

Investigating Molecular Motors Step by Step

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Recent discoveries begin to answer how dyneins, kinesins, and myosins actually work

By Jeffrey M. Perkel

The audience, several hundred biophysicists strong, was not expecting a James Brown impersonation. But there he was: Physiologist Yale Goldman, keynote speaker on motility at the Biophysical Society's annual meeting, doing his "asymmetric hand-over-hand motility dance with a limp" to tinny strains of "Papa's Got a Brand New Bag." And while Goldman, who eschewed Brown's trademark, over-the-top couture for understated, Ivy League-issue khakis and blue blazer, won't star on MTV any time soon, he still has reason to celebrate.

The last few years have been exciting times for molecular gearheads like Goldman, researchers who are dedicated to understanding the protein motors that transport material throughout the cell. Using single-molecule techniques that have, by all accounts, transformed biophysics, researchers now know how two motors sashay their way down their protein tracks (they use a hand-over-hand mechanism, hence the title of Goldman's dance). And they are beginning to piece together just how a third, and previously recalcitrant class of motors, operates.

On one level, these findings reveal a surprising degree of consistency. Says Steven Block, a professor of biological sciences at Stanford University, "There's a wonderful unanimity that's emerging." But beneath the veneer of uniformity lies considerable confusion. As is so often the case in biology, these studies

generate more questions than answers. How the motors convert chemical energy into force, for instance, still confounds, as does the issue of coordinating their motion. And though this current crop of work is remarkable, it applies only to the specific proteins studied; hundreds of variants may or may not operate by the same principles.

"The recent discoveries, as exciting as they are, are only the beginning of our understanding of what's going on there," says Jeff Gelles, a biochemist who focuses on kinesin at Brandeis University, Waltham, Mass. "Biology is always more complicated than our simple models of it capture."

THE NEED FOR MOTORS Eukaryotic cells mirror society, explains Nobutaka Hirokawa, chair of the cell biology and anatomy department, University of Tokyo. Just as countries exchange goods and services, cells synthesize, assemble, and package proteins and protein complexes into membranous vesicles and organelles, for use throughout the cell.

To move this cargo, the cell uses a variety of molecular motors. Navigating an intracellular interstate system built of proteinaceous segments of actin and tubulin, the Big Three of the cellular auto world—myosin, kinesin, and dynein—harness the power of ATP hydrolysis to transport loads vastly larger than themselves across relatively vast distances. They move

everything from RNA molecules to whole chromosomes, and from tiny vesicles to mitochondria. "This is analogous to an ant [that] can crawl across a picnic table with a giant potato chip on its back," explains Block.

Several key findings laid the groundwork for understanding how the motors work. First, scientists already knew that conventional kinesin and myosin V both have two heads, each of which can bind to its track (microtubules and actin, respectively) and hydrolyze ATP. They also knew that the enzymes require both heads for processive motion.

Further, they knew that kinesin and myosin V do not move continuously (like a bead sliding on a string) but rather in discrete steps of 8 nm or 37 nm, respectively. As the molecules move, they consume one molecule of ATP, the motor's molecular fuel, per step. Scientists also knew that these motors would continue to advance, even against a backwards-pulling force, meaning that the molecules never let go of their track.

These facts in aggregate support three basic models, says Block. The molecule might move, like a person climbing a ladder, by always moving one hand first and then the other (see diagram at right). In this "inchworm" model, one hand always leads, while the other always lags. The molecule also could move, like a child on monkey bars, by alternating leading and lagging hands, in an "asymmetric hand-over-hand" motion. A third model, called "symmetric hand-over-hand," posits movement akin to that of a cartographer's compass. As the mapmaker marks off distances on the map, he fixes one point and rotates the instrument about that axis, thereby alternating the leading and lagging tip without changing its configuration.

