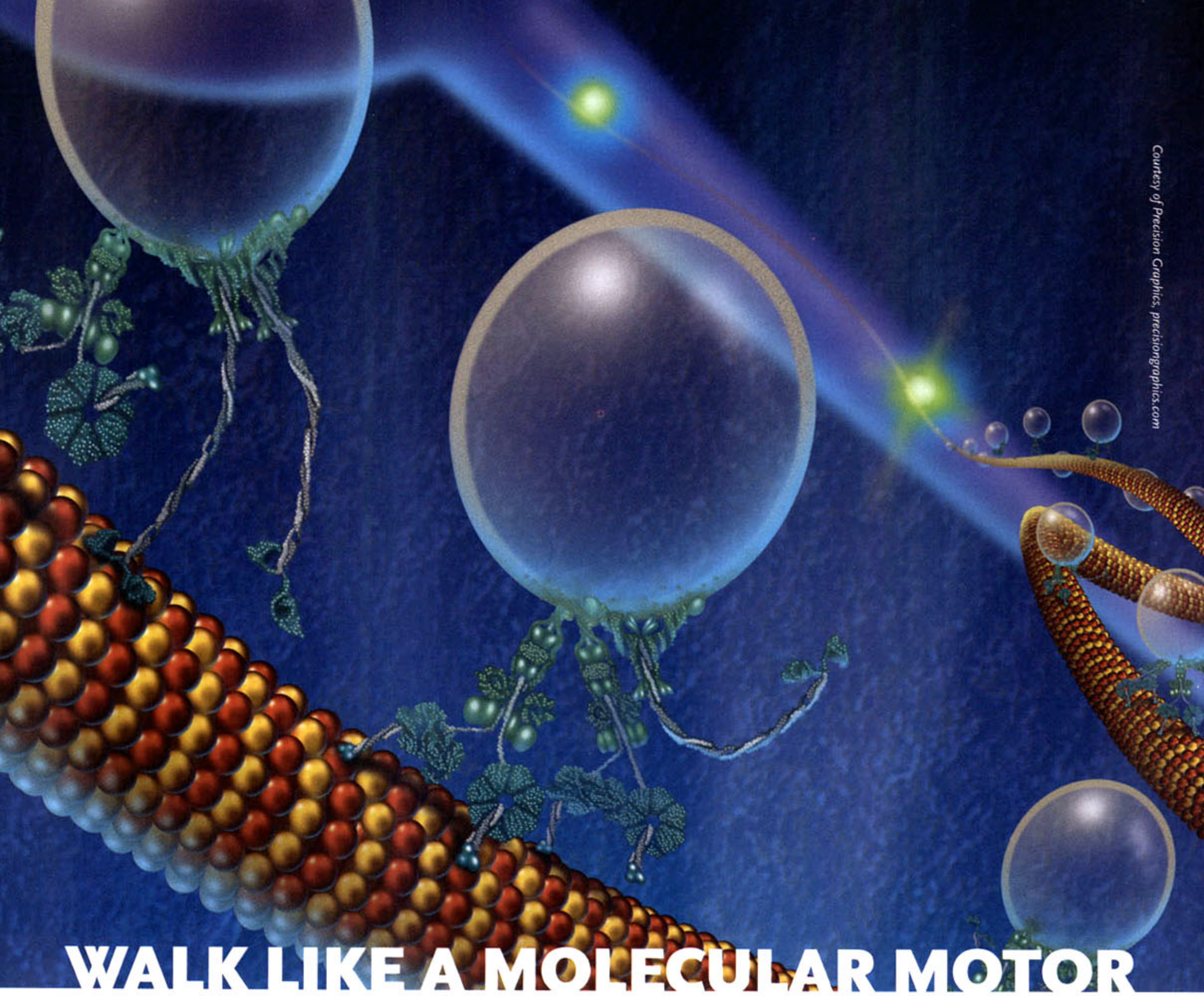

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WALK LIKE A MOLECULAR MOTOR

| VISION | *A molecular biophysicist weighs in on kinesin, dynein, and a molecular tug-of-war*

By Paul R. Selvin

Cells are a riot of activity. When a cell divides, chromosomes replicate and segregate into two daughter cells; flagella wiggle around to move sperm; cilia beat so mucous doesn't accumulate in the lung; and nerve cells fire by vesicles moving around and releasing their neurotransmitters. But how does such coordinated motion happen?

The universal answer, it turns out, is molecular motors: proteins that convert the energy in adenosine triphosphate (ATP), the cellular food source, to produce a force or torque on the cargo, moving it some distance. Sometimes these motors move things a short distance (a few microns in a typical cell), and sometimes far (a meter in the sciatic nerve). But how these motors operate, in some cases, at near 100% efficiency, is a subject of intense interest.

