Measured Steps Advance the Understanding of Molecular Motors

The origin of motion within our bodies has been traced to certain molecules that function as tiny motors. Recent experiments have been able to study individual motor molecules and to measure the size of the discrete, unidirectional steps they take and the force they exert.

Our bodies contain myriad motors to produce mechanical work. We're only too aware of the muscles that propel our limbs or pump our hearts; less noticed are the minuscule molecular motors at the cellular level that accomplish such chores as transporting organelles. All these actions involve motor proteins, enzymes that convert the chemical energy stored in the molecule adenosine triphosphate—known as ATP—into mechanical energy. Exactly how they channel the energy into rectified motion remains a compelling mystery.

Early attempts to study the molecular mechanism behind such actions as muscle contraction were confounded by the millions of molecules that act in concert. But researchers can now study *in vitro* individual motor proteins moving on polymeric filaments. New experimental techniques allow them to measure nanometer displacements and piconewton forces at millisecond rates. Such measurements have confirmed the expectation that the motor proteins move in discrete, unidirectional steps. Quantitative determinations of the lengths of those strides, together with results from biochemical, molecular biological and crystallographic studies,[1] are consistent with prevailing, though still sketchy, ideas of how motor molecules work.

Stepping motion

Motor molecules act by moving along a polymeric filament or causing the filament itself to move. In muscles, myosin molecules packaged into filaments pull on actin fibers to achieve contraction. Within a cell, kinesin molecules carrying cellular organelles step along tubulin polymers known as microtubules. These myosin–actin and kinesin–microtubule systems have been the most extensively studied among nearly a hundred motor proteins that have been identified by now.

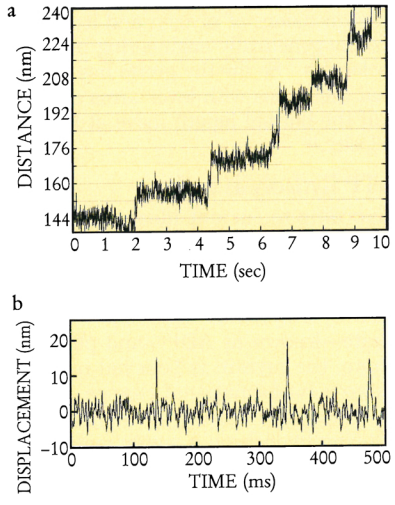
A single molecule of either myosin or kinesin has a pair of "heads" connected to a long "tail." Despite the nomenclature, the heads of kinesin are thought to function somewhat as legs, stepping along the microtubules. And the heads of myosin are viewed as grabbing briefly onto the actin filaments and pulling; actin moves in response to millions of such "flicks."

An early picture of the motion originated with the so-called "swinging crossbridge" model of the myosin–actin system proposed[2] in the 1960s.

One difference between myosin and kinesin is that the former binds to actin only during a small fraction of its stepping cycle, whereas the latter holds onto the microtubules through most of its stepping cycle.

Measuring the step size

In vitro study of motor proteins is done with motility assays.[3] In a study of kinesin, for example, one might coat a quartz or glass surface with a layer of the enzyme and then add a solution of microtubules. The kinesin motors would grab the microtubules and move them unidirectionally across the surface. Motility assays usually involve many proteins working at once on the same filament, but in 1989 Jonathon Howard (University of Washington) and two colleagues showed that one can reduce the concentration of kinesin in a motility assay low enough to discern the movement of single molecules.[4]

