Mitotic kinesins in action: diffusive searching, directional switching, and ensemble coordination

Allison M. Gicking[a], Weihong Qiu[a,b,*], and William O. Hancock[c,d,*]
[a]Department of Physics and [b]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331; [c]Department of Biomedical Engineering and [d]Intercollege Graduate Degree Program in Bioengineering, Penn State University, University Park, PA 16802

ABSTRACT **Mitotic spindle assembly requires the collective action of multiple microtubule motors that coordinate their activities in ensembles. However, despite significant advances in our understanding of mitotic kinesins at the single-motor level, multi-motor systems are challenging to reconstitute in vitro and thus less well understood. Recent findings highlighted in this perspective demonstrate how various properties of kinesin-5 and -14 motors—diffusive searching, directional switching, and multivalent interactions—allow them to achieve their physiological roles of cross-linking parallel microtubules and sliding antiparallel ones during cell division. Additionally, we highlight new experimental techniques that will help bridge the gap between in vitro biophysical studies and in vivo cell biology investigations and provide new insights into how specific single-molecule mechanisms generate complex cellular behaviors.**

Monitoring Editor
Keith G. Kozminski
University of Virginia

Received: Feb 2, 2018
Revised: Mar 15, 2018
Accepted: Mar 16, 2018

INTRODUCTION

Consider the forces that drive vesicle transport and mitotic spindle assembly in eukaryotic cells. These forces are generated by teams of microtubule motors that work either in concert, to achieve long-distance directional transport, or in opposition to control mitotic spindle morphology (Derr *et al.*, 2012; Furuta *et al.*, 2013; Shimamoto *et al.*, 2015). Despite the many advances in our understanding of motor mechanisms at the single-molecule level, how multiple motors of same or opposite directionality work in teams is still poorly understood due to a lack of techniques for studying motor ensembles in vitro. Kinesin-5 motors are antiparallel tetramers that provide outward pushing forces to separate duplicated poles during spindle formation and inhibition of kinesin-5 blocks spindle formation (Kapoor *et al.*, 2000). Kinesin-14 motors are dimers containing C-terminal motor domains that stabilize microtubule bundles, and mutants alter mitotic spindle structure and chromosome movements (Hatsumi and Endow, 1992). Although their structures and directionalities differ (Figure 1), motors in the kinesin-5 and kinesin-14 families share the property of working in teams to achieve their physiological roles of cross-linking and sliding microtubules. In this perspective, we explore mechanical properties unique to these two families that allow them to work effectively in teams and highlight new methods for further understanding motor actions in ensembles.

DIFFUSIVE BEHAVIOR

Processive motors, such as conventional kinesin, step along microtubules by alternating power strokes in each head that enable them to move to the next binding site. However, it is established that some motors can achieve long-distance motility without exclusively taking multiple discrete steps via this hand-over-hand mechanism. For example, the Hirokawa lab showed that a monomeric kinesin-3/KIF1A could achieve processive motility through a combination of ATP-fueled steps and diffusion along the microtubule enabled by the positively charged Loop12 of the motor interacting with the negatively charged C-terminal tail of tubulin (Hirokawa, 1998). Dynein/dynactin complexes have been shown to diffuse along microtubules under some conditions through similar electrostatic interactions (Culver–Hanlon *et al.*, 2006). Members of the kinesin-5 family are primarily processive but can also exhibit diffusive behavior, likely mediated through the microtubule-binding tail domain

DOI:10.1091/mbc.E17-10-0612

*Address correspondence to: William O. Hancock (wohbio@engr.psu.edu) or Weihong Qiu (Weihong.Qiu@physics.oregonstate.edu).

Abbreviation used: ATP, adenosine triphosphate.

