

How Molecular Motors Move

Ahmet Yildiz

Myosin and kinesin motor proteins use the energy obtained from adenosine triphosphate (ATP) hydrolysis to transport organelles and vesicles by moving along the cytoskeleton. Structurally, these motors are dimeric, having two motor heads, two legs, and a common stalk. The head regions bind to actin or microtubule filaments and power the forward movement. The central question was how the two heads are coupled so that the motor can processively move along its track. In the hand-over-hand model (1), ATP binding and hydrolysis creates a conformational change in the forward head (head 1) and this conformation pulls the rear head (head 2) forward, while head 1 stays fixed on the track. In the next step, head 2 stays fixed and pulls head 1 forward. Alternatively, in the inchworm model (2) only the forward head catalyzes ATP and always leads while the other head follows (see figure below).

In both of these mechanisms, the motor needs two heads to be able to stay on the track as it moves and its step size depends on the length of the legs. However, myosin VI with short legs (8 nm) was observed to take the same long steps (30 nm) as myosin V. Moreover, a single-headed processive motor has suggested that two heads are not necessary for processive motion. These observations lead to another mechanism: biased diffusion of the motor along the actin/microtubule lattice (3). The bias is provided by the initial push of the power stroke, and the motor most likely attaches to the next binding site in the forward direction. Understanding motor protein movement is a fundamental step in understanding how cargo transport works within a cell, but despite intensive research, the mechanism underlying movement remained highly controversial.

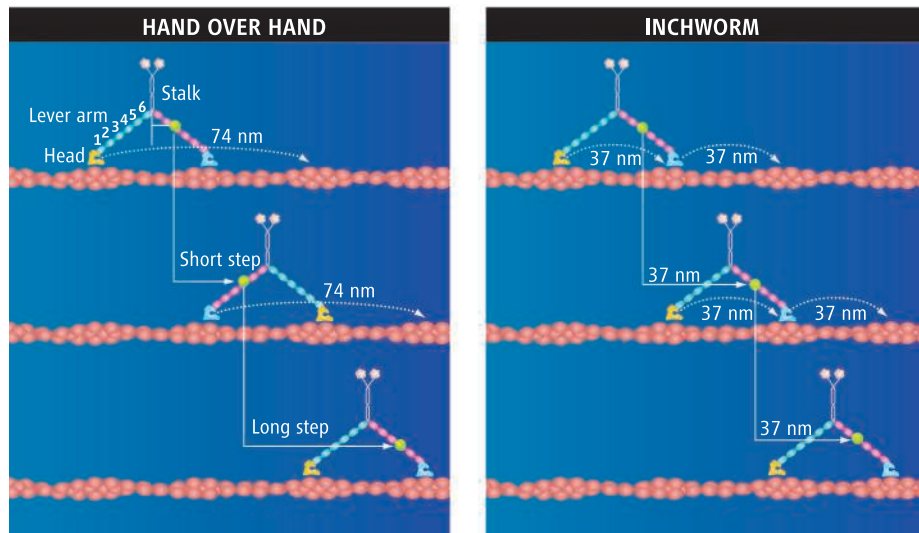
The most direct way to distinguish among these models is to measure how much each head moves when the motor walks. The hand-over-hand model predicts that a head alternately moves twice the stalk displacement and stays stationary in the next step while the other head takes a step (see figure, left panel). In contrast, the inchworm model predicts that both of the heads move forward the same distance as the stalk (see figure, right panel). The diffusion model states that heads randomly bind to the track. Current nanometer-precision tracking techniques (optical traps and cantilever probes)

cannot readily be used to watch the head movement, because they use a large probe (>100 μm) that might hinder the movement of the motor's tiny heads (5 to 10 nm). What is needed is to track a nanometer-sized probe (such as organic dyes) attached to a motor head with single-nanometer precision.

