Measuring Single Protein Motors at Work

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As engineers aspire to create smaller and smaller micromachines, one cannot help but marvel at the elegant design of biological motors. These motors are remarkably compact proteins (kinesin's motor domain is 350 amino acids) that convert chemical energy from adenosine triphosphate (ATP) hydrolysis into work with great efficiency (>50% in the case of myosin). Understanding the mechanism by which biological motors work has been one of the great puzzles in biophysics for the last century, and, contrary to what might be inferred from reading a biology textbook, the mechanism of myosin motility is far from being solved. Muscle fibers have been the primary experimental system used to study biological motors, but individual force-generating events have proven difficult to measure because of the large number of motors ($>10^{11}$) involved in contraction. Inspired by successful studies of single ion channels by patch-clamp recordings, investigators are now developing technologies to study motility at the level of single molecules. The report by Kuo and Sheetz in this issue of *Science* (*1*), as well as work by others, demonstrate the feasibility of making high-resolution mechanical measurements of single motor molecules and show that important insights can come from such analyses.

Protein motors are responsible for the various animated qualities of living organisms. By exerting tension upon or moving along actin filaments, members of the myosin superfamily produce muscle contraction, cytokinesis, organelle transport, and probably cell migration. Dyneins and kinesins are microtubule-based motors that generate organelle movements, force-generating events in mitosis, and ciliary and flagellar beating. In addition to the cytoskeletal motors, a variety of proteins, such as helicases, move unidirectionally along DNA and RNA polymers.

To understand how motors work, one must decipher the details of their chemomechanical cycles (Fig. 1A). All known motors bind to and hydrolyze ATP and then release the products [first phosphate (P_i) and then adenosine diphosphate (ADP)]. The motor must also participate in a defined mechanical cycle in which it binds to a polymer, undergoes a force-generating step, releases, and then rebinds to a new site further along the polymer. According to the rotating cross-bridge model, which has dominated thinking in the myosin field for the last 25 years, the myosin head undergoes a large conformational change that alters its angle relative to actin, thereby eliciting a net displacement of ~10 nanometers (nm) and a force exceeding 2 piconewtons (*2*). This conformational change is thought to occur once per ATP hydrolysis cycle in conjunction with phosphate release, the step corresponding to the greatest free energy change in the chemical cycle.

Recently, the notion that motility is produced by a large conformational change has come into question. First, a variety of methods have failed to detect a conformational change in the myosin head capable of eliciting a 10-nm displacement. Alternative models have been put forth that do not require large conformational changes but instead propose that motors use ATP energy to harness Brownian motion into unidirectional motion (*3*, *4*); however, these models also stand without solid experimental support. Second, the dogma of one mechanical step per adenosine triphosphatase (ATPase) cycle has been debated. On the basis of measurements of sliding velocity and ATPase rates, some studies have indicated that myosin can travel along actin for distances greater than the dimensions of its force-generating head (>20 nm) during one round of ATP hydrolysis (*5*). Such results imply that the motor can take several steps along the actin filament as a consequence of splitting one ATP. However, smaller "step sizes" consistent with the conventional model have been measured by other laboratories (*6*).

To circumvent the difficulties of interpreting such experiments, investigators are attempting to make mechanical (force, displacement) and chemical (ATP hydrolysis, heat) measurements on individual motor

