

Roop Mallik Profile: Nature was there first: Modeling a nanoscale gear mechanism within the dynein molecular motor

By Geoffrey Montgomery



In May 2004, on route to the 4th Annual HFSP Awardees' meeting in Hakone, Japan, HFSP fellow Roop Mallik went back to his hometown in India to visit his father, a retired mechanical engineer. His father's main interest had been machine design; he had specialized in gears. "And when I was a little kid," Roop remembers, "I would go to my father's workshop and try to understand, to visualize, how does this work, how does that work? Many things were beyond my understanding," he laughs, "but at least I made the attempt."

Now Roop Mallik's father had some questions about machine design for Roop. That February, Mallik had published a remarkable paper in *Nature* with Steven Gross, the head of his University of California at Irvine lab, a paper which Roop had sent his father in his hometown of Allahabad. His father understood in a general sense that Roop studied a giant protein, dynein, one of three special classes of protein nanomachines that act as "molecular motors" to transport cargo around cells. And if the idea of his son's study of evolutionarily-designed motors that operate at the scale of nanometers was not enough to warm a retired machine designer and gear specialist's heart, the title of his son's *Nature* paper was tuned to strike an even deeper chord of paternal pride and wonder. The title read: "Cytoplasmic dynein functions as a gear in response to load." In this paper in the world's leading science journal, his son and Steve Gross were claiming that the dynein nanomotor contains a self-regulating gear that allows it to adjust the size of its "steps" and the force it generates as it tugs cargo along cellular tracks called microtubules. This was an unprecedented hypothesis, one that is already beginning to open up new avenues of research not only into the biological architecture of natural nanoscale machines, but also into the underlying dynamic logic of the complex molecular interactions governing cellular life, the central challenge of 21st century biology.

"Nice paper, good work. You're earning your money," said Roop's father when they sat down on a portable cot under some trees outside his father's house. Yet as Roop had often struggled as a boy to visualize the connecting elements, the intermeshing motions, of the machines in his father's workshop and factory, now his father was struggling to penetrate the paper's biological jargon and to picture the self-regulating gear of the dynein nanomotor. Spur gears, bevel gears, worm gears—the interlocking toothed wheels of different sizes and orientations Roop's father had worked with all his adult life: that was how he pictured a gear. "My father looked at how a gear might work from an engineer's point of view," says Mallik, "and because he's in a small city in India he didn't have access to scientific journals, he couldn't read up on molecular motors."

"I don't understand this, how can it be?" his father said to Roop as they sat under the trees with his dynein paper. "Explain this to me."

And so Roop Mallik went over with his father the chain of evidence and inference—of biological reverse engineering—that had led him to envision his unprecedented model of a molecular gear's design.

A Physicist Turned Biologist

Roop Mallik had only the vaguest picture of molecular motors himself when in 2000, while working at the TATA Institute for Fundamental Research in Bombay, he was forwarded a copy of an e-mail written by Steve Gross. Mallik had received a Ph.D. in physics from the Institute a year before, specializing in the strange electronic properties of heavy fermion magnetic systems. Yet while Mallik's career as a young physicist had been extraordinarily productive, he had found himself increasingly attracted to the biological phenomena he read about in *Nature* and *Science*. "I would understand very little, but I would try to go through the News and Views to gather as much information as I could." Indeed, he had taken up a post-doctoral position at the Institute to build an instrument designed to study the properties of green fluorescent proteins at extremely fine time-scales. "My interest was in instrumentation, to try to make instruments which you can use to ask questions in biology. That's the idea [for a scientific career] I came up with." Steve Gross's e-mail said that he was setting up a new lab at Irvine to study molecular motors, and that he was looking for a post-doc with a background in physics to work with him.

