ONE FOOT IN FRONT OF THE OTHER INSIDE CELLS, SPECIALIZED PROTEINS MARCH DOWN A NETWORK OF HIGHWAYS CARRYING MACHINERY AND MESSAGES. RESEARCHERS ARE LEARNING REMARKABLE DETAILS OF HOW THEY MANAGE IT.

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To get from Boston to San Francisco, a person has a few choices: drive a car, hop on a bus, fly in an airplane, hitchhike from city to city, maybe even ride a bike. Circumstances will dictate which way works best. If the traveler is in a hurry, the bus might take too long. Carrying a large suitcase makes a bike impractical. And if the person is short on cash, a plane ticket may be too expensive.

In every type of living cell, materials jet around in a similar variety of manners. The way cellular cargo travels depends on its size, where it’s headed, how quickly it must arrive, and how much energy is available. Some chemicals circulate passively through a cell, with no need for energy or a road, but others—like building materials needed at the end of a growing cell or chemical messengers that must reach the nucleus—need to move quickly toward a set destination.

Beyond that, some of the goods must first be packaged and then picked up by vehicles that follow an ever-changing highway to the right destination.

In the past half-century, scientists have revealed how cells build these thoroughfares, and they’ve uncovered specialized proteins that walk along the roads’ lengths carrying freight. But plenty of questions remain: How does the cell control the transportation? How do the walking proteins coordinate their steps to keep grounded on their tracks? How can materials hitch a ride on cellular freeways if there isn’t energy to spare?

“It’s become clear that there is an enormous platter of movements that have to be executed by the cell,” says HHMI investigator Ronald Vale of the University of California, San Francisco. “Chromosomes have to be separated, a cell has to pinch in two, materials made in one place have to be delivered to another place in the cell. All of those features of life are dependent on physical motion.”

That physical motion is generated by three types of molecular motors that can walk down tracks inside cells: myosin, which walks on actin filaments, and two microtubule motors—kinesin, which carries cargo from the center of a cell outward, and dynein (the largest and least understood), which carries cargo from the periphery toward the cell’s center. Most of the motors in the cell have two “feet,” which alternate steps as they move. But each protein also has distinct quirks in its movement, a unique form of regulation, and a different role in keeping cells alive.

Research by HHMI investigators and others has revealed that when any of the molecular motors fails, it causes not only traffic jams and lost messages but also faulty construction and demolition of the cells’ roads, and that can lead to disease. Understanding the process better, scientists think, can help them learn how to rev up the engines of the motors, keep their steps on track, and rebuild the transport systems that are needed to keep a cell alive.

**THE RIGHT MOTOR FOR THE JOB**

Since the dawn of microscopy, scientists peering into the innards of cells have seen many moving parts. The earliest experiments on mobile proteins studied muscle cells, an obvious place to look for molecular movement. More than 50 years ago, scientists isolated two proteins—myosin and actin—from muscle cells. Andrew Huxley and Hugh Huxley (no relation) independently proposed that actin filaments slide across myosin thick filaments in the presence of the cellular energy molecule ATP. As this idea gained traction, it also became clear that isolated molecules of myosin could walk along actin filaments, suggesting a way that materials in the cell could...
be transported as well as providing a way to study myosin motors (see Web Extra sidebar, “Stepping Back in Time”).

By the mid-1980s, scientists at Stanford University were using a microscope to watch myosin carry plastic beads along actin filaments in non-muscle cells. Vale, a graduate student at Stanford at the time, got caught up in the excitement of seeing cellular movement and wanted to try the same experiments on proteins from nerve cells, where materials could be seen moving through the cells’ long axons. He expected to turn up myosin as the vehicle responsible for this transportation. Instead, Vale, together with Mike Sheetz and Tom Reese, isolated another molecular motor—kinesin—and they began focusing their attention on it.

Within 10 years, Vale and colleague Robert Fletterick had solved the structure of kinesin, helping to explain the molecular underpinnings of how the molecule walks along microtubules, dynamic tubes that run throughout cells. And he showed that the three-dimensional arrangement of atoms that make up kinesin was highly comparable to that found in myosin. “By using what was known about myosin,” says Vale, “we could bootstrap experiments and apply prior knowledge on myosin to understand the workings of kinesin, the newer kid in town.”

Kinesin and myosin have globular heads that attach to their tracks and flexible tails that extend outward and carry cargo. The proteins’ heads break down ATP to convert energy into work. For each ATP consumed, the motor protein takes a step forward (see Web Extra animation).

