New and Notable

A Slippery Walk to the Microtubule-End

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Members of the kinesin superfamily of proteins are widely expressed microtubule-based motors that play important roles in intracellular motility and cell division, and have other vital cellular functions. Kinesin motor domains share a high sequence homology, while the tails are very diverse. This striking variability of tails has led to the suggestion that the motors are workhorses targeted to different cargos and cellular locations by their different tails. This picture, however, is now known to be too simplistic. Tailless kinesins show remarkable functional diversity: the dimerized motor heads can determine on their own which direction to walk on the microtubule (MT) lattice, as well as how fast and how far. Minor differences in their structure may also define how they maintain force and work in teams, and whether they walk in a straight path or twirl around the microtubule (1,2).

For more than two decades, single molecule techniques have been used to look under the hood of kinesin motors. Sophisticated laser trapping experiments by Jannasch et al. (3) in this issue of *Biophysical Journal* have opened another fascinating page in this quest. The motile properties of two members of the kinesin-8 family, Kip3 from budding yeast and human Kif18A, have been mechanically interrogated as they walk under opposing or assisting loads. Previous work has established that kinesin-8 molecules are dual-function motors, combining features of the classical MT-based transporter, kinesin-1, and the mitotic depolymerase, kinesin-13, which has completely lost its ability to walk (4). On the microtubule wall, kinesin-13 becomes locked in the ADP-bound state and undergoes one-dimensional diffusion, reaching the ends faster than it would by three-dimensional diffusion in solution. Apparently, this strategy is not good enough for kinesin-8, which, similarly to kinesin-13, can destabilize MT-ends (5). Although Kip3-ADP can diffuse on the MT lattice, the motor reaches the plus-end of microtubules by directional ATP-dependent walking. Unlike kinesin-1, which runs along microtubules for only 1–2 μm before detaching, kinesin-8 molecules travel 10–12 μm, giving this depolymerizing motor "first prize" for distance walking among all of the kinesins. Previous work showed that such processivity is facilitated by the MT-binding tail, but the tailless kinesin-8 is still twofold more processive than kinesin-1 (6,7).

The work by Jannasch et al. (3) now reveals that kinesin-8 walks processively via a series of mini-runs, switching between a regular walking mode and a diffusive mode with weak microtubule affinity. The latter state was not observed previously in the absence of a mechanical load, but it has now been revealed as fast force-induced slipping. The previously characterized processive kinesins usually respond to hindering forces (i.e., applied toward the MT minus-end), by slowing, stalling at 4–7 pN, and detaching. Kinesin-8 also slows down, but under loads even $<$1 pN, it frequently starts slipping backward at 4–10 μm/s, 100-times faster than its normal rate of walking. After slipping for tens of nanometers, the motor then regains its strong binding and resumes walking toward the plus-end. Larger forces can also cause the motor to step backward; forces applied toward the plus-end of the microtubule accelerate the walking, but in both cases the motor continues to slip occasionally in the direction of the force.

Amazingly, during such repeated cycles of slipping and walking, the motor remains attached to the MT, so that Jannasch et al. (3) were able to apply an entire range of loads, up to 3–5 pN, to a single motor during single stick-slip runs. This interesting adaptation of its motility may hold a key to understanding kinesin-8's cellular role, which does not appear to involve transport of any large cargoes. Kinesin-8 is an important regulator of microtubule dynamics, and some members of the family can induce catastrophe in a MT length-dependent manner (5,8). The ability to walk processively to the MT-ends is an essential feature for this regulation, the so-called antenna effect (5). Further molecular and biological aspects of this model will certainly be tested in many future studies. In particular, it remains to be established whether some differences in force-velocity relationships for Kip3 and Kif18A are related to their different efficiencies in depolymerizing stable polymers, and whether these activities can be traced to the specific structural features, such as length and composition of the neck linker or loop 2 within the motor domain (4).

Future work is also required to dissect the molecular mechanism of slipping. According to Jannasch et al. (3), the frequency of slipping is not affected by ADP, so it is unlikely that slipping occurs when both heads have bound ADP. However, the duration of slipping does not depend on load, implying that the slip-state is linked with some intrinsic biochemical transition. Because the slip time distribution could not be fit with a single exponent, several molecular events are involved in switching this mode off. Another interesting finding is that the slip distances occur in increments of 8-nm, the size of the tubulin dimer and the kinesin's footprint on the microtubule

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wall. This suggests that, after recovering from slipping, kinesin-8 lands on its canonical binding site. The slipping velocity, however, was too fast to discriminate if it involved any specific steps.

