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Kinesin-5: A Team Is Just the Sum of Its Parts

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How the cell builds a spindle remains an open question. In this issue of *Developmental Cell*, Shimamoto, Forth, and Kapoor (2015) show that kinesin-5 motor ensembles can exert sliding forces that scale with microtubule overlap length. This behavior could allow microtubule architecture-dependent modulation of force and contribute to spindle self-organization.

The bipolar structure of the spindle is critical to its function: two poles dictate chromosome segregation into two groups, for two daughter cells. How do the cellular-scale forces that shape this bipolar structure emerge from molecular-scale forces? The tetrameric motor kinesin-5 slides microtubules outward at antiparallel overlaps to push spindle poles apart, promoting bipolarity; it also crosslinks parallel microtubules near spindle poles. Beautiful single-molecule work has revealed detailed kinesin-5 motor mechanics (Kapitein et al., 2005, 2008; van den Wildenberg et al., 2008), but it remains poorly understood how the functions of a single kinesin-5 motor scale up with multiple motors working at long microtubule overlaps. Indeed, understanding how molecular-scale forces give rise to diverse cellular-scale architectures—and are regulated by them—remains a frontier due to the large gap in scale. New work by Shimamoto, Forth, and Kapoor (2015) uses well-defined microtubule overlaps (Figure 1) to show that kinesin-5 ensembles can generate forces that scale linearly with the length of microtubule overlap and motor number. This scaling provides a mechanism by which spindle architecture can regulate force generation—a key ingredient for spindle self-organization.

A large gap persists between cellular and single-molecule studies of kinesin-5. For example, in cells kinesin-5 acts at both antiparallel and parallel microtubules and as part of protein complexes, and we do not have the tools to separate the contributions of these different kinesin-5 populations in vivo (Uteng et al., 2008). Conversely, it is difficult to map in vitro work on individual motors and single microtubules onto our understanding of a spindle structure containing thousands of both. Working to close this gap from the bottom up, single-molecule studies of kinesin-5 crosslinking and sliding two microtubules in a microtubule "sandwich" (Kapitein et al., 2005, 2008; van den Wildenberg et al., 2008) brought us closer to biological geometry than motor-bead experiments. However, we do not understand how ensembles of multiple crosslinking motors work together in microtubule overlaps of different lengths. Here, Shimamoto, Forth, and Kapoor (2015) address this question with a high level of technical control: they use "control dials" to dynamically modulate the architecture of microtubule sandwiches (orientation, overlap length, relative velocity) and develop a method to precisely measure the number of motors mechanically engaged within a sandwich. One microtubule in the sandwich is attached to an optical trap, allowing the authors to measure, for example, the pushing force generated by three versus six motors sliding antiparallel microtubules, or the braking force generated by six motors crosslinking parallel microtubules moved at different velocities.

This assay allows Shimamoto, Forth, and Kapoor (2015) to probe feedback between microtubule architecture—both geometry and velocity—and motor function. They find that ensembles of kinesin-5 motors within antiparallel microtubule pairs push microtubules apart with force that scales with motor number and microtubule overlap length (Figure 1, green). When antiparallel microtubules are pulled apart by the authors more quickly than motors can step, motors instead oppose microtubule motion, and this braking force increases linearly with overlap length. When parallel microtubules are pulled apart, motors similarly resist this sliding (Figure 1, red); braking force increases with overlap length (and, thus, number of motors) and decreases with microtubule speed. Motors can bind and step effectively within stationary parallel microtubule overlaps, producing no processive microtubule sliding but constantly creating active fluctuations. The authors develop a computational model that recapitulates their major findings and suggests that kinesin-5 does not change its stepping kinetics when crosslinking microtubules in different orientations. Microtubule architecture does not regulate individual kinesin-5 motor mechanochemistry, but it regulates the total force applied back to the structure by an ensemble of motors.

