

« Take a walk with the two-motor domain kinesin protein as it moves stepwise along the cellular roadway known as a microtubule. Microtubule binding energy and a second energy source, ATP, fuel kinesin as it repeatedly slings its rear “foot” around to take the lead position, moving the molecule along in a ratcheted manner.

# WE GET A Kick FROM KINESINS

By Paul Muhrad

Illustrations by Graham Johnson

Under the hood of the cell, researchers get their hands dirty exploring the motors that propel molecular cargo along cellular superhighways.

AT A RECENT SEMINAR, HHMI investigator Larry Goldstein flashed a slide of Godzilla, the monster of Japanese sci-fi, towering over a cityscape, devouring a string of railroad cars. The next slide showed Arnold Schwarzenegger as Conan the Barbarian, bedecked in fur loincloth and sword, muscles bulging. Goldstein’s point was to remind his audience that size matters: An organism’s size can impose some daunting challenges on the cells it contains. ¶ Conan’s massive legs, Goldstein said, contain individual axons—wiry projections from nerve cells, or neurons—that from the tips of his toes to the base of his Barbarian spine span more than 1 meter. At one end of that

axon are nerve endings that would alert Conan if, say, a bad guy stepped on his foot. But those nerve endings are assembled from proteins manufactured in the cell body, at the other end of the long cell. How, Goldstein asked, do those molecules get from one end of the neuron to the other? And if Conan's nerve endings get injured, how can the cell notify its central command center, back in the cell body, so that Conan can respond appropriately?

One short answer is "kinesins."

Along with their kin from the dynein and myosin families, kinesins are motor proteins that the cell uses to propel molecular cargo. In recent years Goldstein, who is at the University of California, San Diego, and other investigators have developed high-tech methods for watching how these molecular motors move. They have produced a dazzling gallery of photographs and videos revealing the inner world of cells in motion. And their discoveries have uncovered links between malfunctioning molecular motors and some destructive human diseases.

### GIANT AXONS OF THE SQUID

Ronald D. Vale entered the molecular-motor field in the early 1980s at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, where, as a graduate student, he studied squid giant axons. These nerve wires, which trigger squids' rapid escape from danger, are close to a millimeter in diameter, about 100 times thicker than mammalian axons. Under an ordinary light microscope, Vale and his Woods Hole collaborators Mike Sheetz, Bruce Schnapp, and Tom Reese could see individual filaments running down the length of the axon. Video-enhancement methods, developed independently by Robert Allen, of Dartmouth College, and Shinya Inoue, of the MBL, enabled the researchers to follow the smallest visible features, tiny organelles (cellular components) traveling along the filament tracks.

"Our prejudice was that actin and myosin were the major motile system," as they are in muscles, recalls Vale, now an HHMI investigator at the University of California, San Francisco (UCSF). But electron-microscope examination showed that the filaments were microtubules—hollow fibers best known for forming the spindle that chromosomes traverse during cell division—and



myosin motors do not ride on microtubule tracks.

So Vale began isolating the proteins from squid axons in search of the organelle-transporting mechanism. Assuming the motor protein probably was bound to the organelles, he mixed various protein combinations from squid axons with organelles and microtubules and then viewed the mixtures under a microscope, hoping to find one that would cause the organelles to glide along the microtubules. One late night in the lab, Vale ran a set of experiments that left out the organelles. "We just wanted to make sure that nothing was happening if we didn't have the organelles there," he explains. But something *was* happening—one of the protein mixtures stuck to the glass microscope slide and sent the microtubules gliding along the surface,

## Conducting the Choir

"There's a whole universe of other kinds of motor proteins out there," says Anna Marie Pyle, an HHMI investigator at Yale University's school of medicine. Pyle's lab studies RNA helicases, which traverse RNA strands rather than protein cables. Pyle's lab recently measured the movements of the NS3 helicase, which hepatitis C virus uses to smooth out its RNA genome as part of its replication cycle. Instead of observing individual helicase molecules under the microscope, Pyle and postdocs Victor Serebrov and Jane Kawaoka devised innovative enzyme-mixing experiments to demonstrate that the helicase operates just like those pliers you use to separate speaker wires.

"You attach that little tool onto one of the wires and pull it through the hole, and then the other strand gets stripped off. And just like your hand has to let go and then come closer to the pliers as you pull the wires through, that's how these proteins appear to behave," Pyle says.

Pyle's analysis showed that the helicase plows through exactly 18 base pairs with every rip, and then pauses to regain leverage. The researchers credit the unprecedented accuracy of their measurements to the fact that they were able to synchronize the helicase molecules with extreme precision, allowing them to time the motions of many motors simultaneously.

"We think single-molecule experiments are great, and we are doing them, too. But bulk enzyme experiments are often discounted by people who say, 'Oh well, you can't hear the notes if everybody's singing together,'" Pyle jokes, defending her different approach. "But that's not true if you have a good choir. You can hear them perfectly well, and you can often hear them louder." —PAUL MUHLRAD



Scientists now recognize kinesin as one of the most prevalent proteins in cells—having found it in just about every organism and cell type in which they have looked.

even without any organelles. “That was a complete, after-midnight, ‘can’t-believe-I’m-seeing-this’ result!” Vale recalls.

