of high stress throughout metaphase, as it both pulls on the substrate and resists osmotic swelling[4]. These forces are likely to function in a similar way within a tissue context, as *in vivo* cells generally round-up and divide along their interphase long axis, as they do *in vitro*. Therefore, a change in the balance of tensile forces in a tissue that leads to a distortion in interphase cell shape, for example, through the anisotropic localization of a cortically localized myosin motor[16], can orient the plane of cell division. Moreover, cell–cell[14] and cell–substrate[15] adhesions have been shown to guide spindle positioning in model organisms. Until now, however, direct evidence for a role for external forces acting on the mitotic cell itself has been lacking. It is tempting to speculate based on the results of Fink *et al.* that the forces that induce cell elongation in interphase also function in metaphase to guide spindle positioning in multicellular animal tissues, perhaps through direct distortion of mitotic cell shape. This would enable mitotic cells to rapidly react to changes in the force distribution in tissues undergoing active morphogenesis, causing them to divide along the axis of highest tension to relieve tissue stress. Whether or not this is the case, the study by Fink *et al.* shows that the mitotic actin cortex is a remarkable physical structure which combines mechanosensitivity with morphological robustness to achieve two tasks at once: buffering spindle formation from mechanical perturbations while remaining sensitive to anisotropies in external force to ensure correct spindle alignment.

COMPETING FINANCIAL INTERESTS
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1. Fink, J. *et al. Nat. Cell Biol.* **13,** 771–778 (2011).
2. Heald, R. *et al. Nature* **382,** 420–425 (1996).
3. Kunda, P. & Baum, B. *Trends Cell Biol.* **19,** 174–179 (2009).
4. Stewart, M. P. *et al. Nature* **469,** 226–230 (2011).
5. Toyoshima, F. & Nishida, E. *EMBO J.* **26,** 1487–1498 (2007).
6. Thery, M. *et al. Nature* **447,** 493–496 (2007).
7. Mitsushima, M. *et al. J. Cell Biol.* **191,** 453–462 (2010).
8. Mori, M. *et al., Curr. Biol.* **21,** 606–611 (2010).
9. Woolner, S. *et al. J. Cell Biol.* **182,** 77–88 (2008).
10. Siller, K. H. & Doe, C. Q. *Nat. Cell Biol.* **11,** 365–374 (2009).
11. Redemann, S. *et al. PLoS One* **5,** e12301 (2010).
12. Kimura, K. & Kimura, A. *Proc. Natl. Acad. Sci. USA* **108,** 137–142 (2011).
13. Hamaguchi, M. S. & Hiramoto, Y. *Dev. Growth Differ.* **28,** 143–156 (1986).
14. Gibson, W. T. *et al. Cell* **144,** 427–438 (2011).
15. Fernández-Miñán, A. *et al. Curr. Biol.* **17,** 683–688 (2007).
16. Mao, Y. *et al. Genes Dev.* **25,** 131–136 (2011).

Multi-talented MCAK: Microtubule depolymerizer with a strong grip

Stefan Diez

Microtubule-depolymerizing motor proteins regulate microtubule dynamics during chromosome segregation, but whether they can independently grip the ends of shrinking kinetochore microtubules has remained unresolved. MCAK, a member of the kinesin-13 motor protein family, is now shown to grip microtubules on its own and harness the forces of microtubule disassembly.

The mechanics underlying the faithful segregation of genetic material during cell division rely on the mitotic spindle, which is created by the attachment of microtubules emanating from opposite spindle poles to the chromosome kinetochores. After alignment at the spindle equator, chromosomes segregate by being pulled polewards through their attachment to the ends of the shrinking microtubules. Microtubule shrinkage is thought to occur through 'poleward flux', which involves microtubule depolymerization at the minus ends (at the spindle poles), and through the kinetochores 'chewing up' the microtubules at their plus ends (described as the 'Pac-Man' model)[1]. Among the motor proteins involved in the depolymerization of kinetochore microtubules are members of the kinesin-13 family. As mitotic centromere-associated kinesins (coining the term MCAK[2]) these motors have received increased attention in *in vitro* experiments, where they were shown to diffuse along the microtubule lattice to target both microtubule ends[3], and to induce conformational changes that lead to microtubule catastrophes.

