore microtubule remains dynamic throughout mitosis: the plus ends stochastically grow and shorten (polymerize and depolymerize) through the rapid addition and loss of many individual tubulin subunits[4]. These kinetochore microtubule dynamics are likely to contribute to the chromosome oscillations that are observed in many cell types[5]. However, correctly oriented kinetochores appear to remain stably attached to a population of dynamic microtubule plus ends despite the chromosome oscillations.

In this issue, Gudimchuk *et al.*[6] examine the role of the kinesin-7 CENP-E in associating kinetochores with dynamic microtubule plus ends during metaphase. The authors first ascertained that CENP-E was present at the kinetochore during metaphase in mammalian cells, as it co-localized with the kinetochore marker CENP-A in metaphase-arrested cells. They then treated cells with a small-molecule inhibitor of CENP-E which locks the protein in a non-moving microtubule-bound state and observed that a small number of metaphase chromosomes in each cell were severely misaligned, suggesting that motile CENP-E may have a role in maintaining proper end-on kinetochore attachment to microtubules during metaphase. To investigate how motile CENP-E motors could interact with the plus ends of dynamic microtubules to maintain end-on kinetochore attachment, Gudimchuk *et al.* performed *in vitro* studies in which they demonstrated the interaction of purified full-length CENP-E–GFP with dynamic microtubules. As expected, full-length CENP-E dimers processively walked to dynamic microtubule plus ends[7]. However, once at the microtubule plus ends, CENP-E dimers remained associated with both growing and shortening microtubule plus ends for many seconds, with average tracking durations of 11.6 ± 1.4 s for shortening and 17.9 ± 1.3 s for growing microtubules. Therefore, CENP-E has the unique property of being both a processive plus-end-directed microtubule motor and also a plus-end tip tracker for both polymerizing and depolymerizing microtubules. Furthermore, beads coated with full-length CENP-E could also follow depolymerizing microtubule plus ends, suggesting that CENP-E may be able to couple microtubule dynamics to cargo motion.

The authors used *in vitro* experiments to discern a possible molecular mechanism for CENP-E's unexpected double function of plus-end-directed motility and microtubule plus-end tip tracking. Specifically, truncated CENP-E motor constructs, comprising the motor domain and short stalk segment only, were generated to assess the consequences of motor tail loss for CENP-E motility and tip tracking. Although the plus-end-directed motility of CENP-E on the microtubule lattice was similar between the full-length and the tail-truncated constructs, there was a remarkable difference in their ability to track depolymerizing microtubule plus ends. Whereas ~70% of the full-length CENP-E motors tracked depolymerizing plus ends, almost none of the tail-truncated CENP-E motors were successful in tracking the shortening microtubule ends, providing strong evidence that the motor tail

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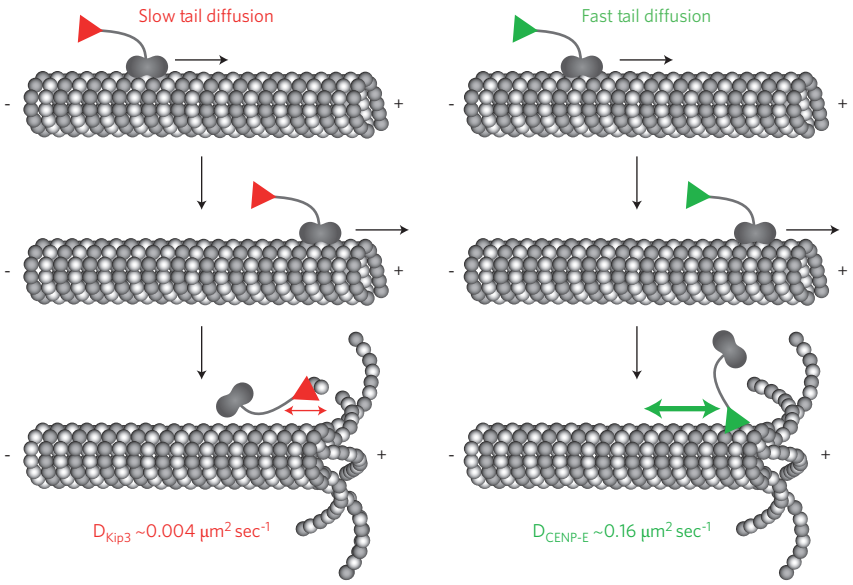

Figure 1 A potential model for the influence of lattice diffusion rate on plus-end-directed molecular motor tip tracking of depolymerizing microtubules. Plus-end-directed molecular motors processively move towards the plus end of a growing microtubule (top and middle). If the microtubule tip switches into a shortening state, a slow-diffusing molecular motor such as Kip3 (tail domain shown as red triangle) may release from the microtubule tip (bottom left). In contrast, a fast-diffusing molecular motor such as CENP-E (green triangle, tail domain) is able to track the depolymerizing microtubule tip, because rapid biased diffusion allows the motor to move away from the shortening tip before dissociating with the departing tubulin subunits (bottom right). Microtubules are in light grey, with tubulin subunits in alternating shades of grey. Plus-end-directed walking motor heads and stalk are in dark grey. Red and green arrows denote diffusion magnitudes and direction.

is critical for CENP-E tracking of depolymerizing microtubule plus ends.