Shortly after Vale’s discovery of kinesin, he and his colleagues found evidence for another motor that moved along microtubules in the opposite direction. Two years later, Richard Vallee discovered that this second motor protein was dynein, which had been identified decades earlier for its role in flagella—whiplike tails that can propel entire cells, such as sperm. This newly discovered dynein—called cytoplasmic dynein—transports cargo along microtubules, serving a different function from the dynein that propels cells. The key difference between cytoplasmic dynein and most kinesins is the direction of transport: dynein molecules move along microtubules pointed at the cell’s center; kinesin walks outward. Having a distinct molecule for each task lets cells fine-tune traffic control.

Cytoplasmic dynein, though, looks different from myosin and kinesin. It has a very large wheel-like motor domain that binds multiple ATPs and a more complex tail region to connect to cargo. Because of its large size and complex structure, it has been harder to study than myosin and kinesin. For Vale, the challenge is enticing—since 2002, his lab has primarily focused on studying dynein.

“One of the really intriguing parts of dynein is that the key nucleotide binding site is a very large distance from the microtubule binding site,” says Vale. The distance between dynein’s ATP-binding site and its feet is four times the size of the whole kinesin molecule, he says. So how does the energy-generating portion of the protein communicate with the walking feet?

In 2011, Vale’s team solved the crystal structure of dynein, more than a decade after the structure of kinesin, and got some hints as to how the long-distance communication could work. They discovered a buttress—a section that supports the lanky top of the protein—that may be involved in transferring information from the ATP-binding head to the protruding feet that bind to the microtubule track. But questions remain about how dynein takes steps with a structure that’s so different from the other two motors.

“At this point, we can’t articulate a complete model of how dynein produces motion,” says Vale.

This much is known: when dynein or kinesin stops carrying goods across the cell, the cell stops functioning. Mutations in kinesin have been linked to kidney disease and an inherited neuropathy. Mutations in dynein are involved in motor neuron degeneration and can cause chronic respiratory infections (because the movement of mucus through the respiratory tract is inhibited). In 1997, Vale along with three colleagues launched a drug-development company, Cytokinetics, based on their research on molecular motor proteins. Their first drug, omecamtiv mecarbil—an activator of cardiac myosin—is in phase 2 clinical trials for the treatment of heart failure. The company also is conducting early tests of a treatment for amyotrophic lateral sclerosis, or Lou Gehrig’s disease.

At Children’s Hospital Boston, HHMI investigator Elizabeth Engle has stumbled upon another class of diseases linked to...
mutations in molecular motors and their roadways. She studies inherited eye disorders in which the muscles of the eye don’t develop properly. The root of the problem, her lab has discovered, is that dynein and kinesin don’t properly carry messages up and down the microtubules of growing neurons. The consequence: the neurons don’t connect to the correct muscle tissues. In genetic screens designed to pinpoint the cause of this problem, Engle’s lab has revealed mutations in the proteins that make up microtubules as well as in a specific type of kinesin. Now, they’re probing how the kinesin mutation changes the motor protein’s function.

“These human genetic studies are highlighting amino acid residues vital to specific functions of both kinesins and microtubules,” says Engle. “Thus, we can translate the human findings backward to enlighten more basic studies of these proteins and their interactions.”

TALES FROM THE ROAD
What about the other side of the cell cargo story, the roads themselves? Within the cell, road construction—and destruction—is an around-the-clock, nonstop job. To control the flow of goods between its neighborhoods, a cell is as likely to shut down or open new roads as it is to impose changes on vehicles.

“Microtubules are often considered the passive track for movement to occur on,” says Eva Nogales, an HHMI investigator at the University of California, Berkeley. “But in the cell they are extremely dynamic.”

As a model for understanding how microtubules shrink and grow, Nogales studies mitosis, the process by which a cell copies and divides its genetic material to separate into two cells. Once the cell divides up its chromosomes—the structures that contain the genetic material—microtubules pull the chromosomes to opposite sides of the cell. Kinetochore proteins enable this movement by linking microtubules to a special region in each chromosome and then remaining attached as the microtubules shrink or grow. When the microtubules shorten at the proper time, the two copies of each chromosome—each attached to one set of tubules—separate.

Nogales wants to know how the cell regulates this process and how the many other steps in mitosis are coordinated with microtubule arrangement and movement. Using cryoelectron microscopy, her lab group has shown how kinetochore proteins bind to microtubules and allow movement of chromosomes.

“Somehow there has to be a feedback between the checkpoint that allows mitosis to proceed and the microtubules,” says Nogales. That feedback is mediated by phosphorylation of the protein complexes that tether microtubules to the chromosomes. To visualize the effect of this phosphorylation on the microtubule-chromosome attachments, Nogales has turned again to cryoelectron microscopy.

“Our studies are ultimately just snapshots in a movie that is very dynamic,” says Nogales. As technology evolves, she says, the process will become even clearer (see Web Extra movie).