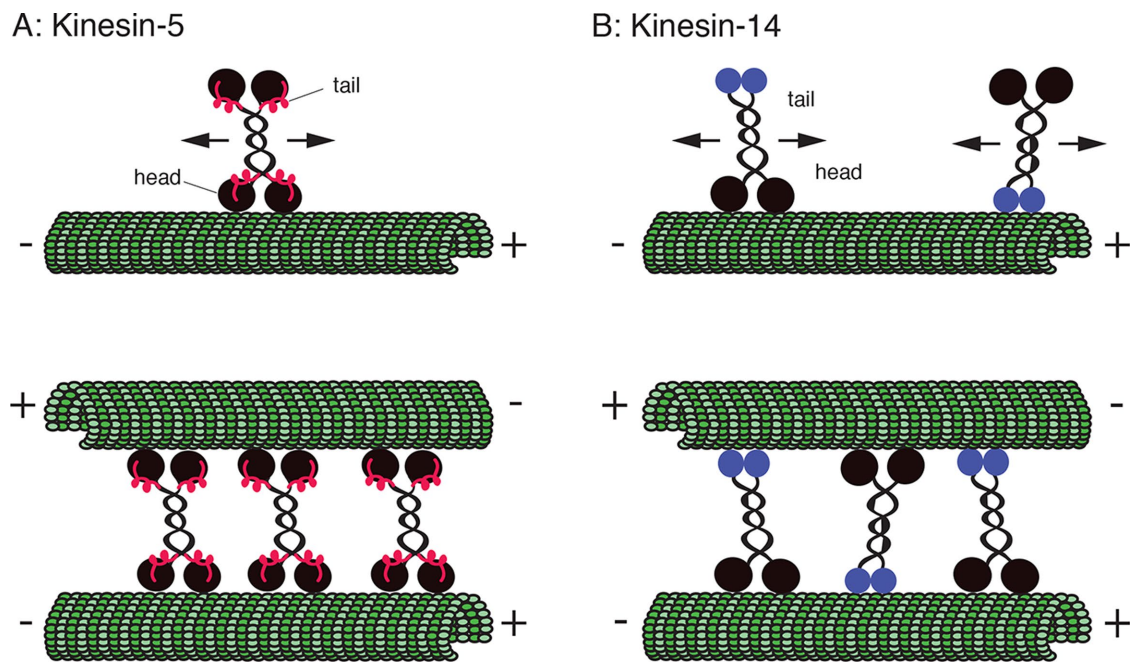

FIGURE 1: Activities of kinesin-5 and kinesin-14 motors in isolation and in teams. (A) Individual kinesin-5 interacts with microtubules through one pair of heads with possible contribution of tail domains. Vertebrate kinesin-5 displays processive plus-end movement, but some fungal kinesin-5s display minus-end motility as single molecules. Teams of kinesin-5 slide antiparallel microtubules apart with plus-end directionality. (B) Individual kinesin-14 motors can diffuse along microtubules through either their head or microtubule-binding tail domains. In teams, kinesin-14s cross-link parallel bundles and slide apart antiparallel bundles through the action of the heads interacting with one filament and the tails interacting with the other filament.

(Kapitein *et al.*, 2008; Weinger *et al.*, 2011). Individual kinesin-14 family motors are generally nonprocessive, which is commonly attributed to a lack of coordination between the two motor domains (deCastro *et al.*, 2000). However, two kinesin-14 motors have been shown to exhibit processive motility: Kar3, which forms a heterodimer with noncatalytic subunits Vik1 or Cik1 and achieves processive minus-end-directed motility through diffusive activity of the noncatalytic motor domain (Molodtsov *et al.*, 2016), and KlpA, which achieves processive plus-end-directed movement through diffusive tethering of an N-terminal microtubule-binding tail (Popchock *et al.*, 2017). Thus, even at the single-molecule level, combining diffusive nonmotor domains with force-generating motor domains provides novel motor properties.

Recent work provides an emerging picture of how diffusive properties of kinesin-5 and kinesin-14 are related to their function of cross-linking and sliding microtubules within the mitotic spindle. Because kinesin-5 is an antiparallel tetramer, each end contains motor and tail domains in close proximity (Figure 1A). Work from the Kapoor lab suggests that the nonmotor microtubule-binding tail of kinesin-5 functions to maintain the motor-microtubule interaction while the motor domains diffusively search for optimal binding orientation (Weinger *et al.*, 2011). Consistent with this, Eg5 diffuses on single microtubules but switches to ATP-dependent directional motion once it is engaged between two microtubules (Kapitein *et al.*, 2008). For the kinesin-14 Ncd, the motor and tail domains, which reside on opposite ends of the homodimer, can either bind to the same microtubule or interact with two different microtubules (Figure 1B). In bundles, the motor is able to both slide apart antiparallel microtubules and cross-link parallel microtubules (Braun *et al.*, 2009; Fink *et al.*, 2009). Furthermore, when a Ncd-laden microtubule encounters a new microtubule, the motors are able to redistribute themselves to the overlap zone, thus initiating and strengthening intermicrotubule links. This plasticity provides an explanation for the observed biophysical properties of the motor—because the heads are non- or minimally processive and the tail binds diffusively and reversibly to the microtubule, they can dynamically detach and reattach to the same or different microtubule in a bundle. The kinesin-5 tail likely provides an analogous function of maintaining association of the heads with the microtubule to maximize its cross-linking abilities. Thus, diffusive binding by the tail domains appears to be vital to the intracellular function of kinesin-5 and -14 motors.

