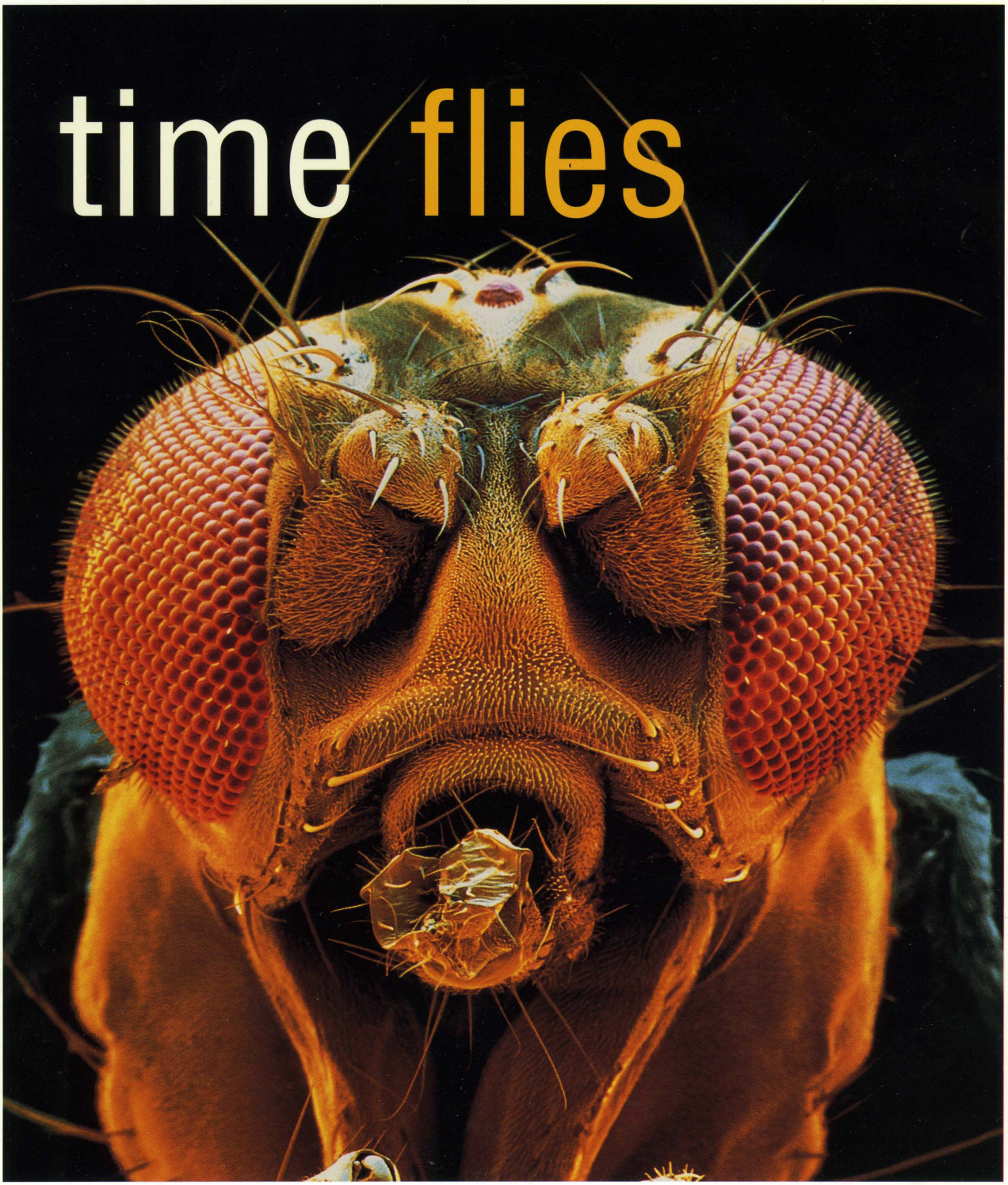
Spring 1998

SEARCH MAGAZINE 1998, SPRING

The Rockefeller University

SEARCH

THE ROCKEFELLER UNIVERSITY MAGAZINE • SPRING 1998



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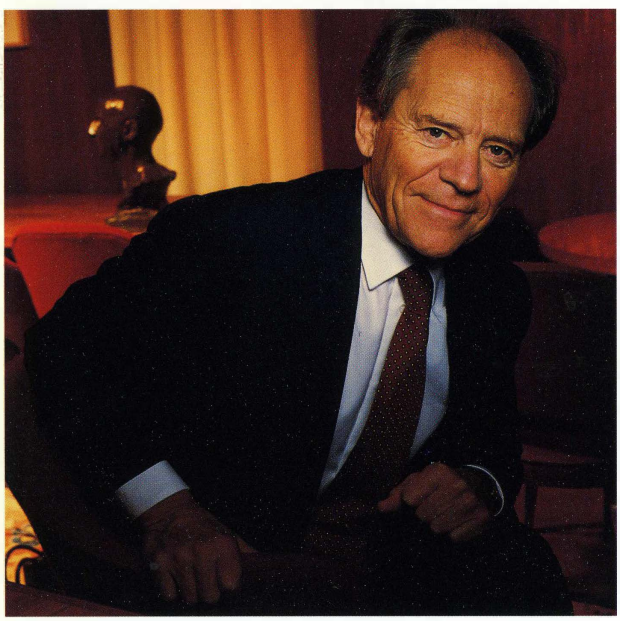
Support for research reported in SEARCH was provided in part by the National Science Foundation and the National Institutes of Health.

FRONT COVER: The fruit fly *Drosophila melanogaster* is a powerful tool for genetic studies. Professor Michael Young, head of the Laboratory of Genetics, investigates the genes that control circadian rhythms in the fruit fly.
OLIVER MECKES/PHOTO RESEARCHERS, INC.

Message from the President

Many of the most intriguing scientific discoveries raise more questions than they answer. Indeed, reading this issue of *Search*, one is struck by how major new findings and lines of investigation at The Rockefeller University have their roots in puzzles that first arose as a result of discoveries made decades ago.

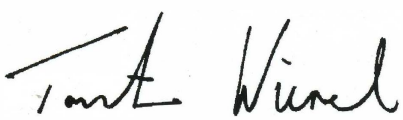
In 1911, for example, Rockefeller scientist Peyton Rous discovered the first virus that could cause cancer. It was only last year, however, that the laboratory of John Kuriyan was able to visualize the 3-D structure of a protein that, when defective, enables the virus to trigger tumor growth. The structure now allows researchers to ask far more precise questions about how the protein works in both its normal and cancerous forms.



ROBIN THOMAS

In 1952, the British physiologists Hodgkin and Huxley postulated that nerve impulses were generated by the movement of sodium and potassium ions through specialized filters, or "channels," embedded in the surface of nerve cells. Earlier this year, in a historic achievement, the laboratory of Roderick MacKinnon used X-ray crystallography to solve the 3-D structure of a potassium ion channel protein. This finding resolves the basis of the selectivity of the channel for potassium ions. At the same time, it raises new questions about how ion channels generate the ordered electrical impulses that underlie our perceptions, movements and thoughts.

Three other articles in this issue describe critical studies of gene replication, genetic control of sleep–wake cycles and the signals that form the nervous system during embryogenesis. These discoveries, as in the two examples mentioned above, are simultaneously yielding answers to classic scientific enigmas and are serving as gateways for fundamental new explorations into life and disease processes.



