New and Notable

Drunk or Sober? Myosin V Walks the (Quantum) Dotted Line in Cells

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Targeted trafficking of vesicles, RNA, mitochondria, and many other organelles in cells requires the coordinated activity of myosin, dynein, and kinesin molecular motors. Our understanding of these motors has progressed rapidly due to advances in biochemistry, structural biology, single-molecule force measurements, and super-resolution optical tracking in vitro (1–3). Two articles in the *Biophysical Journal*—one previously published (4) and one in this issue (5)—report advances in high-resolution tracking of the nanometer-scale motions of myosin V in live cells.

Myosin V is a favored subject for biophysical studies because it is relatively abundant in tissues, especially the brain; it transports vesicles, organelles, and mRNA in many cell types; and it exhibits robust processive motility in vitro. Isolated, two-headed myosin V molecules carry artificial cargos along actin in runs of successive 37 nm steps, averaging up to 1–2 μm (i.e., 50–60 steps). The walking mechanism is hand-over-hand, so a head stepping from the trailing to the leading position moves 74 nm, twice the distance of the center of mass.

Transport activity by myosin V in live cells was previously studied by tracking cargos such as membrane-bound vesicles labeled by multiple fluorophores, melanin, or endocytosed gold nanoparticles or quantum dots (Qdots) (6–12). It is difficult to discern the actions of individual motors by watching these cargos because their complement of motors is not evident. Long-range transport on microtubules and handoff to actin at the cell periphery imply that neuronal vesicles and melanosomes are carried by several types of motors regulated by complex modulators. Discrepancies between organelle velocity and motor activity in vitro have been reported. The dynamics of the cytoskeletal tracks also strongly influence vesicle motions (13,14). In several studies, investigators were able to watch individual kinesin molecules in cells by labeling them with multiple fluorescent proteins (15) or Qdots (16,17).

Pierobon et al. (4) and Nelson et al. (5) attached myosin V molecules to Qdots and incorporated them into HeLa or COS-7 cells using pinocytosis and then osmotic shock to break the pinosomes. Both groups produced evidence that the incorporated complexes were free in the cytoplasm, and therefore the motions of the Qdots reflected the properties of the motors. Qdots have an advantage over organic fluorophors in that they are much brighter and less subject to photobleaching, although they do blink. The stoichiometry of labeling was low enough to produce Qdot complexes with single motors (as well as many free Qdots).

Qdots bound to myosin V in these cells showed a mixture of random and directed motions. Steps of 74 nm were observed during the short runs of directed motion, indicating motor activity at the single myosin level, as expected for labeling near the head. Velocities and run lengths were comparable to those in vitro, addressing the afore-mentioned discrepancy. The motors also displayed a high proportion of apparently random motions. This behavior is quite reasonable considering the dense nature of the actin cytoskeleton in these cells. A platinum replica electron micrograph of a HeLa cell (Fig. 1) shows the high density of the actin cytoskeleton in the cell interior. We pasted cartoons of a sphere and a myosin V onto the electron micrograph, roughly to scale, to help us imagine the environment that a 20 nm Qdot would encounter attached to the motor. Two other objects that may be myosins are also indicated. There are many cytoskeletal crossings and barriers even in this detergent-extracted HeLa cell. The distance between nodes in filament matrix is a few tens of nanometers in the largest gaps and much less in filament bundles. The rapid changes in Qdot direction and velocity observed in these studies would be hard to avoid in such a dense zone.

Nelson et al. (5) raised the question as to whether the apparent chaotic motions they observed were completely random. They produced evidence that part of these tracks have statistical properties (slope of the mean squared displacement versus time) above the expectation for completely random diffusion, indicating directed motions specifically over timescales shorter than 200 ms. This result suggests processive run lengths of approximately three successive steps before the motor switches over to a neighboring actin filament in the network. The analysis and conclusions are convincing; however, at labeling densities that produce Qdots associated with single myosin Vs, many of the Qdots should have no myosin bound. Consistent with that idea, Pierobon et al. (4) found that the movement of most Qdots in HeLa cells showed characteristics of completely random diffusion. Surprisingly, in COS-7 cells (5), many more Qdots exhibited directed motion than expected, suggesting that motor activity is necessary for Qdots to enter the cortical matrix and that diffusion is insufficient.

The ability to insert Qdots containing several types of motors into cells would enable researchers to study the bidirectional motions and track switching that are characteristic of native

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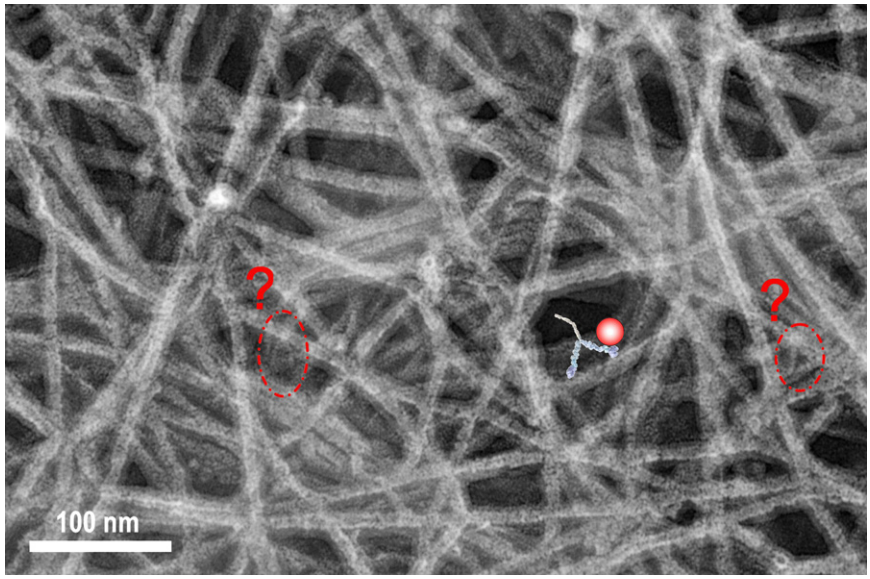

FIGURE 1 Electron micrograph of a HeLa cell platinum replica after detergent extraction, fixation, and critical-point drying. The density of the cytoskeletal network is apparent. A cartoon of myosin V and a 20 nm Qdot are drawn approximately to scale. The dashed ovals with question marks indicate possible myosin V molecules in the cell. The electron micrograph was provided by Dr. Tatyana Svitkina (University of Pennsylvania, Philadelphia, PA).

cargos (6–12). The behaviors of motors and cargos at intersections between filaments and in complex filament matrices can be studied in vitro as well (18). It will be of great interest to watch the progress of these ''top down'' and ''bottom up'' approaches as they converge, and to evaluate motor and cargo behaviors in artificial geometries emulating the interior of the cell with the actual characteristics in ''real life''.

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