In this respect, it is interesting that many MT-binding proteins (MAPs) exhibit MT-dependent diffusion, which is likely to rely on different mechanisms. Some MAPs are thought to diffuse in a footprint-free and step-free manner, sliding within a continuous MT-wall associated potential valley, akin to that suggested for the DNA-scanning enzymes (9). The energy landscape of the microtubule surface, however, is likely to be rougher than for the DNA polymer, where the subunit size is only 0.3 nm, so this mechanism of MAP diffusion is controversial (10). Even nondimeric MAPs have been reported to diffuse well on microtubules, assisted by elongated tubulin tails, but the step size and molecular mechanism for these random walks have not yet been established. For a slipping kinesein-8, Jannasch et al. (3) favor a different model in which the load triggers the motor's transitions between the canonical binding sites in a hand-over-hand manner. Thus, it remains to be determined how the slipping of weakly bound motors differs from the diffusion of nonmotor MAPs.

This study highlights the substantial diversity of functions encoded within even quite similar kinesin motor domains, equipped with varying loops, necks, and patches. It also illustrates that, as of this writing, it is not possible to predict how different motors will respond to mechanical loads and biochemical milieu. Earlier work has produced tremendous progress in understanding many biomechanical aspects of classical kinesin-1, but every time one of its relatives is conjugated to beads for a laser trapping assays or is examined by other increasingly sophisticated single molecule methodologies, researchers are almost guaranteed to be surprised by new features and findings. Other recent exciting discoveries include the ability of one kinesin-5 to switch from diffusive motion on single microtubules to directed motion on microtubule bundles (11), and of another kinesin-5 isoform to switch its direction of motion when working alone versus in a team (12). Dynein has also been shown to move bidirectionally, although the physiological switching mechanism is not understood (13,14). Thus, detailed and technically sophisticated interrogations of single kinesin molecules under force and in various environments remain the most straightforward approach to learn how such similar motors have been tailored by nature to carry out their very different cellular functions.

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REFERENCES

1. Marx, A., A. Hoenger, and E. Mandelkow. 2009. Structures of kinesin motor proteins. *Cell Motil. Cytoskeleton.* 66:958–966.
2. Gilbert, S. P., and H. S. Sardar. 2012. Kinesin structure and biochemistry. *In* Comprehensive Biophysics, *Vol. 4*. Elsevier, Dordrecht, the Netherlands, pp. 321–343.
3. Jannasch, A., V. Bormuth, …, E. Shaffer. 2013. *Biophys. J.* 104:2456–2464.
4. Su, X., R. Ohi, and D. Pellman. 2012. Move in for the kill: motile microtubule regulators. *Trends Cell Biol.* 22:567–575.
5. Varga, V., J. Helenius, …, J. Howard. 2006. Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat. Cell Biol.* 8:957–962.
6. Stumpff, J., Y. Du, …, R. Ohi. 2011. A tethering mechanism controls the processivity and kinetochore-microtubule plus-end enrichment of the kinesin-8 Kif18A. *Mol. Cell.* 43:764–775.
7. Su, X., W. Qiu, …, D. Pellman. 2011. Mechanisms underlying the dual-mode regulation of microtubule dynamics by Kip3/kinesin-8. *Mol. Cell.* 43:751–763.
8. Tischer, C., D. Brunner, and M. Dogterom. 2009. Force- and kinesin-8-dependent effects in the spatial regulation of fission yeast microtubule dynamics. *Mol. Syst. Biol.* 5:250.
9. Cooper, J. R., and L. Wordeman. 2009. The diffusive interaction of microtubule binding proteins. *Curr. Opin. Cell Biol.* 21:68–73.
10. Grishchuk, E. L., J. R. McIntosh, …, F. I. Ataullakhanov. 2012. Force generation by dynamic microtubule polymers. *In* Comprehensive Biophysics, *Vol. 4*. Elsevier, Dordrecht, Netherlands, pp. 93–117.
11. Kapitein, L. C., B. H. Kwok, …, E. J. Peterman. 2008. Microtubule cross-linking triggers the directional motility of kinesin-5. *J. Cell Biol.* 182:421–428.
12. Roostalu, J., C. Hentrich, …, T. Surrey. 2011. Directional switching of the kinesin Cin8 through motor coupling. *Science.* 332:94–99.
13. Ross, J. L., K. Wallace, …, E. L. Holzbaur. 2006. Processive bidirectional motion of dynein-dynactin complexes in vitro. *Nat. Cell Biol.* 8:562–570.
14. Walter, W. J., M. P. Koonce, …, W. Steffen. 2012. Two independent switches regulate cytoplasmic dynein's processivity and directionality. *Proc. Natl. Acad. Sci. USA.* 109:5289–5293.