TORSTEN N. WIESEL, PRESIDENT

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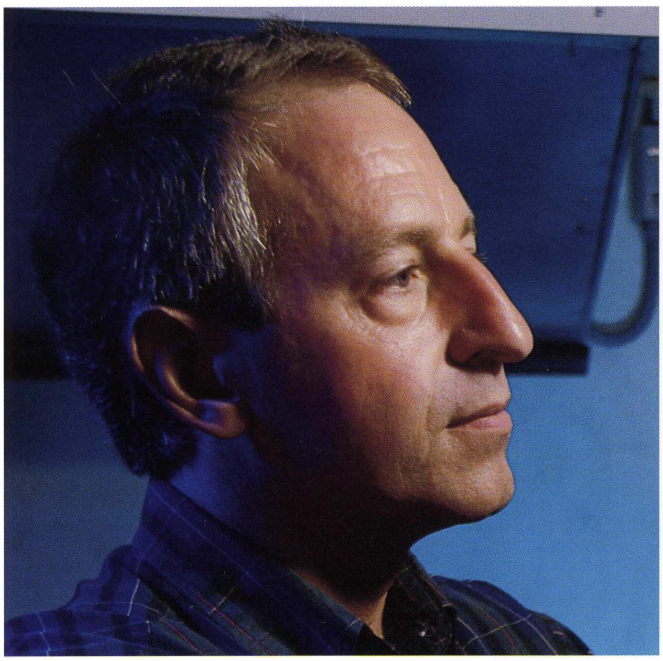

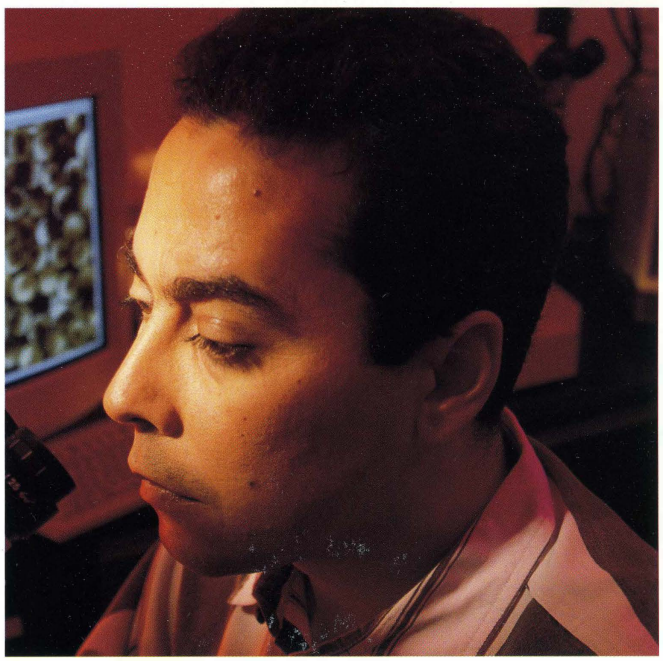

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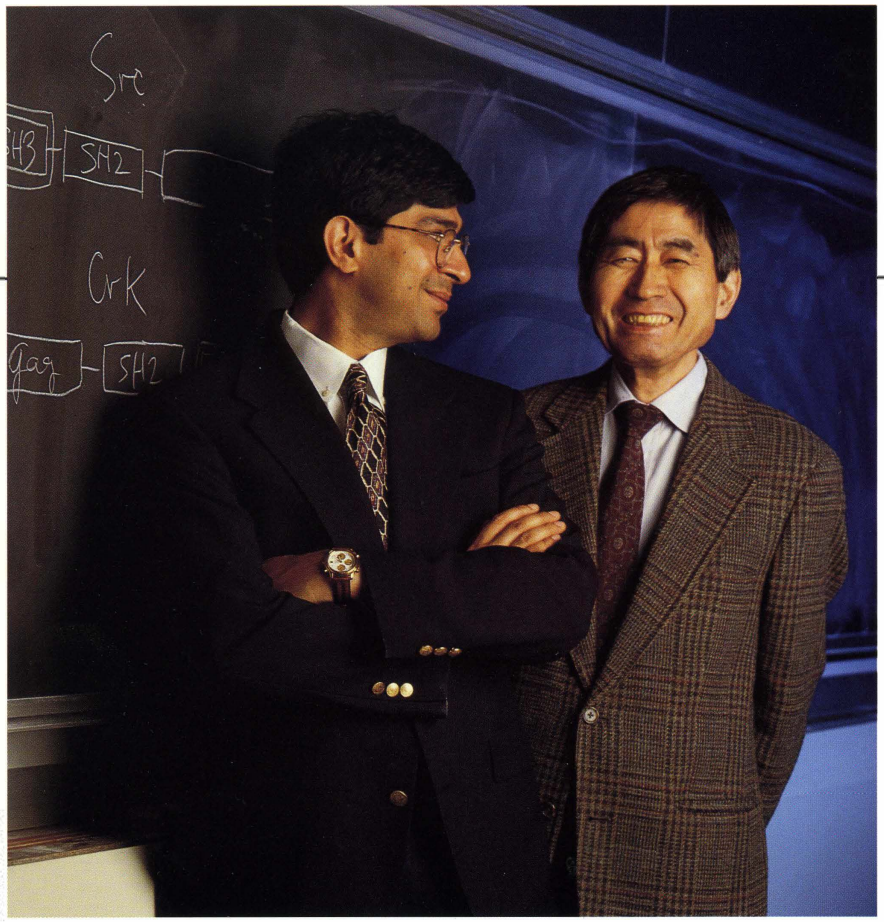
BY URMILA RANADIVE

CLOCKWISE FROM TOP LEFT: *Michael O'Donnell, Roderick MacKinnon, Ali Hemmati-Brivanlou.*



MICHAEL DAMES

LEFT: *Professors John Kuriyan (left) and Hidesaburo Hanafusa.*

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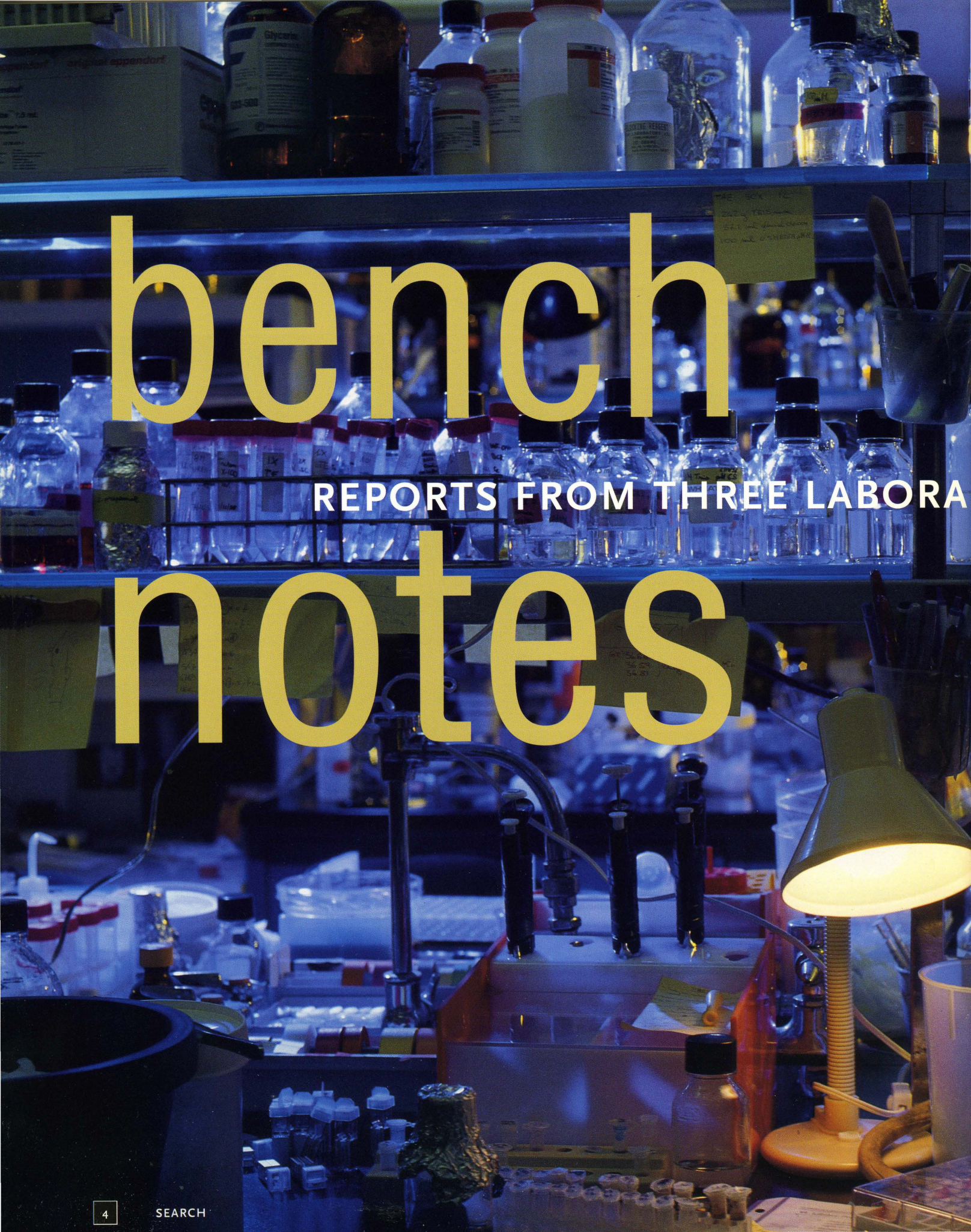


