Hybrid four-headed myosin motor engineered with antagonistic motor domains

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Bidirectional, or saltatory, motion is the back-and-forth movement of organelles as they are transported on their filamentous tracks (1, 2). It occurs when plus-end– and minus-end–directed motor proteins colocate on a cargo and there is an alternation in which motor dominates. Bidirectional transport has been observed for many organelles—exosomes, endosomes, mitochondria, melanosomes, neural axon vesicles, and viral particles—and it can involve all motor protein families: kinesin, dynein, and myosin (3–5). In PNAS, Ali et al. (6) present an exciting, new in vitro study of bidirectional transport mediated by myosin V and myosin VI that gives insight into the mechanisms underlying the tug of war between individual motor proteins.

Just as in the game of tug of war, antagonistic teams of motor proteins bound to the same cargo can pull against each other in opposite directions. There are three possible outcomes:

1. Win outright: one team overpowers the other by forcing the opposing motors to detach from the track or detach from the cargo (analogous to letting go of the rope or slipping on the grass). This may happen if load forces accelerate a motor's detachment from the filament or cargo; in this case, as one team begins to lose, its motors begin to let go, and the force is redistributed to a smaller number of remaining motors. This positive feedback leads to the detachment of all the motors on the losing side (7). The winning motors end up winning outright. Such force dependence is thought to underlie mitotic oscillations (8, 9), the oscillatory beat of sperm (10), and meiotic oscillations (11).
2. Draw: one team anchors itself to the track and halts the motion. This can occur if the motors have a latch state: increasing load force decreases the detachment rate, as observed for smooth muscle myosin II (12) and cytoplasmic dynein (13).
3. Compromise: both teams continuously pull on each other. This is the result if the motion can be characterized by the linear superposition of each motor's force-velocity curve (7). There are still winning and losing teams, but the win is not outright; rather, it is fought to the end.

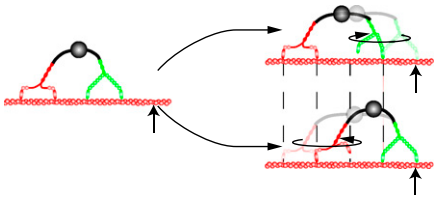

Fig. 1. Pathways for the coupled model given that myosin V takes a forward step. *Left*: Complex in the low intermotor tension state. Myosin V (green) and myosin VI (red) are both bound to the actin filament (red). Assume myosin V takes a forward step, its trailing head moves to the binding location indicated by the arrow pointing up and increases the intermotor tension. The tension can be relieved by myosin V taking a back step (*Upper Right*), returning the complex to its original position, or myosin VI taking a back step (*Lower Right*), advancing the complex 36 nm. After either pathway, the complex is returned to the low-tension state (*Left*).

A number of experimental studies have addressed the mechanisms of bidirectionality. Bidirectional transport was reconstituted in vitro by observing microtubules gliding over surfaces coated with a mixture of kinesin-1 and cytoplasmic dynein (14), or with mutant Ncd motors (in the kinesin-14 family) that lack directionality (15). More recently, kinesin-1 teams acting antagonistically on cross-linked pairs of oppositely polarized microtubules showed slow gliding movement (<20% of the speed of a single microtubule; consistent with a compromise) or fast gliding movement (>85% of the speed of a single microtubule; consistent with an outright win) (16). The disadvantage of these studies is that the number of motors is uncontrolled and unknown; an important contribution of Ali et al. (6) is the establishment of a well defined preparation in which a tug of war can be studied between just two motors.

Other studies have taken theoretical approaches to bidirectional transport. Early work showed that a collection of motors bound to a rigid backbone can generate a net force on a filament, even under symmetrical conditions in which equal numbers pull in opposite directions; the key requirement was that the transition rates of the motors between their bound and unbound states depended on position with respect to the binding sites on the filament (i.e., the strain in the motor) (17). Such a mechanism can account for the bidirectional transport of the Ncd mutant (18). The idea of strain-dependent binding and unbinding was extended to force-dependent unbinding, which, in turn, was used to model mitotic oscillations (8, 9) and bidirectional organelle transport (19). Recently, a unified model has been developed in which coupling can vary from "rigid" (strain-dependent binding) to "soft" (force-dependent binding) (20).

