Advances in understanding Earth’s magnetic field behavior require continuing improvements in data distribution, quality, and accessibility. The use of stringent laboratory procedures is critical for mapping regional differences in field behavior and obtaining temporal resolution of a few thousand years. Continuous long-core relative paleointensity measurements and high-quality absolute paleointensity measurements have led to a substantial increase in sediment and lava flow data. A promising avenue for future paleointensity work avoids the heating of samples through use of microwaves.

Until now, limited data sets have led to a somewhat artificial separation of studies of paleodirection and paleointensity. The availability of colocated, contemporaneous records of intensity and direction with better temporal information promote a different approach: that of analyzing the full vector evolution of the geomagnetic field.

Perhaps the most exciting implications of the improved data sets and models are the suggestion of lower mantle influence on the dynamics of the outer core, and the claimed detection of orbital periodicities in geomagnetic records. The arguments in favor of such interactions may be qualitative appealing but are not yet supported by strong theoretical arguments. Addressing these questions will require improved understanding of geomagnetic field variations and close integration with research in paleoclimate, orbital dynamics, and geophysical studies of deep Earth.

References

BIOPHYSICS

Myosin Motors Walk the Walk
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Myosins are a diverse protein family comprising 18 different classes (2), of which muscle myosin II is the best characterized. Myosin II works by converting small structural rearrangements at the catalytic site within the motor domain into a large swing or power-stroke of the light-chain binding domain. This serves as a flexible lever arm, transferring force to the object that is being moved. In this model, the presence of nucleotide (ATP or ADP and inorganic phosphate) at the catalytic site is tightly coupled both to the affinity of myosin for actin and to the lever-arm position. The “power-stroke” must occur when myosin is firmly attached to actin, and a “recovery-stroke” when it is detached. If the two heads of a double-headed myosin molecule cycle asynchronously, then they could move along the actin filament processively (that is, in a series of steps (3, 4). However, for this system to work, at least one of the two myosin heads must be bound to actin at all times. Thus, either the two heads must work in a coordinated fashion or each myosin head must spend most of its cycle time attached to actin (having a high “duty-cycle” ratio).

Biochemical studies have shown that myosin V is a motor with a high duty-cycle ratio (5). Furthermore, the light-chain binding domains of myosin V (each carrying six
calmodulin light chains compared to two for muscle myosin II) are of sufficient length to span the F-actin (filamentous actin) helical repeat, enabling its two heads to walk along actin with minimal distortion (6). But it took an optical trapping study (7) to provide unequivocal evidence that myosin V does indeed move processively along actin filaments in a series of successive 36-nm steps. Because molecular motors work at a very low Reynolds number (8) and because biochemical processes are stochastic, they exhibit jerky stepwise movements. Detailed kinetic analyses of single molecules of myosin V have shown that the dwell periods between steps are due to the rate-limiting release of the product ADP (9), such that the motor must pause at this point during each cycle of ATP hydrolysis. Further studies using single-headed myosin V molecules that move nonprocessively have shown that the power-stroke is smaller (25 nm) than the step size of the intact molecule (36 nm). Together these data lead to a minimal three-state model (3, 10) (see the figure). A recent study using a doublet of micrometer-sized beads bound to a single myosin V molecule showed that the complex takes a gentle helical path as it movesforward, making one complete turn around the actin filament for each 2 μm advanced (11). If we assume a 28/13 helical geometry for F-actin (that is, 167° rotation per monomer) (12), this would introduce the observed left-handed bias to the movement.