With more experiments, he isolated the motor protein from the mixture and named it kinesin. Scientists now recognize kinesin as one of the most prevalent proteins in cells—having found it in just about every organism and cell type in which they have looked.

From that summer at Woods Hole, Vale was hooked on motors. “The whole field is so captivating,” he says. “Watching movement created by protein molecules under a microscope—it doesn’t get any more interesting than that.”

#### POKING UNDER THE HOOD

Since those pioneering experiments, cell biologists have become even bolder in their quest to understand the effects of motor proteins. Once satisfied merely to see organelles and microtubules in motion, now they want to observe the machinations of the proteins themselves—and of their individual parts.

In 1996, Vale and Robert Fletterick, a colleague at UCSF, probed the very depths of kinesin. Using x-ray crystallography—a technique for studying protein structures—they mapped the three-dimensional structure of the protein’s motor domain, the part that contacts microtubules. Before solving the structure, Vale and Fletterick had assumed that kinesin must move in a fundamentally different way than the better-characterized myosin motor. After all, myosin “hops” along actin filaments, falling off after every jump, while kinesin takes many steps along microtubules before falling off. Also, the central core of the kinesin “engine”—the motor domain—is less than half the size of myosin’s, and the sequence of amino acids in the two proteins is completely different. But surprisingly, the x-ray pictures showed that the motor domains of myosin and kinesin had practically identical shapes. “That really changed our thinking,” Vale recalls. “These are not two completely unrelated [proteins], but they’re actually variations, in many ways, of a similar basic machine.” That realization, Vale says, led his research team to develop new experiments to understand the next part of the puzzle: how the motor works.

As much as the x-ray crystallography advanced scientists’ understanding of kinesin, it still could not explain how the motor moves. The x-ray images were static snapshots of the protein, posed in only one of its many contortions made during its travels. So Vale and his colleague Ronald A. Milligan, of the Scripps Research Institute in La Jolla, California, turned to electron microscopy to collect a set of action shots. That technology would not let them directly watch the proteins in motion either. Instead, they took freeze-frame pictures of individual kinesin motors walking along microtubules. To achieve that goal, they combined kinesin molecules with various chemical analogs, or look-alikes, of ATP (adenosine triphosphate), the



⚡ Taken nearly two decades ago, this classic image of kinesin motor proteins in repose was captured with an electron microscope.

molecule that provides the energy kinesin needs to move. The researchers knew that kinesin underwent a shape change when it bound ATP, another change when the

ATP converted to ADP, and still another alteration when it released the energy-spent ADP. With structures and chemical properties close to but not quite the same as ATP, the analogs served as monkey wrenches tossed into the gear works, locking the motor in one or another of those positions.

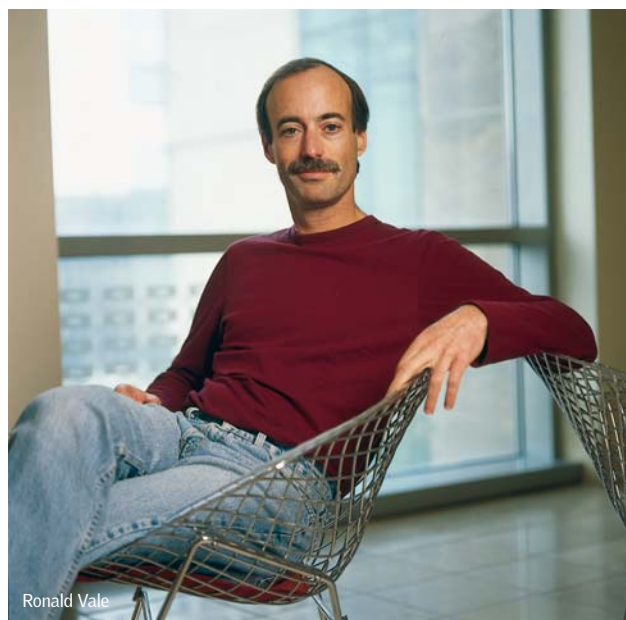
The complete kinesin protein, it turns out, is composed of two ball-shaped motor domains—the “feet”—tethered by short strands to a rod-shaped torso. Vale and Milligan suspected that the tethers, called the “neck-linker regions,” were the critical hinges that controlled kinesin’s movement. By attaching minuscule gold beads to parts of the neck linkers, the researchers flagged those positions of the molecule to make them clearly visible under the electron microscope.

The microscope images confirmed Vale’s suspicions that kinesin’s neck linker makes a series of swinging motions as it cycles between ATP binding, breakdown, and release. When ATP binds the motor domain, the neck linker momentarily snaps down toward the microtubule and throws its partner motor domain to the next step along the microtubule. As the ATP changes to ADP, the neck linker and motor domain relax and release their footing, poised to take the next step. The cycles for each linker and motor domain are coordinated, so that when one foot steps down, the other steps up.

