Counting Motors by Force

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Properties of the microtubule motor protein kinesin-1 have been well characterized in vitro but not in the viscous environment of a cell. By measuring the force that kinesin-1 exerts on lipid droplets in fly embryos, Shubeita et al. (2008) determine the number of active motors per droplet and find unexpected differences between motor regulation in vivo and in vitro.

Motor proteins in the kinesin and dynein families play essential roles in intracellular trafficking and cellular organization by transporting vesicles, organelles, and mRNAs along polarized microtubule networks. Both kinesin-1 and dynein are "two-headed" dimers that use the energy of ATP hydrolysis to move in a hand-over-hand fashion along the microtubule. There is always one head of the pair attached to the microtubule, thus enabling the motor to perform hundreds of consecutive steps without detaching from the microtubule track and thereby moving cargo processively over long distances. The basic mechanisms of kinesin-1 movement have been elucidated by elegant single-molecule experiments in vitro, which have revealed that a single motor can generate a force of 5–7 picoNewtons (pN) and move processively for distances of about a micron (Howard, 2001). However, most of these experiments have been performed in nonviscous media. It is thought that motors in vivo experience much more drag because the cytoplasm is estimated to be 1000 times more viscous than buffer (Luby-Phelps, 2000). The drag may therefore exceed the force that a single motor can generate, suggesting that multiple motors are required to move larger cargoes. In this issue, Shubeita et al. (2008) count the number of active motor proteins on a single cargo (a lipid droplet) in the early embryo of the fly *Drosophila melanogaster* and in doing so shed light on the regulation of motor proteins in vivo.

A number of vesicles and organelles have been observed to move processively over distances of several microns in vivo. This argues in favor of multiple motors being associated with a single cargo. For example, beads with more than one motor attached cover much greater distances in vitro because the second motor maintains attachment to the microtubule when the first motor detaches (Vershinin et al., 2007). Movement of a cargo along microtubules in vivo is often much faster than the maximum motor speeds measured in vitro, suggesting that multiple motors share the load. These theoretical considerations have led to attempts to measure the number of motors engaged in moving specific cargoes in vivo. Counting motors is not an easy task because one cannot merely quantify the number of associated motors. Not all motors will be active at the same time or be in the appropriate orientation to contact the microtubule simultaneously. Several groups have attempted to indirectly measure motor number by determining the velocities of cargoes moving along microtubules in vivo. These measured velocities best fit a multimodal distribution, with peaks at regular intervals (Kural et al., 2005; Levi et al., 2006). Because

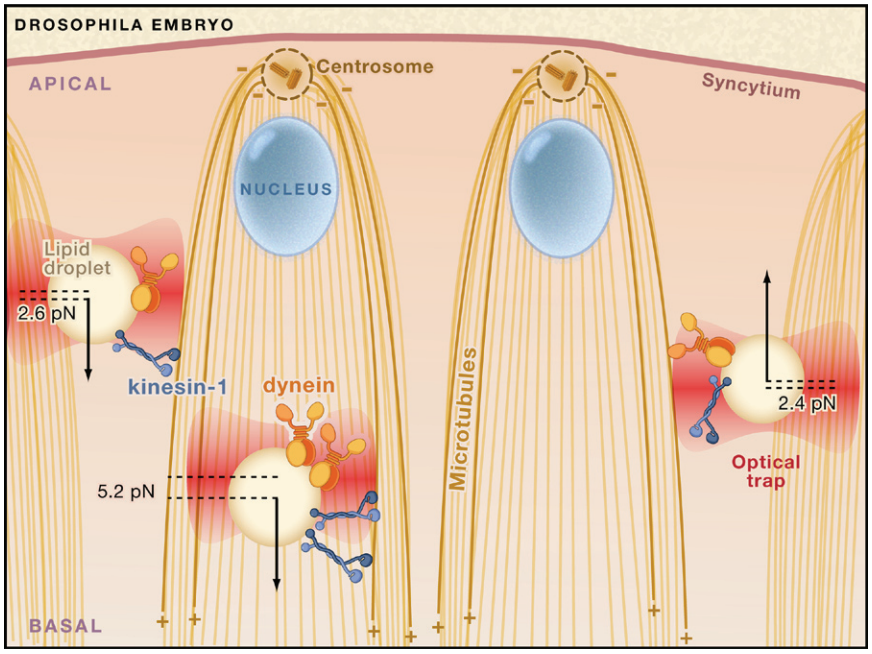

Figure 1. Stall Force Measurements Reveal the Number of Active Motors on Individual Lipid Droplets
Lipid droplets undergo bidirectional movements along microtubules powered by kinesin-1 (blue) and dynein (orange) in the early *Drosophila* embryo (syncytium). Using a custom-built optical trap with an infrared laser (red), Shubeita et al. (2008) measured the forces required to stall the movement of lipid droplets along microtubules. This enabled them to count the number of active motors per droplet and to correlate this number with droplet dynamics.

adding more motors should increase the speed of movement (assuming some drag due to viscosity), each peak in the velocity distribution has been interpreted as representing the addition of one extra motor. However, this indirect measurement has been challenged on theoretical grounds, and the reason for the discrete velocity steps remains to be resolved.