Steven Gross was also a physicist-turned-biologist; as an undergraduate and graduate student he had worked in the laboratory of non-linear dynamic ("chaos") pioneers Albert Libchaber and Harry Sweeney. But by the time Gross was finishing his own Ph.D. work, he says, "it seemed clear that the methodologies of non-linear dynamics and chaos worked," that the overarching issues in the field had been settled, and that it was now a question of "finding yet other systems that could be described in the same way. And that didn't seem so interesting to me." By then, his former mentor Libchaber had switched to biophysics and "was singing the praises of the cytoskeleton"—the dynamically self-assembling and disassembling polymer filaments that shape cells and serve as the tracks along which molecular motors carry cargo. "And I thought Libchaber was a pretty smart fellow, so I decided that maybe I should learn some more about that." At the time Gross wrote the e-mail from Irvine that was forwarded to Roop Mallik, he had completed a post-doc at Princeton with Steven Block and Eric Wieschaus, primarily studying the behavior of dynein motors *in vivo* in *Drosophila* embryos.

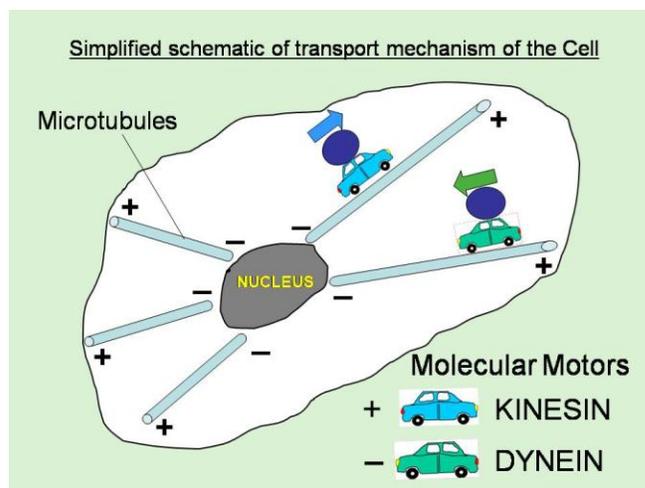
Gross's e-mail described the kind of work he wanted to do in his Irvine lab: to look at how molecular motors, especially dynein, work both individually and in collaboration with each other and a host of associated regulatory factors. Gross wanted his lab to serve as a bridge between physics and biology; between studies of single molecular motors and the *in vivo* dynamic complexity of actual living cells.

"I was looking for a biophysicist," says Gross, "someone with a quantitative background in physics, and with experimental expertise so they could do things like build optical traps" to study the motion and force generated by molecular motors. "But more importantly, I think we're at a crossroads as far as trying to figure out how you do cross-disciplinary science—and I think this is the belief of Human Frontiers also. How do you integrate physics and biology so that you go beyond each individual discipline?" Gross had met many physicists working on biological problems whom he found very smart and technically adept, but who really "wanted to do physics, and didn't want to learn enough about the details of the biology and to be guided by that knowledge. ...So I wanted someone who was interested in learning the biology, and in trying to come up with some new synthesis of how you do this work at the interface" of the physical and life sciences.

Roop Mallik fit the bill. "He had an amazing number of publications from his Ph.D. work," says Gross, "and we had some e-mails back and forth, which suggested that not only was he smart, but that he was really interested in biology."

Mallik liked the idea of working with a mentor who was also a physicist-turned-biologist. “I thought, if I go there, we can be on the same wavelength.” But when he read Gross’s first e-mail, Roop’s picture of dynein and the two other classes of molecular motors, kinesin and myosin, was quite abstract. “I knew these things move around in cells, nothing more than that.” He started reading up on molecular motors, e-mailed Gross, who sent more references. Roop appreciated from high school biology that the cell was a highly organized structure, with many different compartments or “organelles” with different functions—the nucleus with its DNA Library of Genetic Instructions, the power plants of mitochondria, the protein synthesis and packaging factories of ribosomes, endoplasmic reticulum and golgi apparatus, the cellular city gates of the plasma membrane.

As he read up on molecular motors, he appreciated that in order to organize itself, the cell used these motors running along cytoskeletal tracks to transport material such as messenger RNA and vesicles between organelle-compartments and down the long axons of neurons, to pull apart chromosomes during cell division, among many other functions. Roop would later draw a cartoon of how the kinesin and dynein motors are involved in transport within cells, depicting kinesin as a “car” of one color that moves along microtubule tracks in one direction: from the microtubule’s slow-growing “minus-end” near the nucleus, to its fast-growing “plus-end” towards the cell’s periphery. He depicted the dynein motor as a car of a different color moving along these polar tracks in the opposite “minus-end” direction, towards the nucleus.