bench notes

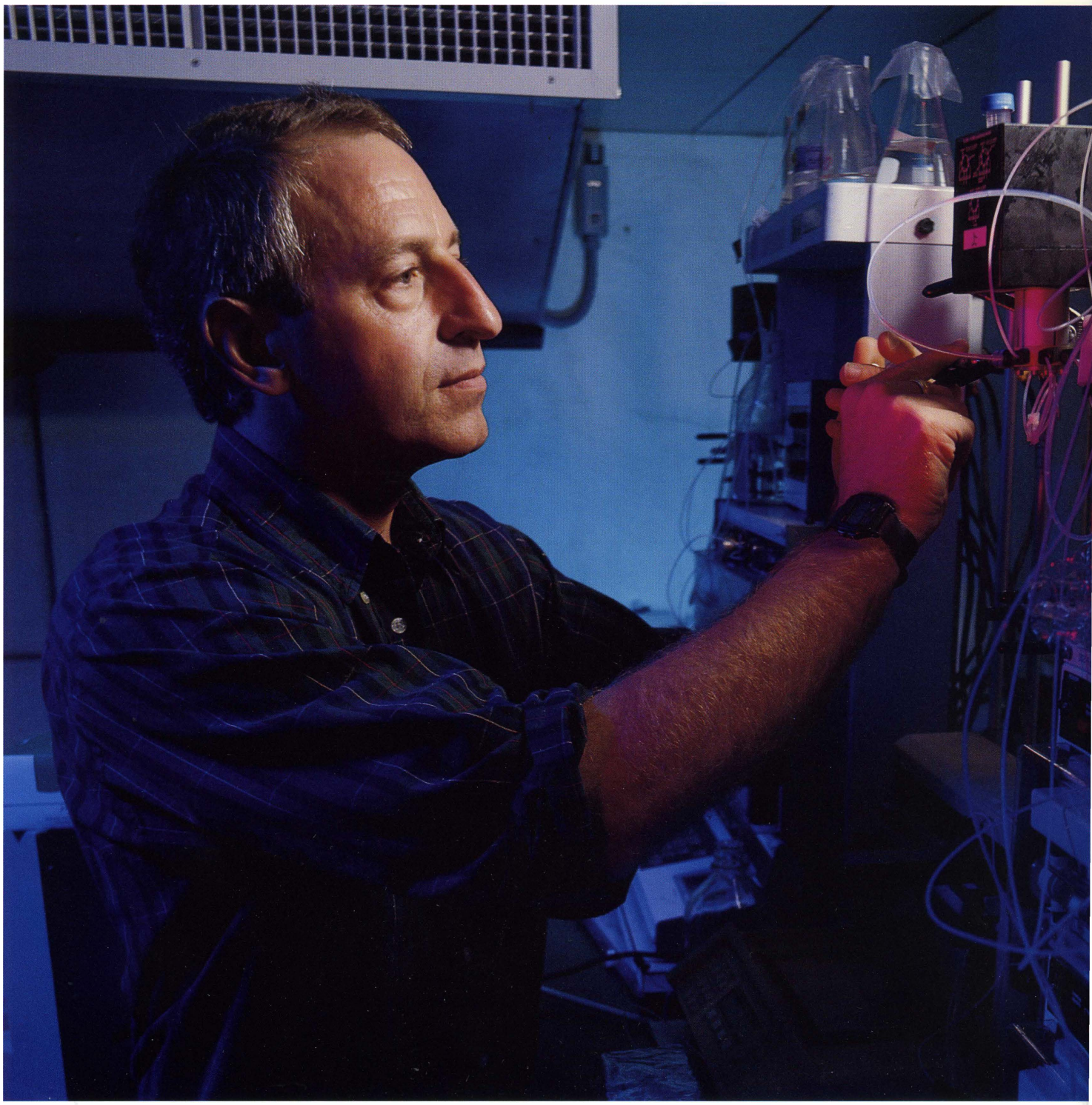
REPORTS FROM THREE LABORA

TORIES AT THE ROCKEFELLER UNIVERSITY

Since the institution's earliest days, investigators at The Rockefeller University have pushed the boundaries of science to expand our knowledge of the living world, making seminal contributions in such areas as neuroscience and immunology and pioneering new fields such as cell and molecular biology. Today, university researchers continue to seek answers to many basic questions, from how healthy cells turn cancerous to why certain cells form one tissue but not another.

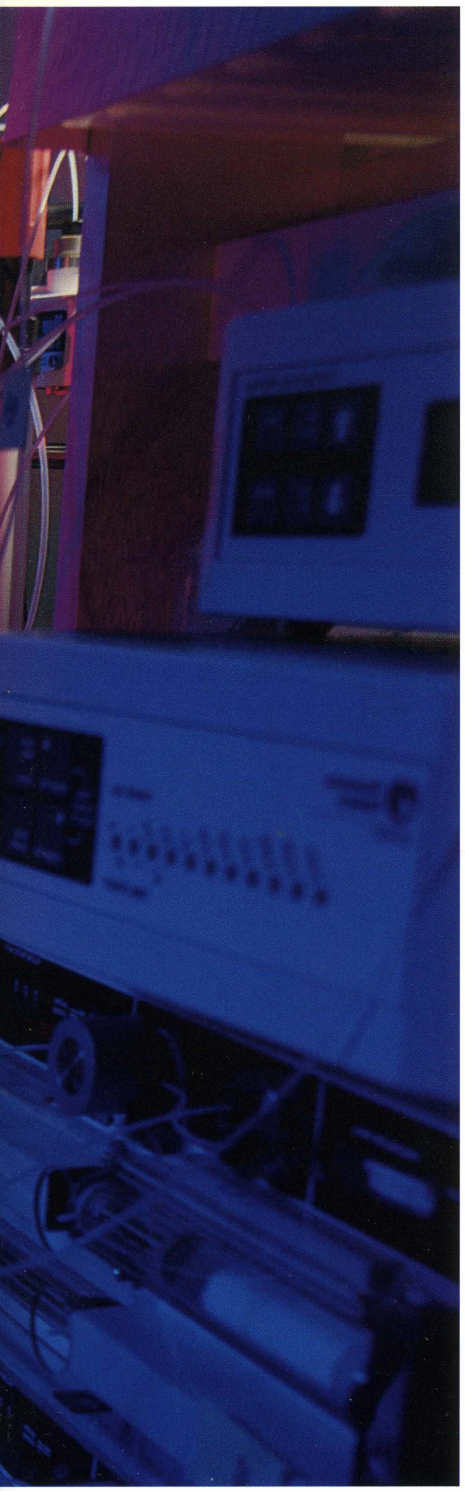
From its inaugural days as The Rockefeller Institute for Medical Research, the university has grown to emcompass more than 80 laboratories, headed by some of the world's leading scientists. Highlighted here is the work of three of them.