BIDIRECTIONAL MOVEMENT

The canon of kinesin directionality is that kinesins with N-terminal motor domains are exclusively plus-end directed and those with C-terminal motor domains are minus-end-directed (Henningsen and Schliwa, 1997). This canonical view broke down with recent findings that fungal kinesin-5s can move bidirectionally despite having an N-terminal motor domain (Gerson-Gurwitz *et al.*, 2011; Roostalu *et al.*, 2011; Edamatsu, 2014). Studies of Cin8 from *Saccharomyces cerevisiae* demonstrated that whereas individual Cin8 molecules are minus-end directed, ensembles generate plus-end-directed motility (Gerson-Gurwitz *et al.*, 2011; Roostalu *et al.*, 2011). It is suggested that in dividing yeast cells, the result of minus-end movement of individual motors toward the poles is, when microtubules from the opposite pole are captured, the clustered motors switch to plus-end motility to drive spindle pole separation (Shapira *et al.*, 2017). A recent study on Cut7 from *Schizosaccharomyces pombe* showed similarly that small teams in gliding assays generated minus-end-directed movement, whereas large teams generated plus-end movement (Britto *et al.*, 2016). On the basis of this observation, Britto *et al.* (2016) propose a lattice-crowding model in which motion

toward the minus end, which dominates at low motor densities, becomes increasingly constrained by neighbors under crowded conditions, and forces the motors to switch directions. Relevant to this crowding model, recent work from the Surrey lab showed that Cin8 ensembles in a gliding assay configuration are able to generate forces in both plus- and minus-end directions, implying that bidirectional stepping is an active process inherent to the motor domain and not simply a consequence of diffusive activity (Fallesen *et al.*, 2017). There is no structural explanation for this motor activity to date, but the finding that the Eg5 neck linker domain can dock in both forward- and rearward-facing configurations may provide a clue (Goulet *et al.*, 2014).

Interestingly, directional switching also extends to the kinesin-14 family. KlpA from *Aspergillus nidulans* displays canonical minus-end-directed motility in multimotor assays but switches to plus-end-directed processive motility on single microtubules (Popchock *et al.*, 2017). Thus, like fungal kinesin-5, the canonical directionality of KlpA is achieved only when motors are working in groups, and it is only under single-molecule conditions where the opposite directionality is observed. Considering their mitotic role in cross-linking and sliding spindle microtubules, it may be desirable that the directionality of these mitotic kinesin-5 and -14 motors are sensitive to changes in motor density, as it provides enhanced functionality that can be easily regulated via concentration. This interplay between motor structure and motor coupling in bidirectional movement will be an important area of study for understanding the molecular mechanism and physiological role of this behavior.

METHODS FOR STUDYING MOTOR ENSEMBLES IN VITRO

A typical single-molecule in vitro assay, in which individual motors walk along microtubules with no load, provides valuable information about the inherent motor properties but is not an accurate representation of their group behavior in vivo. Ensemble behavior can be investigated using microtubule-gliding assays, where the cargo-binding tail is immobilized on a glass coverslip and the heads are free to walk, but this assay provides no external load. More recently, force measurements of motor ensembles have been made by immobilizing one microtubule on the surface, attaching a bead held in an optical trap to a second microtubule, and allowing motors to cross-link and slide the microtubule pair. One finding from these studies is that teams of the mitotic motor kinesin-12 (KIF15) generate ensemble forces very similar to its single-molecule stall force, whereas kinesin-5 (Eg5) teams can work together to generate much larger ensemble forces (Shimamoto *et al.*, 2015; Reinemann *et al.*, 2017).

However, a drawback of this ensemble approach is the difficulty in controlling the number and orientation of bound motors. A technique that provides a more precise tool for arranging defined numbers of motors in a defined geometry is DNA origami. These structures can be as simple as a DNA linker between two motors or as complex as a bundle of helices containing multiple single-stranded DNA overhangs to which motors can be attached (Derr *et al.*, 2012; Furuta *et al.*, 2013; Feng *et al.*, 2018). A recent study found that for Ncd, the average stall force scaled with the number of motors bound to the scaffold, whereas it did not for kinesin-1 in the same geometry (Furuta *et al.*, 2013). Additional work on the ensemble behavior of other kinesin families, as well as how the relative motor geometry affects collective kinesin transport remains to be done.