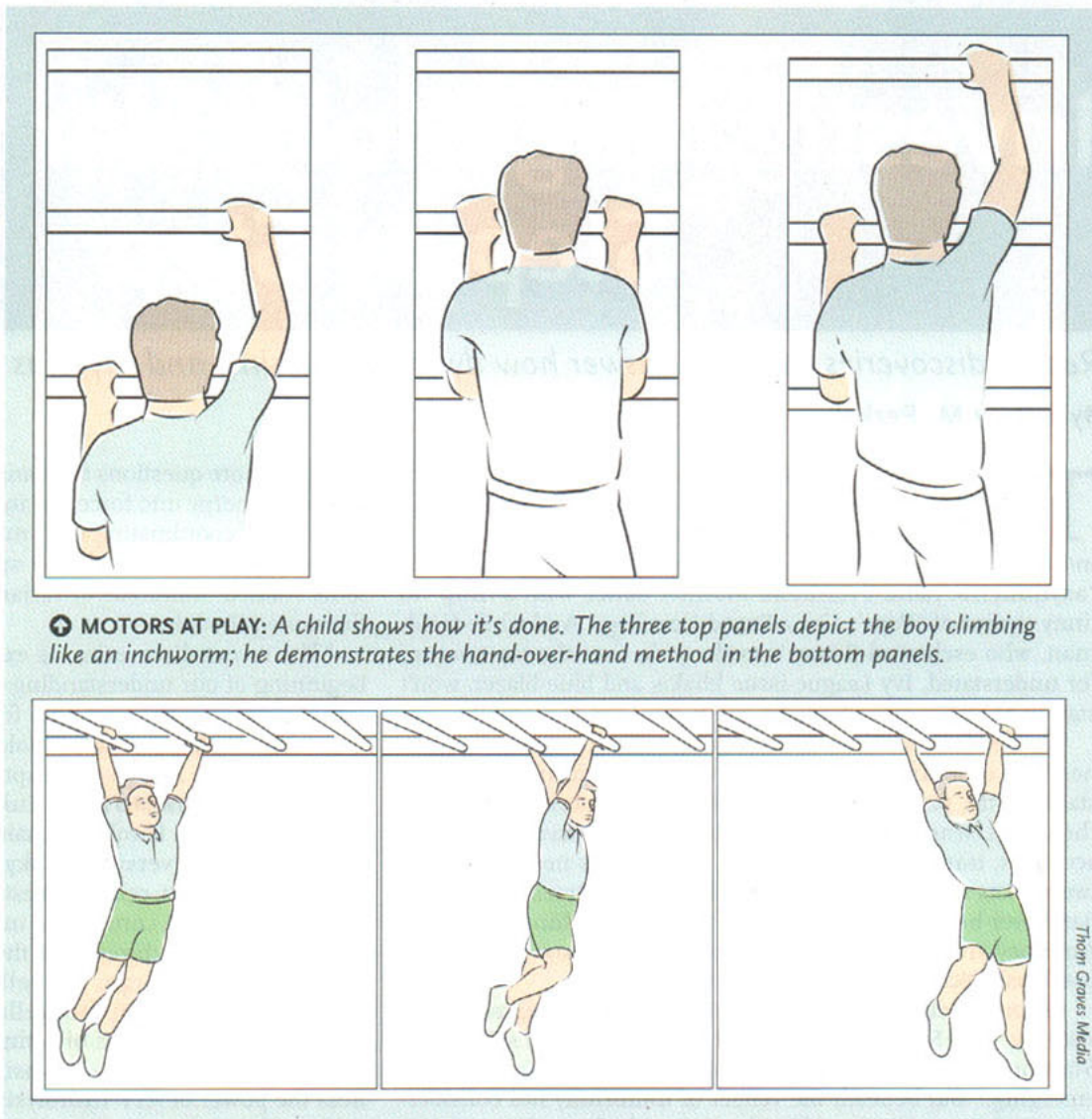
HAND OVER HAND Gelles' group provided the first clue in 2002. Realizing that the three models, particularly the symmetric hand-over-hand model, imply different amounts of rotation of the enzymes as they move, Gelles' graduate student Wei Hua and postdoc Johnson Chung affixed a kinesin molecule to a surface and watched as microtubules inched their way across it. Observing no rotation of the filaments, the team concluded that only the asymmetric hand-over-hand and inchworm models were likely.¹

Early in 2003, Goldman's team, along with Taekjip Ha's and Paul Selvin's groups at the University of Illinois, Urbana-Champaign, set out to determine which of the two models applies to myosin V. Both groups pinned a fluorescent dye on one of the enzyme's legs and then monitored that tag as the enzyme moved.

