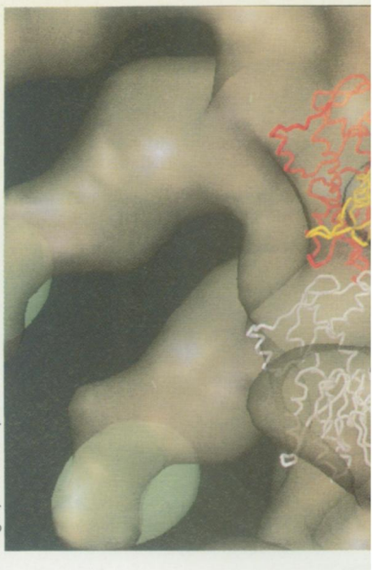
Minimizing Molecular Motor Mysteries

Physics and biology work together to unravel the basis of movement

By GABRIELLE STROBEL



Milligan/Michael Pique

Viewed through a microscope, a live, cultured cell doesn't seem to do much except lie placidly in its dish.

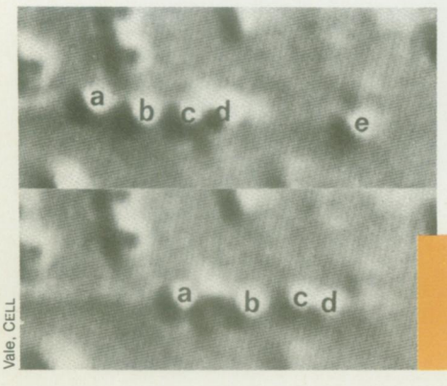
But that's just a facade. The inside of the cell looks like Grand Central Station at rush hour: dizzying traffic, frantic motion. At any given moment, countless objects in the cell are zipping along thread-like tracks, traveling to the cell's distant reaches and back.

Scientists have long known that certain enzymes, called molecular motors or motor proteins, somehow power these and other movements in all living things, from the wiggling of sperm to an organism's final breath. Existing independently of the nervous system, these enzymes are present in each cell of every organ, setting up an intracellular transport network without which no cell can go about its business.

These locomotive enzymes burn a cellular fuel called adenosine triphosphate (ATP). They translate that chemical energy into a series of changes in their shape that ultimately propels them forward.

That feat goes far beyond enzymes' traditional role of speeding up biochemical reactions, and it puzzles scientists. In fact, even though the first motor protein was discovered in 1864, scientists have only recently begun to understand just how these molecules work.

Technological advances in biology and physics are revving up research on kinesin and myosin, the two major kinds of motor proteins. Building on research during the last three decades, several groups are now "setting the stage to solve the mechanism by which these biological motors move," says Steven M. Block, a biophysicist at the Rowland Institute for Science in Cambridge, Mass.



Vale, CELL

Latex beads (150 nanometers across) being transported along a microtubule (25 nanometers thick) by the motor protein kinesin (not visible). In the cell, kinesin carries vesicles in the same manner.

The development 10 years ago of *in vitro* motility assays — techniques that enable scientists to take motor proteins out of cells and study them on glass slides — "blew the lid off the field," recounts Block. It allowed researchers to remove motor molecules from their complex environment — the cell — and study their movements under simpler, more tractable conditions. "This [was] a physicist's dream: We [could] literally mix isolated proteins from test tubes and produce movement under the microscope," explains Block gleefully.

Prior to the early 1980s, scientists could only study whole muscle fibers or motor proteins in solution. The sheer number of motor molecules in such preparations — one muscle fiber contains billions — obscures the action of individual motors. Together with the *in vitro* assays, that research led to ambiguous results that still provoke heated discussions about how the molecules work.

Today, microscope technology has advanced to the point where researchers can examine movement at the level of individual molecules, opening the door to a resolution to the debate.

Playing a key role are microscopic "optical tweezers," which use laser beams to make possible the study of single molecules. Another tool, electron microscopic and crystal-structure images of myosin (SN: 7/3/93, p.4), allows researchers to picture the interaction of myosin with other proteins in almost atomic detail. Together, these techniques promise to solve the old riddle of how some proteins move.

Motor proteins travel along protein filaments that span the cell, forming an intricate, railway-like transport system. They perform myriad functions. Kinesin carries vesicles, tiny storage sacs containing chemicals or cellular waste material, from one processing site to the next. Mitochondria, the cell's tiny power plants that produce ATP, hitch a ride on kinesin, which takes them to where energy is needed.