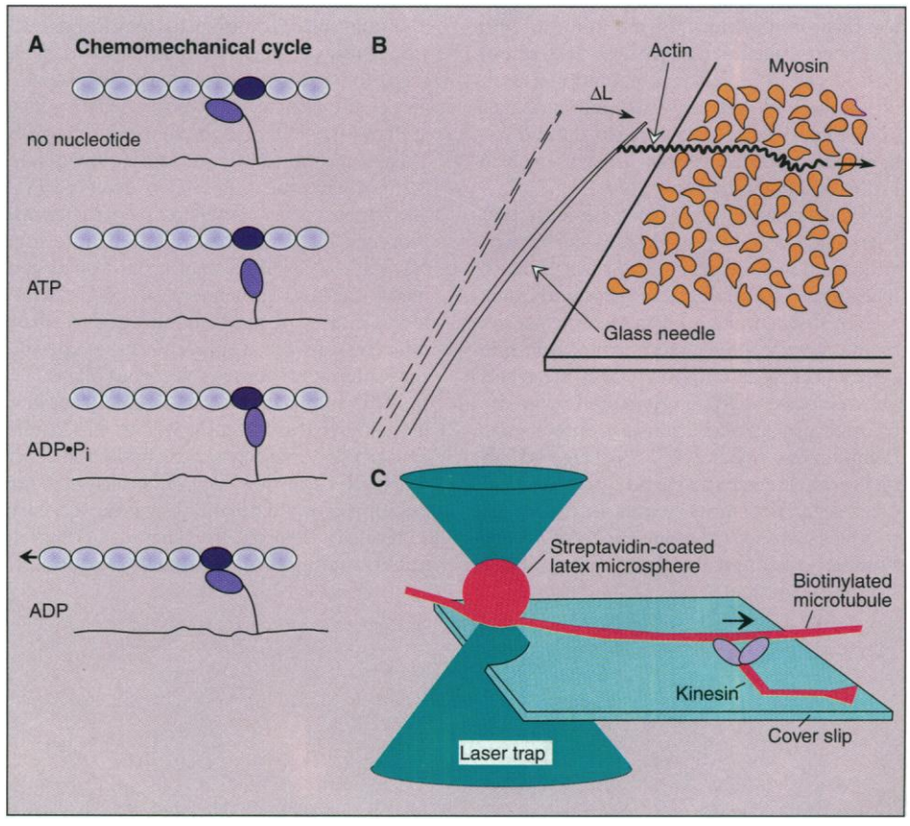

Fig 1. (**A**) The classical rotating cross-bridge model of protein motors in its most simplified form. In the absence of ATP, myosin (only one of its two heads is shown) is bound tightly to actin at an angle of 45°. Myosin dissociates from the filament when ATP binds, and, after ATP hydrolysis, the myosin head rebinds with a 90° orientation. In conjunction with phosphate release, the myosin head reverts to its 45° orientation, thereby driving the filament forward by a distance of 5 to 15 nm. (**B**) To estimate the forces associated with these proposed mechanical events, Yanagida and co-workers have measured the bending of a small needle attached to an actin filament that has been "cast" over a myosin-coated surface [adapted, with permission, from (*13*)]. (**C**) Kuo and Sheetz (*1*) have used radiation pressure from a high-intensity optical trap to measure kinesin forces.

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proteins. In vitro motility assays have contributed greatly in this regard. The most widely used assays involve adsorbing myosin, dynein, or kinesin onto a microscope slide and observing the transport of the complementary polymer across the surface (Fig. 1B) or attaching the motors onto a small latex bead that is transported along the surface of a stationary polymer. Low density variations of these assays have been used to measure motility produced by a single kinesin motor (*7*, *8*) and possibly by a single myosin motor (*6*).

Since the development of single motor assays, the challenge has been to design microscopes with detection systems capable of measuring the small forces (piconewtons) and displacements (nanometers) produced by individual motors with a temporal resolution in the millisecond range (an ATPase cycle is ~30 ms). Because the motor itself cannot be visualized, the object transported by the motor—the cytoskeletal polymer, a latex bead, or a glass needle—must be tracked. Although the resolution limit of visible light is approximately 200 nm (the spacing between two diffraction-limited objects that allows them to be distinguished as separate), the relative position of an object can be followed with nanometer accuracy by means of a photodiode detector (*9*, *10*) or computational methods that track the object's center of mass (*11*). Dual-beam interferometry can be used to extend motion detection limits to the resolution of a few angstroms (*12*).

Traditionally, force measurements have been made by holding a muscle fiber at either end in a tensiometer; constant forces can be maintained by feedback control and quick-release steps can be used to measure tension recovery with millisecond timing. More recently, Yanagida and co-workers have been able to assay the forces generated by myosin by attaching an actin filament onto a small, flexible glass needle (*10*, *13*) (Fig. 1B). By measuring the maximal bending of the needle caused by the motors and knowing the needle's stiffness, the isometric force of myosin can be estimated.