Goldman's team measured its angle with respect to the actin filament.² "We thought, if it is doing this hand-over-hand mechanism, the orientation in space of the light chains should be different, depending on whether it's the leading or trailing head," explains Goldman, who runs the Pennsylvania Muscle Institute at the University of Pennsylvania. And they were right: The fluorophore alternated rapidly between two discrete angles as the molecule stepped, suggesting a hand-over-hand mechanism.

Following this finding, Selvin's team measured the motor's motion directly.³ Block says that it's like watching a person walking across a field on a moonless night who has a flashlight attached to one foot. You cannot see the person, but you can see the light move, and you can measure the distance it advances with each step.

Previous measurements of myosin V's step size recorded how



MOTORS AT PLAY: A child shows how it's done. The three top panels depict the boy climbing like an inchworm; he demonstrates the hand-over-hand method in the bottom panels.

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far the molecule's cargo moved, not the heads themselves. But in a hand-over-hand model, the molecule's center moves by half the length of the stride, (see figure, this page) so a 37 nm step requires that the head move 74 nm. Molecules moving like an inchworm, on the other hand, advance the moving head by only 37 nm per step.

With step sizes of 74 nm, Selvin's data established that myosin V walks hand over hand. "Between Yale's [Goldman] paper and Paul's [Selvin] paper, it's nailed the mechanics of stepping, as far as what the gross behavior of the heads on the actin filament are," observes H. Lee Sweeney, chairman of the physiology department, University of Pennsylvania. In September 2003, Sweeney published a crystal structure of myosin V without bound nucleotide.⁴ "I would say that we understand myosin V more than any myosin now, because we have data on every level with that [enzyme]."

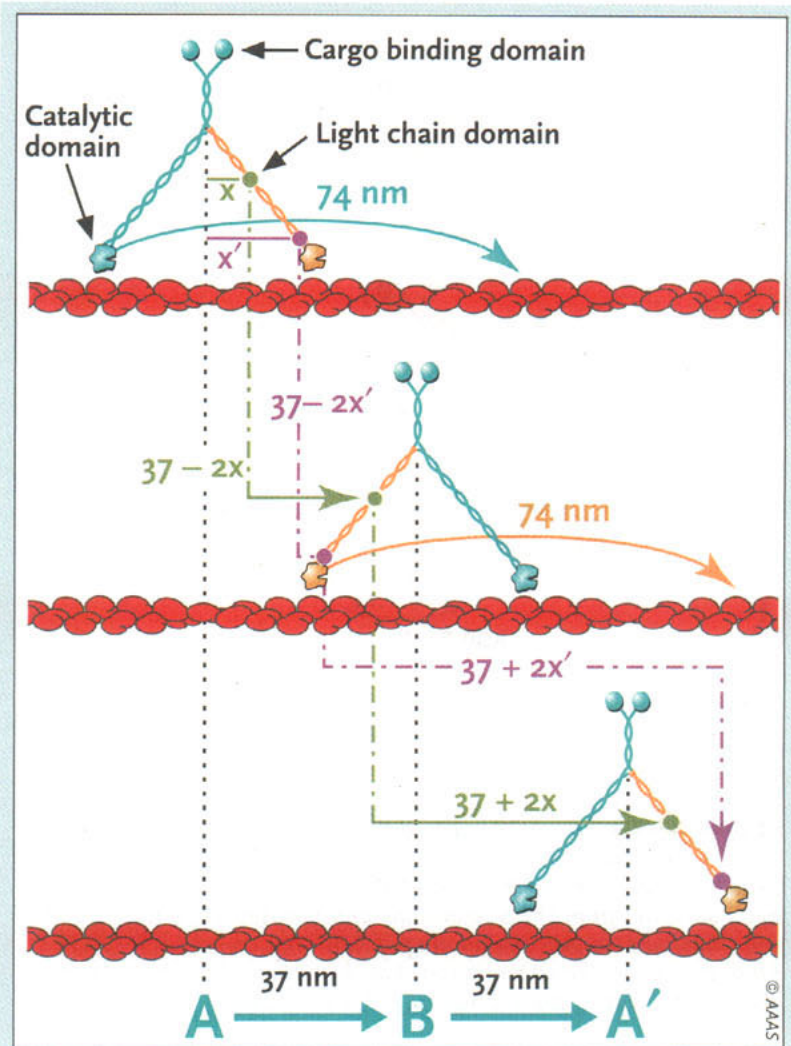
Attention next turned to kinesin. In December 2003, three labs, each using a different, single-molecule detection approach, arrived at the answer nearly simultaneously. Block's group measured the motion of a truncated tail domain mutant of kinesin, using an "optical tweezers" apparatus to hold steady a bead coupled to the motor.⁵ They observed that this bead, and hence the motor, appeared to limp. It would take a step and wait, and then take another step and wait, on average six times longer, before taking another step. Block says that the kinesin is alternating between two configurations, supporting the asymmetric hand-over-hand model. Selvin, applying his work on myosin V to kinesin, concurred.⁶