Different proteins serve particular routes. For example, kinesin ferries neurotransmitters down the extensions of nerve cells, whereas dynein, another motor protein, shuttles cargo in the opposite direction, from the nerve terminal back to the center of the cell, says Ronald D. Vale, a cell biologist at the University of California, San Francisco.

Myosin, the best-studied molecular motor, works with actin filaments to bring about muscle contractions. Both motors play a role in cell division: Kinesin helps sort out the chromosomes, and myosin then pinches the furrow between the cells, gently forcing them apart.

Besides moving particles within cells, molecular motors can drive whole cells, sometimes for long distances. Lymphocytes use the motors to speed them to an infection site; embryonic cells use them to migrate to their final destination in a developing organism.

"The definition of life is movement," says James A. Spudich, a biochemist at Stanford University. "Molecular motors are that fundamental."

Recently, Block and his collaborators have for the first time "watched" the gait of individual kinesin molecules. While directing the spotlight of a laser beam onto single

Electron-microscope-based computer graphic shows contours of muscle filament (gray). Image of myosin's crystal structure is docked into filament.

molecules, the researchers found that kinesin actually walks along its protein rail, or microtubule, in discrete little steps of 8 nanometers each. They describe their study in the Oct. 21 NATURE.

"Does kinesin slide along smoothly, does it walk in a stepwise fashion, or herky-jerky? This fundamental question has never been answered adequately until now," says Block.

"It's a brilliant piece of work," comments Vale, who discovered kinesin in 1984. "It clearly shows kinesin making distinct steps, something people in the field have tried to see for a long time."

The measurement of kinesin's "stride" dovetails nicely with current understanding of the structure of microtubules, thread-like protein polymers, says Vale. When a microtubule forms, its individual protein units line up in such a way that a binding site for kinesin appears every 8 nanometers — exactly the distance Block's group has measured between steps. Vale likens kinesin's movement along a microtubule to walking across a pond on evenly spaced stepping-stones.

While these measurements represent a quantum leap for kinesin research, they raise the question of exactly how kinesin moves. The detailed structure of the molecule remains unknown, but researchers do know that it has two domains that generate movement. Attached to each of these domains is a long, thin tail, and the two tails wind around each other. Eight nanometers, ordinarily a tiny distance, is huge for kinesin, whose motile region measures only 9 nanometers across. "Just bending kinesin's motor domain won't move it by 8 nanometers," says Vale.

Based on this sketchy structure, researchers assume that kinesin, a two-legged protein, prances along a microtubule, explains Vale. But, he adds, this notion rests on little hard evidence because of the relative dearth of data on kinesin.

No lack of data plagues myosin researchers. They drew up a detailed model describing its mode of action 25 years ago. But since then, supporting experimental evidence "has not materialized in quite the way people had hoped," says Block. Consequently, the theory has come under attack in recent years.

The hypothesis holds that myosin molecules, arranged in long filaments, latch on to binding sites along actin filaments and perform a "power stroke" that pulls the actin filament past the myosin filament. They then let go of the actin and grab on to another binding site, farther downstream, to crank out the next stroke. Developed in the 1960s and elaborated ever since, the model suggests that myosin has a lever-like arm that enables it to ratchet its way along the actin filament.

This scenario is featured in every biochemistry textbook, but the structural changes in the molecule that it proposes have turned out to be difficult to prove. Though many researchers still regard the model as correct, others doubt it. Toshio Yanagida of Osaka University in Japan has called for an entirely "new conceptual framework" to account for myosin's mode of action.

Much of the disagreement concerns the motor's mileage, or how far it can go per unit of fuel. While the standard myosin model proposes a step of about 10 nanometers per molecule of ATP, Yanagida's studies indicate that a myosin motor can leap up to several hundred nanometers per ATP molecule and suggest that the exact distance can vary, depending on how hard the muscle works.

The debate stems from experiments involving large numbers of motor molecules. Since larger numbers mask the action of individual molecules, Spudich and his co-workers turned to optical tweezers to try to settle the dispute. Over several years, they adapted optical tweezer technology — also known as laser light traps — to the study of myosin. Then earlier this year, while observing single myosin proteins at work, the Stanford researchers recorded the discrete steps that a single myosin molecule takes along actin.