Ali et al. (6) developed a minimal assay for studying bidirectional transport by engineering differently colored quantum dot labels on myosin V and myosin VI and complexing them together via a third quantum dot as a cargo. By using total internal reflection fluorescence microscopy, they tracked the position of each motor with 6 nm precision. This study is exciting because the number of motors bound to the cargo and the filament are known unambiguously, and the geometric arrangement of the motors can be accurately determined, creating a tractable problem for analysis.

Their first major finding is that complexes with one myosin V and one myosin VI usually move to the plus, or barbed, end of the actin filament, i.e., myosin V wins. The speed is only 30% that of myosin V on its own, so the win is not outright. This is an example of a tug of war resulting in a compromise because the antagonistic myosin VI evidently exerts a continuous resistive force on myosin V. Because myosin V is considerably slowed, the stall forces of these motors must be quite similar, with that of myosin V only slightly exceeding that of myosin VI.

The second major finding is that the forward stepping of the winning motor occurs almost simultaneously with the backward stepping of the losing motor. This synchrony suggests that information about one motor's stepping is communicated to the other motor

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mechanically through the cargo. Presumably the mechanical communication consists of changes in intermotor tension caused by stretching the elastic intermotor link upon stepping. It would interesting to measure the link stiffness and compare it with the that of the individual motor domains. This might determine whether the motor complex is in the soft or rigid regime (20). Ali et al. (6) report that no complexes reversed direction during a run; this likely indicates that the myosin V–myosin VI system is sufficiently asymmetric to ensure that there is only one winner.

The last major finding is that, in the presence of ADP, myosin VI remains bound to the actin filament whereas myosin V takes multiple futile forward and backward steps. This is a change the outcome of the tug of war from a compromise to a draw.

The study of Ali et al. (6) demonstrates the power of single-molecule experiments to reveal the molecular details of motor proteins in a tug of war. That the tug of war results in a compromise suggests the transport of this complex might be described by the linear superposition of myosin V and myosin VI's force–velocity curves:

$$\left[F_{\text{complex}}(v) = F_{\text{V}}(v) + F_{\text{VI}}(v)\right] \qquad [1]$$

where the velocity of each motor is equal because they are rigidly linked. Although this simple compromise model might account for the resultant velocity of the complex, it fails to consider the mechanochemical synchrony.

These experimental results challenge theorists to extend their models of individual motors to hybrid motors.

To account for the synchrony, the entire complex needs to be treated as one hybrid four-headed motor. The simplest such model assumes that the myosin V and myosin VI motors are coupled through an elastic element. The transport is governed by the probability that, when one motor steps forward, increasing the intermotor tension, either it will step back or the other motor will step back to relieve this tension (Fig. 1). By comparing the transition rates in the hybrid motor with those in the individual motors, Ali et al. (6) estimate how much tension the motors can generate. In addition to tension, the transitions could also be altered by nucleotide concentration, protein binding, or protein modification, and in this way directionality could be regulated. These experimental results challenge theorists to extend their models of individual motors to hybrid motors. The extended model would then describe the geometry and chemical state of both motors in the filament bound and unbound, nucleotide hydrolyzed and nonhydrolyzed, and motor pre- and post-power stroke states.

The approach developed by Ali et al. (6) could help explain subcellular transport systems consisting of cargos carried by teams of motors, intraflagellar transport, and other tug-of-war scenarios involving rigid filaments like flagellar beating, cell division, and centrosome centering. If the mechanochemical properties of individual motors can be combined in motor complex models, then system-level properties of these phenomena such as force–velocity and force–detachment relationships might be understood from the bottom up.

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