name: Michael O'Donnell



laboratory: DNA Replication

area of study: Mechanisms of DNA replication in bacteria and humans

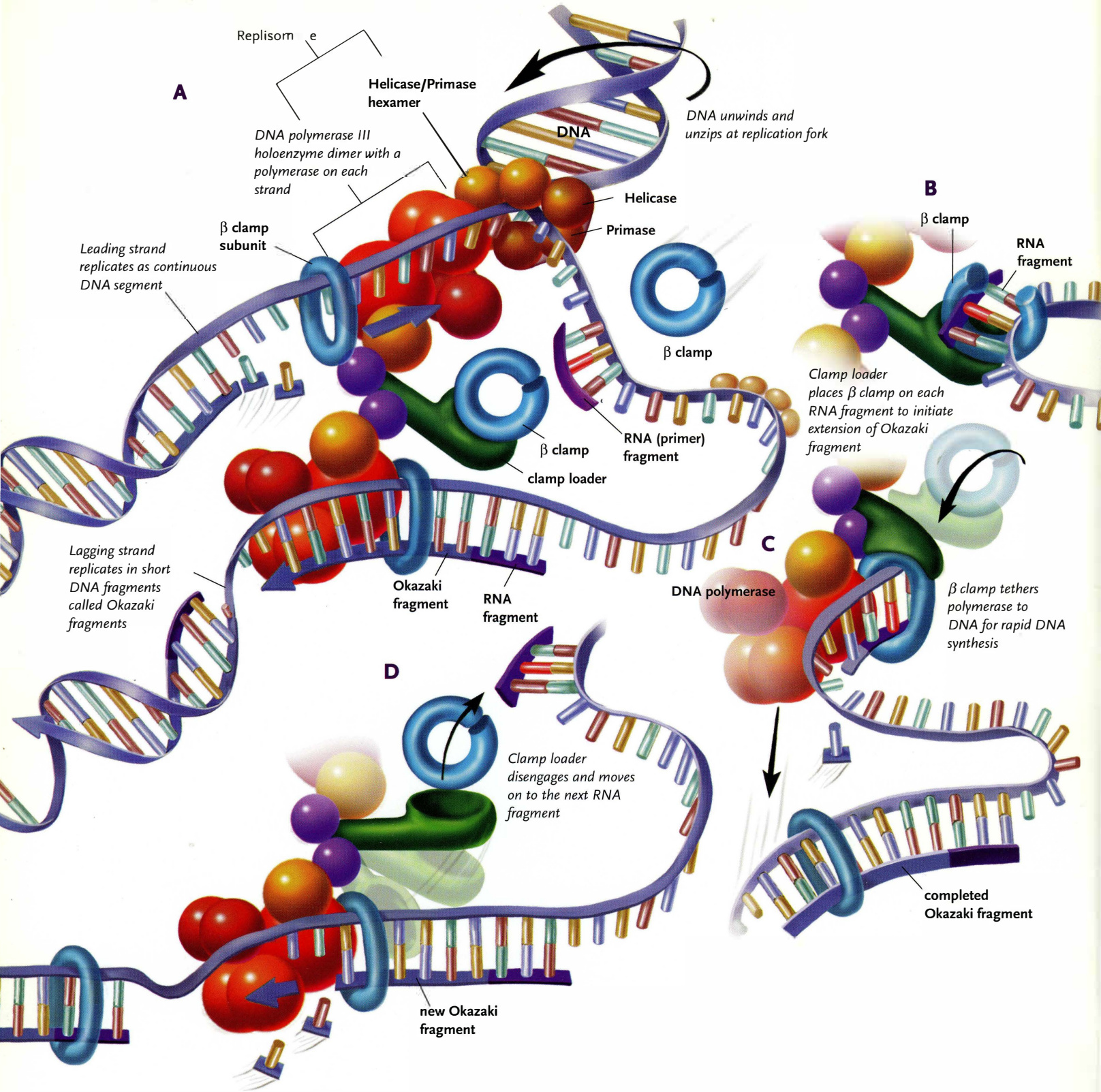


tinkering WITH THE GEARS IN THE machinery OF life

In the world of proteins, form follows function, as these molecules bend and twist into the varied shapes that enable them to perform some of the most crucial operations in the living cell. Professor Michael O'Donnell, head of the Laboratory of DNA Replication and an investigator with the Howard Hughes Medical Institute, studies the various proteins that direct perhaps the most important function in the cells of bacteria and humans—the copying of DNA during cell division—to gain new insights into the development of abnormal cells and to identify molecular targets for halting diseases such as cancer.

Before any cell divides, it must accurately copy its DNA and the genetic information carried within it, giving each new cell a complete set of instructions on how to live and function. "The elegantly simple structure of DNA initially suggested that the replication process should also be simple," says O'Donnell. "But over time, the study of this process has shown that nothing could be further from the truth." →

By Joseph Bonner



The structure of DNA—the so-called double helix that James Watson and Francis Crick teased out in 1953—is made up of four building blocks called nucleotides.

Represented by the letters A, T, G and C, the nucleotides pair—A with T, C with G—to form limitless combinations along each mirror-image strand of the double helix. The sequence of the letters in this alphabet determines the biological messages that differentiate all living organisms.

With the discovery of DNA's complementary structure, scientists suggested that for DNA to copy itself into new cells, the double helix might separate into individual strands, with each strand acting as a template for the creation of a new

ILLUSTRATION BY TERESE WINSLOW

complementary strand.

Little was known about the mechanisms behind DNA replication until the late 1950s, when Stanford University's Arthur Kornberg isolated an enzyme from the baterium *E. coli* that he called DNA polymerase. (For this discovery he shared the 1959 Nobel Prize in physiology or medicine.)

Polymerase was long thought to be the main enzyme responsible for replication in *E. coli*, but scientists now know that more than a dozen proteins, known collectively as the replisome, work in concert to rapidly and accurately duplicate bacterial chromosomes.

The replisome comprises many enzymes, including the primase, the helicase and DNA polymerase *(see figure)*. In bacteria, replication begins at a distinct point in the chromosome called the origin, where several proteins gather to unwind and unzip the DNA into separate strands.

The helicase encircles the replication fork, a Y-shaped region, or growing point, that translocates the parental DNA helix as it unwinds. The polymerase moves with the replication fork, but the DNA polymerase only replicates in one direction, and as a result, only one strand, called the leading strand, is copied in one continuous segment.

The other strand, called the lagging strand, is replicated in short pieces called Okazaki fragments. At the replication fork, the primase produces a short stretch of RNA to "prime" the synthesis of each Okazaki fragment. A protein assemblage called a clamp loader places the β subunit on each fragment. Dubbed a "sliding clamp," the β subunit completely encircles the fragment's double helix and freely slides along the DNA surface, tethering the polymerase machinery to the DNA for rapid synthesis.

The clamp loader quickly moves from fragment to fragment, placing a clamp on each primed segment. The polymerase comes in and works with the clamp to extend the fragment. The clamp loader disengages and moves on to the next primed fragment.

