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# Movers and Shapers: [Dr. David Luck]

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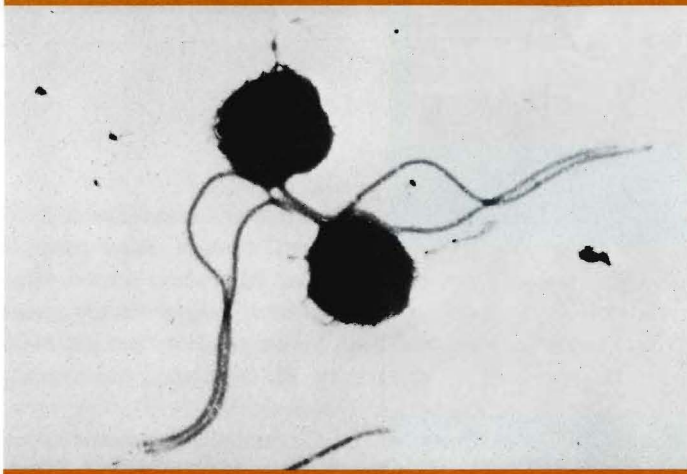
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*Chlamydomonas mating*

# THE ROCKEFELLER UNIVERSITY RESEARCH PROFILES

SUMMER 1986

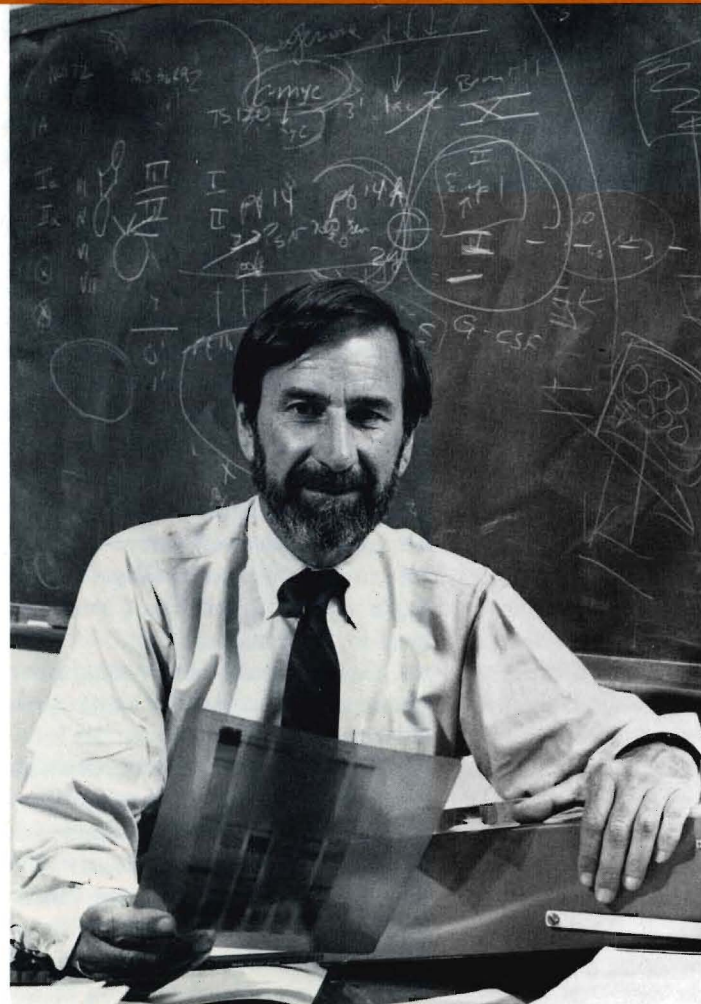
## Movers and Shapers

*Chlamydomonas reinhardtii* is a plant that swims, a microscopic member of the algae family equipped with a pair of hair-like projections, called flagella, with which it breaststrokes around in ponds and streams and in test tubes in David Luck's laboratory. Dr. Luck is neither a botanist nor a freshwater ecologist. He is a cell biologist. What interests him about *Chlamydomonas* are its microtubules—slender, hollow tubes inside its single-celled body and its flagella.

