

## 2012 Albert Lasker Basic Medical Research Award

**Michael Sheetz**  
**James Spudich**  
**Ronald Vale**

For discoveries concerning cytoskeletal motor proteins, machines that move cargoes within cells, contract muscles, and enable cell movements.

The 2012 Albert Lasker Basic Medical Research Award honors three scientists for their discoveries concerning cytoskeletal motor proteins, machines that move cargoes within cells, contract muscles, and enable cell movements. By developing systems that allow reconstitution of motility from its constituent parts, **Michael Sheetz** (Columbia University), **James Spudich** (Stanford University School of Medicine), and **Ronald Vale** (University of California, San Francisco) established ways to study molecular motors in detail. These accomplishments enabled the discovery of the motor protein kinesin and unveiled the steps by which these engines convert chemical energy into mechanical work. The miniscule motors underlie numerous vital processes, and the landmark achievements of Vale, Spudich, and Sheetz are driving drug-discovery efforts aimed at cardiac problems as well as cancer.

Since 1774, when microscopist Bonaventura Corti discovered “torrents” of fluid inside plant cells, scientists have known that even tiny units of life bustle with motile activity. By the mid 1900s, researchers had observed chromosome separation during cell division and discovered that material travels long distances within nerve cells, but existing methods could not untangle these processes. Throughout most of the 20<sup>th</sup> century, the study of biological movement focused on muscle contraction, an activity that is fueled by the energy-rich molecule ATP.

In 1954, two British scientists, Hugh Huxley and Andrew Huxley (unrelated), independently proposed that muscles contract when filaments of two types—made of the proteins actin or myosin—slide past each other. Hugh Huxley later proposed that motion occurs when myosin molecules repeatedly grab actin, exert a stroke, and then let go. In this scenario, the mechanical force results from a precise set of structural changes in myosin when it binds ATP and breaks the high-energy bond. This model remained hard to test, however, because scientists lacked an experimental system that allowed them to piece together the reaction from its parts.

Fascinated by the mechanism with which ATP drives cellular activities, Spudich joined Hugh Huxley’s laboratory as a postdoctoral fellow. He was struck by the inability to reconstruct muscle contraction in a test tube. Spudich also learned that scientists had discovered actin and myosin inside nonmuscle cells; they had proposed that these proteins power intracellular movements, yet little was understood about the processes. When

Spudich set up his own lab in 1971 (UCSF), he embarked on two quests: to establish a way to study movement in a test tube and to explore nonmuscle motility.

### **First test-tube system**

Over the next dozen years, Spudich laid the groundwork for developing a test-tube—or *in vitro*—system. Among many challenges, he needed to find a source of actin filaments that faced the same way. Actin's subunit proteins possess directionality and form fibers that “point” in a certain direction. Inside cells, these tracks foster directed movement. Spudich positioned actin on glass slides and then added myosin-coated beads. Although some of the beads jiggled, they didn't reliably trek along the filaments, presumably due to insufficient actin alignment.

In 1982, Sheetz (then at the University of Connecticut Health Center) began a sabbatical in Spudich's lab (now at Stanford) and the researchers decided to try working with the alga *Nitella* because it contained long and well oriented actin filaments. They slit the cells, splayed them open to expose the actin fibers, and added myosin-coated fluorescent beads. The beads streamed in a single direction along chloroplasts, which mark actin fibers. Spudich and Sheetz had, for the first time, created an *in vitro* assay for myosin movement. In 1983, they published a description of this system.

This innovation was momentous, but the *Nitella* cell innards were ill defined, and the work did not directly prove that myosin was moving on actin. Freshly invigorated, the researchers returned to the effort of building an actin scaffold on glass—much as nature did in the *Nitella* cells—on which they could assemble the minimal components for movement.

In 1985, Spudich, his student Stephen Kron, and Sheetz achieved this feat, creating an assay that allowed reconstitution of the process from scratch. They used the system to show that purified actin, purified myosin, and ATP could support myosin movement at rates consistent with the speed of muscle contraction.

### **A new motor protein**

In the meantime, Vale had enrolled at Stanford as a neurobiology graduate student. The Sheetz and Spudich work captivated him: Perhaps, he thought, the actin-based system carried chemical-filled lipid bubbles, or vesicles, inside axons, the long protrusions of nerve cells.