Another strategy for applying a counteracting force in a microscopic assay is by means of an intense laser light source forced through a microscope objective with a high numerical aperture (Fig. 1C). This so-called "optical tweezing" method [reviewed in (*14*)] can be used to "grasp" and manipulate microscopic objects, and a very strong laser illumination can generate a force sufficient to stall microtubule transport produced by a single kinesin molecule. By gradually reducing the illumination, Kuo and Sheetz (*1*) have determined the threshold at which kinesin can power the microtubule out of the confines of this optical trap. Nanometer-scale, motor-induced movements of objects within the trap can also be followed with photodiode detectors, and the forces experienced by the motors can be rapidly altered on a millisecond time scale by modulating the light source (*15*). Through such procedures, force fluctuations, as well as the classical tension transient and force-velocity profiles, can now be examined at the level of single motors.

It is comforting that the high-resolution measurements of single motors are yielding results that are generally consistent with earlier work conducted on muscle fibers. The maximal force of myosin measured with whole muscle fibers is similar to that measured for myosin in vitro with glass needles and to the optical trap "escape" force generated by kinesin. Force fluctuations that probably correspond to quantal force-generating events have also been detected (*10*) and appear to fit well with a model for myosin proposed by Sir Andrew Huxley more than 30 years ago. High-resolution tracking of motor-induced movement at maximal velocity, however, has thus far failed to detect discrete stepwise displacements, suggesting that some surprises may yet be in store.

Will single motor mechanical measurements have the dramatic impact that patch-clamp recordings have made in the ion channel field? The new instrumentation is just beginning to yield data, so it is far too early to render judgment. In addition, a complete understanding of motility will require linking mechanical measurements to events in the chemical cycle. Such studies demand new technologies capable of measuring ATP hydrolysis from single molecules—a difficult but perhaps not impossible task.

The current fervor of in vitro motility measurements should not diminish the importance of muscle as a system for investigating the mechanics and energetics of motility. Recent work by Lombardi and co-workers (*16*), in which they demonstrated a rapid regeneration of the power stroke before myosin has time to enter a new ATPase cycle, also indicates how exciting new results can be obtained by making thoughtful permutations on classic experimental paradigms. Clearly, one must integrate information from different motile systems and experimental techniques and be ready to embrace new ideas. To quote Huxley (*17*): "The whole history of muscular contraction during the last half century shows that even when a set of ideas seems to be well established, there is a large chance that it will be overthrown by some unexpected discovery."

References

1. S. Kuo and M. P. Sheetz, *Science* **260**, 232 (1993).
2. A. F. Huxley and R. M. Simmons, *Nature* **233**, 533 (1971).
3. N. J. Cordova, B. Ermentrout, G. F. Oster, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 339 (1992).
4. R. D. Vale and F. Oosawa, *Adv. Biophys.* **26**, 97 (1990).
5. Y. Harada *et al.*, *J. Mol. Biol.* **216**, 49 (1990).
6. T. Q. P. Uyeda, H. M. Warrick, S. J. Kron, J. A. Spudich, *Nature* **352**, 307 (1991).
7. S. M. Block, L. S. Goldstein, B. J. Schnapp, *ibid.* **348**, 348 (1990).
8. J. Howard, A. J. Hudspeth, R. D. Vale, *ibid.* **342**, 154 (1989).
9. S. Kamimura and R. Kamiya, *ibid.* **340**, 476 (1989).
10. A. Ishijma, T. Doi, K. Sakurada, T. Yanagida, *ibid.* **352**, 301 (1991).
11. J. Gelles, B. J. Schnapp, M. P. Sheetz, *ibid.* **331**, 450 (1988).
12. W. Denk and W. W. Webb, *Appl. Opt.* **29**, 2382 (1990).
13. A. Kishino and T. Yanagida, *Nature* **334**, 74 (1988).
14. S. M. Block, *ibid.* **360**, 493 (1992).
15. J. Finer, R. Simmons, S. Chu, J. Spudich, personal communication.
16. V. Lombardi, G. Piazzesi, M. Linari, *Nature* **355**, 638 (1992).
17. A. F. Huxley, *J. Physiol.* **243**, 1 (1974).