Microtubules are present in virtually all plant and animal cells from *Chlamydomonas* to man. They play critical roles in cellular activities. "What makes microtubules particularly interesting," says Dr. Luck, "is that they play different roles at different stages of a cell's life cycle as well as special roles in specialized types of cells."

Among their general tasks, microtubules form the skeletons that hold cells in shape. Then, when a cell is preparing to divide in two, in the process known as mitosis, they disassemble and rearrange themselves into a spindle on which the chromosomes, the bearers of the cell's genes inside the nucleus, line up like soldiers. When mitosis is completed, microtubular scissors snip the offspring cells apart.

In the nervous system, within the long, thin threads that nerve cells send out to communicate with other cells, chemical messages are relayed along microtubular rails. Microtubules power the wiggling tails of sperm cells as well as the flagella of free-swimming organisms like *Chlamydomonas*. They help to supply motive force to cilia (densely packed forms of flagella),



David Luck in his laboratory examining a film showing axonemal proteins separated by the process of electrophoresis.





Dr. Luck and Zenta Ramanis in the temperature-controlled room where *Chlamydomonas* cultures are grown.

such as the cilia on cells of the respiratory tract that brush away debris and the cilia of the oviduct, which churn up waves that float the egg to the uterus.

Dr. Luck wants to find out how microtubules do all the things they do. He uses *Chlamydomonas* as his experimental model because it's easy to cultivate in the laboratory, it reproduces rapidly, and its flagella provide a more accessible and simpler microtubular system for study than the systems inside cell bodies. Which is not to say that it is simple.

Stripped of its membranous covering and viewed in the electron microscope, a *Chlamydomonas* flagellum displays an internal structure, called an axoneme, composed of a cylinder of nine pairs of microtubules—doublets—surrounding two central tubules. Embellishing the doublets are delicate wing-shaped outer and inner "arms." Within the cylinder are radial spokes and bridges. Intricate and elegant architecture measured in millionths of a millimeter.

## AN OLD STUDENT

To see structures as small as microtubules requires an electron microscope. To study their biological activity—their biochemistry—requires being able to get them out of the cell intact. In the 1940s, the late Albert Claude, working at what was then The Rockefeller Institute for Medical Research, introduced electron microscopy to biology. He also developed gentler methods of cell fractionation. Biochemists extract cell parts—their organelles—by whirling cell mixtures in a centrifuge, which separates organelles by weight and density. Before Claude, organelles often were destroyed in the process.

Among Claude's proteges, George Palade, now at Yale, spent twenty-seven years at The Rockefeller, years in which cell researchers, at Rockefeller and elsewhere, building on Claude's innovations, characterized all of the cell's organelles, many of which had not been seen before or even imagined. Among his own numerous contributions, Dr. Palade isolated and described ribosomes, the cellular factories where proteins are made. In 1974 Claude, Palade, and another Rockefeller cell researcher, Christian de Duve, discoverer of lysosomes, the cell's digestive organelles, shared a Nobel Prize for their contributions to cell biology.

Dr. Luck joined Dr. Palade's laboratory at Rockefeller in 1958 as a Ph.D. student. "An old student," he says. After medical school at Harvard, service in the Air Force, and several years on the staff of Massachusetts General Hospital, he was returning to basic science, which had been his original destination as an undergraduate at the University of Chicago before being "waylaid by the seductions of the clinic."