My lab has spent the better part of 10 years addressing this issue. Being a physicist implies I use things like lasers, detectors, and optics to solve problems; being a biophysicist means I apply these tools to understand biology. We, and others, have already shown how it is that two motors, myosin and kinesin, move about in vitro. Now we have turned these techniques to the cell itself, watching vesicular cargo move to and fro on microtubules in vivo.

Our results shed light on how multiple motors compete for activity on their protein scaffolds. We find, surprisingly, that kinesin and another motor, dynein, operate in large gangs to produce more than 10 times the speed that is expected from a single molecule. Though it remains to be seen whether this finding is reproducible in other cells, it suggests that the regulation and activity of molecular motors is even more subtle than previously thought.

WALK, DON'T CRAWL Three classes of molecular motors operate in mammalian cells. Myosins, of which there are 18 different subclasses, traverse a roadway called actin. Myosins tend to be good for short-range motion because the actin within a cell is randomized in all directions, and therefore doesn't extend very far. Kinesins, of which there are about 15 families, walk on microtubules, which tend to be good for long-range motion because they are arranged radially. Dynein, a massive, multi-subunit protein complex, also walks on microtubules. But where kinesins move from near the nucleus to the cell periphery, dynein moves from the periphery to near the nucleus.

Most of these motors—at least the processive ones—are homo-

dimers, each part having a foot that attaches to its particular "road," a central body that can hydrolyze ATP, and a stalk, which holds the two halves together and connects the motor to its cargo. But does the dimer walk like humans walk, in a "hand-over-hand" fashion, or does the dimer walk much like an inchworm crawls? In the case of hand-over-hand motion, the rear foot moves forward twice the center, while the front foot remains stationary. In the inchworm model, all parts of the motor move uniformly forward.

To study this question, we needed a way to watch individual motors moving. This is easier said than done, as the step size of individual motors is a mere 37 nm for myosin V and 8.3 nm for kinesin.[1] The diffraction limit of light, moreover, means it isn't possible to resolve objects separated by less than 250 nm using a light microscope.

Undeterred, a student of mine named Ahmet Yildiz developed a technique called Fluorescence Imaging with One Nanometer Accuracy (FIONA),[2] which achieved a spatial resolution of 1.5 nm. Using FIONA we discovered that myosin V, kinesin, and myosin VI, all move in hand-over-hand fashion. Indeed, it looks like all motors—at least the dimeric ones—"walk," and don't crawl.

MOVEMENT IN VIVO Or at least, they do in vitro. The question was, do they act the same in vivo? Another student of mine, Comert Kural, collaborators Hwajin Kim and Vladimir Gelfand, and I, tested peroxisome movement inside a fruit fly cell by the molecular motors, kinesin and dynein, and came up with some surprising results.[3]

Peroxisomes are little compartments that are involved in cleaning up the cell's garbage. We loaded these vesicles with green fluorescent protein (GFP) to make them easy to see. Because myosin V is also involved in moving peroxisomes, in addition to kinesin and dynein, we also added latrunculin, which depolymerizes actin. As an added benefit, latrunculin also causes the cells to grow long, thick, microtubule-rich processes that project from the cell; we were able to image peroxisome motion within these processes.

The goal of the project was to capture the motion of individual kinesins or dyneins, so in addition to FIONA's spatial resolution, we also needed excellent temporal resolution. That's because the intracellular ATP concentration is fairly high (on the order of 5 mM), meaning the molecular motors, and attached peroxisomes, will move rapidly. Fortunately, there are cameras that can take images a thousand times per second and produce images sharp enough to allow us to watch peroxisomes moving by a single motor.

Our observations agreed well with published in vitro figures. When kinesin moved the peroxisomes, it moved them an average of 8.6 nm (in vitro, 8.3 nm), and when dynein moved them, they moved an average of 8.9 nm. Previous in vitro results said dynein moved with 8 nm steps,[4] if the cargo was under significant load, as one might imagine it to be with the relatively high viscosity inside a cell.

These results imply that kinesin and dynein are not fighting each other in a molecular tug-of-war. If one motor was pulling against the other, we might expect that it would lead to a series of extremely small steps, owing to "compliance" in the peroxisome/motor linkage. For example, if kinesin pulls, kinesin will move 8 nm (the minimal step-size forced by the repeat of the microtubule), the "spring" (i.e., the stalk of the kinesin plus the lipid or any other compliant region connecting the motor to the cargo) stretches by 5 nm, and the peroxisome moves by only 3 nm.