The group's findings land smack in the middle of the controversy surrounding myosin's mechanism. "We asked the question whether myosin jumps along actin filaments in big leaps or marches in sequential little steps, and we observed very clear, small steps," says Spudich. "When myosin grabs the actin filament and pulls on it, we record 10-nanometer blips." His group also used light-trap technology to measure the horsepower of the minute motor and found it to be "about 5 piconewtons per stroke."

The 10-nanometer stride length makes sense from a structural point of view, Spudich says, because studies have shown the myosin protein to sport a thin, extended "neck." That neck could cause a 5- to 10-nanometer displacement when snapping backward in the large structural change that researchers think is at the heart of the mysterious power stroke.

Thus the Stanford researchers' findings provide direct support for the conventional model. Moreover, the results of the Rowland group are consistent with it, says Block. Looking at kinesin, a different molecular motor, Block's co-workers measured small steps rather than big leaps and report evidence that, under certain experimental conditions, kinesin advances one step per molecule of ATP burned.

However, "our data only describe what kinesin does," Block cautions. Regarding myosin, he adds, "the techniques that were available previously were not sensitive enough to resolve the controversy."

The optical tweezers that are now boosting molecular motor research resemble the "tractor beam," a beacon of light that captures, stalls, or moves objects around on "Star Trek." The real-life cousin of the fictitious tool is too weak even to hold a pinhead. But when shone through a microscope, the tiny, piconewton forces that an intense laser beam can exert prove ideal for manipulating objects in cellular space. The molecules aren't damaged because they don't absorb the wavelength used.

First developed by physicists at AT&T Bell Laboratories in Holmdel, N.J. (SN: 3/10/90, p.148), the technique enables researchers to trap single molecules such as kinesin or myosin.

Block's and Spudich's groups independently developed the laser trap further to apply it to the study of motor proteins. A graduate student in Block's laboratory, Karel Svoboda, combined the trap with an interferometer, a device that measures minute differences in the wave pattern of light diffracted by a particle in its path. This marriage yielded a new instrument, dubbed the "optical trapping interferometer," that can detect "the displacement of a tiny object to within an angstrom, which is the diameter of a hydrogen atom," explains Block. It does so in a thousandth of a second.

The researchers paid a price for that precision, though. "It took us several years to insulate our measurements from all kinds of disturbances," says Block. For example, Brownian motion, the random movement of water molecules in solution, jostles the tiny objects. In studying specimens as small as a single molecule, says Block, Brownian motion can cause spu-

rious signals that are larger than the signals Svoboda sought – namely, the 8-nanometer strides of kinesin.

Moreover, airborne vibrations proved notorious troublemakers, Block recounts. Since the interferometer is sensitive enough to pick up the sound waves of a conversation, the researchers conducted many of their experiments at night, when the lab was quieter, he says.

Once everything was still, they could record the gait of one kinesin molecule, but only after shifting it into first gear. In a cell, kinesin speeds at about 1 micrometer per second, the equivalent of a person running at 55 miles per hour. To measure its movement reliably, the investigators had to slow it down to about 0.01 micrometer per second, Block explains.

While the Rowland researchers stayed up late with their laser beam, Spudich's group across the country also spent nightly vigils with lasers. They devised a method of stretching a normally floppy actin filament taut between two laser beams and then lowering the filament to touch a single myosin molecule, Spudich explains. With the two laser beams holding the ends of the actin filament fast, the researchers could measure the force exerted on the actin by the myosin molecule. They did so by adding a feedback loop to the laser trap. That allowed them to record the force with which the actin filament tried to escape the grip of the laser beams, says Spudich.

Optical tweezers "seem to be the way to go to answer many questions," says Vale. But, he adds, "they do not tell us the entire story. They reveal nothing about the structural changes in the motor molecules that accompany the steps. That area is extremely important but still a black box for kinesin."

Again, researchers studying myosin have made some headway in assembling data from structural biology and optical tweezers into a complete picture. The crystal structure of myosin – its three-dimensional makeup – has been unveiled recently and imaged with near-atomic resolution, summing up what Block calls "20 years of biochemistry on myosin."

In a report accompanying the publication of myosin's crystal structure, a team of researchers collaborated to produce a detailed model of the sequence of events that brings about myosin's power stroke. It involves the alternate opening and closing of myosin's several clefts and pockets. To develop the model, the researchers also used the previously discovered structure of actin and computer models illustrating how myosin and actin bind (and function) together in the elaborate superstructure of a muscle filament.

