Motor Molecules on the Move

The cell is not just filled with randomly moving parts but is well equipped with special proteins that power internal movements

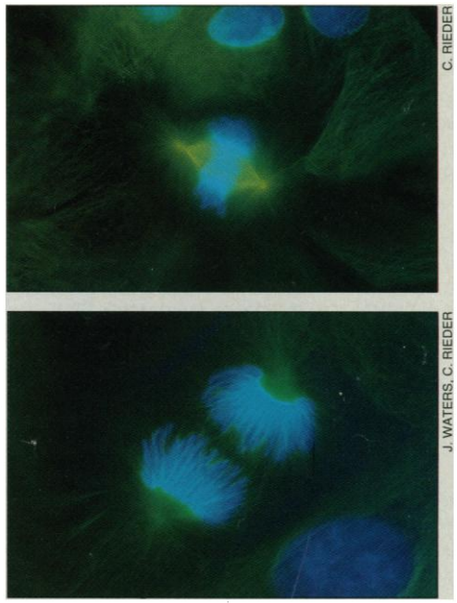
For 100 years, biologists had studied the tiny structures that form the anatomy of the living cell without realizing that they move in a purposeful way. Only within the past decade or so have researchers realized that the structures—sometimes called "organelles"—do not passively float from place to place within the cell but are actively transported along well-laid out tracks, much the way the cars are propelled along an old-fashioned roller-coaster. This discovery has been of more than theoretical interest. As Harvard University molecular biologist Lawrence Goldstein points out, "We should not lose sight of the tremendous value [in understanding intracellular movement] for human health issues."

The abnormal movement of chromosomes during cell division might, for example, cause some of the chromosomal abnormalities underlying cancer development and birth defects such as Down's syndrome, as well as lead to certain types of infertility. So the question of what precisely drives the chromosomes and other organelles from place to place is one of the most exciting issues in cell biology today. And while researchers by no means have a complete answer yet—if anything, their recent results suggest that the movement puzzle is going to be very complicated indeed—the work has launched a new cell biology subspecialty by uncovering a veritable menagerie of "motor molecules" that power organelle movements. "Seven years ago we only had myosin, now we have at least 19 different kinesin molecules and several different dyneins," says Goldstein, referring to the three main types of proteins currently known to serve as motor molecules.

Motors everywhere

Together these motor proteins play a role in many of the cell's most fundamental activities. They may help orchestrate the dance of the chromosomes as they separate into the two daughter cells during cell division—hence the possibility that a defect in the proteins might lead to the chromosomal abnormalities of cancer or birth defects. They guide the migrations of the small membrane-bound vesicles that carry the enzymes that synthesize neurotransmitters to the nerve terminals where the transmitters are made and released. And they may shuttle into place the protein filaments needed for the assembly of large internal cellular structures such as the endoplasmic reticulum, which is where many proteins are assembled.

But while all the motor molecules are important, none has received more enduring attention than myosin, as Goldstein's comment suggests. It's been the focus of biological inquiry for at least 40 years—ever since the two Huxleys, Andrew and Hugh, recognized that muscle contractions are brought about when two types of protein filaments, one consisting of myosin molecules and the other of actin, slide past each other. And it was while studying myosin's action in 1982 that cell biologist Michael Sheetz, who's now at Duke University Medical Center, developed an assay with members of James Spudich's lab at Stanford that was to pave the way for much of the motor molecule research that has come since.



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Getting there. The chromosomes *(blue)* are somehow pulled to the poles of the dividing cell along the spindle fibers *(green)*.

At the time, Sheetz was at Stanford on sabbatical from the University of Connecticut. Then, as now, the exact details of the interaction between the myosin and actin filaments during muscle contraction were hotly debated. The evidence indicated, however, that the motor that powers the sliding of the myosin filaments along the actin filaments is in the globular "head" of the myosin molecule.