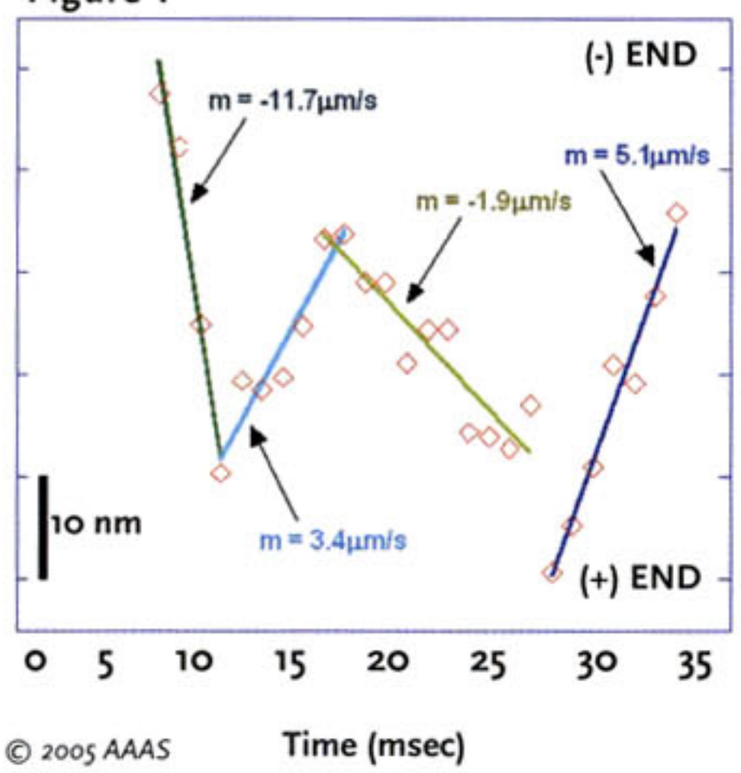
In fact, no such short steps were seen, and we therefore think that when one motor is pulling, the other is inactive. This could be due to an as yet unknown small regulatory molecule, but this explanation seems unlikely to us, because a step between motors takes less than a millisecond. Whether a regulator can turn on and off motors that quickly is debatable. One possible regulator could be physical strain in the system.

Another observation, even more surprising, was that the peroxisomes moved significantly faster than cargo ferried by single motors in vitro. Kinesin moves at no more than 1 µm/sec in vitro, no matter how many motors are attached to a cargo. But we saw peroxisomes in the cell racing along at up to 12 µm/sec. One experiment, for example, saw the peroxisomes being moved by kinesin at 11.7 µm/sec, followed by dynein at 3.4 µm/sec, and then kinesin at 1.9 µm/sec. After another short kinesin jump, the peroxisome takes a final ride on dynein, at 5.1 µm/sec (see Figure 1).

To delve into the questions raised by these new findings, we first will look at other systems to see if they also undergo rapid motion. The nerve cell line, PC12, in which membrane vesicles appear to move faster (4 µm/sec) is one possibility.[5] Or, returning to the peroxisome system, we can use an optical tweezers (see related story, p. 26) to trap the vesicles and see how much force is necessary to prevent either kinesin or dynein from moving it. If there are two kinesins pulling on it, the stall force, the force necessary to prevent motion, should be 14 pN, twice the normal stall force of 7 pN per kinesin. Of course, these studies require that one can trap the peroxisomes in a cell—a nontrivial task, although other subcellular organelles have been trapped before.[6]

Another possibility is to extract the peroxisomes and see how fast they move on microtubules in vitro. According to standard lore, they should move at less than 1 µm/sec. But this would mean observing peroxisomes outside their native environment, and membrane connections may be critical in determining peroxisome movement. In any case, answering these questions, and trying to unravel how these molecular motors really work, will likely keep me busy for some time to come. ⊗

Figure 1



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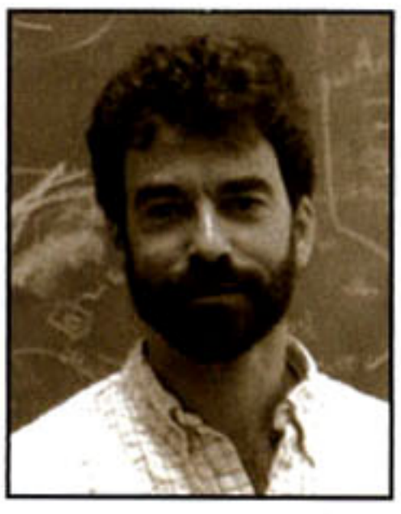

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