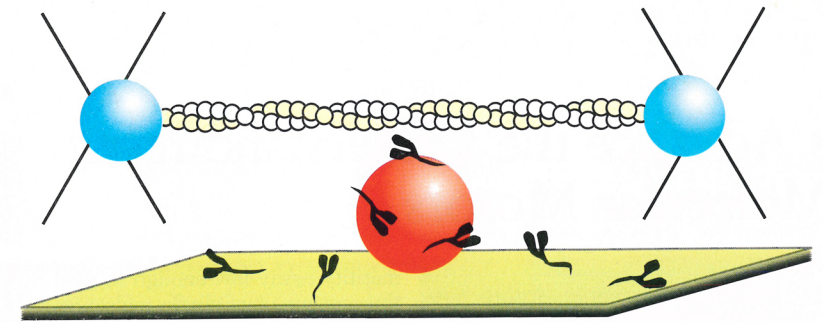


DISCRETE STEPS taken by motor molecules. **a:** Displacement of a silica bead carried by a kinesin motor molecule has jumps that are integral multiples of 8 nm. (Adapted from ref. 6.) **b:** Longitudinal displacement of an actin filament pulled by myosin. Steps, averaging 11 nm, are seen as peaks above the Brownian motion. (Adapted from ref. 9.)

Another advance was the use of optical traps (sometimes called optical tweezers) to facilitate quantitative measurement of the motion of individual motors. An optical trap,[5] which is a strongly converging laser beam, can hold a dielectric particle at its focal spot because the light gradient creates a force in the direction of greatest intensity. The laser beam can serve not only to confine small dielectric spheres but also to measure their positions. When either the motor proteins or their filaments are attached to such spheres, their motion can be tracked.

In 1993 a group working at Harvard and at the Rowland Institute for Science in Cambridge, Massachusetts, combined these tools to study the motion of kinesin.[6] The group consisted of Karel Svoboda (now at AT&T Bell Labs, Murray Hill, New Jersey), Christoph Schmidt (now at the University of Michigan), Bruce J. Schnapp (Harvard Medical School) and Steven Block (now at Princeton). First they added kinesin to 0.6-micron silica beads at a concentration so low that, on average, less than one molecule of kinesin clung to each bead. Next they coated a substratum with microtubules and placed a kinesin-carrying bead on a microtubule, using the optical tweezers.

To detect the position of the bead, Block and his colleagues used a technique of differential interferometry developed by Winfried Denk (now at AT&T Bell Labs, Murray Hill) and Watt Webb at Cornell University.[7] In this technique, a polarized laser beam is split into two beams having orthogonal polarizations, and both beams are focused to overlapping



MEASURING THE MOTION OF ACTIN. The ends of an actin filament (yellow) are attached to polystyrene beads, which are held in place by optical traps. One of the myosin molecules (black) coating the glass bead is in position to grab and pull on the actin. The displacement of one end of the filament is sensed optically. (Adapted from ref. 9.)

spots at the center of the trap. If the bead is displaced slightly away from the center, it will introduce a relative shift in phase between the split beams, giving the recombined beam an elliptical rather than circular polarization. The degree of ellipticity is a measure of the displacement of the bead.

In the experiment, kinesin tended to drag a bead away from the center of the trap. Because the trap forces grow in proportion to the displacement, the kinesin would eventually reach a point where it could no longer pull against the load and it would then detach from the microtubule. The kinesin and its cargo would slip rapidly back to the center of the trap and start over again. When the trap forces were small, Brownian motion jostled the bead, obscuring the discrete steps. But a statistical analysis of the distance traveled revealed peaks at 8 nm and integral multiples of it. This step size is just about the length of one repeating unit in the molecular structure of a microtubule, suggesting that it is the distance between binding sites for kinesin.

When the researchers reduced Brownian motion by increasing the trapping forces, they could discern individual steps in the displacement of the kinesin-driven bead (see the top panel of the figure on page 17).

Block and his colleagues have subsequently measured[8] the forces exerted by kinesin (5–6 pN maximum) and the force–velocity curve.

Myosin step size

To measure the step sizes of single myosin molecules, which remain anchored to actin for only a small fraction of the stepping cycle, researchers used optical traps to capture the filaments rather than the motor molecules.[9] Working at Stanford, Jeffrey Finer, Robert Simmons (King's College, London) and James Spudich attached polystyrene balls to both ends of an actin filament and held each ball in place by an optical trap. Then they affixed silica beads to a microscope coverslip and coated them with myosin molecules at a low enough density that only one motor on each bead was likely to be in a position to grab and move an actin filament. (See the figure above.)