The position of a diffraction-limited spot can be localized very precisely by determining the center of its emission pattern. However, organic dyes are not very bright and the signal disappears quickly by permanent photobleaching. This limited previous single-molecule tracking experiments to a precision of around 30 nm (4). I have extended the photostability and brightness of single organic dyes 20 times by effectively deoxygenating the assay solution and using reducing agents, and I have achieved 1.5-nm localization and collected 1.4 million photons from single organic dyes. The technique, named fluorescence imaging with one-nanometer accuracy (FIONA), has improved spatial resolution in single molecule fluorescence by ~20-fold.

Using FIONA, I tracked the movement of the motor proteins myosin V, kinesin, and myosin VI, which were labeled with a single dye in the head region as follows.

Myosin V. Bifunctional rhodamine (Br)-label-



Myosin V: Walking or inchworming? Predicted movement for the heads and a dye molecule label (green dot) on the lever arm in the hand-over-hand model (left) and the inchworm model (right). The FIONA assay has revealed that myosin V, along with kinesin and myosin VI, walks hand-over-hand.

The author is in the Department of Cellular and Molecular Pharmacology, University of California at San Francisco, San Francisco, CA 94107, USA. E-mail: yildiz@cmp.ucsf.edu



GE Healthcare and *Science*/AAAS are pleased to present the prize-winning essay by Ahmet Yildiz, a regional winner for North America who is the Grand Prize winner of the Young Scientist Award.

ed calmodulins were exchanged into the myosin V lever arm, where the calmodulin can potentially exchange at any of six calmodulin-binding sites (IQ domains). The inchworm model predicts a uniform step size of 37 nm regardless of the position of the labeled calmodulin. The hand-over-hand model predicts alternating short and long steps, depending on the in-plane distance of the dye from the midpoint of the myosin. The trajectory of moving spots created three classes of steps. I observed 74- or 0-nm displacements for dye on the first IQ domain, alternating 52- and 23-nm steps for

dye on the fifth IQ domain and alternating 42- and 33-nm steps for dye on the sixth IQ domain (5) (see figure below, left).

Kinesin. A human kinesin was specifically labeled on the head region with a single Cy3 molecule. As the stalk took 8-nm steps, the head was observed to take alternating 16-nm and 0-nm steps (6).

Myosin VI. Myosin VI was labeled with a single Cy3 molecule on a calmodulin-binding site. Again, the labeled head alternately moved twice as far as the stalk moved and stopped as the other head moved (7). Unexpectedly, Cy3-calmodulin showed significant flexibility when it had ATP bound, whereas it was immobile in

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the nucleotide-free and ADP states. This implies that in some part of myosin VI's ATPase cycle, the lever arm uncouples from the motor, which could arise from elongation of the lever arm. Lever arm elongation may provide the long step (30 nm) of myosin VI with a short lever arm (8 nm).

Thus we have established a new, single-molecule fluorescence technique, FIONA, which is

able to resolve steps of a few nanometers taken by molecular motors. FIONA assays on myosin V, myosin VI, and kinesin have revealed that these motors move by walking hand over hand, not by "sliding" like an inchworm, nor by "diffusing" along the cytoskeleton. FIONA is also a broadly applicable technique in other fields of molecular biology, such as DNA sequencing and particle tracking in vivo.

References

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2005 Grand Prize Winner >>

Ahmet Yildiz, the author of the prize-winning essay and a North American regional winner, grew up in Sakarya, Turkey. In 2001, he received a bachelor's degree in physics from Bogazici University, Istanbul, and started his graduate studies in biophysics at the University of Illinois Urbana-Champaign. Working in the research group of Dr. Paul Selvin, he developed the technique of fluorescence imaging with one-nanometer accuracy (FIONA). This work was recognized with a Foresight Institute Distinguished Student Award in 2003. He went on to use FIONA to study the molecular walking mechanism of the motor proteins myosin V, myosin VI, and kinesin. Dr. Yildiz received his Ph.D. in 2004, and his thesis was awarded the Gregorio Weber International Prize in Biological Fluorescence. In 2005, he moved to the University of California, San Francisco, where he is a postdoctoral fellow in the research laboratory of Dr. Ronald Vale. He is currently studying the structural mechanism of cytoplasmic dynein.



adapts its adherence properties to fit predominant patterns of gastric mucosal cell surface glycosylation. During this time, she also collaborated with the group of Dr. Douglas Berg, Washington University, St. Louis, Missouri. Dr. Aspholm defended her Ph.D. thesis in 2004 and now holds an EMBO long-term fellowship and is a research scientist in the laboratory of Dr. Michael Koomey at the University of Oslo, Norway.