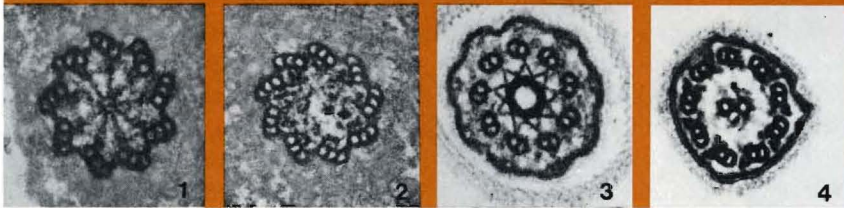
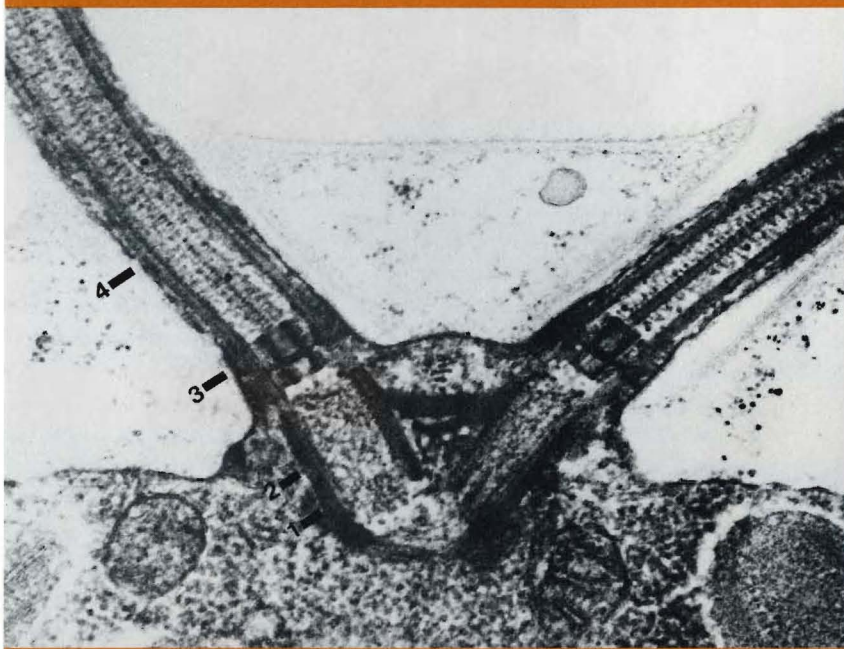
At Harvard and Massachusetts General, he had heard about the "impressive" Porter and Palade groups at Rockefeller. Keith Porter, along with George Palade, was an early colleague of Claude's in the development of electron microscopy. It was Porter in fact, some years later at Harvard, who identified microtubules as the element at work in the different microtubular systems.

"I had a hard time persuading George to take me on," Dr. Luck says. "Science had done a lot of marching ahead while I was in medicine. The combined Porter and Palade approaches were so fertile that it seemed as if each day's coffee-break discussion brought to light a new discovery. Unfortunately, my thesis didn't become one of them. It was a study of certain membranes of the liver cell, which turned out not to be doing what we had thought they were doing. But I learned to use the technology, and I guess I must have been doing something right because George began to talk to me."

One of the things they talked about during Dr. Luck's last year as a student was mitochondria, the organelles in cells where food is converted to chemical energy. "I had been wondering how mitochondria are produced," Dr. Luck says, "and I had an idea how to find out. I had finished my thesis so I thought I'd give it a try." To pursue his idea, he needed to learn how to study genes and how to cultivate microorganisms, in which a simpler genetic composition than those in higher organisms facilitates research. "One of the great things about being in this place," he says, "is that you can walk across the hall and ask someone, 'How do you do this?'" He walked across the hall to the laboratory of Nobel laureate Edward Tatum and learned to grow *Neurospora*, a simple mold. He also learned "the power of genetics."

In collaboration with biochemist Edward Reich, Dr. Luck discovered that *Neurospora* mitochondria contain their own





A diagram of the mitotic spindle. Pairs of chromosomes (black) lined up on a central plane are connected to perpendicularly arranged microtubules (orange). Other microtubules originate from two pairs of centrioles—the poles of the spindle. At later stages of mitosis, the chromosome pairs will separate and move toward opposite poles, thus assuring that every chromosome will be represented in the two daughter cells. (In the micrographs to the right, the central panel shows this structure in an actual cell.)