At the time Mallik arrived in Steve’s Gross’s Irvine lab in 2001, there had been intensive analysis of the kinesin motor, as well as the myosin motor that moves along actin filament-tracks. It was known that kinesin and myosin motors are members of the same family of proteins, and work similarly; that dynein was a member of different protein family, was ten times bigger and much more complicated structurally, and had many more regulatory factors with which it interacted. “It was difficult to investigate this motor because its very complex,” say Mallik. “In some

sense dynein is a monster protein.” Steve Block and others had placed the smaller and simpler kinesin motor in “optical traps”—tiny, focused laser beams whose photons exert a graded force against the molecular motor as it pulls a small plastic bead along a microtubule attached to a glass slide. Such optical traps had enabled biophysicists to measure the maximum force that a single kinesin molecule generates as it moves and carries cargo: about 6 piconewtons. Like dynein, a kinesin nanomotor uses two head-domains as “legs” to push back from the microtubule and “walk” along it processively, and optical trap measurements had shown that kinesin walked with a fixed “step-size” of 8 nanometers, no matter how much load it was pulling. But in 2001, no one had succeeded in getting cytoplasmic dynein, so monstrously big and biochemically fragile, to move in an optical trap. No one had ever made force or step-size measurements of single cytoplasmic dynein motors.

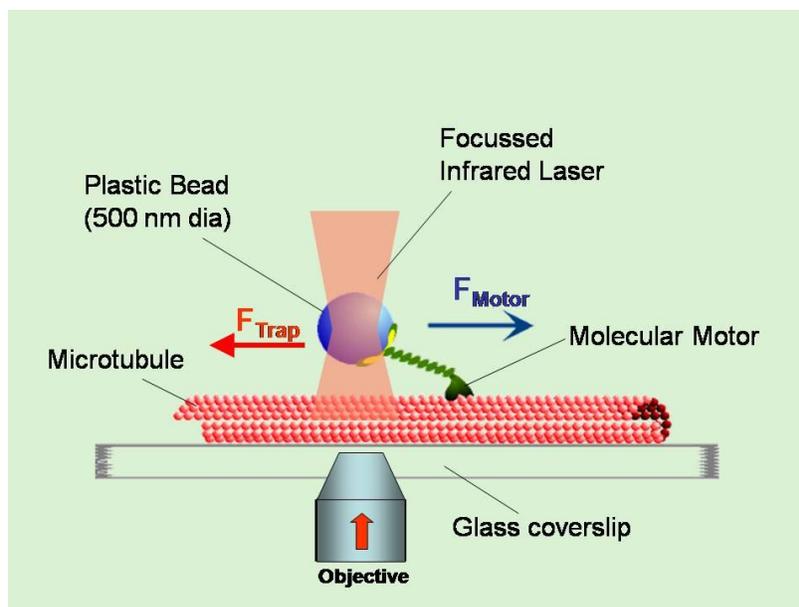
An Optical Trap Springs a Surprise

In his cartoon, Mallik would depict dynein and kinesin as two differently-colored cars running in opposite directions on microtubule tracks, indicative of common architectural features shared by both motors despite large differences in size and complexity: two head domains that act like legs, and a “tail domain” that attaches to the different cargoes the motors carry. Moreover, unlike gasoline, diesel or electric cars, dynein and kinesin “cars” burn the same fuel: ATP. The head domain of both motors have an ATP-binding site where molecules of ATP fuel become bound and then hydrolyzed, releasing energy that results in a conformational change propagated through the head domain and thus a power-stroke of the motor’s leg. For both kinesin and dynein, cycles of ATP binding and hydrolysis drives processive walking along the microtubule track.

But even in their interactions with ATP, the universal energy currency of cells, kinesin and dynein motors display a puzzling difference. Kinesin heads each have one site where ATP is bound and hydrolyzed; dynein has an equivalent fuel-burning site, plus three more sites arranged in a half-ring where ATP can be bound but not burned. No one knew the function of these extra ATP-binding sites. “It was a real mystery why dynein would be so complicated,” says Gross.