#### PROTEINS WITH HEADLIGHTS

If crystallography and electron microscopy gave molecular gearheads like Vale a chance to get their hands greasy tinkering with kinesin’s engine, then fluorescence microscopy offered a broader view of the vehicles in motion.

Researchers in Goldstein’s lab monitor traffic patterns inside nerve axons by essentially equipping motor proteins with headlights. They have devised



Ronald Vale

ways to attach fluorescent molecules to the proteins or their cargoes, which illuminate them as they travel through the cell, looking like cars cruising along a dark highway. And the scientists have seen some cellular freeway snarls rivaling the rush-hour traffic outside their La Jolla lab.

Goldstein first became interested in cellular traffic flow after his lab cloned a number of kinesin genes from fruit flies and began studying mutants. Examining the neurons of mutant flies with dysfunctional kinesin motors, his group saw a striking effect: They accumulated clogs of organelles and vesicles throughout their axons. This paralleled earlier work by Daryl D. Hurd and William M. Saxton, who reported similar effects in other kinesin mutants. Goldstein recognized that such clogs represented a fairly general defect associated with cellular transport problems.

It came as no surprise that defective kinesin could slow traffic, but Goldstein had not quite appreciated the significance of his observation until he read up on Alzheimer's disease at the university library. Coming across some electron micrographs of brain tissue that illustrated markers of Alzheimer's disease called dystrophic neurites, he realized that the diseased brain cells looked exactly like the clogged nerves in his fly mutants. It dawned on Goldstein that motor-driven cell congestion might be at the root of this devastating neurodegenerative disease.

Thinking back to Conan's meter-long leg neuron, Goldstein put the problem into perspective. "If you convert microns to feet, you have a 30- to 50-foot room (the cell body) where all the synthesis happens; and this long tube (the axon) that's 200 miles long that you have to move all these things you built down to the synapse." And some of those cargoes are not much narrower than the axon itself. "It looks like the Achilles heel of the cell," Goldstein says. So he and his colleagues immediately began searching for a link between kinesin and Alzheimer's disease, and before long they found one. They discovered that amyloid precursor protein (APP), which leads to the "amyloid plaque" deposits that litter the brains of Alzheimer's patients, appears to work like a tow hitch, helping to latch kinesin motors to many of the cargoes they haul across the cell. When researchers in Goldstein's lab illuminated the APP in fruit flies by fusing it with a fluorescent protein, they saw tiny yellow spots cruising down the axons in the fly. But when Shermali Gunawardena, a postdoc in the Goldstein

Researchers have devised ways to attach fluorescent molecules to the proteins or their cargoes, which illuminate them as they travel through the cell, looking like cars cruising along a dark highway.

lab, introduced excessive levels of APP in the fly, she saw the same type of axonal traffic jams as occurred in the kinesin mutants, reinforcing the connection between kinesin-driven nerve traffic and Alzheimer's disease.

Goldstein's lab has also been looking into the role of motor proteins in other human diseases and has uncovered some tantalizing leads. Gunawardena recently discovered that pathogenic forms of huntingtin, the protein associated with Huntington's disease, another genetic neurologic disorder, also causes axon traffic jams.

#### TAKING A HIKE

Vale, together with collaborators in Paul R. Selvin's lab at the University of Illinois, recently took fluorescence-imaging technology back to kinesin's individual moving parts, directly watching the motor taking steps along microtubules by mounting a single fluorescent molecule onto one of its feet.

A debate had been simmering about the protein's "stride." Vale's group had proposed a normal gait, each foot moving past the other with every step. Others had envisioned a model more like an inchworm or a wedding march: one foot always advancing first, with the other following and then meeting it in place.

To settle the argument, Vale and Selvin took the approach of a track coach affixing reflective dots to a runner's feet to analyze the stride. They attached an individual fluorescent molecule to one of the two motor domains on kinesin molecules and then watched the motors walk along microtubules. To detect the faint light and discern the incredibly small steps made by the motor domains—strides of only a few nanometers (nm, or millionths of a millimeter)—the team developed a sophisticated microscope that could track a single dyed domain traveling a fraction of a kinesin step. And to slow down the motors so they could carefully capture every step, the scientists starved the molecules by supplying precious little ATP fuel.

If the motor used the inchworm walk, its illuminated foot would have moved 8.3 nm with every stride. But after measuring hundreds of kinesin steps, the team found that each foot moved about 17 nm per step—the distance predicted by the normal gait model, in which the foot travels from 8.3 nm behind the "torso" to 8.3 nm in front of it.

Now, as part of his quest to understand kinesin's motions on a more basic level, Vale has experiments in the works to revisit the moves of the critical neck linker that he initially outlined with electron microscopy. "We'd like to look directly at what the neck linker is doing as the molecule is walking, and we're trying to put little fluorescent sensors into the molecule that are sensitive enough so that we can measure those motions." ■



The online version of this story contains links to sites that visualize the liveliness of kinesins. Visit [www.hhmi.org/bulletin](http://www.hhmi.org/bulletin) to connect to kinesin-related animations, movies, and still images.