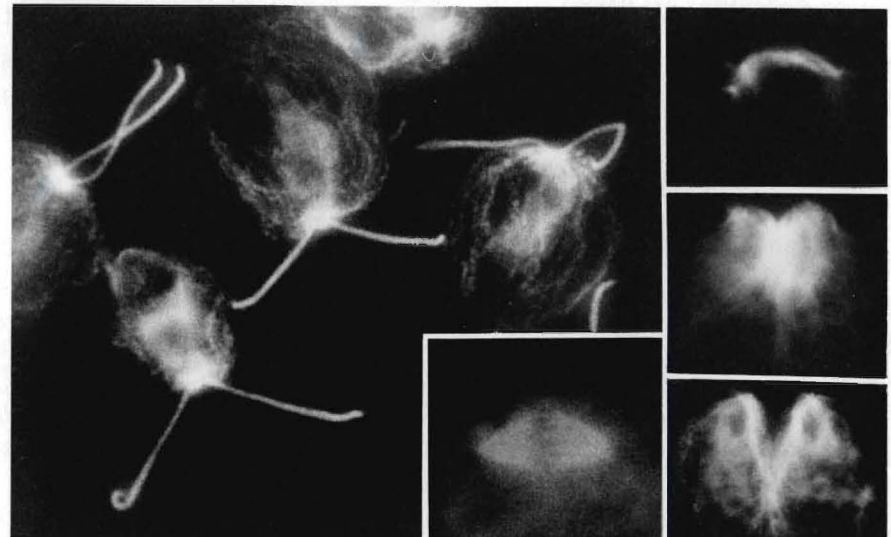
The electron micrographs to the left, made from cell slices so thin that the results resemble draftsmen's drawings, illustrate the extraordinary micro architecture of the bases of *Chlamydomonas* flagella, the structures called basal bodies. (Only a small portion of the flagella themselves are shown.) The larger micrograph is a longitudinal section. The smaller images are cross sections taken at the sites indicated by corresponding numbers in the longitudinal section.

Cross sections 1 and 2, from deepest in the cell, show nine triplet microtubules arrayed pinwheel fashion within a circle. This part of the structure cannot be distinguished from a mitotic apparatus centriole. (See diagram below.) In cross section 3, the triplets have given way to doublets and in the center is the beautiful star-shaped image that characterizes components unique to basal bodies called transition zones. The doublet microtubules of the transition zone are continuous with the nine doublets that are the major components of the cylindrical shaft of the flagella, as shown in cross section 4.

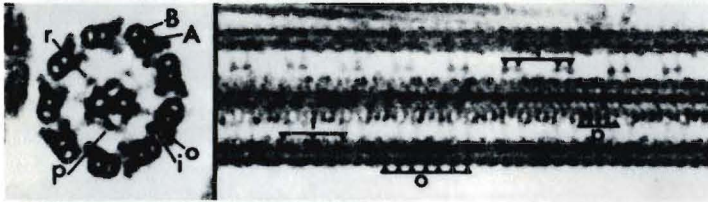
In dividing *Chlamydomonas* cells the flagella and transition zones disassemble. The remaining triplet microtubular structures act as centrioles for the mitotic spindle.

These structures have been magnified 65,000-times. To give some idea of how incredibly small they are, at the same magnification the diameter of a penny would measure more than three-quarters of a mile.

*Chlamydomonas* cells at various stages of their life cycle. The cells are stained by means of a highly specific antibody to tubulin, one of the major building blocks of all microtubules. The stained components represent different microtubular structures. In non-dividing cells, left, the structures are flagella and intracellular scaffolding. Early in cell division, center, the stain shows the mitotic spindle. In later stages, as on the right, the microtubules act as a knife to cut the daughter cells apart. (The micrographs on this page and on page 4 were made by Dr. Bessie Huang, a former member of Dr. Luck's laboratory who recently joined the Research Institute of Scripps Clinic in La Jolla, California.)







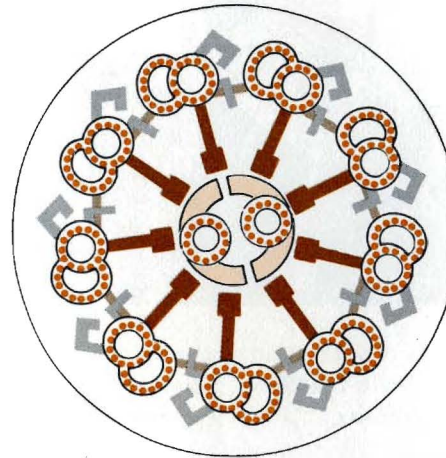
genes, distinct from the genes in the cell nucleus, a phenomenon later confirmed in cells of higher organisms as well. States Gianni Piperno, a current colleague of Dr. Luck's, "It was a seminal contribution to cell biology."