Regardless of the mechanism of microtubule depolymerization, chromosome segregation only proceeds efficiently when substantial forces are generated and when the kinetochores attach strongly to the shrinking microtubule plus ends. In spite of experimental and theoretical progress on these issues (reviewed in refs 4–7), the load-bearing elements in this process remain unknown. On page 846 of this issue, Oguchi *et al.* (ref. 8) now show that MCAK can depolymerize stabilized microtubules while firmly gripping both shrinking ends, thereby potentially contributing to the force required for chromosome segregation during mitosis.

The two prevailing candidate mechanisms for force generation at the kinetochore are based on the biased diffusion and conformational wave models[4–7]. In the biased diffusion model, the kinetochore consists of an array (the 'sleeve') of diffusive anchors where each individual element undergoes rapid binding and unbinding on the microtubule lattice. Because sliding off the microtubule end would mean breaking some or all of these bonds, it is energetically more favourable for the sleeve to bias its diffusion away from the microtubule tip. During microtubule depolymerization, energy gained from this process can thus be converted into mechanical work performed at the kinetochore. In the conformational wave model, kinetochore movement is driven by conformational changes at the microtubule tip, where protofilaments are known to 'curl' as they depolymerize. This curling creates a conformational wave on which the kinetochores can 'surf' by means of ring- or fibril-based coupling structures. Again, energy stored in the straight conformation of tubulin in the microtubule lattice is harnessed to perform work at the kinetochores. Both models allow the estimation of the potential force available from microtubule depolymerization. In

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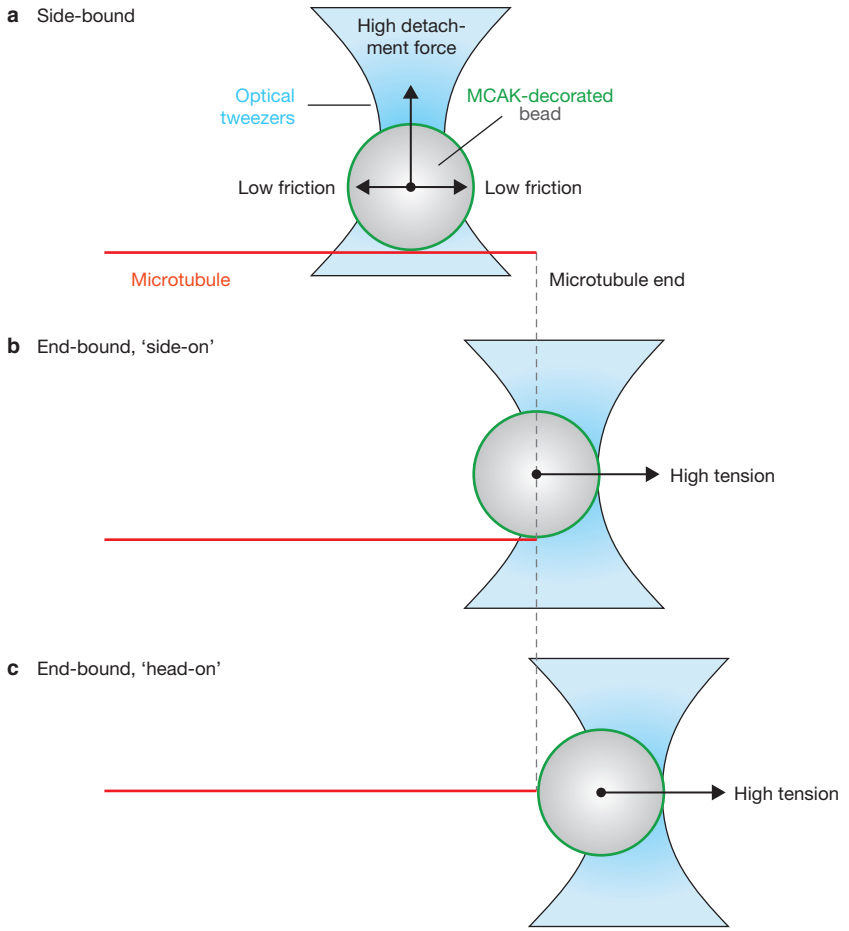