Traditional microtubule-gliding experiments also fail to account for the diffusive nature of the cargo-tail interaction. A large proportion of kinesin-driven transport in cells is that of membrane-bound vesicles and organelles, where the cargo-binding tail domain is able to diffuse in the plane of the bilayer; behavior that is distinct from both static attachment of tails to glass and one-dimensional diffusion of tails along other microtubules. In recent work, diffusive tail interactions have been reproduced in vitro by reconstituting motility assays on immobilized lipid bilayers. Interestingly, in contrast to traditional gliding assays on glass, the gliding velocity of microtubules driven by teams of kinesin-1 motors bound to an immobilized lipid bilayer increases with increasing motor density (Grover *et al.*, 2016). In this two-dimensional geometry, the size of the motor team matters because there is a balance between the viscous drag forces of the microtubule moving through the solution and the motors moving in the opposite direction through the lipid bilayer; increasing motor densities means greater motor viscous forces and thus faster microtubule speeds. Deformation of lipid vesicles in three dimensions is similarly an interplay between motor forces and membrane rigidity. When a motor-functionalized giant unilamellar vesicle (GUV) is placed on a network of microtubules, the ability of motors to pull out thin membrane tubes depends on coordination of multiple motors working asynchronously on multiple protofilaments (Campàs *et al.*, 2008). Recently, the GUV system was expanded to using both myosin and kinesin motors, where it was found that motor deformation involved coordination of the two motor types to generate antagonistic forces and that adding lipid binding proteins or increasing membrane fluidity led to greater deformations and membrane tube formation (McIntosh *et al.*, 2018). Overall, these new methods highlight how new in vitro approaches are increasingly able to capture key features of cellular systems and narrow the gap between in vitro biophysics and cell biology.

LOOKING TO THE FUTURE

In vitro studies of the mitotic kinesin-5 and kinesin-14 families reveal two common features of their motility: diffusion and directional switching. These behaviors appear to be advantageous for their respective roles in microtubule cross-linking and sliding, which require cooperation of multiple motors. Moreover, these behaviors are likely not unique to these two families. Some outstanding questions include: What aspects of the mechanochemical cycles are tuned to allow motors to switch directions? What aspects of the mechanochemical cycle allow some motors to sum their forces in teams while others cannot? How are binding affinities of tail domains tuned to achieve both diffusive characteristics and the ability to sustain mechanical forces? Answering these questions will require a combined effort of in vitro reconstitution and cellular investigations, as well as the application of new experimental tools for understanding motor ensembles. To understand how teams of motors carry out their cellular functions, it will be important to understand both how assemblies of similar motors work together and also how structurally divergent motors coordinate their activities to generate multimotor microtubule sliding, cross-linking, and transport.

ACKNOWLEDGMENTS

W.Q. is supported by the National Science Foundation (MCB-1616462), and W.O.H. is supported by the National Institutes of Health (R01 GM076476).

REFERENCES

Braun M, Drummond DR, Cross RA, McAinsh AD (2009). The kinesin-14 Klp2 organizes microtubules into parallel bundles by an ATP-dependent sorting mechanism. Nat Cell Biol 11, 724–730.

Britto M, Goulet A, Rizvi S, von Loeffelholz O, Moores CA, Cross RA (2016). Schizosaccharomyces pombe kinesin-5 switches direction using a steric blocking mechanism. Proc Natl Acad Sci USA 113, E7483–E7489.