"This is where the polymerase really begins to look like a machine," says O'Donnell. "You can think of it as a ratchet, where it produces the strand by making a fragment, popping off the helix, going back, making a fragment, popping off, again and again."

A major part of O'Donnell's research involves determining what the gears in the DNA replication machinery look like. Since the early 1990s, when he was at Cornell University Medical College, O'Donnell has collaborated with RU Professor and HHMI Investigator John Kuriyan to solve the three-dimensional structures of many of the proteins that replicate DNA in bacteria. To date, the two scientists and their colleagues have determined the structures of six replication proteins, including the ring-shaped β subunit and subunits of the clamp loader.

O'Donnell is working on understanding how the bacteral replication machinery interfaces with other proteins that perform such processes as the repair of DNA. He also works on piecing together the replication process in humans. Because evolution conserved the molecular machinery in bacteria, yeast and people, many of the components are similar albeit more complex. "The polymerase in humans is very similar to the one in *E. coli*, but there are important differences," says O'Donnell. "Several of the human replication proteins have yet to be discovered. For example, it is still uncertain what enzyme performs the helicase function, although most scientists expect one will be found."

O'Donnell also expects to find differences in the events that take place at the origin. Yeast chromosomes, like those in humans, are linear rather than circular. Scientists have identified specific origins in yeast, but the human origin complex remains elusive.

As O'Donnell and other scientists uncover more details about human DNA replication, new weapons will emerge in the fight against diseases like cancer. Some may target specific proteins in the replication machinery, acting likea molecular monkey wrench in the gears.

But cancer is a tricky disease, cautions O'Donnell. "If you tried to inhibit the polymerase, for example, you would also inhibit normal cells that divide, like the cells in the lining of the gut or those that make blood cells," he says. "It is important in cancer therapy to find drugs that are much more sophisticated, that will target only the cancer cell." ■

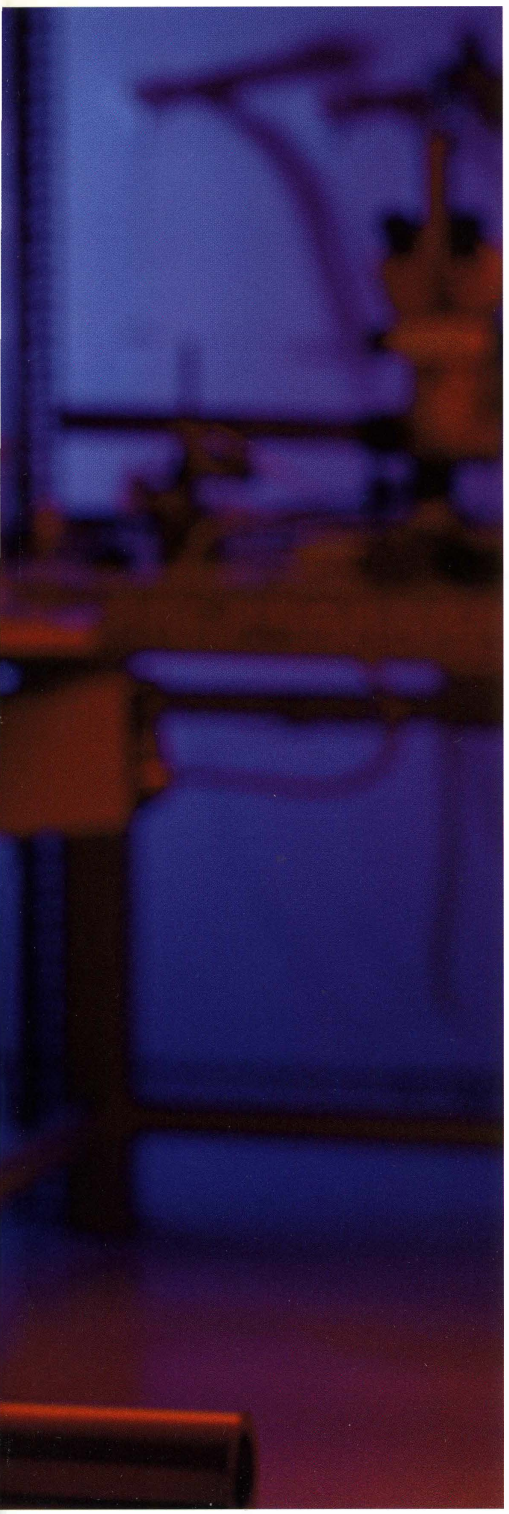
"The elegantly simple structure of DNA suggests that the replication process should also be simple. But nothing could be further from the truth."

—MICHAEL O'DONNELL

name: # Roderick MacKinnon

laboratory: Molecular Neurobiology and Biophysics

area of study: Structure and function of ion channels



poring over THE MYSTERIES OF ion channels

Professor Roderick MacKinnon set up his Laboratory of Molecular Neurobiology and Biophysics at Rockefeller with a specific goal in mind—to obtain a high-resolution three-dimensional structure of a potassium ion channel protein. He fully expected that his quest for the structure would be a long-term undertaking, requiring at least a few years. But less than two years after his arrival here, MacKinnon has already accomplished his mission, as evidenced by the image of the X-ray crystallographic structure of an intact potassium channel protein on the cover of the April 3, 1998 issue of *Science*.

MacKinnon, who was appointed an investigator with the Howard Hughes Medical Institute last year, modestly attributes his success to "some very lucky breaks and extremely hard work on the part of my entire laboratory."

Outside experts, however, are far more forthcoming with their praise for this achievement—"A remarkable accomplishment," proclaimed Clay Armstrong of the University of Pennsylvania, who →

By Neeraja Sankaran

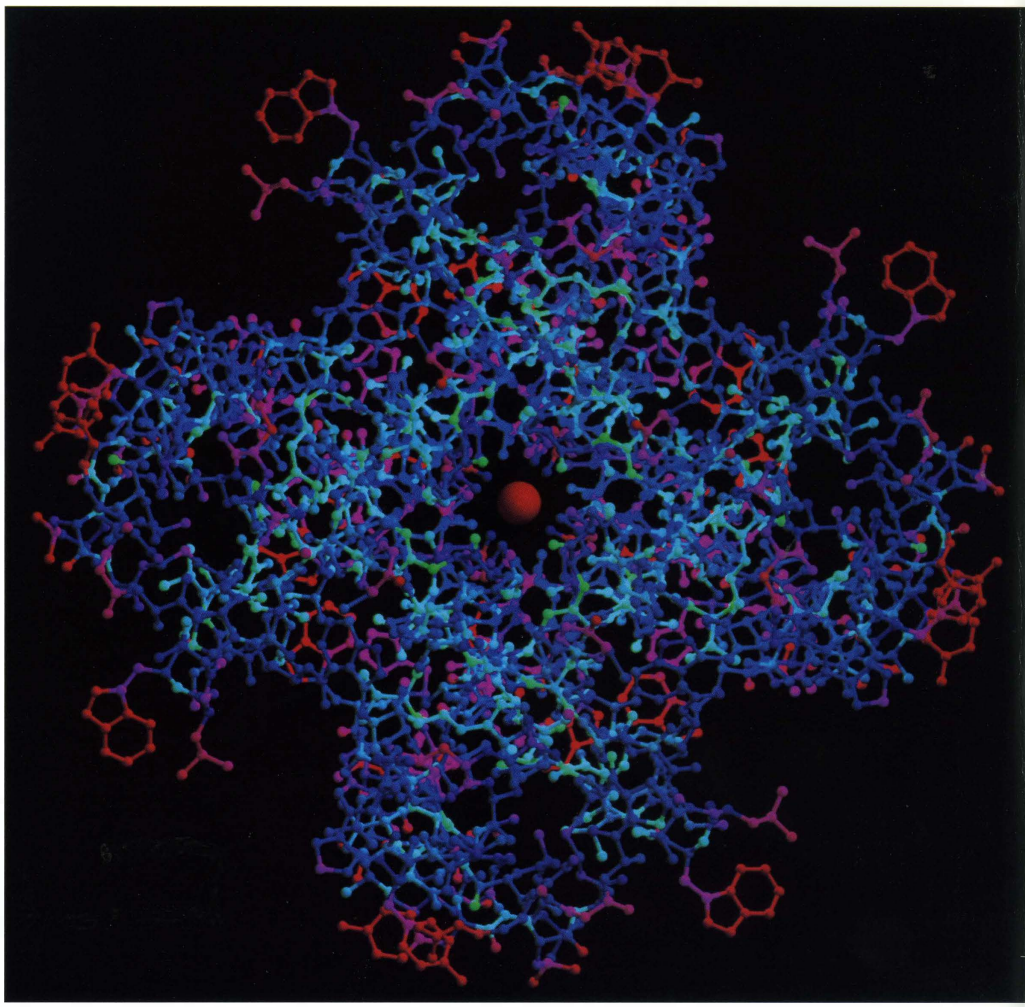
reviewed MacKinnon's paper in the same issue of *Science*. "It is a dream come true for biophysicists."