Figure 1 Experimental configuration to measure the forces related to GMPCPP-stabilized microtubule depolymerization by MCAK in the presence of ATP. Optical tweezers are used to manipulate and track MCAK-decorated beads interacting with the lattice or the end of microtubules. **(a)** Side-bound MCAK beads readily slide along the microtubule lattice with low friction, but detach from the microtubule with difficulty in a perpendicular direction. **(b, c)** Two distinct geometrical configurations of MCAK-decorated beads generating high tension through their attachment to the microtubule end. 'Side-on' attachment (**b**) allows bead interaction with the microtubule lattice, whereas 'head-on' attachment (**c**) promotes interaction mainly with the microtubule tip. The number of MCAK molecules capable of microtubule interactions may be up to five times higher in the 'side-on' configuration.

accordance with thermodynamic arguments considering the on and off rates of tubulin dimers during the shrinking of dynamic microtubules, possible stall forces of 30–50 pN have been estimated. Similar force values for individual kinetochore–microtubule interactions were measured *in vitro* and *in vivo*. The alternative idea that strong kinetochore–microtubule attachment and force generation is achieved by the direct action of ATP-hydrolysing, microtubule-depolymerizing motor proteins residing at the kinetochore, has been long discussed, but not directly tested.

Oguchi *et al.*[8] now show that MCAK can grip both ends of shrinking microtubules generating forces of about 1 pN per motor. The authors observed that MCAK-decorated beads held by optical tweezers strongly interacted with the lattices of microtubules reconstituted *in vitro* in the presence of GMPCPP, a slowly hydrolysable GTP analogue (see Fig. 1). The beads were difficult to detach in a direction perpendicular to the long axis of the microtubules, whereas weak external loads applied on-axis forced them to readily slide on the microtubule lattice in either direction. On reaching the microtubule ends, the beads tightly captured these regions and facilitated microtubule disassembly. Detailed analysis of bead displacement associated with microtubule ends revealed a behaviour characteristic of molecular motors under load: the velocity gradually decreased with load and the bead–microtubule attachment ruptured at a force characteristic of the tension maximally generated by the depolymerizing activity of MCAK. In an impressive set of elegant measurements involving polarity-marked microtubules, the authors showed that: (i) the generated tension increased with the number of MCAK molecules per bead, (ii) detachment occurred more frequently from microtubule minus ends than from plus ends and (iii) the depolymerization force was larger at the minus than the plus end. Performing the experiments in 30% glycerol, to stabilize microtubules and to lower protofilament curling, increased the depolymerization force, but significantly lowered the depolymerization rate. These results provide clear evidence that MCAK is capable of performing both tasks required for chromosome segregation: depolymerizing microtubules from both ends and holding on to them, thereby generating force.

The authors propose that the observed tension is generated when an individual MCAK head attaches to the microtubule end and induces protofilament curling. A hypothetical conformational power stroke within the head, related to microtubule binding and/or ATP hydrolysis, then generates tension. To reach processivity, at least one other head needs to attach to the microtubule end before the first one detaches with a bound tubulin dimer. As MCAK is dimeric, individual MCAK molecules may thus act processively. In this model, no microtubule-disassembly-derived energy would be necessary for chromosome segregation. Instead, the forces generated by MCAK's conformational changes would suffice for kinetochore movement.

Challenging the recent view on force generation at kinetochores, this model provides the basis for an alternative mechanism. However, as is often the case, it also calls for a number of exciting follow-up experiments to test its validity. Indeed, it will be important to characterize the role of ATP hydrolysis in the process of tension generation. Protofilament curling[9] and depolymerization[10] of stabilized microtubules can also occur in the presence of AMPPNP, a non-hydrolysable ATP analogue, suggesting that the microtubule lattice destabilization is derived from the MCAK binding to the microtubule. Moreover, under conditions of low free tubulin, even non-ATP-hydrolysing microtubule-associated proteins have been shown to catalytically depolymerize GMPCPP microtubules[11]. As protofilament curling alone can already generate force, is an additional power