By 1968 the old student was a full professor in charge of his own laboratory. In 1984 he was elected to membership in the National Academy of Sciences. In 1985 he became the University's first Alfred E. Mirsky Professor, a chair named in honor of another pioneering Rockefeller cell scientist.

Mary Rifkin was Dr. Luck's first graduate student while he was still a junior faculty member. (Her thesis helped to demonstrate that mitochondria make their own ribosomes.) "The most important thing I learned from David," says Dr. Rifkin, now herself a member of the Rockefeller faculty, "was an approach to research that says, don't be afraid to try new things, new techniques you're not familiar with. Don't hesitate to follow up a line of investigation even if you have no experience in that area. You never know where it may lead."

## SWIMMERS AND SPINNERS

The molecular building blocks of microtubular structures as well as the enzymes that trigger their biological activity are proteins, the primary molecules of living matter. When Dr. Luck began investigating microtubules, in the 1970s, their characteristic structural proteins, called tubulins, had only recently been identified. "Tubulins account for about seventy percent of the proteins in axonemes," Dr. Luck says, "but it already seemed clear that other proteins had to be interacting with tubulins to produce all the vastly different activities of different microtubular systems." As his laboratory has since established, there are about three hundred different kinds of proteins associated with tubulin in the axonemes of *Chlamydomonas*.



*Electron micrographs of a cross section and longitudinal section through an axoneme, the "motor" of a flagellum, and a diagram of the cross section. The axonemal cylinder is made up of nine doublet microtubules. A doublet consists of an A and a B tubule. A tubules have outer and inner arms (o and i) and radial spokes (r) that extend toward the center. The center of the cylinder contains two microtubules with projections (p).*

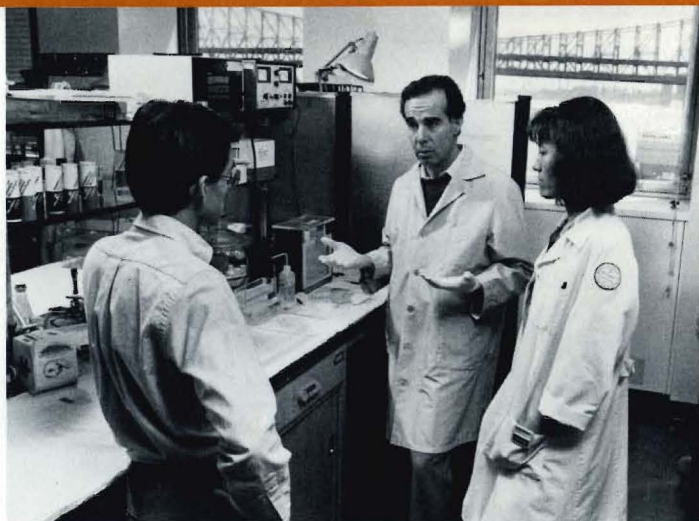
To find specific kinds of molecules within the biochemical soup scientists use chemical probes. Antibodies, the chemicals of the immune system that normally attack foreign invaders such as bacteria or viruses, can be adapted for use as probes. What antibodies "recognize" and bind to are antigens, molecules on the surface of an invader that act as its identifying signature. In recent years, scientists have achieved the ability to make very pure antibodies, called monoclonal antibodies, that bind to target molecules with great specificity. In Dr. Luck's laboratory, Gianni Piperno has devoted much of his effort to the development of antibody probes for microtubular proteins. Another technique the laboratory has adapted very successfully is two-dimensional electrophoresis, in which mixed batches of proteins are separated and sorted on the basis of electrical charge and size.