What Sheetz and Spudich wanted to know was whether the actin filaments, which appeared to have polarity in that the actin subunits that form the filaments are added more rapidly at one end, in any way dictate the direction in which myosin moves along the actin fiber. In fact, many groups were trying to answer that question, and they were all hampered by the difficulty of tracking the movements of a myosin molecule. Electron microscopy, which had proved very useful for observing the actin and myosin structures, couldn't be used to observe a dynamic interaction between the filaments because the technique requires that materials be "fixed," or inactivated, and the myosin heads were much too small to be seen directly with the light microscope.

So Sheetz and Spudich devised a way to track myosin movements indirectly. They attached the protein to plastic beads that could be seen with the light microscope, and then watched what happened when the beads were put in contact with actin fibers of known polarity in cells from the plant *Nitella axillaris*. The result: The beads traveled in one direction only, suggesting that myosin movement is indeed dictated by the polarity of actin. But just as important, Sheetz and Spudich created a motility assay that "revolutionized the field," in the words of biophysicist Steven Block of the Rowland Institute for Science in Cambridge, Massachusetts.

One of the first things the researchers did with the new assay was to connect motor protein research to an earlier observation made in nerve cells by the late Robert Allen of Dartmouth University in collaboration with Scott Brady of the University of Texas Southwest Medical Center in Dallas and Ray Lasek of Case-Western Reserve University in Cleveland. The three researchers used a microscopic method developed by Allen to watch the fast movement of enzyme-containing vesicles down protein fibers called microtubules from the nerve cell body to the nerve terminals. To Sheetz, the vesicle behavior looked just like that of the beads in the assay he developed with Spudich. He naturally assumed that vesicular movement was being propelled by myosin. But when Sheetz, working first with Allen and later with Ronald Vale, Bruce Schnapp, and Thomas Reese of the National Institutes of Health, attempted to apply the myosin-coated bead assay to Allen's transport filaments in squid nerve, the researchers got a rude shock: It didn't work. The reason soon became clear. The transport filaments in nerve cells are made

not of actin but of a different protein, called tubulin, which does not direct the movement of myosin.

The question then became, What was the motor for vesicle movement? It took the team nearly 3 years, until 1985, to get the answer: It was a new motor molecule, which they isolated from the cytoplasm of squid axons and named kinesin. Independently, Brady identified kinesin from chicken brain. "We knew we had the right stuff," says Sheetz, when they attached some of the pure kinesin to plastic beads and showed that the protein propelled the beads along tubulin filaments, just as myosin propels them along actin filaments.

Vesicle traffic in nerve cells is a two-way street, however, since they move both from the cell body out to the terminals and back again. And shortly after the kinesin work, Sheetz's team, and also those of Richard Vallee at the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, and Richard McIntosh of the University of Colorado at Boulder, obtained evidence that vesicle transport in the backward direction is powered by still another motor molecule, a protein called dynein. Although cell biologist Ian Gibbons of the University of Hawaii in Honolulu had discovered more than 20 years earlier that dynein propels the motors of cilia and flagella, those were the first demonstrations that the protein is important in vesicular transport as well.

But at the time, the function of motor molecules was still limited to vesicular transport. Then in 1990, independent work by McIntosh and his colleagues and by Sheetz, who was then at Washington University in St. Louis, and his colleagues connected them to another major area of research—namely cell division.