Ever since their discovery in the 1950s, ion channels have been the subject of intense interest to many scientists because of the key role they play in maintaining some of the body's most vital functions. Seated in the oily layers of the cell membrane that preserves the integrity of each cell, these proteins govern the flow of different ions such as potassium, sodium and calcium into and out of cells. The proper balance of these ions is essential for fundamental operations such as the transmission of nerve impulses throughout the body and brain.

Shaped like tiny doughnuts floating in oil, the ion channels perform the dual functions of gateway and gatekeeper. The holes in the doughnut form the gateway through which the ions flow. However, these holes, or pores, are endowed with special properties that enable different channel proteins to be selective as to which ions they allow passage. Like security guards who check ID before allowing entrance, these molecular gatekeepers only allow specific ions to pass through their pores.

What allows the ion channel proteins to work as they do? This is the question that has engaged MacKinnon for over a decade, beginning with his observation of an experiment monitoring the electrical activity of a potassium channel, when he was a medical student just contemplating a switch to basic science. He joined the laboratory of his undergraduate mentor Christopher Miller at Brandeis University, where, as a postdoc, he began to work on biophysical aspects of channel function. He chose to focus specifically on the protein selective for potassium ions, because it happened to be the target

RODERICK MACKINNON



Ball-and-stick model of a potassium channel from the bacterium Streptomyces lividans *looking down the pore, which has a potassium ion (red sphere) positioned inside it. The protein is composed of four identical subunits surrounding a central pore. The overall molecular architecture is expected to be preserved in all known potassium channels.*

of Miller's lab and also because, at the time, it was the least-studied ion channel, says MacKinnon.

Beginning with electrophysiological and biochemical approaches, MacKinnon studied the interaction of the potassium channel with a specific toxin derived from scorpion venom and deduced that the toxin inhibited the flow of ions by sitting directly on the pore of the channel. This led to questions about which specific regions of the channel proteins bind to ions and the toxins. Investigations along those lines became possible after 1987, when scientists cloned the genes for potassium channels in fruit flies.

In the wake of this development, MacKinnon turned to the field of molecular biology. By systematically mutating the channel gene at specific locations and observing the effects on the channel's ability to bind to the specific ions, he was able to pinpoint ion-binding capacity to a single region of the protein.

"All potassium channel proteins contain a little signature stretch of about 8 to 10 amino acids, which is specific for the ion," explains MacKinnon. "The sequence is conserved across evolution from bacteria to humans. In fact, some of these proteins have no features in common except for this signature.

It's as if biology chose only one way to select for potassium ions."

Meanwhile, genetic evidence suggested that a single potassium channel consisted of multiple protein subunits. MacKinnon determined that a functional channel has four identical subunits that join together like the staves of a barrel around the central hole. Each of the subunits contains the ion-specific signature sequence, "which forms a loop extending into the center of the hole to create a pore selective for potassium ions," he explains.

The pore-forming loops seemed to govern specific functions such as ion selectivity, but without a clear idea of the 3-D structure of the channel, MacKinnon says there was no way to test the truth of this idea. So he came to Rockefeller where he hoped to master crystallographic techniques and solve the structure of the potassium channel.

The very nature of the problem led MacKinnon and others to anticipate slow progress in their work. A basic requirement for protein crystallography is that scientists have sufficient quantities of the starting material or protein, in order to grow crystals for X-ray analysis. But although potassium channels are present in virtually all cells, they are usually produced in low quantities.

Fortunately, this hurdle was overcome when a group of scientists discovered and cloned a potassium channel from the bacterium *Streptomyces lividans*, using the very signature sequence that MacKinnon had discovered a few years earlier.

"The bacterial system cleared the path by giving us a way to express channel proteins in much larger quantities than had been possible," remarks MacKinnon.

Armed with sufficient amounts of channel protein, the scientists could turn their attention to tackling their other problem, namely growing good crystals. Historically, membrane-bound proteins like ion channels have been notoriously bad candidates for protein crystallography, because the detergents used to separate such proteins from lipids—a necessary step in making good protein crystals—often destroy the proteins as well.

"To give an idea of the labor involved in determining the ideal parameters for growing good crystals, we concocted a crystal screen with a total of 900 different conditions at two different temperatures and with 10 detergents," says MacKinnon. "Luckily we hit on the right combination relatively early."

The structure of the potassium channel *(see figure)* confirms MacKinnon's theory about the pore–loop structure determining ion selectivity and also offers an explanation as to why the channel prefers potassium over sodium ions. "Basically the potassium selectivity filter is a cylindrical cavity lined by slightly charged oxygen atoms which, in turn, are held in place by structural elements in the portion of the channel protein that traverses the membrane," explains MacKinnon.

This structure confers a certain rigidity to the cylinder and determines the diameter of the oxygen-lined pore. A charged potassium ion fits neatly into the narrowest configuration of this cylinder. Sodium ions, which are smaller, do not fit properly in the cavity. "The oxygens in the pore cannot get as close to the sodium as they do to potassium. Consequently sodium ions are better stabilized by oxygens in water molecules and do not enter the pore," MacKinnon adds.

The channel structure also suggests an explanation for the seeming paradox of a channel protein's ability to reconcile high ion selectivity with high throughput, or the rapid flow of ions through the channel.