And so did Keiko Hirose's group at Japan's National Institute of Advanced Industrial Science and Technology.⁷ Hirose's team tracked the motion of a heterodimeric kinesin molecule in which one head hydrolyzes ATP more slowly than the other. They observed an alternation between fast and slow steps, akin to the limping that Block's team saw. "That really argues strongly in favor of an alternating hand-over-hand hypothesis," says cell biologist Manfred Schliwa, University of Munich.

GATING THE GAIT "The nice thing about all those articles is each one of them addresses a different aspect of the mechanism," says Gelles. "It's really only taken together that all of them make a very strong case for an asymmetric hand-over-hand mechanism." Which prompts the question: How does ATP hydrolysis fit into that mechanism?

One model suggests that ATPase activity alternates between heads with each step. In support of this, Jonathan Howard of the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, probed the kinesin reaction cycle by examining how excess ATP, ADP, and inorganic phosphate inhibit the enzyme's activity.⁸ He found that, unlike other ATPases, kinesin appears to release ADP before phosphate. "This result is surprising," the authors wrote, "because kinesin's nucleotide-binding pocket is expected to release [inorganic phosphate] before ADP."

Howard and his colleagues propose a three-step, cyclical hand-over-hand model (see figure, p. 22) that begins when the trailing, microtubule-bound head's active site is empty, and the forward, unattached head contains ADP. First, the bound head

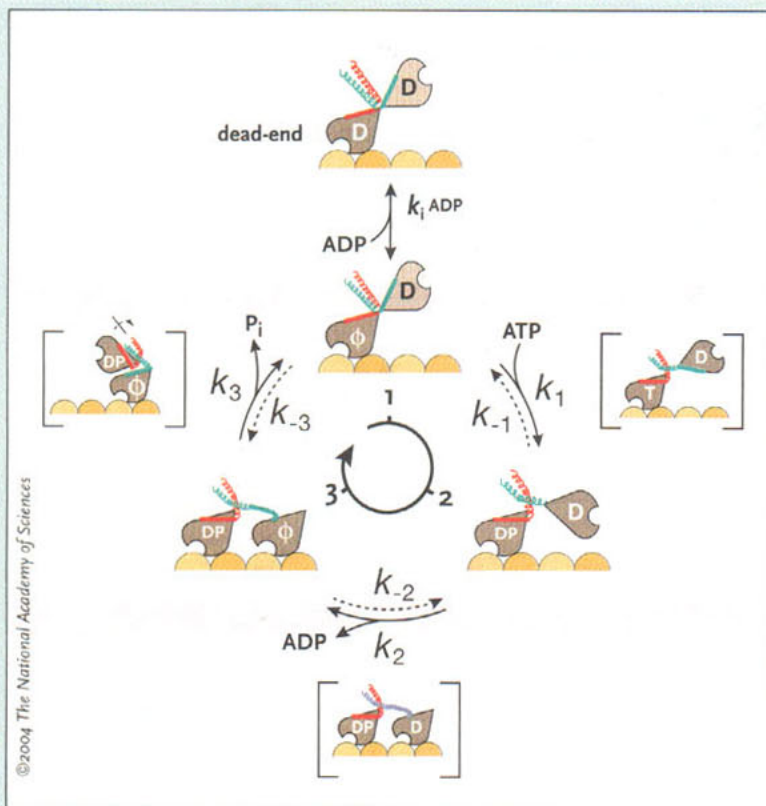


IN STRIDE: Using single-molecule fluorescence, Paul Selvin and his team dissected the motion of myosin V. The critical data showed that the molecule's moving head advances by twice the distance of its stalk, proving a hand-over-hand motion. (From *Science*, 300:2061–5, 2003.)

binds ATP and cleaves it into ADP and Pi. The leading head then releases ADP and binds the microtubule. Finally, the trailing head ejects its inorganic phosphate and detaches from the microtubule to restore the original state.