Altogether, this work indicates that kinesin-5 ensembles can act as a "converter," translating geometric features such as microtubule orientation and overlap length into a defined force signature. Without any specialized regulation, the same kinesin-5 molecule can in principle supply different activities depending on position in the spindle (e.g., whether it crosslinks fast-moving antiparallel bundles in the central spindle or more synchronized parallel bundles near poles). Notably, kinesin-5's ability to produce ensemble forces that scale linearly with the number of engaged motors is unusual among kinesins (Furuta et al., 2013). Linear scaling implies that each

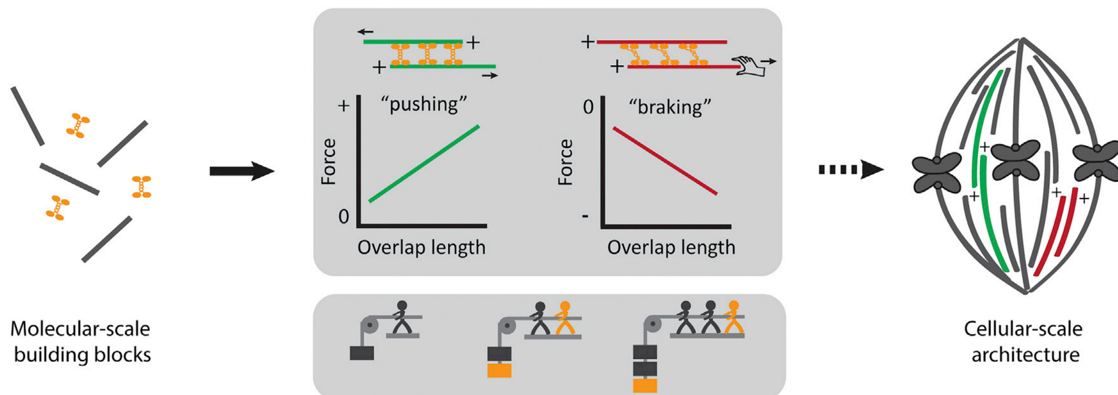

Figure 1. Microtubule Overlap Length Regulates Active Force Generation
Shimamoto, Forth, and Kapoor (2015) use an in vitro assay to map how microtubule architecture regulates force generation by kinesin-5 ensembles, dissecting two minimal architectural modules in the spindle. They show that motors linking antiparallel microtubules (green) generate pushing forces that scale with microtubule overlap and motor number and that motors linking parallel microtubules (red) can exert braking forces with the same scaling. Thus, for the numbers of kinesin-5 (people) probed, the force (weight lifted) generated by each motor is independent of the total number of motors acting in the team.

kinesin-5 in a microtubule overlap does not affect the force production of its neighbors (Figure 1, pulley system where the team is just the sum of its parts) and that kinesin-5's mechanics allow these forces to be transmitted across microns. The modeling work of Shimamoto, Forth, and Kapoor (2015) suggests that kinesin-5's particular detachment rate and the compliance of its tetramerization domain are important for linear integration of forces generated by multiple motor molecules. Moving forward, it will be exciting to experimentally vary these two parameters and probe the effect on force output scaling and on kinesin-5 function in spindles.

The findings of Shimamoto, Forth, and Kapoor (2015) add to a growing list of microtubule length-dependent forces that position and build the spindle: for example, length-dependent pulling forces position centrosomes in large cells (Hamaguchi and Hiramoto, 1986), depolymerizing kinesins can preferentially shorten long microtubules (Varga et al., 2006; Bieling et al., 2010), and non-motor MAPs (microtubule-associated proteins) can resist microtubule sliding in an overlap length-dependent manner (Braun et al., 2011). How length-dependent kinesin-5 force scaling contributes to spindle structure or function remains an open question, but it is tempting to speculate that such regulation could promote homeostatic regulation of spindle mechanics. In cells, however, kinesin-5 force scaling is likely complicated by higher motor numbers and by the network of other motor and non-motor MAPs present in microtubule overlaps. It will be interesting to see how the addition of these other players affects the force "converter" code of kinesin-5 ensembles. Indeed, protein composition will define each "converter" code, allowing specialized regulation of architecture-to-force feedback and opportunities for structural diversity. The work of Shimamoto, Forth, and Kapoor (2015) provides a conceptual and technical framework for future steps toward cellular complexity. Looking forward, the continued development of tunable in vitro cytoskeletal architectures will narrow the gap between molecular- and cellular-scale mechanics and provide insight into how self-organization builds the cell's structures.

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