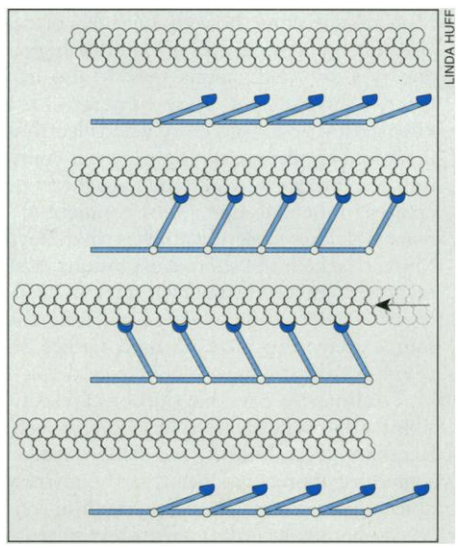
Before a cell divides, the chromosomes first have to be duplicated, then the membrane surrounding the cell nucleus, where the chromosomes are located, disappears, and a complex array of microtubules, called a spindle apparatus, forms. The microtubules, which are made of tubulin and thus resemble transport filaments, extend from each of the cell's ends (or poles) to the duplicated chromosomes, which they somehow pull apart, in the manner of a curtain parting, so that when the two daughter cells form each one gets a copy of every chromosome.

From 1883 until 1960, says Duke University mitosis expert Bruce Nicklas, researchers thought they knew how the chromosomes on their spindle fibers are moved to the cell poles by motors on adjacent filaments called traction fibers. After 1960, however, a number of researchers started uncovering evidence that cast doubt on the traction fiber idea. And then in 1989, Nicklas performed an experiment that pointed to another possibility. He cut the spindle near one pole of a dividing cell, assuming that this would sever the traction fibers as well as the spindle fibers and cause the chromosomes to stop moving. But they didn't stop. They continued to move almost to the tips of the truncated spindle fibers. "We concluded that the molecular motor was within the kinetochore [the site on the chromosome where the microtubules attach] or very close to it. This meant that the chromosomes moved themselves. No one had taken that suggestion seriously before," Nicklas says.

Some researchers have even proposed that motors might not be involved at all in chromosome movement. Take, for example, Tim Mitchison of the University of California, San Francisco (UCSF), who showed in 1986 that microtubules grow and shrink during mitosis. With Marc Kirschner, also at UCSF, Mitchison constructed models in which growth and shrinkage of microtubules could alone account for chromosomal movement. But in 1990 the Sheetz and McIntosh groups showed that dynein is present in the kinetochore, thereby reviving the idea that a motor is needed.

Some researchers now think, McIntosh says, that dynein moves the chromosomes toward the pole ends of the tubulin spindle fibers, and the force generated somehow causes the disassembly of tubulin fibers, causing them to shrink, as Mitchison observed. There is another possibility, however, and that is that dynein merely helps the chromosomes hold onto the spindle, but the actual force of movement is supplied by the disassembly of the tubulin filaments.

While researchers are now convinced that motor molecules are needed to power vesicle movements, and perhaps those of chromosomes as well, they know much less about how the proteins work. Indeed, within the past 2 years, they've had to discard one of their cherished notions about the molecules. This was that kinesin moves vesicles toward one end of tubulin filaments, designated the plus end, whereas dynein moves them toward the other, or minus, end.



Row, row, row your boat. The molecular motors in the myosin heads pull the myosin filaments *(blue)* over the actin filaments.

Kinesin coming and going

Doubt about this idea first sprang up during the late 1980s, the result of work by Sharyn Endow, a geneticist at Duke, who was studying a mutation in fruit flies that causes improper segregation of chromosomes during meiosis, the specialized form of cell division that produces the sperm and eggs. She traced the mutation to the site on the chromosome that encodes claret eye color in the flies and called the gene nonclaret disjunctional (*ncd*). As it turns out, the sequence of the ncd protein is very similar to that of the kinesin, whose gene was cloned and sequenced by Goldstein in 1989. But based on the way the chromosomes move in flies with a mutated *ncd* gene, Endow speculated that this kinesin look-alike would push chromosomes toward the minus ends of tubules, unlike the original kinesin, which directs movement toward the plus ends.

Endow's colleagues, however, didn't see it that way. "Since ncd was so similar to kinesin, they expected it to travel in the same direction," says Endow. "The editors of *Nature* made us remove the statement in our paper speculating that this was a minus-end directed motor." The idea received so much negative feedback that Endow herself lost confidence in it. And Goldstein, who had also come across ncd and was studying its directionality at about the same time, recalls, "We didn't believe our data [showing ncd to be a minus-end directed motor] at first, so we didn't rush to publish."