This model implicitly requires that ATPase activity alternate between steps. But then how that coordination, or "gating" is accomplished becomes an issue. It's the \$64,000 question, Block says: "How is your gait gated?" Claudia Veigel of the Medical Research Council in London presented evidence at the Biophysical Society meeting in support of strain-dependent gating in processive myosin motors. When both myosin V heads are bound, she suggests, there is mechanical strain placed on the molecule. As Block explains, the two heads could communicate with each other through this strain to regulate ATPase activity and keep the heads moving sequentially.

Another Gelles graduate student, Todd Thoresen, presented data of his own, which Block says "is absolutely wild." Thoresen made a heterodimeric kinesin, in which one head hydrolyzes



GIVING IT GAS: An ADP-released-first model for kinesin motility. (1) The attached head binds ATP and hydrolyzes it. (2) The forward head attaches to the microtubule and releases its ADP. (3) The rear head detaches from the microtubule and releases its Pi. Lastly, the top transition denotes the binding of ADP that competes for ATP binding. The bracketed structures show hypothetical transition states. (From Proc Natl Acad Sci, 101:1183–8, Feb. 3, 2004.)

microtubule-binding stalk projects from between domains AAA4 and AAA5, while the cargo-binding stem emerges from domain AAA1 (see figure, p. 23). Steve Gross of the University of California, Irvine, wonders: “Why do you need this amazingly complicated structure for dynein?”

Answers are finally emerging. Early last year, Stan Burgess’ group at the University of Leeds, UK, took electron photomicrographs of the protein frozen at various points in its cycle.⁹ They processed the images with a computer and produced movies of its power stroke that suggested a mechanism. “Microtubule movement,” the authors wrote, “is initiated by tight binding to the tip of the stalk, which promotes a concerted conformational change in AAA1–4, thereby activating release of ADP and phosphate from AAA1, the probable site of ATP hydrolysis.” Vallee, who wrote a “News & Views” article on the work, says, “It was a beautifully executed study.” And a prescient one.

Last month, Samara Reck-Peterson and Ron Vale published data that support this model.¹⁰ Using genetic analysis of yeast dynein, they found that the four AAA domains have different functions. ATP hydrolysis appears to occur only at AAA1, though ATP binding by AAA3 is required for activity. AAA2 and AAA4 appear to play little or no role in the enzyme’s activity.

The same week, Gross published data suggesting one possible explanation for dynein’s complexity.¹¹ Using single-molecule-based techniques, Gross observed that the motor’s force output varies substantially with ATP concentration. This stands in contrast to motors such as myosin and kinesin, Gross says, whose forces are relatively constant over a range of ATP concentrations.

Further, Gross and his team saw that as load is increased on the molecule, its step size decreases, from a high of 32 nm per step to a low of 8 nm. But regard-

less of the ATP concentration, force, or step size, the molecule hydrolyzes only one ATP per step. His explanation for these data: Dynein uses a sort of automatic transmission that allows the molecule to take either big steps and exert low force, or small steps and exert higher force.

Key to this mechanism are the AAA domains, which, says Gross, compact when they bind ATP. ATP hydrolysis occurs in AAA1, and he suggests that as load on the molecule increases, the other AAA domains bind more tightly to ATP. The result is not unlike a coiled spring: Under low load, AAA2–4 bind ATP weakly, the spring is loose, and the step size is large; at high load, ATP binds tightly, and the spring compacts as the step size decreases (see figure, p. 23).

So far, such behavior is singular, says Gross. “It’s like the first four-speed bike in a world of one-speed bikes,” observes Gross’s coauthor, Steven King, University of Missouri, Kansas City.