Mallik and Gross were fortunate to have an excellent source of dynein protein purified from cow brains by their collaborator, Stephen King of the University of Missouri-Kansas City. Indeed, King had shown that this dynein could move and carry cargo in a bead assay. But it was no trivial task to catch single molecules of this dynein protein alive and walking within the laser beam of the optical trap Mallik built. It took about two years of hard work—choosing the right wavelength laser, optimizing conditions for studying this massive and fragile molecule. “One of the most difficult tasks probably was calibration of the instruments,” says Mallik. “Because you’re talking about measuring things happening at the size scale of one nanometer—almost a million times smaller than the width of the hair on our heads.”

Indeed, tracking the precise number of nanometers a dynein molecule walked was everything in these optical trap experiments.



Mallik adsorbed a single dynein motor on a single plastic bead, then used the force field of the optical trap's laser beam as tweezers to place dynein on a microtubule. "What happens is that the dynein motor attaches to the microtubule and starts walking along. But as it walks, it pulls the bead out of the center of the optical trap." This optical trap acts very much like a linear spring, pulling back upon the bead-carrying-motor with a force that increases linearly with distance from the trap's center. Thus by precisely tracking the bead and dynein motor's position as it moves from the center of the trap—by making so-called "displacement records"—Mallik could trace both the motor's motion and the force the trap was exerting on the bead-cargo it was carrying. The further the motor walks from the center of the trap, the greater the force pulling it back: this force can be calculated by multiplying distance from the center by the trap's "spring constant." When it reaches a certain distance from the trap's center, "the motor cannot win against the optical trap and it stalls there," says Mallik. "This stall force is the maximum force the motor can generate."

Roop's first major observation was that the big, complex dynein motor's stall force was 1.1 piconewton, only about a fifth of kinesin's. "That was exciting," says Gross. "First, because the stall force was very low in comparison with kinesin. And second, because it was consistent with previous measurements from the lab in vivo," looking at the movement of individual lipid droplets in *Drosophila* embryos, which Gross had inferred were being carried by multiple dynein motors, and which all had stall forces that were multiples of 1.1 piconewtons.

To measure dynein's stall force, Mallik had concentrated his analysis of dynein displacement records to regions of the trap—"regimes"—where it was under a relatively high amount of load, regimes near the motor's stall force, and therefore relatively far away from the trap's center, out toward its periphery. Also, because Roop was trying to determine the maximum force his dynein "car" could generate, he gave it a lot of gas: a lot of ATP. In this regime of high-load, high ATP, Roop found that the dynein motor walked along the microtubule polymer with a step size of 8 nanometers—the same size steps kinesin made in the opposite direction. "And that was not very surprising," says Gross. "Because the periodicity of the microtubule lattice is 8 nanometers, and the kinesin motor takes 8 nanometer steps. So it was nice to see that we could measure dynein steps"—for the first time ever—"but the result wasn't anything unexpected."

Yet this similarity with kinesin did not address the mystery of dynein's complex molecular architecture: specifically, the three extra ATP-binding sites on dynein's head which kinesin lacked. "What are all those other ATP-binding sites in the dynein head doing?" says Roop. "So we thought: let us starve the molecule of ATP and see what it does."

Starved of ATP as it tried to move out of the center of the optical trap, dynein sprung its first big surprise. Varying the concentration of ATP above a certain minimum value had been found to have relatively little effect on kinesin. But this was not true of dynein. Roop found that dynein's stall force declined as much as 75% when starved of ATP; moreover, there was a linear relationship between stall force and ATP concentration. This is not typically how motors behave as a function of fuel concentration; they behave as kinesin does: above a certain minimum amount, the motors run pretty well. "This made us think that for dynein, ATP might be much more than fuel," says Mallik. "That ATP might also be regulating motor function." Could this regulation be seen in the motor's motion?