Japan: Rikinari Hanayama for his essay, "Impaired Phagocytosis of Apoptotic Cells and Development of Autoimmune Diseases." Dr. Hanayama was born in 1974 and grew up in Osaka, Japan. He obtained an M.D. degree from Osaka University in 1999. After a year as a medical intern, he decided to pursue basic research and joined the laboratory of Dr. Shigekazu Nagata as a graduate student. There he identified a molecule that promotes the phagocytosis of apoptotic cells and showed that the inefficient removal of the apoptotic cells can lead to autoimmune diseases. Dr. Hanayama was awarded a predoctoral fellowship from the Japan Society for the Promotion of Science in 2002 and received his Ph.D. and the Yamamura Award from Osaka University in 2004. After working as an instructor in genetics with Dr. Nagata, he joined the laboratory of Dr. Michael E. Greenberg at Children's Hospital/Harvard Medical School with a long-term postdoctoral fellowship from the Human Frontier Science Program.



He is currently studying the structural mechanism of cytoplasmic dynein.

Regional Winners

North America: Nieng Yan for her essay, "Mechanisms of Programmed Cell Death in *Caenorhabditis elegans*." Dr. Yan was born in Jinan, China, in 1977 and grew up in Beijing. As an undergraduate at Tsinghua University, she developed a strong interest in science and was also deeply influenced by Beijing's unique civil milieu. After receiving a bachelor's degree in biology in 2000, she traveled to New Jersey to pursue graduate training in the Department of Molecular Biology at Princeton University. Under the guidance of Dr. Yigong Shi, she used structural biology and biochemistry techniques to elucidate the molecular mechanisms of cell death regulation. Dr. Yan received her Ph.D. in December 2004 and is currently completing research projects in Dr. Shi's lab. Her goal is to continue in an academic career.



Europe: Marina Aspholm for her essay, "Adaptation of *Helicobacter pylori* Adherence Properties in Promotion of Host Tropism and Inflammatory Disease." Dr. Aspholm comes from Kiruna, Sweden, a city famous for an ice hotel that is constructed anew each winter. Dr. Aspholm studied chemistry and molecular biology at Umeå University and received a Master of Science degree in 1998. She remained at Umeå University for Ph.D. studies through a fellowship from the Swedish Foundation for Strategic Research. Under the guidance of Dr. Thomas Borén, she examined how the gastric pathogen *Helicobacter pylori*

All Other Countries: Jianmin Zhang for his essay, "Establishment of Transcriptional Competence in Early and Late S Phase." Dr. Zhang was born in Tianjin, People's Republic of China. After graduating from Tianjin Medical University, he worked as a research associate at Tianjin Infectious Diseases Hospital. In 1996, he began graduate studies at the Hebrew University of Jerusalem, where he first obtained an M.Sc. under the guidance of Prof. Hagai Ginsburg in the Department of Biological Chemistry and then joined Dr. Howard Cedar's lab at the Hadassah Medical School. Life in a foreign country was made easier by the support he received from Dr. Cedar. His studies on gene repression suggested a mechanistic connection between DNA replication timing and gene expression. Dr. Zhang received his Ph.D. in 2004 and was awarded The Aharon Katzir Prize. He is now a postdoctoral fellow in Dr. Daniel Haber's laboratory at the Cancer Center, Massachusetts General Hospital and Harvard Medical School. Dr. Zhang's life was recently made richer by the arrival of a baby daughter.



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