Previous work has implicated rapid biased diffusion in the ability of microtubule-associated proteins or protein complexes to tip-track depolymerizing microtubule plus ends. Specifically, single yeast kinetochore Dam1 complexes, which bind to and diffuse along microtubules with a rapid stepping rate, were shown to diffuse away from a depolymerizing microtubule tip, rather than dissociate with a departing tubulin subunit[8]. This was a result of the slow net tubulin dissociation rate from the microtubule tip relative to the rapid stepping rate of the diffusion of the Dam1 complex (a key kinetochore component) on the microtubule lattice[9,10]. This type of model predicts that non-walking CENP-E motors would tend to diffuse rapidly on the microtubule lattice to facilitate the experimentally observed CENP-E tip-tracking of depolymerizing microtubule plus ends. Furthermore, because Gudimchuk *et al.* found that the CENP-E motor tail domain was critical for the tip-tracking behaviour, the tail alone could possibly diffuse even more rapidly than the full-length motor. Consistent with this prediction, although CENP-E motion was typically characterized by unidirectionally walking molecules, the diffusion coefficient for CENP-E molecules on the microtubule lattice was measured as 0.16 ± 0.01 μm² sec⁻¹ in the present study, which is even faster than the diffusion coefficient previously reported for individual Dam1 complexes (0.083 ± 0.002 μm² sec⁻¹)[8]. Further, the diffusion coefficient for the purified tail domain of CENP-E was an order of magnitude larger than for the full-length protein (1.6 μm² sec⁻¹). However, the tail protein alone did not show processive association with either growing or shortening microtubule ends, probably because of its extremely rapid diffusive excursions both towards and away from the microtubule tip. Regardless, these results support the intriguing idea that diffusion of microtubule-associated proteins can promote tip-tracking of shortening microtubules through biased diffusion away from the depolymerizing tip[10] (Fig. 1). In general, an examination of published diffusion coefficients for various microtubule-associated proteins or complexes also supports this idea, as proteins or complexes that track depolymerizing microtubule plus ends tend to have coefficients that are much larger than those that dissociate from shortening microtubule plus ends rather than track the shortening ends[8,11–14], as is the case for the Kip3 kinesin-8 family member[11]. An exception to this idea is the Ndc80 complex (a kinetochore component), which has reported diffusion coefficients ranging from 0.03 μm² sec⁻¹ (ref. 14) to 0.17 μm² sec⁻¹ (ref. 15) but seems to require multiple complexes for tip tracking depolymerizing microtubule ends[15].

To integrate the CENP-E single-molecule results into a physically constrained model, Gudimchuk *et al.* developed a computational model that was defined by a 'tethered motor' mechanism for CENP-E, and sought to answer whether the cooperative action of a plus-end-directed motor domain, combined with a rapidly diffusing tail domain, could lead to persistent tip tracking of dynamic microtubule tips. Thus, the computational model explicitly tested the idea that repeated cycles of plus-end-directed walking motion alternating with tail-mediated microtubule lattice diffusion could produce tip tracking of simulated dynamic microtubules. In the computational simulations, the fast tail diffusion led to tip tracking, such that diffusive tail-tethered molecular motors could compensate for detaching motor heads at depolymerizing microtubule plus ends to allow for efficient tip tracking. Moreover, a slowly diffusing tail did not efficiently tip track depolymerizing microtubule ends in the simulation, because microtubule disassembly was too rapid relative to the slowly diffusing simulated motor, and so the motors frequently dissociated along with tubulin subunits.

The combination of single-molecule *in vitro* studies with integrative computational modelling work led Gudimchuk *et al.* to conclude that a plus-end-directed motor head domain combined with a rapidly diffusing tail domain provides the CENP-E molecular motor with ideal characteristics to participate in the maintenance of proper end-on attachments at vertebrate kinetochores. Indeed, further *in vivo* results in the study support the hypothesis that CENP-E is important for the maintenance of end-on kinetochore attachments at microtubule plus ends.

Questions remain regarding the role of multiple CENP-E molecules in regulating the *in vivo* dynamics at kinetochore microtubule plus ends. Future work will involve determining whether multiple CENP-E molecules could contribute to robust kinetochore–microtubule attachments in part by stabilizing microtubule

plus-end dynamics, and how multiple CENP-E molecules are integrated with multiple microtubule attachments and other kinetochore-associated proteins. Nevertheless, the work of Gudimchuk *et al.* represents an important step towards elucidating the mechanism for stable kinetochore attachment during mitosis.

COMPETING FINANCIAL INTERESTS
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