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Kinesin Motors: No Strain, No Gain

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The processive movement of the dimeric motor protein kinesin 1 along microtubules requires communication between the two motor domains. Yildiz et al. (2008) now show that tension between the motor domains not only is necessary for normal processivity but also may be sufficient for motor motility under some conditions.

Members of the kinesin superfamily of motor proteins are remarkable nanomachines. Most kinesins use the chemical energy stored in ATP to produce directed force along microtubule protofilaments, powering critical cellular processes such as vesicle transport and chromosome segregation (Vale, 2003). Other kinesin family members do not act directly as motors but rather regulate microtubule dynamics. Remarkably, some members of the kinesin family, such as dimeric “conventional” kinesin 1, move processively along their protein tracks by coordinating their two motor domains in a hand-over-hand manner. Kinesin 1 is able to take hundreds of 8 nm steps without falling off, even while pulling a substantial load, thus ensuring that diffusion does not remove the motor and its crucial cargo from the track. This processivity is dependent upon one kinesin motor domain being attached to the microtubule at all times. It remains unknown how exactly processive kinesins coordinate the activities of their two motor domains such that one domain always remains attached to the microtubule. In this issue of *Cell*, Yildiz and colleagues present an elegant study

that clarifies this question and uncovers some remarkable features of the kinesin motor.

The molecular architecture of dimeric kinesin 1 partially explains how it might achieve the feat of processivity. Each monomer of kinesin 1 is composed of a core motor domain of some 350 amino acids containing the ATPase catalytic site as well as microtubule-binding sites. Adjacent to the motor domain is the neck linker, a flexible region that has been shown to undergo a nucleotide-dependent transition from a disordered to an ordered structure. The linker is followed by a coiled-coil dimerization domain. Thus, kinesin 1 has two “feet” (the motor domains) connected to each other by a flexible linker that can change conformation and that is long enough to allow the two motor domains to bind to adjacent sites on the microtubule, 8 nm apart. The structure of kinesin 1 allows it to “walk” along the microtubule filaments. Intermotor domain (interhead) communication is known to be necessary for processive movement, but how this communication occurs is unclear. Most theories posit that communication occurs through a “gating” mechanism where a mecha-

nistic step in one head is blocked until a certain step is taken in the other head (reviewed in Block, 2007). Such gating could be chemical in nature, e.g., ATP binding is blocked until a head dissociates from the microtubule (Klumpp et al., 2004; Rosenfeld et al., 2003), or mechanical in nature, e.g., a conformational change in one head pulls, or pushes, the other head off the microtubule (Hancock and Howard, 1999; Spudich, 2006). Of course, it is more than likely that the actual mechanism of interhead communication utilizes both types of gating, as they are not mutually exclusive.

In their new work, Yildiz et al. (2008) used mutant kinesin 1 molecules and optical trapping microscopy to observe how altering the length of the neck linker, and thereby the tension between the heads, affects gating and hence kinesin motility. Several interesting and unexpected results emerged from this study. In the first set of experiments, the authors inserted progressively larger polyproline helices between the linker region and the dimerization domain. When two heads of wild-type kinesin 1 are bound to the microtubule, the native linkers are more or less fully extended.