Subsequent work in the Endow and Goldstein laboratories, as well as that by Edward Salmon at the University of North Carolina, Chapel Hill, has confirmed that ncd is indeed a minus-end directed motor. What's more, McIntosh cites recent work from Manfred Schliwa's laboratory at the University of Munich indicating that dynein, too, can work in both directions. Before, researchers simply assumed that directionality was built into the structure of the motor, but with the new findings, McIntosh says, "The polarity issue has become more complicated than anyone would have ever believed." Mitchison offers the possibility that some protein modification, like phosphorylation, might alter the direction of a motor. "Understanding the basic mechanism of motility would help us understand the directional switches," he says.

But the researchers are still a long way from doing that. Although everyone hopes that myosin will serve as a mechanistic model for kinesin and dynein action, biophysicists are far from a consensus about how even this well-studied molecule works. "We assumed that myosin was a ratchet that went chickety-chickety along the actin fiber," says biophysicist Block. But the 25 July 1991 issue of *Nature* contained three papers with data in

support of three different mechanisms, "all reviewed by intelligent reviewers who couldn't find anything wrong," he says.

A recent refinement by Block of Sheetz's motility assay, which makes it possible to follow the movements of single motor molecules, may help resolve the issue of how motor molecules bring about motility. Using a tool called optical tweezers, Block traps a microscopic silica bead carrying a single motor molecule on its surface with a beam of light and then deposits the bead directly on the appropriate filament where its movements can be monitored. His findings are consistent with the ratchet mechanism, but he has found some interesting differences between myosin and kinesin. The emerging picture is that kinesin spends most of its time bound to tubulin, and only a fraction of the time moving. Myosin, in contrast, spends most of its time unbound from actin filaments.

Block explains the difference by invoking the different circumstances under which each molecule must work. Myosin, he points out, is working as a team, with many heads positioned at regular intervals along an actin fiber, like oarsmen in a scull. As such, each head would do best to "flick lightly and not get in each other's way so as not to jam each other," says Block. But kinesin is more like a rope climber. It operates alone or with very few other molecules to push a vesicle along tubulin. As a result, says Block, kinesin finds itself, "clinging on for dear life to the organelle it's pushing and to the tubule it moves along. If it were to let go, the organelle would float away. To prevent that, kinesin should be unbound for a very short time."

But even that neat picture, portraying an apparent division of labor between myosin and kinesin, has now come under challenge. In the 26 March issue of *Nature*, Sue Lillie and Susan Brown of the University of Michigan Medical School at Ann Arbor report that a member of the kinesin family can substitute for myosin in delivering vesicle-bounded cargo to budding yeast cells.

Whatever their mechanism of action, the story of the molecular motors will probably take a few more twists and turns and changes of direction before it comes to a close. Mitchison predicts that many of the remaining questions will be sorted out by the end of the century, but then he thinks about it and adds, "but with the proliferation of all of these new molecules, the problem is getting more complex, not simpler."

–Michelle Hoffman

Additional Reading

M. Irving, "Biomechanics Goes Quantum," *Nature* **352**, 284 (1991).

T. A. Schroer, "Association of Motor Proteins with Membranes," *Current Opinion in Cell Biology* **3**, 133 (1991).

R. Vallee, "Dynein and the Kinetochore," *Nature* **345**, 206 (1990).

ASTROPHYSICS

Astronomers Bag Another Black Hole

Black holes once lived only in the minds of theoretical physicists and science fiction writers. Now they seem to be everywhere. It's all but certain that gigantic black holes, packing the mass of millions of stars, lurk at the hearts of some galaxies; only the gravitational force of a black hole, many researchers think, could power the titanic energy outputs of "active" galaxies and quasars or pull stars into the dense swarms seen at the centers of some galaxies. Now a new finding suggests that smaller cousins of these monsters, formed when a single massive star collapses at the end of its life, may be equally common.