Roop proceeded to take dynein displacement records—"many, many displacement records"—from different regimes of the optical trap: near the center of the trap, in regimes of low load; closer to the middle of the trap, in regimes of medium load; and closer to the periphery of the trap, in regimes of high load. From these displacement records, he performed a statistical analysis that allowed him to extract the molecule's step-size; and he found something quite amazing and

unprecedented in the molecular motor field. Like a hiker whose step size decreases the steeper the hill the hiker is climbing, the steps the dynein molecule took along the microtubule grew smaller as it walked outward from the center of the trap and into regions where it experienced higher and higher backward-pulling load. Near the center dynein took 24 nanometer steps; then 16 nm steps in intermediate regions; finally, 8 nm steps near the region it stalled out. Nothing like such step-size changes had ever been observed for myosin or kinesin. No matter the amount of load a kinesin molecule was laboring to pull along a microtubule's track—a track that in living cells traverses cytoplasm which sometimes has the viscosity of honey—kinesin invariably took steps of 8 nanometers, no more, no less, up to the load at which it stalled out.

When Roop showed Steve Gross his initial findings that dynein was taking different size steps under different loads, “Steve said, okay, this means that dynein is working like a gear.” Gross was in essence suggesting that the dynein “car” could somehow down-shift from third gear (24 nm) to second gear (16 nm) to first gear (8 nm) as it moved out from the trap's center and “climbed” more and more steeply up the graded “hill” of the trap's backward-applied optical force. “I thought, Aha! We have a gear,” says Gross. “But I didn't have any idea how such a gear might be implemented.”

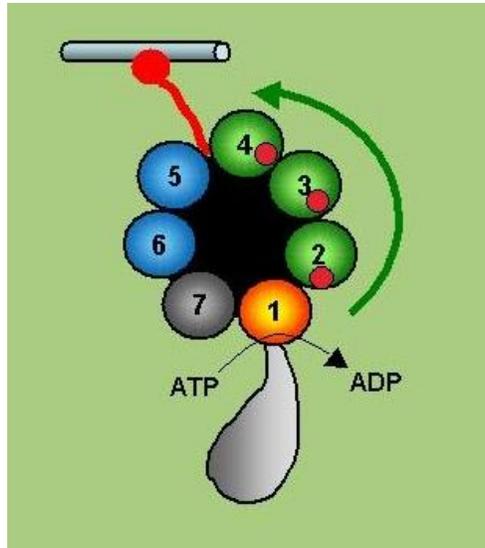
Picturing a Molecular Gear

Roop was at first fairly skeptical about Gross's conclusion. “That was a very unconventional idea,” says Mallik. There was no precedent for a gear-like mechanism operating in any molecular motor or force-generating molecule known to science. But Roop's displacement records continued to firm up the evidence for changes in dynein step-size in response to both changing load and ATP concentration. Most importantly for Roop, he found that whatever its step-size, the physical work performed by the dynein molecule—step size multiplied by stall force—was approximately constant. Roop became a believer in dynein's molecular gear. He adapted low-tech video tracking to show that dynein could take long, loping steps of 32 nanometers when under zero load. Free of any backward-pulling force, dynein seemed to be able to shift into a fourth gear as well.

But how could such an unprecedented gear be embodied in the nanoarchitecture of a protein? “One thing I am probably pretty good at is visualizing things,” says Mallik. Where in this molecular nanomachine was the equivalent of the different size wheels Roop's father used to make gears all his adult life? Where was the “handle” by which dynein's gears could be shifted? It was rather like being a boy again in his father's workshop, trying to visualize the inner workings of some device, except that now Roop was peering inside a cell's workshop, trying to visualize the connecting elements, the intermeshing motions, of a molecular machine assembled by evolution a billion years ago. “You need to get some kind of picture in your mind when you close your eyes,” says Mallik. “And I think that is something I have in me to some extent because of the way I have interacted with my father.”