Notwithstanding the stunning insights provided by such single-molecule mechanical studies, the crucial question of whether myosin V moves by a hand-over-hand or “inchworm” mechanism remains unanswered. Negatively stained electron micrographs (13) favor the hand-over-hand mechanism, whereas a study using a mutant myosin V with an artificially shortened light-chain binding domain (14) leaves room for other possibilities. The Yildiz et al. paper and another recent optical study (1, 15) now provide convincing evidence in favor of the hand-over-hand model. Both studies exploit the high signal-to-noise ratio of total internal reflection fluorescence light microscopy to make measurements from individual fluorophores (16) attached to the light-chain region of the myosin V head. The studies provide complementary information about the angular disposition and spatial location of a single myosin head as the intact motor moves along an actin filament. A single fluorophore, attached to two cysteine residues in a myosin V light chain, reported the location and orientation of the light-chain binding domain. Forkey et al. (15) found that the polarization axis and hence tilt of the light-chain binding domain changed abruptly by 70° for each alternate step taken by the myosin motor. Yildiz et al. discovered that the fluorophore moves stepwise by 74 nm, and that this motion recurs for each alternate step taken by the myosin head (see the figure). Both of these studies are remarkable because they extract a wealth of information from just one fluorophore over a relatively long time scale. Polarization ratios were determined within 30 ms (15), and the spatial location was measured with 1-nm precision within 500 ms over an observation period of 100 s or more (1). The ability to localize an individual fluorophore with a resolution well below that of the light microscope depends on determining the central position of the diffraction-limited spot of collected light (17).

In both studies, the stepping rate of myosin V was slowed artificially by working at very low ATP concentrations, because then ATP binding becomes rate-limiting and the stepping rate is much slower. Furthermore, both studies relied on biochemical kinetic arguments to establish that the head movements occurred once for each alternate step taken by the whole motor. It is unlikely, but nonetheless possible, that myosin V adopts a different stepping pattern at saturating ATP concentrations or when an external load is applied. However, when the results of both studies are combined, they provide compelling evidence for the hand-over-hand mechanism of myosin movement.

Many questions remain to be answered, such as the order and timing of chemical events at the catalytic site and the resulting movement of the light-chain domain and whole molecule. Simultaneously visualizing labeled nucleotide and the position of the light-chain domain with either optical tweezers or a combination of optical probes might help to address this issue. The question of whether myosin V works through a combination of a power-stroke and a thermally driven process could be answered by optical studies performed under very high viscosity conditions.

Details of how myosins drive muscle contraction or move vesicles around the cell will be of great interest to nanotechnologists seeking to build synthetic nanometer-sized motors. Moreover, many of the single-molecule methods devised to probe how molecular motors work hold great promise for broader application in other fields.

References and Notes
1. A. Yildiz et al., Science 300, 2061 (2003); published online 5 June 2003 (10.1126/science.1084398).
4. Processivity is defined as the average number of steps taken per diffusional encounter between a motor protein and its actin filament track.
8. The Reynolds number is a ratio between inertial and viscous forces acting on a moving body. Molecules work at a low Reynolds number and thus stop abruptly when applied forces are removed. Hence their movements are jerky.
17. Such experiments require that the mechanical stability of the microscope is in the nanometer range and that the camera system noise (dark counts and read-out noise) is trivial compared to the photon counting noise. Accuracy in determining the centroid of a diffraction-limited spot is then simply a matter of counting statistics: The standard error in estimating the mean position is related to the spot size (about 150-nm radius at half height) and the number of photons collected (14,000 per half-second bin). Yildiz et al. had a resolution of about 1.5 nm.

The myosin three-step. A three-state model for how the two heads of myosin walk along an actin filament, consuming one molecule of ATP per step. The “power stroke” of the leading head drives the transition from state 1 to state 2 with the hydrolysis of ATP and the production of ADP and inorganic phosphate (yellow); the trailing head now becomes fixed to the ground. The stepping rate of myosin V was slowed artificially by working at very low ATP concentrations, because then ATP binding becomes rate-limiting and the stepping rate is much slower. Furthermore, both studies relied on biochemical kinetic arguments to establish that the head movements occurred once for each alternate step taken by the whole motor. It is unlikely, but nonetheless possible, that myosin V adopts a different stepping pattern at saturating ATP concentrations or when an external load is applied. However, when the results of both studies are combined, they provide compelling evidence for the hand-over-hand mechanism of myosin movement.

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