In a paper submitted to the *Astrophysical Journal*, Ronald Remillard of the Massachusetts Institute of Technology, Jeffrey McClintock of Harvard University, and Charles Bailyn of Yale University report that one unseen member of the binary star system known as Nova Muscae 1991 has its companion in a gravitational grip so powerful that the mystery object must be a black hole. Other astronomers think their case is strong; says Harvard astronomer Jonathan Grindlay, "A black hole is the simplest and most economical way to explain Nova Muscae." The discovery brings the number of well-supported "small" black hole candidates to five, and it suggests that astronomers are becoming efficient hunters of these once-exotic objects.

It was only a year and a half ago, after all, when Nova Muscae first drew astronomers' attention by emitting a massive outburst of x-rays and gamma rays. Spotted by x-ray satellites including the Japanese probe Ginga, Nova Muscae remained one of the brightest objects in the x-ray sky for several weeks after the January 1991 outburst. Such an x-ray nova, astronomers believe, implies the presence of either a black hole or a superdense stellar cinder known as a neutron star; they are the only objects with the gravitational pull needed to yank material off their companions and heat it to temperatures high enough to generate an x-ray burst.

Remillard, McClintock, and Bailyn had grounds for thinking that a black hole was the more likely culprit in the outburst. Recalls Bailyn, "People looked at it and said 'My God, this looks just like A0620-00,'" another binary strongly suspected of harboring a black hole. Like A0620-00 and V404 Cygni, a probable black hole identified since then (*Science*, 14 February, p. 794), Nova Muscae's spectrum showed a huge flux of soft (low-energy) x-rays and a "tail" of higher energy x-rays and gamma rays. It also featured a bright spot at the energy—511 kiloelectron volts—given off when electrons meet and annihilate their antimatter counterparts, positrons, which are thought to be created in the high-energy environment of a black hole. Just as significant was what Nova Muscae lacked: the shorter explosions of x-rays often emitted by neutron stars. In keeping with theorists' picture of a black hole, the source seemed to have no hard surface on which infalling material could explode.

To clinch the case that the object responsible for the outburst was a black hole, though, Remillard, McClintock, and Bailyn needed something more: evidence that the mystery object was too massive to be a neutron star. Neutron stars, according to Einstein's theory of general relativity, cannot have masses greater than about three times that of the sun; such objects inevitably collapse into black holes. The upper limit of stability for neutron stars may actually be much lower, depending on how the protons and neutrons crushed together in a neutron star behave. "A more comfortable upper limit is about 2 to 2.5 solar masses," says Clemson University theorist Donald Clayton.

In their *Astrophysical Journal* paper, the trio reports that no matter which upper limit you assume, Nova Muscae is above the threshold. By using the 4-meter telescope at Cerro Tololo, Chile, to measure tiny Doppler shifts in the spectrum of the sun-like companion star, Remillard, McClintock, and Bailyn found that it circles the mystery object every 10.4 hours, at a speed of at least 400 kilometers a second relative to Earth. Drawing on a little help from Isaac Newton, they calculate that to hold the companion star in this frenetic orbit, the mystery object must be at least 3.1 times as massive as the sun—and more massive still if the companion star's orbit is tilted away from the line of sight.

That finding adds Nova Muscae to a tally of probable black holes that may grow rapidly in the future. Stanford University physicist Roger Romani estimates that as many as 500 of the x-ray emitting binaries that inhabit our galaxy may be powered by black holes. Astronomers think that when they apply their black hole tests to additional transient x-ray sources—Ginga has already found a dozen or so—the objects may prove an easy quarry. "We have just begun taming black holes," Bailyn declares. "They are becoming everyday things rather than some kind of weird, exotic beasts."

–Ray Jayawardhana

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