OPEN QUESTIONS Dynein researchers want to know the functions of dynein’s accessory proteins, dynactin and Lis1. Now that Gross has a functioning *in vitro* system established, he says that he can start addressing this question.

Another mystery: how dynein connects to its cargo. “When the motors were first discovered, the idea was that there would be a direct receptor, a dynein receptor on vesicles,” says Erika

ATP 200-fold more slowly than normal, and found that the molecule was still processive. Moreover, it was only seven to nine times slower than the wild-type molecule. “This is a surprising result,” says Gelles, “and it is contrary to this hypothesis that says that alternating ATP hydrolysis by the two heads is essential for this processive, high-duty-ratio movement.”

CHECK THE TRANSMISSION Dynein, a minus-end directed, microtubule-based motor, has not been equally investigated because the enzyme is more difficult to work with. “It’s a monster protein,” says Columbia University’s Richard Vallee, who studies dynein.

Dynein differs substantially from myosin and kinesin. Its heavy chain, at 500 kDa, is more massive than kinesin’s nearly 120-kDa counterpart. The complete complex has a variety of integral and associated regulatory polypeptides and measures more than one million Daltons. Further, while the myosin and kinesin motor domains bind one ATP molecule, each dynein motor domain can bind four. And, dynein’s motor domain does not directly contact its track; instead, a small stalk emerging from the motor domain does.

Dynein’s motor domain contains a series of six so-called AAA modules (“ATPase associated with various cellular activities”), which are predicted to form a ring structure when folded. The

Holzbour, associate professor of physiology at the University of Pennsylvania. "But the reality seems to be much more complicated than that."

Finally, says Vallee, there's the question of how dynein knows where it is needed in the cell. The process, he says, must involve a remarkable degree of regulation by other factors, which can react to such things as cell-cycle state and movement. "The current perception is that we have only begun to break the surface on this problem."

As for kinesin and myosin, researchers wonder how the conformational change induced by ATP hydrolysis is amplified to create force. Vale observed in 1996 that under the hood, as it were, motor proteins share striking structural similarities, despite their sharing virtually no sequence homology. "Conformational changes that are generated in the core structure in response to ATP hydrolysis are very similar," says Schliwa. "But then they are translated into different conformational changes in myosin and kinesin."

Also up for debate: Will the models established for myosin V and conventional kinesin be applicable to their larger superfamilies? Genome sequencing projects have revealed that these families are extensive; humans have 45 kinesins, for instance, and *Arabidopsis* has 61. "Motors as a group of proteins are an incredible example of what evolution has done with one single idea," says Ron Milligan, professor of cell biology at the Scripps Research Institute, La Jolla, Calif.

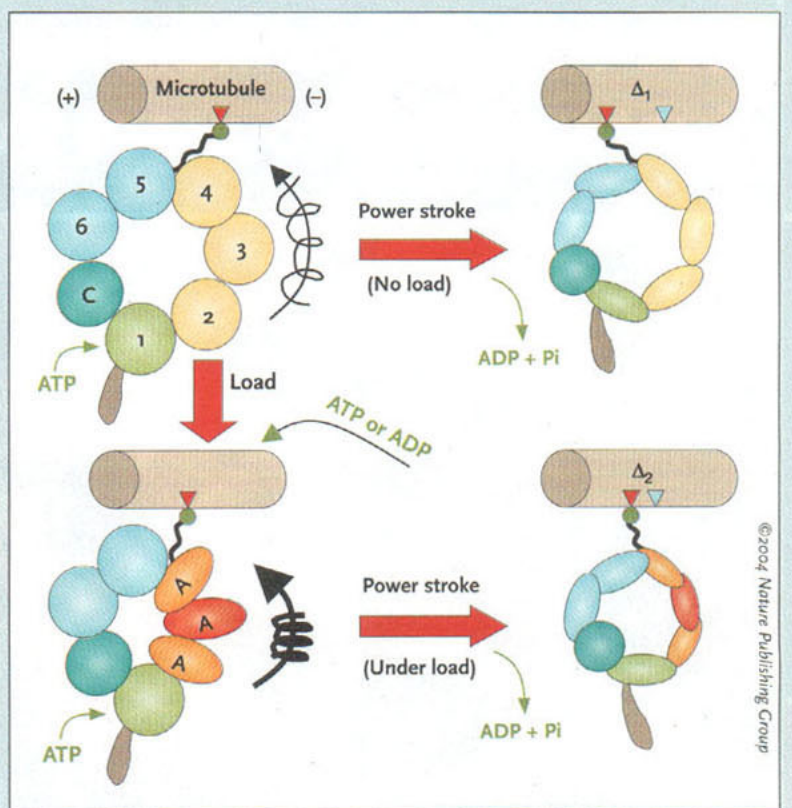
Some motors, such as KIF-1a, have only one head, yet are still processive. Other kinesin family members are not even transport motors at all. MCAK, a mitotic myosin studied in Tim Mitchison's lab at Harvard, does not move along microtubules like its cousins do; instead, the protein chews up microtubules, much like a "Pac-Man," Mitchison says. Still others, such as myosin VI, move in the opposite direction from most of their family members.