ATP as both motor fuel and gear-regulator; the backward pulling spring of Roop's optical trap countered by the changing “tension” of an ATP-regulated spring within the dynein molecule itself; a molecular spring whose transmitted force increased as step-size decreased in response to higher loads: these were the pieces of the puzzle-picture Mallik visualized and assembled in his mind. These were the essential elements of the nano-gear model that Roop explained to his father under the trees in Allahabad. Dynein's head domain, which functioned like a leg, had the shape of a ring with four ATP-binding sites. ATP bound at the first site was burned as fuel, transmitting a conformational change through sites 2, 3 and 4, which collectively could be pictured as “a big floppy spring,” says Mallik. With no ATP bound to this site, this big floppy

spring can take big, loping 32 nanometer steps. But as load is applied to dynein, the three sites on the spring begin to bind ATP, making the spring shorter and stiffer, leading to smaller but more forceful steps. Indeed, the ring-like head of a related protein called NSF had been shown to grow more compact upon ATP-binding—a finding crucial to Roop's visualization of how dynein's gear might work.



Moreover, Mallik noticed that all the steps sizes were multiples of 8 nanometers, and that there was a beautiful numeric harmony between the number of step-size changes and ATP-binding sites. No ATP bound to any of these three regulatory sites: dynein takes 32 nm steps; ATP bound to one site: 24 nm steps; ATP bound to two sites: 16 nm steps; ATP bound to all three sites: 8 nm steps. In Mallik's model—which Mallik stresses is only a model, not yet proved—dynein contains a self-regulating gear that down-shifts itself in response to increasing load, not by shifting to toothed-wheels of larger sizes, but by changing the size and tension of a kind of force-transmitting molecular spring.

"We use gears all the time in our daily life," says Mallik, "and the idea that Nature has 'thought' of that a long, long time ago, has implemented a gear at the level of a few nanometers—that's very exciting to me." Mallik's model of a nanoscale molecular machine involves no exotic physics, no quantum weirdness. "The principle is quite simple—I think that's the beauty of it." Once Roop had described the essential features of the dynein motor and his gear model to his father, his father's experience as a machine designer and gear specialist perfectly equipped him to comprehend the underlying principle. Mallik's measurements show that whatever its step-size, the work performed by the dynein molecule per step is approximately constant. "That's a basic physical principle, that the work done is constant. And Nature has found a way to use that... Work per step is force multiplied by distance. You reduce distance, you increase force." You take a shorter but more forceful powerstroke. Roop's father now had a picture of the dynein nanomachine's underlying principles, how its molecular gears functioned and shifted. "And that was very satisfying to me, because he's someone who understand these things."

From a Model of a Molecular Gear to the Challenge of Biological Complexity

Mallik plans to return to India in 2006 to start his own lab and continue his pathbreaking studies of molecular motors. He has already received an attractive offer of a position at a newly-expanding cross-disciplinary research institute in India and has good prospects of others. Utilizing a relatively new feature of HFSP long-term fellowships, Roop is deferring the third year of his fellowship support to use in India upon his return, where he will also apply for an HFSP Career Development Award. "I think it's important that science spreads out," says Mallik. "And I want to give something back to my country also."

During his time at Irvine, "Roop's really become a peer," says Steve Gross. "And I think one of the major things Roop is going to be doing in his own lab is to build from the [single-molecule] foundation that he's established here and try to push the notion of how do you build complexity."

"Trying to understand complexity in biological systems, that is my main goal," says Roop. "In vivo, inside the cell, we know that there are multiple motors which are working together and maybe coordinating and collaborating with each other—but we don't know how that's happening. My feeling is that we need to take things to the next level of complexity"; to analyze a few motors acting simultaneously on a single cargo along perhaps with a regulatory factor such as dynactin, a giant protein complex that is crucial to dynein function in vivo but whose role remains quite mysterious. Roop thinks that the next generation of experiments, which he has already begun in Gross's lab, "should be bottom-up, but aimed at a level which is in between single molecule studies and very complex systems—complex but not too complex. That is the next step which I want to attempt to take."

Mallik is also continuing to test his dynein gear model on two fronts. First, he is trying to track dynein step-size "when it is moving under constant load for a long interval of time." This means developing a second generation optical trap that moves its laser beam through a feedback mechanism, trailing the dynein-attached-bead at a constant distance as it walks. (Similar optical traps have been used to study kinesin.) Second, Mallik is collaborating with two Irvine theoretical physicists, Clare Yu and Manoranjan Singh, to create Monte Carlo simulations of his gear model on a computer. "The core idea of this simulation is the gear model in the Nature paper," says Gross. "And the beauty of these Monte Carlo techniques is that they assign a probability of a particular event occurring, and because of that, you can very easily model branched enzymatic pathways, you can sort of gradually add complexity. And Roop's been very active in understanding the ramifications of theory, of modifying theory and really making it fit with the experiments, and then using theory to guide experiments as well."