The Stanford team monitored the position of one end of the actin filament by projecting its image onto a quadrant photodiode detector. They recorded the components of motion along the direction of the actin filament and at right angles to it. The parallel trace revealed that the actin filament took periodic jumps forward, with an average step size of 11 nm (see the bottom panel of the figure on page 17). No such spikes were seen above the Brownian motion in the perpendicular direction.

The 11-nm step size for myosin is consistent with the length of the lever arm on which the head of myosin pivots in the swinging cross-bridge model. Spudich and his colleagues found that the average force exerted by a single myosin molecule was 3–4 pN.

The Stanford group is currently exploring the relation between the physical dimensions of myosin and its step size by genetically engineering myosin molecules. So far the group has found that molecules with lever arms only half the normal length move at half the velocity.[10]

ATP hydrolysis cycle

According to current ideas about myosin, the stepping cycle begins when ATP binds to a site located on the head of a motor molecule. As ATP hydrolyzes to adenosine diphosphate—known as ADP—and phosphate, the myosin is believed to undergo a conformational change, such as a sideways motion of the head. (During hydrolysis a water molecule dissociates and the hydroxyl ion binds to ADP.) The subsequent release of energy may trigger a power stroke, such as the swinging of the head back to its original position. According to this picture, the step size is expected to be commensurate with the length of the lever arm on which the head swings. After the power stroke, ADP releases from the motor, making way for a new ATP to bind. Not enough is yet known about the other motor molecules to construct similar scenarios.

The myosin stepping cycle implies that the motor takes one step for each ATP that is hydrolyzed. Some believe, however, that each ATP powers several steps. The optical-trap experiments did not see any bunching of steps, as expected for a multiple-step scenario, but the question is not yet settled.

The exact structures of the motor proteins provide essential insight into the details of the motion. Ivan Rayment and his colleagues at the University of Wisconsin, Madison,[11] determined the structure of the myosin head in 1993 and are now trying to compare it to the shape of the same molecule when bound to ADP, to see what conformational change, if any, occurs. Robert Fletterick, Elena Sablin and their colleagues at the University of California, San Francisco, are just completing their structural studies of the head of a kinesin-related molecule called ncd. Although they have not yet determined the coordinates of ncd, Fletterick told us that its longest dimension is 8 nm.

Models of the motion

A number of fairly simple models have been proposed to account for the dynamic measurements made to date. For example, Stanislas Leibler (Princeton) and David Huse (AT&T Bell Labs) focus on the states among which the motor molecules cycle, with the variables being the rates of transition between states (such as the rate of ATP hydrolysis).[12] Charles Peskin of the Courant Institute of Mathematical Sciences at New York University and George Oster of the University of California, Berkeley, have proposed a model for the stepping motion of kinesin in which the hydrolysis rates of ATP in the two heads of kinesin are coupled, causing the head in the back to take the next step.[1]

Recently a number of theorists have proposed thermodynamic, rachet-type models to explain how thermal energy might be channeled into unidirectional motion. They build on the Brownian-motion machine described by Richard Feynman 30 years ago, involving a paddle coupled to a rachet (at a different temperature) and sitting in a box of gas. The fluctuations in this case are

not those of a pawl moving along the asymmetric teeth of a physical rachet but those of a particle in an uneven, sawtooth potential.

BARBARA GOSS LEVI

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Chromium Surrogate Sun Confirms That Solar Neutrinos Really Are Missing

There may still be some who doubt that the missing-solar-neutrino problem is real. But an impressive experiment with a radioactive solar surrogate recently completed at the Gallex solar neutrino detector in Italy makes it difficult to sustain such skepsis.[1] The new experiment supports the reports over the last several years that Gallex and Russia's SAGE, the other large gallium detector, see only about 60% of the solar neutrino signal confidently predicted by astrophysical models. (See PHYSICS TODAY, August 1992, page 17.) Thus it does much to bring the observational features of the solar neutrino puzzle into clearer focus.

As we hear early reports of neutrino oscillation in an accelerator beam, an experiment with a surrogate Sun lends credence and clarity to the solar neutrino puzzle, the oldest of the anomalies that point to exotic neutrino metamorphosis.