AN EXCITED STATE Much of the data collected over the past year used single molecule approaches like optical traps or single molecule fluorescence detectors. "The field is really growing spectacularly because of the power of these techniques," says Gelles. But to advance even farther, Block says, they will need to couple these methods, complementing the resulting information with structural data.

Block's lab recently developed an instrument he says will allow them to measure, for instance, how close together two parts of a molecule are, as well as where in the stepping cycle the molecule is.¹² "I think this is the wave of the future for this kind of work," he says, adding, "When we can finally tell the story, from the point where an ATP binds the molecule, to what part of the molecule changes, and how that communicates to other parts of the molecule, and how that leads to force and displacement ... then we can say we really understand the molecular mechanism of motor proteins. Until we can do that, we ought not to congratulate ourselves too heavily."

Nevertheless, says Dietmar Manstein, director of the Institute for Biophysical Chemistry in Hannover, Germany, biophysicists are ebullient. "People around me are really excited about what has been happening over the last 12 months, and all these new possibilities that are opening up. We don't know in every case what the application will be, but it's great fun to be doing it."

The question is, do they feel like dancing? ☉



☉ **PUT IT IN GEAR:** A model for an ATP-regulated gear. The dynein head is shown as six sequential AAA domains (numbered spheres) and one carboxy terminal subdomain (C) connected in a ring conformation with a projecting microtubule-binding stalk (blue). See text (p. 22) for details. (From *Nature*, 427:649–51, Feb. 12, 2004.)

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References

1. W. Hua et al., "Distinguishing inchworm and hand-over-hand processive kinesin movement by neck rotation measurements," *Science*, 295:844–8, 2002.
2. J.N. Forkey et al., "Three-dimensional structural dynamics of myosin V by single-molecule fluorescence polarization," *Nature*, 422:399–404, 2003.
3. A. Yildiz et al., "Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm resolution," *Science*, 300:2061–5, 2003.
4. P.-D. Coureux et al., "A structural state of the myosin V motor without bound nucleotide," *Nature*, 425:419–23, Sept. 25, 2003.
5. C.L. Asbury et al., "Kinesin moves by an asymmetric hand-over-hand mechanism," *Science*, 302:2130–4, Dec. 19, 2003.
6. A. Yildiz et al., "Kinesin walks hand-over-hand," *Science*, published online, doi:10.1126/science.1093753, Dec. 18, 2003.
7. K. Kaseda et al., "Alternate fast and slow stepping of a heterodimeric kinesin molecule," *Nat Cell Biol*, 5:1079–82, December 2003.
8. W.R. Schief et al., "Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism," *Proc Natl Acad Sci*, 101:1183–8, Feb. 3, 2004.
9. S.A. Burgess et al., "Dynein structure and power stroke," *Nature*, 421:715–8, 2003.
10. S.L. Reck-Peterson, R.D. Vale, "Molecular dissection of the roles of nucleotide binding and hydrolysis in dynein's AAA domains in *Saccharomyces cerevisiae*," *Proc Natl Acad Sci*, 101:1491–5, Feb. 10, 2004.
11. R. Mallik et al., "Cytoplasmic dynein functions as a gear in response to load," *Nature*, 427:649–52, Feb. 12, 2004.
12. M.J. Lang et al., "Combined optical trapping and single-molecule fluorescence," *J Biol*, 2:6.1–6.4, Feb. 24, 2003.