These theoretical simulations, now being submitted for publication, have strongly supported and expanded upon Mallik's original gear model. Moreover, Roop's model has served as the nucleating center for a new perspective on how cells organize the tremendously intricate and dynamic complexity of motor-driven transport. This new perspective is described by Mallik and Gross in a recent review article [Current Biology (2004) 14: R971-R982] entitled, "Molecular Motors: Strategies to get along." Why is dynein the only known motor whose step-size varies under the control of a hypothetical gear? Why is dynein ten times bigger than kinesin and much more complicated structurally and in its regulatory partners, yet capable of generating only one-fifth kinesin's cargo-pulling force? "In the world of cellular transport, is there any advantage of having a poor performer that requires external help?" write Mallik and Gross. "We hypothesize that inherent in the complexity of dynein is the opportunity for regulation at multiple levels." For instance, a single microtubule track serves as a highway for many dynein and kinesin motors carrying cargo in opposite directions, making it susceptible to molecular motor "traffic jams" which

have been associated with human diseases such as forms of neuronal degeneration. Mallik and Gross propose that the smaller and simpler kinesin and myosin motors be considered as “performance oriented ‘lean and mean’ workhorses of transport.” On a given microtubule, for instance, kinesin can “bulldoze” past dynein, whose more complicated architecture “may have adaptations giving it a ‘yield when necessary strategy,’” enabling dynein to step aside and avoid traffic jams and crashes.

“You need a motor that can be fine-tuned,” says Mallik “If every motor in the cell was a bulldozer, it would just take cargo and go, that’s probably not a situation you would like to have.” Roop’s gear model has served as the theoretical seed for viewing the complexity and fine-tuning of intracellular transport in a new way. Indeed, such bottom-up molecular models are critical for understanding cellular complexity in general, says Steve Gross. “One of the things we’re seeing in many different areas of biology is that we have a lot of different molecular interactions and we can map each individual interaction. But then to make sense of how they all fit together we need some sort of theory,” a theory that can determine which specific interactions are most important; “a theory that can go from single molecule properties, and gradually be altered or increased to deal with all these other complexities.”

When Roop would visit his father’s workshop as a boy, “I kind of thought I wanted to go into machine design and those kind of things. But I suppose I’m doing that in a different way right now.” Mallik is a reverse engineer of natural selection’s nanoscale machinations. And while humans can learn about life’s nanomachines and think about how to implement this knowledge in man-made devices, “I think its still too far in the future to talk seriously about how to build nanomachines” that, for instance, might work therapeutically in the human body.

Nevertheless, as Roop discussed his dynein gear model with his father under the trees outside his father’s house in Allahabad, “we had some quite interesting discussions about what are the parameters by which you can tell whether a gear is a good gear or not. And these are probably questions which we will need to think about in the future, from an engineering point of view.” Did his father’s life-long experience as an engineer and gear specialist give him any ideas about how the dynein gear might be more optimally designed? “No—at least not that he told me,” says Roop with a laugh. “I think he was quite impressed by how Nature has managed to do it.”

June 2005

Additional References

Mallik R, Petrov D, Lex SA, King SJ, Gross SP..Building complexity: an in vitro study of cytoplasmic dynein with in vivo implications. *Current Biol.* 2005 Dec 6;15(23):2075-85.

Reck-Peterson SL, Yildiz A, Carter AP, Gennerich A, Zhang N, Vale RD. Single-molecule analysis of dynein processivity and stepping behavior.*Cell.* 2006 Jul 28;126(2):335-48.

Update September 2007: Roop has in the meantime established his laboratory back in India at the Department of Biological Sciences, Tata Institute of Fundamental, Mumbai (<http://www.tifr.res.in/~roop/>). He has also written a “Layman’s version of molecular motors” (his own words) available at <http://www.tifr.res.in/~roop/NaturesNanotech.htm>.