One way to get a handle on how a biological mechanism works is to compare normal organisms with organisms in which the mechanism doesn't work, or works peculiarly. In Dr. Luck's laboratory there are *Chlamydomonas* that can swim only backward. There are slow swimmers and jerky swimmers. There are some, missing one flagella, that spin like tops.

Some earlier studies of flagellar axonemes had shown, through comparisons of normal and defective structures, that



Laboratory member Gianni Piperno, center, a pioneer in the application of biochemistry to the study of microtubular proteins. Working with him, doctoral students Michel LeDizet, left, is investigating the process by which an enzyme made in the cell modifies tubulin to increase the stability of microtubules, and Xiao-jia Chang, has been preparing monoclonal antibodies with which she is identifying and comparing the proteins of axonemes with those of other cellular components.



specific substructures correlate with specific function. For example, the arms on the doublet tubules are the essential elements in flagellar locomotion. "As we identified the axonemal proteins," says Dr. Piperno, "by comparing the proteins we had isolated from normal structures and seeing what proteins were missing or altered in the defective structures we were able to correlate substructures with specific proteins."

Dr. Piperno has been one of Dr. Luck's principal collaborators since 1972. They met at a scientific conference. "It was held on an island off the coast of France," Dr. Piperno says, "and right away David and I discovered a common mission—to get away from the meeting every moment we could to go to the sea and swim."

Originally from Italy and originally a chemist, Dr. Piperno was then completing a second doctorate, at the University of Paris, in biology. "I was studying mitochondria," he says, "so of course I knew David's work. I expressed my desire to come to The Rockefeller and learn more cell biology." But when the invitation came, it was to study microtubules not mitochondria. "So I had to deal with a new country, a new language, and a new line of research."

A few years ago, Dr. Piperno began an independent investigation. Abandoning *Chlamydomonas*, he has been developing antibody probes against axonemal proteins in sea urchins to see if he can find comparable proteins within cells. He is using the sea urchin because it has axonemes in its sperm tail that can be obtained in quantity and it is possible to isolate the mitotic apparatus from dividing sea urchin egg cells. He is concentrating on dyneins, the class of proteins found in the

doublet arms. "Since we knew that dyneins generate movement in the axoneme," he says, "my question was whether they might be dyneins functioning in the same way in mitosis."

Dr. Piperno has found dyneins in the mitotic apparatus, but he is not certain they are functioning in the mitotic process. "We haven't yet been able to isolate pure mitotic apparatus. There's always some contamination from other proteins in the cell. Since later in its development the embryo will be ciliated, we may be encountering a pool of dynein intended for future cilia. A further complication arose when we learned that other microtubular proteins besides dyneins are involved in movement. That's a very recent finding. So we've started experiments to approach the problem in different ways."

Since genes control the structure and function of proteins, a major part of Dr. Luck's research is aimed at understanding the genetics of *Chlamydomonas*. Genes reside on chromosomes, with each gene occupying a specific spot on a specific chromosome. Genes linked together on the same chromosome form what is called a linkage group. Mutant organisms are ones in which the normal gene arrangement has been disrupted. Mutations can be induced in organisms by exposing them to chemical or environmental insults.

In addition to being able to reproduce by simple cell division, *Chlamydomonas* has two sexual forms, allowing cells to exchange genes through mating. Thus it is possible to mate mutants with so-called wild type or normal organisms and, by comparing their offspring, make a map of the genes and see what genes are responsible for what proteins by seeing what proteins are lacking or altered when genes are lacking or altered. "But we were killing ourselves trying to get the experiments to work," says Dr. Luck, "until Zenta came. In minutes, it seemed, Zenta became the great 'enabler,' turning experimental plans into realities."