Campàs O, Leduc C, Bassereau P, Casademunt J, Joanny J-F, Prost J (2008). Coordination of kinesin motors pulling on fluid membranes. Biophys J 94, 5009–5017.
Culver-Hanlon TL, Lex SA, Stephens AD, Quintyne NJ, King SJ (2006). A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. Nat Cell Biol 8, 264–270.
deCastro MJ, Fondecave RM, Clarke LA, Schmidt CF, Stewart RJ (2000). Working strokes by single molecules of the kinesin-related microtubule motor ncd. Nat Cell Biol 2, 724–729.
Derr ND, Goodman BS, Jungmann R, Leschziner AE, Shih WM, Reck-Peterson SL (2012). Tug-of-war in motor protein ensembles revealed with a programmable DNA origami scaffold. Science 338, 662–665.
Edamatsu M (2014). Bidirectional motility of the fission yeast kinesin-5, Cut7. Biochem Biophys Res Commun 446, 231–234.
Fallesen T, Roostalu J, Duellberg C, Pruessner G, Surrey T (2017). Ensembles of bidirectional kinesin Cin8 produce additive forces in both directions of movement. Biophys J 113, 2055–2067.
Feng Q, Mickolajczyk KJ, Chen G-Y, Hancock WO (2018). Motor reattachment kinetics play a dominant role in multimotor-driven cargo transport. Biophys J 114, 400–409.
Fink G, Hajdo L, Skowronek KJ, Reuther C, Kasprzak AA, Diez S (2009). The mitotic kinesin-14 Ncd drives directional microtubule–microtubule sliding. Nat Cell Biol 11, 717–723.
Furuta K, Furuta A, Toyoshima YY, Amino M, Oiwa K, Kojima H (2013). Measuring collective transport by defined numbers of processive and nonprocessive kinesin motors. Proc Natl Acad Sci 110, 501–506.
Gerson-Gurwitz A, Thiede C, Movshovich N, Fridman V, Podolskaya M, Danieli T, Lakämper S, Klopfenstein DR, Schmidt CF, Gheber L (2011). Directionality of individual kinesin-5 Cin8 motors is modulated by loop 8, ionic strength and microtubule geometry. EMBO J 30, 4942–4954.
Goulet A, Major J, Jun Y, Gross SP, Rosenfeld SS, Moores CA (2014). Comprehensive structural model of the mechanochemical cycle of a mitotic motor highlights molecular adaptations in the kinesin family. Proc Natl Acad Sci USA 111, 1837–1842.
Grover R, Fischer J, Schwarz FW, Walter WJ, Schwille P, Diez S (2016). Transport efficiency of membrane-anchored kinesin-1 motors depends on motor density and diffusivity. Proc Natl Acad Sci USA 113, E7185–E7193.
Hatsumi M, Endow SA (1992). The Drosophila ncd microtubule motor protein is spindle-associated in meiotic and mitotic cells. J Cell Sci 103, 1013–1020.
Henningsen U, Schliwa M (1997). Reversal in the direction of movement of a molecular motor. Nature 389, 93–96.
Hirokawa N (1998). Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279, 519–526.
Kapitein LC, Kwok BH, Weinger JS, Schmidt CF, Kapoor TM, Peterman EJG (2008). Microtubule cross-linking triggers the directional motility of kinesin-5. J Cell Biol 182, 421–428.
Kapoor TM, Mayer TU, Coughlin ML, Mitchison TJ (2000). Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. J Cell Biol 150, 975–988.
McIntosh BB, Pyrpassopoulos S, Holzbaur ELF, Ostap EM (2018). Opposing kinesin and myosin-I motors drive membrane deformation and tubulation along engineered cytoskeletal networks. Curr Biol 28, 236–248.e5.
Molodtsov MI, Mieck C, Dobbelaere J, Dammermann A, Westermann S, Vaziri A (2016). A force-induced directional switch of a molecular motor enables parallel microtubule bundle formation. Cell 167, 539–552.e14.
Popchock AR, Tseng K-F, Wang P, Karplus PA, Xiang X, Qiu W (2017). The mitotic kinesin-14 KlpA contains a context-dependent directionality switch. Nat Commun 8, 13999.
Reinemann DN, Sturgill EG, Das DK, Degen MS, Vörös Z, Hwang W, Ohi R, Lang MJ (2017). Collective force regulation in anti-parallel microtubule gliding by dimeric Kif15 kinesin motors. Curr Biol 27, 2810–2820.e6.
Roostalu J, Hentrich C, Bieling P, Telley IA, Schiebel E, Surrey T (2011). Directional switching of the kinesin Cin8 through motor coupling. Science 332, 94–99.
Shapira O, Goldstein A, Al-Bassam J, Gheber L (2017). Possible physiological role for bi-directional motility and motor clustering of the mitotic kinesin-5 Cin8. J Cell Sci 130, 725–734.
Shimamoto Y, Forth S, Kapoor TM (2015). Measuring pushing and braking forces generated by ensembles of kinesin-5 crosslinking two microtubules. Dev Cell 34, 669–681.
Weinger JS, Qiu M, Yang G, Kapoor TM (2011). A nonmotor microtubule binding site in kinesin-5 is required for filament crosslinking and sliding. Curr Biol 21, 154–160.