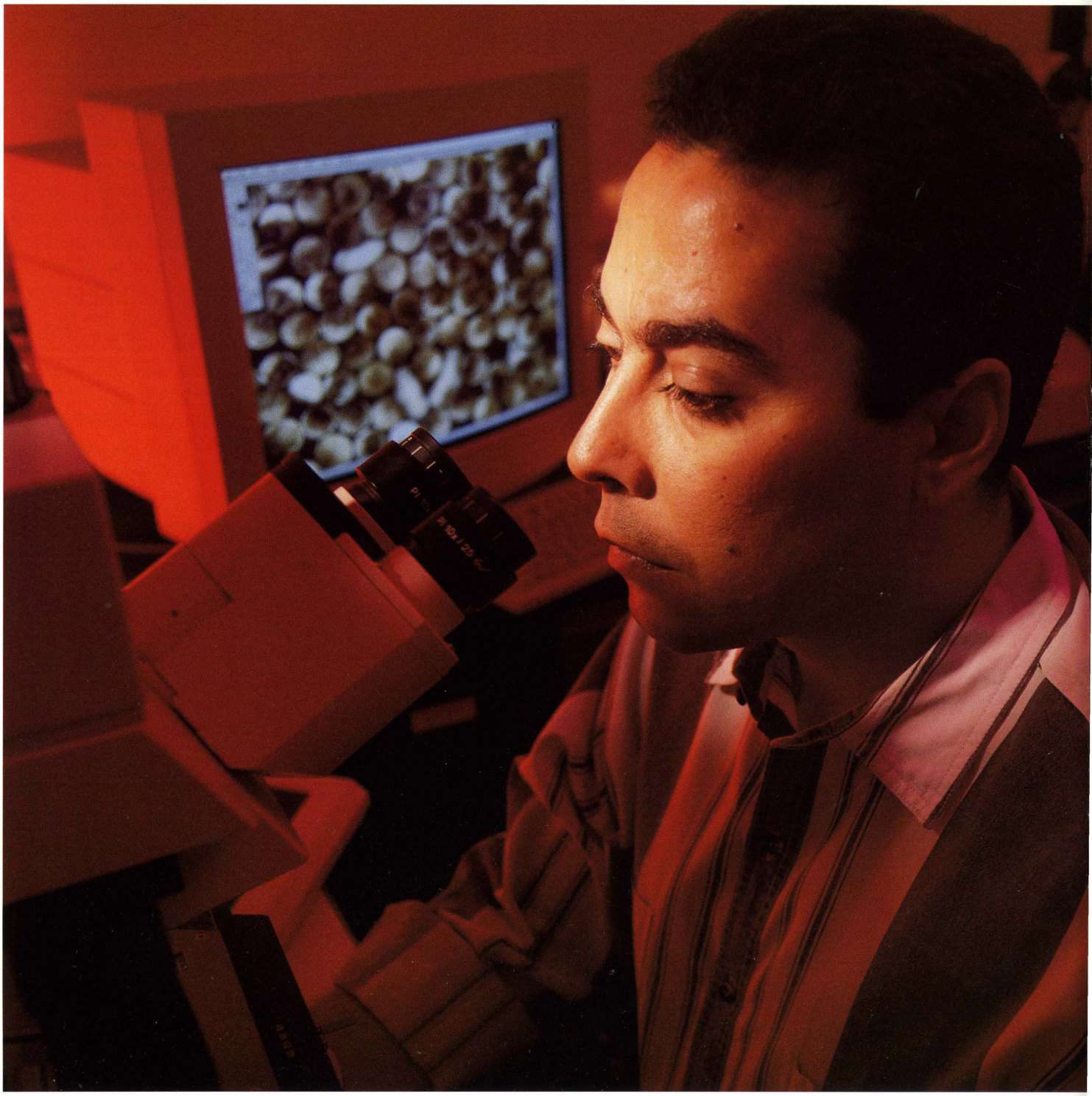
"The two properties seem incompatible because high selectivity implies that the interaction between the channel and ion must be a strong one, which would not be conducive to letting the ions escape past the protein into the cell," MacKinnon says.

The channel structure also suggests an explanation for the seeming paradox of a channel protein's ability to reconcile high ion selectivity with high throughput, or the rapid flow of ions through the channel.

But it appears as though the selectivity pore resolves this paradox by placing two ions near each other where they experience mutual repulsion by virtue of their electric charge. "This suggests that the strong attractive forces between the potassium ions and channel are counteracted locally by the repulsive forces between two positively charged potassium ions, which allows each ion to fall through at a rapid rate," he adds.

Having solved the elusive structure of the channel, MacKinnon plans to obtain information about channel structure at still higher resolutions and probe other aspects of their function. Ultimately, he says, "My aim is to figure out how channel proteins function as the electrical impulse generators in biology." ■

name: Ali Hemmati-Brivanlou



laboratory: Molecular Vertebrate Embryology

area of study: formation and patterning of the vertebrate embryo

challenging THE CONCEPT OF EMBRYONIC cell fate determination

What causes cells taken from a particular region of an early embryo to become nerve cells in some situations and skin cells in others? This is a question that has puzzled embryologists throughout most of the 20th century. The answer, arrived at by Associate Professor Ali Hemmati-Brivanlou, head of the Laboratory of Molecular Vertebrate Embryology at The Rockefeller University, holds the potential to yield new methods to inhibit tumor formation, treat neurodegenerative diseases and improve plastic surgery.

As early as the 19th century, the search was on for the answers to this intriguing puzzle. Even without the aid of the powerful imaging technology currently available, embryologists were able to draw astonishingly accurate fate maps, detailing embryonic development and cell migration. One such fate map showed that cells from the dorsal, or back side, of the outermost →

By Urmila Ranadive

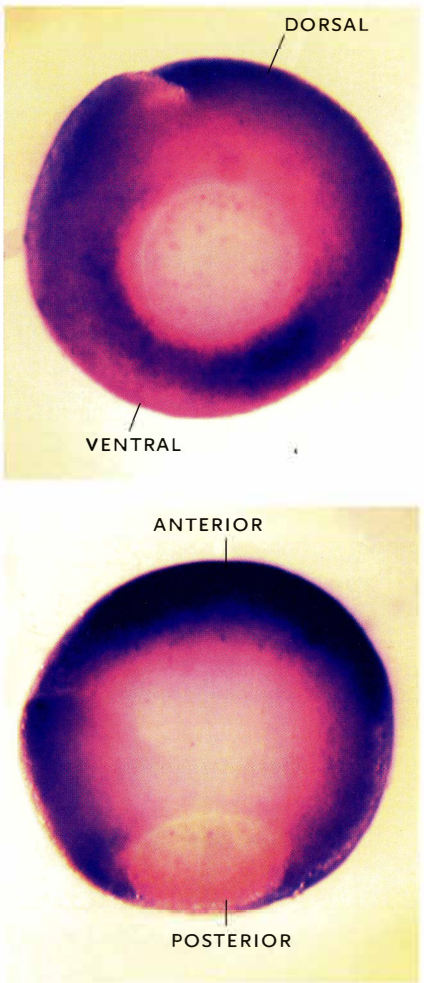
layer of an early embryo were destined to become the nervous system, while cells from the ventral, or front side, would differentiate into the epidermis, or skin.

In 1924, German embryologists Hans Spemann and Hilda Mangold used these potentially neural and epidermal cells to perform experiments that would garner Spemann a Nobel Prize. Spemann and Mangold took a small number of cells from the dorsal-equatorial region of an early embryo and implanted them into the ventral side of a different embryo. What they got was an embryo with two heads and two main body axes, including two complete nervous systems. Because the second nervous system derived from cells that would normally have become epidermis, the scientists concluded the transplanted dorsal cells were sending signals telling the host ventral cells to be neural. The researchers called the transplanted signaling region an organizer. Embryologists spent the next 70 years trying unsuccessfully to find the molecule that told epidermal cells to become neural.

Following on a project that he started during his postdoctoral studies with Douglas Melton at Harvard University, Hemmati-Brivanlou finally solved the problem at Rockefeller. He realized that Spemann and Mangold had worked under an incorrect assumption. Cells, even from the ventral side of an embryo, do not become epidermal by default. Rather, without any external signal, cells become neural.

A decade ago three groups independently observed an interesting phenomenon that proved key to Hemmati-Brivanlou's research. They found that if cells of an early embryo are removed and grown in such a way that they do not contact each other, they become neural tissue.

In considering these results,



Eight-hour old frog embryos stained for the expression of the neural inhibitor BMP4 (blue). At this time, as a first step toward the establishment of neuronal progenitors, BMP4 is removed from the dorsal side of the embryo, which is the future side of the central nervous system.