Zenta Ramanis, an emigre from Latvia, had begun medical studies in Europe. In New York, with a baby to care for and a husband struggling to get established, there was no money for medical school. "But I wanted to do something," she says, "so I took science courses at night at Brooklyn College. My genetics professor recommended me to Ruth Sager at Hunter College, who was investigating mechanisms of inheritance in





*Serial photographs of a single Chlamydomonas cell taken with rapidly flashing stroboscopic light, which freezes the stages of the flagellar bending cycle so that the shape of the flagellum can be visualized at various points during the one-sixtieth of a second required to execute a complete stroke and recovery. This particular cell is a mutant with one flagellum instead of the normal two. It rotates rather than swims. This method of analysis permits study of altered flagellar bending in mutants with axonemal defects.*

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*Chlamydomonas.*" When Dr. Sager moved to Harvard in 1975, Mrs. Ramanis joined Dr. Luck's group. In addition to keeping the laboratory's *Chlamydomonas* population healthy and preparing specimens for experimentation, she does genetics. She was the major contributor in a study of the spinning mutant, the one with only one flagellum, which led to the discovery of a new *Chlamydomonas* linkage group.

## BUILDING A VOCABULARY

Will studies of disorders in a tiny plant one day help to cure human disorders of the nervous system? Or lung diseases? Or infertility? Or cancer, which is a problem of runaway cell division? Dr. Luck doesn't know. What he says is, "There are some ready relationships to human disease, for example, in the condition called Kartagener's syndrome. People who have the disease suffer from respiratory trouble and infertility. Electron micrographs of cells from different affected men show a collection of defects in the cilia of their lung cells and in their sperm tail axonemes that are indistinguishable from defects in different *Chlamydomonas* mutants—dynein arms are missing, or central tubules are missing, and so on. We know, also, that many of the chemotherapeutic agents used to kill cancer cells work by disrupting microtubules, especially those involved in mitosis. But that doesn't take us very far. The vocabulary for understanding cancer and a lot of other diseases is incomplete because the vocabulary of biology is incomplete. My laboratory is one of many laboratories working from different perspectives to try to provide the missing words. I have no idea whether what we're doing will lead to medical applications.

"I embarked on this research in order to understand a cellular system that is central to life and enormously complex. Take the radial spoke, just one substructure of the axoneme. When we compared mutants in which the radial spoke was missing with normal organisms we found that in the mutant there were seventeen proteins missing—seventeen separate building blocks. A defect in only one of them led to an assembly disaster, failure to assemble the entire spoke. Functionally, the lack caused the flagella to be paralyzed, even though the dynein, the major motive element, was intact. Then we isolated another group of mutants, also with missing radial spokes, but the

mutation involved different genes. In these organisms the flagella could beat, although not very well. So then we knew that radial spokes were not essential for movement but that they interacted in some kind of complicated regulatory network."

When *Chlamydomonas* finds itself in hot water—which happens, quite literally, if the light is too bright or concentrated, a situation that can have deadly consequences—it backs up and scoots, reversing its normal breaststroke. Rosalind Segal, a recent student of Dr. Luck's, studied some *Chlamydomonas* locked by mutation in a backward swimming pattern. In a series of investigations Dr. Luck describes as extraordinarily beautiful, she identified the flagellar proteins that appear to mediate the reversal response, among them one protein she thinks may be the switch that alters the pattern of flagellar bending.

Dr. Segal uncovered a biochemical mechanism involving calcium-stimulated phosphorylation. (Phosphorylation is a process in which phosphates attach to molecules.) Her work aroused considerable interest among researchers because of its possible relevance to a number of other biological circumstances in which calcium acts as a biochemical stimulus. This spring she completed a medical degree under the joint Rockefeller University-Cornell University Medical College M.D.-Ph.D. program and has begun a training program in neurology at Harvard. Her long-range plan is to apply the techniques she learned in Dr. Luck's laboratory to the study of inherited neurological disease.

Through biochemical and genetic analysis—and a lot of trial and error—Dr. Luck and his colleagues have accounted for many of the structural and regulatory components of axonemes.

"The way we viewed these various structures when we began seems incredibly naive now," he remarks. "The control of assembly and function of the different kinds of arrays depends on a great number of different molecules; on chemical modifiers of the tubulin building block itself and certainly on organizing structures involving centrioles and basal bodies, which are structures in cells that act as templates or focal points for the formation of microtubule arrays. (See illustrations, page 3.) There is probably an interesting genetic mystery associated with these structures. We've learned a lot, but the things that remain to be learned are legion."