In their elegant new study, Shubeita et al. directly measure the number of motors on a single cargo. They examine the motor-driven transport of lipid droplets and measure the stall force required to prevent the droplets from moving along microtubules in the early *Drosophila* embryo. Lipid droplets are large (~0.5 μm diameter) transparent structures and so can be easily visualized at fast frame rates using video-enhanced DIC microscopy (Welte et al., 1998). The key feature of Shubeita et al.'s study is the development of optical traps that can be used to apply precise forces to moving droplets in vivo. Optical traps have been used extensively for in vitro motility experiments with motor proteins bound to beads. In the optical trap, light deflected by refraction from a spherical bead allows a focused infrared laser beam to exert force on the bead. Lipid droplets are transparent spheres that have a higher refractive index than the surrounding cytoplasm, enabling them to be manipulated by optical traps, as Shubeita et al. demonstrate. Because the droplets are stationary when the force is measured, the effects of viscosity are eliminated, and the stall force equals the counterbalancing force exerted by the motors on the droplet.

In the early fly embryo, lipid droplets undergo bidirectional movements along microtubules of uniform polarity. The minus ends of these microtubules are nucleated from the apical centrosomes, and the plus ends extend basally into the interior of the embryo (Figure 1). The authors analyzed the plus-end movement of the droplets, as only motion toward the microtubule plus ends varies. Shubeita and colleagues first determine that kinesin-1 is the sole motor required for plus-end movement. They show that either null mutations in the kinesin-1 heavy chain (*Khc*) gene or injection of kinesin-1 antibodies that block motor function into the embryos abolish all plus-end droplet movement. Furthermore, *Khc* mutations that slow kinesin movement reduce the velocity of plus-end transport without affecting minus-end transport. Shubeita et al. then set out to assay the force exerted by these motors on moving lipid droplets.

Measurement of the stall forces for droplet motion powered by kinesin-1 reveals a bimodal distribution, with peaks at 2.6 pN and 5.2 pN. Because the force exerted by multiple motors on the same bead in vitro is additive, Shubeita et al. hypothesized that the first peak represents droplets being moved by a single motor, whereas the second peak represents droplets with two active motors. To confirm this, they examined *Khc*/+ mutant fly embryos that have half as much kinesin-1 associated with lipid droplets as wild-type embryos. In the mutant embryos, the average stall force was reduced by half and the 5.2 pN peak disappeared. This proves that the stall force is proportional to the number of active motors and provides an unambiguous demonstration of multiple motors pulling the same cargo in vivo. More importantly, this allows the authors to correlate the number of active motors with the run length and velocity of the droplets, revealing that a single motor actually moves droplets slightly greater distances and about 5% faster than two motors.

These unexpected results have a number of important implications. First, they demonstrate that the drag imposed by viscosity on droplet movement must be much smaller than the force of a single motor, as droplets borne by a single motor do not move more slowly than those carried by two motors. This indicates that the cytoplasmic viscosity experienced by lipid droplets is orders of magnitude lower than suggested by previous studies. Second, because droplets carried by two motors move shorter distances than those carried by a single motor (in contrast to what is observed in vitro), it seems that motor run length in vivo is not determined by the innate properties of kinesin-1 and thus must be regulated at a higher level. Indeed, the authors find that both the plus-end kinesin-1 motor and the minus-end dynein motor are highly coregulated during lipid droplet movement. Dynein-dependent minus-end motility is abolished in embryos lacking kinesin-1, and the average number of active dyneins per droplet is reduced by almost half in *Khc*/+ embryos. This suggests that the two motors are loaded together in pairs onto droplets. In addition, the activity of the motors must be coordinated to regulate the developmental changes in droplet dynamics and to ensure that they do not engage in a tug of war (Gross et al., 2003).

The work of Shubeita and colleagues reveals the complexities of motor protein regulation in vivo. It also raises the question of why the moving organelles observed in previous studies showed a regular series of velocity steps, as Shubeita et al.'s results suggest that each successive step is not due to an extra motor moving the cargo faster through viscous cytoplasm. Whether the motor number effects observed in this study are generally applicable to other cargoes in the cell remains an exciting area of future exploration.

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