stroke necessary? It would therefore be interesting to test different nucleotide conditions, including AMPPNP. It will also be essential to clarify whether the same MCAK molecule facilitates both microtubule depolymerization and tension build-up. To rule out that MCAK operates by a work-sharing principle, where some molecules facilitate depolymerization and others provide the necessary grip onto the microtubule end, single molecule experiments are indispensible. Tethering individual MCAK molecules to a bead with optical tweezers, for example via a DNA-based linker[12], may be one interesting route to pursue. Together with single-molecule fluorescence measurements, possibly under conditions where only a small fraction of motors are labelled, such experiments would also elucidate the processivity of individual MCAK molecules: earlier studies indicate that each MCAK may remove several tubulin dimers from the microtubule end[3,13]. Finally, it will be important to study the requirements on geometry and compliance of the MCAK attachment to the kinetochore. The authors' results do not necessarily mandate a strict 'head-on' attachment of the MCAK-decorated beads to the microtubule ends (Fig. 1c). If such a mechanism were at work, one would expect the extrapolation of the microtubule centre line to always go through the middle of the bead. In contrast, the present data could also be interpreted to support a 'side-on' attachment (Fig. 1b), which could explain how individual MCAK molecules, bound to just one layer of antibodies on the bead, can reach to the outside of a curling protofilament, located potentially tens of nanometres away from the microtubule tip. The rather high MCAK to bead ratios required here, compared with other studies[14], suggest that in the applied geometry MCAK is a relatively poor coupler, perhaps due to a too short and/or too rigid linkage to the beads.

Until these issues are resolved, the results of Oguchi *et al.* may also be consistent with a biased diffusion model, similar to recent reports for Ndc80 (ref. 14) and the heterodimeric fission yeast kinesin-8 KLP5/6FL (ref. 15). Both of these proteins, coupled to beads, were shown to retain load-bearing attachment to the depolymerizing plus ends of dynamic microtubules. In the case of the present results on non-dynamic GMPCPP microtubules[8], individual MCAK molecules may destabilize microtubule ends by forcing them into a curled conformation on binding. Completion of the ATP hydrolysis cycle may then be crucial to release the detached tubulin subunits from the MCAK molecules, allowing MCAK to operate in a catalytic manner. The same MCAK molecules — or others attached to the same bead/kinetochore — may retain the microtubule end attachment by biased diffusion. Nevertheless, in spite of these remaining mechanistic questions, the data of Oguchi *et al.* indicate that MCAK alone is capable of generating force at both microtubule ends, suggesting the exciting possibility that it may contribute to the force-producing systems at the kinetochore–microtubule interface.

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1. Rogers, G. C. *et al. Nature* **427,** 364–370 (2004).
2. Wordeman, L. & Mitchison, T. J. *J. Cell Biol.* **128,** 95–104 (1995).
3. Helenius, J., Brouhard, G., Kalaidzidis. Y., Diez, S. & Howard, J. *Nature* **441,** 115–119 (2006).
4. Civelekoglu-Scholey, G. & Scholey, J. M. *Cell. Mol. Life Sci.* **67,** 2231–2250 (2010).
5. McIntosh, J. R., Volkov, V., Ataullakhanov, F. I. & Grischuck, E. L. *J. Cell Sci.* **123,** 3425–3434 (2010).
6. Asbury, C. L., Tien, J. F. & Davis, T. N. *Trends Cell Biol.* **21,** 38–46 (2011).
7. Joglekar, A. P. & Hunt, A. J. *Biophys. J.* **83,** 42–58 (2002).
8. Oguchi, Y., Uchimura, S., Ohki, T., Mikhailenko, S. V. & Ishiwata, S. *Nat. Cell Biol.* **13,** 846–852 (2011).
9. Desai, A., Verma, S., Mitchison. T. J. & Walczak, C. E. *Cell* **96,** 69–78 (1999).
10. Moores, C. A. *et al. Mol. Cell* **9,** 903–909 (2002).
11. Brouhard, G. J., *et al. Cell* **132,** 79–88 (2008).
12. Guydosh, N. R. & Block, S. M. *Nature* **461,** 125–128 (2009).
13. Cooper, J. R., Wagenbach, M., Asbury, C. L. & Wordeman, L. *Nat. Struct. Mol. Biol.* **17,** 77–82 (2010).
14. Powers, A. F. *et al. Cell* **136,** 865–875 (2009).
15. Grissom, P. M. *et al. Mol. Biol. Cell* **20,** 963–972 (2009).