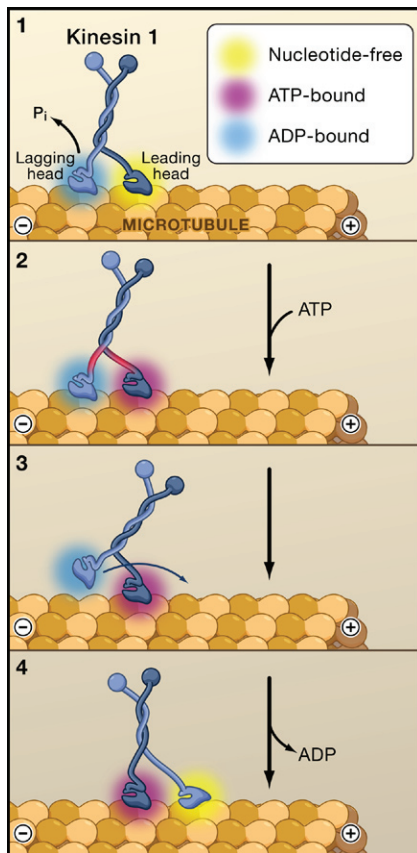


Figure 1. Interhead Tension Is Critical for Kinesin Stepping

Kinesin 1 motility cycle during a processive run along a microtubule protofilament. (1) Release of inorganic phosphate (P_i) from the lagging motor domain (lagging head) of the dimeric kinesin 1 results in a head that is weakly bound to the microtubule. (2) ATP binding to the leading head results in a conformational change (neck-linker docking) that generates interhead tension across the linker regions (red). (3) This tension promotes the detachment of the lagging motor domain (lagging head) from the microtubule and a biased diffusion-based search (arrow) for the next microtubule-binding site. (4) When the heads have swapped positions on the microtubule, the new leading head binds tightly to the forward microtubule-binding site and releases its bound ADP. The lagging head undergoes ATP hydrolysis (step not shown) and releases P_i to continue the cycle.

Thus, it is thought that tension between the two heads could provide a gating mechanism. By extending linker length, tension between the heads should be diminished or eliminated. If linker tension alone is responsible for ensuring processivity, one would expect that the mutant kinesin molecules with longer linkers

would no longer be able to walk processively. Surprisingly, the authors still observe processive movement by the mutant kinesin 1 molecules, although movement along the microtubule is much slower. This suggests that linker tension is required for efficient movement. The decrease in movement speed could be due to an increase in the number of futile “nonsteps” or in the number of abnormal steps, such as steps of the wrong size or direction or steps along a different protofilament.

Interestingly, the motility of these crippled kinesin 1 molecules can be rescued by pulling them in the correct direction. Using an optical trap, the authors applied external tension to a single kinesin molecule, pulling it along the microtubule. Under these conditions, the velocity of even the longest linker mutants now resembled that of wild-type kinesin 1. This result provides strong evidence that tension between the two heads is critical for efficient processive movement (Figure 1). Furthermore, due to the geometry of the optical trap, the applied external tension is felt more by the lagging head than the leading head, suggesting that during the normal mechanochemical cycle of kinesin 1, the lagging head is pulled off the microtubule by the leading head.

Another remarkable result presented by Yildiz et al. is that kinesin can take discrete steps in the absence of ATP if pulled along with an external load. Although this seems improbable given that kinesin requires ATP hydrolysis in order to power movement, it is becoming clear that ATP binding and hydrolysis serve only to bias the direction of the kinesin steps—formation of a tight motor-microtubule interface provides the majority of energy for force generation. In the absence of ATP, an externally applied load (the optical trap) can fulfill the direction-biasing role of ATP. Further, formation of the motor-track interface still occurs in the absence of ATP and the external tension generated by the optical trap also mimics the natural interhead strain developed during the normal ATP hydrolysis cycle. Thus, just as one can roll-start a car, pulling kinesin along its microtubule track can get its motor running.

The work of Yildiz and colleagues provides clear and compelling evidence that interhead tension is required for normal kinesin motility. It also suggests that a conformational change in the leading head, probably triggered by ATP binding, increases this tension and causes release of the lagging head. The lagging head also must have a role in this motility cycle, as under normal conditions, it needs to hydrolyze its bound ATP and release phosphate before it can be pulled off the microtubule. This makes sense as the ATPase cycles of both motor domains must be held in the correct relative phase if the kinesin 1 molecule is to move efficiently. Are these results inconsistent with other chemical gating theories? Not necessarily, although those theories will need to be modified to take into account the role uncovered by Yildiz et al. of interhead strain in motor movement. Also, it remains to be determined why the mutant kinesin 1 molecules with extended linkers remain processive. How can these mutant motors with independent, noncommunicating motor domains still take steps more often than not in the correct direction? Why do their ATPase cycles remain coordinated such that both heads do not detach from the microtubule at the same time? Regardless of the answers to these questions, Yildiz et al. have taken an impressive step toward understanding the mechanism of kinesin motility, although intergroup strain will likely assure that vigorous debate continues in the field.

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