Like research on kinesin and dynein, understanding the role of microtubules in mitosis has applications in human health. For example, stopping the rearrangement of microtubules during cell division, or the separation of chromosomes, is one way to halt the out-of-control growth seen in cancer cells. Already, the drug Taxol (paclitaxel) is being used to treat some types of cancer, where it stops microtubules from rearranging, thus blocking mitosis and cell division. The company Cytokinetics is investigating additional drugs that target kinesin motors for treating cancer.

And as Nogales has probed deeper into the biological details of microtubules, she’s realized that their processes for shrinking and growing provide an interesting means of intracellular transportation of cargo. Her goal is to understand these processes at the molecular level.

“In addition to microtubules enabling the movement of these motor proteins, they’re also growing and shrinking themselves at the same time,” explains Nogales. “So the cell can actually couple this growing and shrinking with the movement of materials. They do this by a process that we are barely starting to understand.”

Scientists have shown, she says, that as microtubules grow, some proteins hop on and off the tips of the developing roads. It’s like getting a ride across the country by grabbing onto the back of a cement roller as it builds a new road, rather than paying for a bus. Many of the proteins that hitch a ride in this way are ultimately involved in contact with the outer membranes of cells. By riding the tip of a growing microtubule, a protein is assured a prime spot at the membrane when the microtubule reaches it. The process, Nogales thinks, could allow proteins to move to a distinct location without using energy.

CELLULAR GPS
Proteins at the growing ends of microtubules also likely help the microtubules find their targets. At the University of California,
San Francisco, HHMI investigators Lily Jan and Yuh Nung Jan have discovered one apt example. The Jans study ion channel proteins located in neurons’ plasma membranes, which selectively allow potassium in and out of the cells. They knew that, after the channel proteins were produced inside the cell, the proteins were delivered to the membrane by hitching a ride on kinesins that stepped along microtubules to the cell’s edge. But they were puzzled to see that the channel proteins were always delivered to clusters of the proteins specifically located in the axons of neurons. How did the microtubules know in which direction to head?

“In central neurons like the motor neurons, across the board evolutionarily, the channel goes down to the same spot,” says Lily Jan. “In giant squid, in humans, in mice. So we wanted to know how this destination is reached.”

The Jans discovered that a protein called EB1 is key—without it, the microtubules guiding the channels’ paths don’t reach the right spot. Their lab group has gone on to show that EB1 is also important in guiding channel proteins to the right spot in heart cells.

Not all neuronal proteins, of course, are delivered to the channel cluster that the Jans study. In fact, most neurons are heavily dotted with another common delivery site: synapses—the structures between cells that neurons use to communicate. So microtubules and motor proteins must find an astonishing array of locations.

“You can imagine kinesins as cars driving the length of microtubule roads,” says HHMI investigator Kang Shen of Stanford University. “But not every car will drive to the end of the road; they’ll get off at different exits.” Shen has discovered that kinesins, not surprisingly, are critical to placing the synapses at different exits along the roads that traverse a neuron’s axon. Kinesins carry synaptic vesicle precursors—sacs containing the components of synapses—along microtubules and then distribute them at different points on the axon.

“We’ve found that when kinesins have some mutations, they appear to drop off their cargo too early,” says Shen. “Other mutations do the opposite, traveling the whole length of the road without depositing the cargo.” In his latest work, Shen has shown that kinesins also carry microtubules to ensure they are oriented correctly in the dendrite, where neurons receive chemical messages. This finding, slated to be published soon, illustrates the diverse jobs that molecular motors have, he says, and the complexity of their functions. He plans to use synapse placement as a system to study how kinesins are regulated in the cell to carry cargo to precise locations.

Throughout mammalian cells there are 45 types of kinesins, 40 versions of myosin, and at least 14 different dyneins. Each carries goods—ranging from entire organelles, such as mitochondria and large chromosomes, to signaling chemicals—to a distinct destination. Yet the various motor proteins display small differences in how they process ATP, how they step along actin or microtubules, what cargo they can carry, and how their function is regulated. Now that the basic structure of each has been elucidated, and the complexity of their pathways revealed, scientists are primed to delve into questions about these differences and the regulation of each.

“We have these 45 kinesins that are involved in an enormous range of biological activities,” says Vale. “And for the vast majority of them, we don’t understand how they’re deployed or targeted within cells.”

But as the implication of gaining knowledge about these motor proteins becomes clearer, the interest in them is growing, says Vale. “This field has broadened as many more scientists have become involved,” he says. “People studying cancer or signaling or developmental biology often encounter some kind of molecular motor that is relevant to their research problem. I think that will continue to be true.” The road to understanding molecular motors, he says, is far from over.

Eva Nogales studies how proteins hitch a ride on microtubules as they shrink and grow. Kang Shen studies the way kinesins deliver cargo essential to nerve signaling.