Neutrino oscillation

Keen interest in solar neutrinos extends far beyond the astrophysics community. If the discrepancy between what the detectors see and what the solar models predict is real, the best explanation at the moment is "neutrino oscillation," an exotic but quite plausible speculation of the elementary-particle theorists. The neutrinos we know about come in three "flavors." What the solar core puts out, and what the detectors are designed to see, are the *electron* neutrinos (ν_e) created in beta decay and hydrogen fusion. If at least one of the three neutrino varieties has a nonvanishing mass, it is possible that solar neutrinos defy detection by oscillating between different flavors.

Tentative evidence for neutrino oscillation also comes from an apparent shortage of muon neutrinos (ν_μ) in atmospheric cosmic-ray showers, reported by various groups since 1988. (See PHYSICS TODAY October, page 22.)

Very recently Hywel White and colleagues at Los Alamos created considerable stir with informal reports that their experiment with a neutrino beam at Los Alamos gives evidence of $\nu_\mu \rightarrow \nu_e$ oscillation, with a ν_μ mass of a few electron volts. As of this writing the jury is still out, pending the appearance of a preprint.

But even if all these tantalizing hints of neutrino oscillation turn out to be right, they appear to require different sets of oscillation parameters (mass-squared difference and mixing angle). That's no problem if each of these phenomena involves a different pair of oscillation partners: Perhaps the atmospheric muon neutrinos are oscillating with tau neutrinos (ν_τ), the third known variety, while the solar neutrinos oscillate with a speculative species of "sterile" neutrinos that are impervious to the standard weak interaction.[2] The accumulating evidence of neutrino oscillation would of course be more compelling if the different observational regimes were converging on the same parameters.

Trusting the radiochemical detectors

At this juncture it becomes all the more important to determine once and for all whether the solar-neutrino deficit, the oldest of the reported anomalies, is real. Like Ray Davis's pioneering chlorine detector in South Dakota, which gave the first evidence of a solar neutrino shortfall in the early 1970s, Gallex and SAGE are radiochemical detectors that attempt to extract something like a dozen alien, neutrino-generated atoms every few weeks from many tons of detector material. In Gallex, for example, solar neutrinos raining down on 30 tons of gallium transmute less than one gallium nucleus per day into a radioactive germanium nucleus. To believe that such experiments are really showing a significant deficit of solar neutrinos, one must have confidence that the experimenters can, with sufficient reliability, chemically extricate one atom from among 10^{28} others and then detect its decay.

The radiochemical groups have taken exquisite pains over the years to examine and avoid the many imaginable pitfalls. They carefully studied, for example, "hot-atom chemistry" issues: whether the unusually energetic atoms created by neutrino collisions might not form unusually stubborn bonds. "We had tested all the individual steps of our solar neutrino detection," says Gallex spokesman Till Kirsten (Max Planck Institute for Nuclear Physics, Heidelberg). "But it was essential to have an overall performance test. In such a complex experiment there could always be systematic error we hadn't thought of. And besides, we had to address the hand-waving skepticism that clouded all the radiochemical results."

A surrogate Sun

It was fairly obvious how such a comprehensive test should be done, but it would be an expensive, demanding task. The idea was to insert into the Gallex detector a calibrated radioactive neutrino source so powerful that it would subject the detector to a flux an order of magnitude greater than that coming from the Sun. Its energy spectrum had to be appropriate. Neutrinos of about 800 keV are particularly desirable for addressing what has become the most urgent issue—the almost complete disappearance, or so it seems, of the neutrinos from the decay of beryllium-7 in the solar core. (See the figure on page 20.)

Several years before Gallex began running in 1991, the group had already concluded that the radioisotope ^{51}Cr, produced by activating chromium in a reactor, would make the best surrogate Sun. With a convenient halflife of 28 days, ^{51}Cr decays to vanadium-51 by electron capture, usually emitting a neutrino of 0.75 MeV. One time in ten it decays to an excited ^{51}V state, emitting a neutrino of only 0.43 MeV.