In December, 1982, a paper appeared that stoked Vale's interest in this question. Using a video camera and computer hooked up to a microscope, Robert Allen, Scott Brady, and colleagues had peered inside the squid's giant axon and witnessed an unexpected flurry of activity. Myriad particles—presumably organelles and vesicles—zipped through the cytoplasm. This behavior resembled that of myosin-coated beads in *Nitella*, so Sheetz and Vale decided to explore whether the particle movements in the squid's giant axon relied on actin.

In 1983, they went to the Marine Biological Laboratory in Woods Hole and teamed up with microscopists Bruce Schnapp and Thomas Reese. The researchers harvested the innards of squid axons—including elements of the cytoplasm's filamentous framework, or cytoskeleton. In the presence of ATP, organelles moved along tracks of unknown identity. When Schnapp and Reese turned the electron microscope on the samples, they found that the structure and composition of the filaments typified not actin, but microtubules—a different cytoskeletal protein. Much to the researchers' surprise, they were not studying myosin-based movement.

Now knowing that transport depended on microtubules, they could trade the mixed filament system in the squid for purified microtubules as they teased apart the reaction's components. Reasoning that the organelle surfaces carry the engine that propels them, the researchers next added purified organelles and ATP to pure microtubules. Few organelles budged. When they added soluble proteins from the cytoplasm, the organelles whizzed along the fibers.

Additional experiments suggested that an abundant motor protein inhabits the cytoplasm—and that it fortuitously sticks to glass. When the investigators exposed glass to the soluble proteins and added microtubules, the glass-attached motors transported the filaments across the surface.

This new assay helped Vale and Sheetz purify the motor protein. In 1985, they named it kinesin, after the Greek root "kinein," which means "to move." Cow brains held a similar molecule, so it was not peculiar to squid. The team showed that kinesin differs from the only other protein known to bind microtubules and do work, dynein, suggesting that kinesin belongs to a novel class of force-generating molecules.

Like actin, microtubules possess directionality because their subunits attach in a head-to-tail fashion—and inside the cell, the microtubule filaments are arranged in a characteristic way. The investigators showed that kinesin movement corresponds to traffic from the center of a nerve cell to its axonal tips—and a different motor in the squid's cytoplasm, which was subsequently identified as dynein, travels in the opposite direction.

In 1986, Kron and Spudich worked out a scheme—similar to the one developed for kinesin—for visualizing actin filaments gliding along myosin-coated surfaces. Together, this work from 1983-1986 established new and powerful ways to study movement *in vitro*, and these systems remain the gold-standard assays today.

### **Molecular motor mechanisms**

With the assays for cytoskeletal motor activity in hand, researchers all over the globe began analyzing the molecules in detail. In 1987, Spudich discovered that myosin's head constitutes that protein's engine, an observation that focused scientists on that portion of the molecule. Vale established that an individual kinesin molecule can pull a microtubule and that this motor, unlike muscle myosin, can take many steps on its filament without

detaching. Sheetz, Spudich, Vale, and others showed how myosin and kinesin harness ATP's energy to generate the power strokes. For instance, Spudich measured the force and step size produced during one myosin reaction cycle, and Vale found that the parts of myosin and kinesin that bind ATP and drive movement strongly resemble each other structurally, even though their amino acid sequences are not similar.

Now we know that humans have dozens of kinesins and myosins. The motors differ in their cargoes and mechanistic details, but they share a strategy for converting small perturbations at the ATP-binding site into a force that provokes movement.

### **Motors in medicine**

Myosin and kinesin operate in a broad range of physiological activities, and Spudich, Vale, and Sheetz's breakthroughs have laid the foundation for many potential medical applications. Defects in genes for cardiac myosin can produce a disorder called hypertrophic cardiomyopathy, a leading cause of death in young athletes. Agents that might improve cardiac myosin performance are under investigation for this and other heart conditions. Flawed kinesin has been implicated in at least one neurological illness and, because kinesin is essential for cell division, which runs amok in cancer, chemotherapeutic drugs that target kinesins are being developed.

At every step, Vale, Sheetz, and Spudich devised robust experimental systems and ideas with which to attack stubborn problems. Through their vision, ingenuity, and persistence, these scientists opened the study of molecular motors and illuminated crucial features of a fundamental biological process.

*By Evelyn Strauss, Ph.D.*