To understand how myosin moves past actin filaments, one needs to know how "the high-resolution crystal structures of actin and myosin fit together to form the large assembly of a muscle filament," says Ronald A. Milligan, a partner in the collaboration.

To find that out, Milligan, a cell biologist at Scripps Research Institute in La Jolla, Calif., began with electron-microscopic images of a muscle filament. Processing these images with computerized image-analysis techniques yielded "three-dimensional maps," which show the assembly's contours, complete with all its bulges and grooves.

Into these contours Milligan and his coworkers then docked the crystal structure of myosin. Marking reference points on the proteins with the element gold, they were able to see in which orientation myosin slips into actin. With that information, they constructed an image of how all the individual proteins come together to form the working assembly found in the cell, he explains. Armed with such detailed knowledge of how actin and myosin interact, the team was able to draw up a step-by-step scenario for myosin's power stroke.

That scenario isn't entirely proved, nor do the new mechanical measurements make an airtight case. But with motor research on the move, "the pieces of the puzzle may soon be put together," says Spudich. "For us, that is a dream come true." □

Continued from p.315

nate plant for a San Diego medical center.

For the highest efficiencies of all – up to 70 percent – alkaline fuel cells have proved the winners so far. Yet these cells, which use alkaline potassium hydroxide as the electrolyte, are also the most expensive to make. NASA and the Defense Department have spent heartily on these lean, pricey systems.

Until recently, civilian applications for alkaline fuel cells looked preposterous. But several companies are seeking to slash production costs and design better methods for storing pure hydrogen, given the alkaline's intolerance to impurities. Soon, even alkaline cells may jockey for position in the commercial fuel-cell market.

The problem of storing hydrogen has plagued fuel-cell advocates from the start. A highly reactive, explosive gas, hydrogen does not lend itself to safe containment. Engineering advances, though, have improved that picture. Other than compressing hydrogen in canisters or cooling it to a liquid, the gas can be extracted as needed from hydrogen-rich compounds, such as methane or ethanol.

Newer systems attempt to hold hydrogen in a metal hydride matrix or activated carbon. As water holds hydrogen well, a more venturesome tack tried by H Power involves controlling the oxidative reduction – rusting – of sponge iron in a cycle that liberates hydrogen as needed. Meanwhile, at the University of California, Riverside, researchers are splitting water molecules with sunlight, using a 12-cell electrolysis unit hooked to a 3.5-kilowatt photovoltaic array.

The major disadvantage of fuel cells – this seeming panacea for energy production – stems from engineering hurdles rather than inherent system weaknesses. Economics, too, have held fuel cells back. Until recently, they've been too expensive to build and operate, costing upwards of $3,500 per kilowatt versus the $1,000 to $2,000 cost of conventional fossil-fuel turbines.

But lately, the economic picture has changed. Better materials and production methods now make fuel cells competitive with gas and oil generators, especially if the expense of an electric grid figures into the equation. Overhead power lines cost $50,000 to $1 million per mile to build, plus maintenance expenses. Fuel cells could potentially make power lines obsolete, with small modular systems running neighborhoods and individual homes.

In fact, DOE is studying the feasibility of fuel cells for commercial and residential buildings, according to Ronald J. Fiskum, a DOE fuel-cell program manager. "We're not looking to reinvent the wheel," he says, "but to see the best way to integrate fuel cells into residential and commercial buildings. Micro-cogeneration – supplying heat and power – is a natural."

Lest fuel cells seem like the final answer to U.S. energy needs, it's worth keeping in mind the technical hurdles researchers must still leap. Cells still suffer material degradation. The life span of commercial stacks must exceed five – sometimes 10 – years to offset the initial capital expense. Current output must hold up steadily for long stretches. And consumers must get accustomed to a hydrogen-based power supply.

Fuel-cell advocates have heralded their solution before. However, where big talk once provoked skepticism, it now calls forth construction contracts.

"Virtually everyone agrees we should move from fossil-fuel dependence toward renewable energy sources," says Martin Gutstein, director of the Fuel Cell Institute in Washington, D.C. "But with fuel cells there's a vicious circle. You can't get cost down until production comes up, and you can't get production up until the cost comes down. The Japanese have taken action here. We've done very little. Now, perhaps, we'll see a turnaround in this country." □