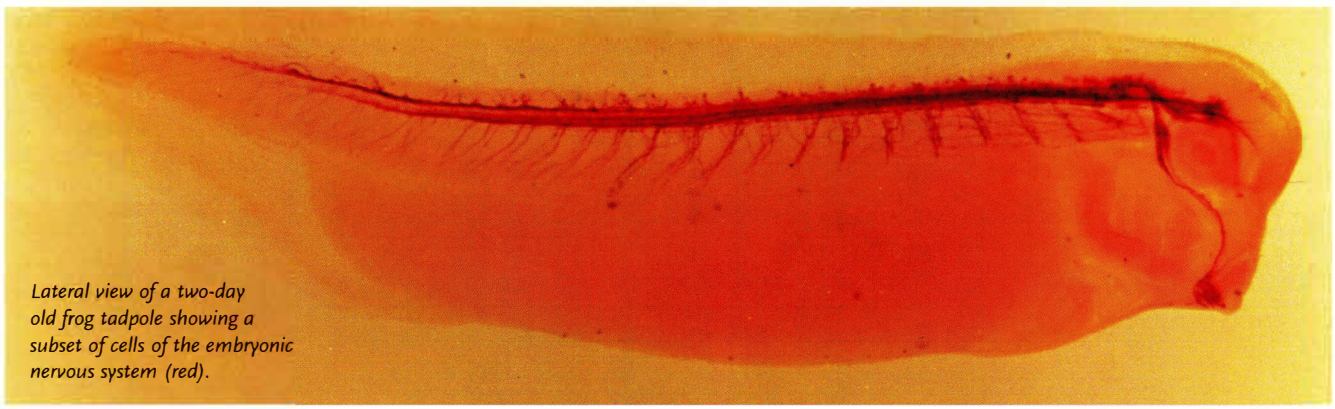
Hemmati-Brivanlou and then-RU Research Associate Paul Wilson recognized that the consequence of separating embryonic cells was the same as that of blocking communication within an intact group of such cells. "When the cells were dissociated or couldn't communicate they became neural," says Hemmati-Brivanlou. "But in the embryo, where the cells contact each other and communicate, they can become epidermal. For the first time we realized that to get epidermal cells we should look for a negative signal, one that told the cells not to become neural."

Hemmati-Brivanlou works with the cells of the frog *Xenopus laevis* to track embryonic development. Its 1-mm diameter egg is one of the biggest cells in the world, making it an ideal candidate for study. Using *Xenopus* cells, Hemmati-Brivanlou identified the bone morphogenetic protein, BMP4, as a signal inhibiting neural development. This molecule, for which Hemmati-Brivanlou received a patent, delivers two messages simultaneously: First, it tells the cells not to become nerve cells, and second, it tells them to become skin cells. Since the discovery of BMP4, two related neural inhibitors, BMP2 and BMP7, have been identified by Atsushi Suzuki, a postdoctoral fellow in the Hemmati-Brivanlou laboratory.

But a further riddle appeared in the form of an embryological catch-22. In the embryo, ectodermal cells sometimes become neural—even if they remain intact and are able to transmit the BMP4 signal to one another. So Hemmati-Brivanlou reasoned that to get neural cells, you must inhibit the neural inhibitor, BMP4.

So far, three inhibitors of neural inhibitors have been identified. These are called neural inducers, and all three are secreted factors expressed primarily in Spemann's organizer. The Hemmati-Brivanlou lab discovered and cloned one of the neural inducers, known as follistatin, for which it also received a patent. The other two neural inducers are noggin and chordin. Noggin was discovered and cloned by William Smith, together with Richard Harland, Hemmati-Brivanlou's doctoral advisor, at the University of California at Berkeley. Chordin was discovered and cloned by Yoshiki Sasai and Eddy De Robertis of the University of California at Los Angeles.

Noggin and chordin have been found to bind directly to BMP4, preventing it from getting to its recep-



ALI HEMMATI-BRIVANLOU

Lateral view of a two-day old frog tadpole showing a subset of cells of the embryonic nervous system (red).

tor on ectodermal cells. The finding that noggin and chordin generate neural fate by eliminating the activity of BMP4 provided independent evidence for the claim originally forwarded by Hemmati-Brivanlou. Follistatin's mode of action is still under investigation.

Identifying these factors is only the beginning. Now the Hemmati-Brivanlou lab must determine the window of time and space in which the ectodermal cells can respond to these factors. BMP4, for instance, induces ectodermal cells to become skin cells in the early embryo. But in adult cells, that same BMP4 is involved in forming bone. The time frame during which cells will become skin in response to BMP4 has not yet been determined.

Defining the window of opportunity for neural inducers is even more difficult. "How can one put a time limit on a default fate, one that would occur in the absence of all other molecular factors?" Hemmati-Brivanlou asks.

Epidermal or neural induction can occur on many levels. Factors from outside or inside the cell may influence transcription—the synthesis of RNA out of DNA—and translation—the conversion of an RNA sequence into proteins. The Hemmati-Brivanlou laboratory aims to understand what happens downstream of BMP4's activity. How does BMP4 tell a cell not to become neural? How does it tell the cell to instead become epidermal?

Scientists know that BMP4 binds to a receptor on the surface of a cell. The binding acts as a trigger for a signal that gets passed from the receptor to the nucleus. "Like runners in a relay race," explains Hemmati-Brivanlou, "signal transducing proteins called SMADs pass the signal baton along until it reaches the nucleus."

SMADs can also work as signal blockers. So even if BMP4 binds to the surface of a cell, certain SMADs can act as internal inhibitors, reversing the fate of the cell back to neural.

But if the epidermal inducing signal does get transduced all the way to the nucleus, it affects the transcription of DNA. Inside the nucleus, the signal turns on the genes involved in skin formation and turns neural genes off.

Postdoctoral Fellow Atsushi Suzuki has identified *msx1* as the first gene to get turned on when the BMP4 signal to make epidermis arrives at the nucleus. Another postdoctoral fellow in Hemmati-Brivanlou's lab, Daniel Weinstein, has identified and cloned a gene, called *eIF-4AIII*, which regulates translation of BMP4's signal. The *eIF-4AIII* gene had never been cloned before in an animal and is in the works as another patent for the Hemmati-Brivanlou lab.

In addition to adopting a given fate, embryonic cells also have the continuous task of maintaining their identity. Maintenance can be achieved through positive feedback loops or by extracellular signals, provided by neighboring cells.

Though their focus is basic research, the Hemmati-Brivanlou laboratory's findings have many potential applications. Their patent on BMP4 is essentially a patent for epidermal induction. The ability to create skin promises to be beneficial for plastic surgery, burn victims and wound healing.

The other side of the epidermal induction coin is neural inhibition. BMP4 is part of the TGFβ, or transforming growth factor-beta family, and acts as a strong neural inhibitor, explains Hemmati-Brivanlou, "so brain tumors or spinal tumors are obvious targets for BMP4 treatments." Some of the SMADs are neural inhibitors that work downstream of BMP4 and could be used to prevent the growth of tumors of neural origin.

Finally, neural inducers like follistatin, noggin and chordin represent promising treatments for neurodegenerative diseases like Alzheimer's and Parkinson's, in which the regeneration of dying neural tissue would be beneficial.

For now, Hemmati-Brivanlou says he will leave the applications of his findings to other scientists and clinicians, while he continues to focus on basic research. ■

CIRCADIAN RHYTHMS

Keeping Time with Biology

Biological clocks are ubiquitous in nature, influencing everything from a tree's tendency to shed its leaves in autumn to a bird's decision to head south for the winter. A master clock ticks within each of us as well—a biological timepiece that guides us through the daily, 24-hour circadian cycle of sleep and wakefulness. It is this clock that leaves us fighting jet lag when we travel across time zones, then forces us to sleep no matter how much work we have left to finish. The biological clock also maintains an indirect control over other cyclic processes in the body, regulating, among other things, the daily waxing and waning of body temperatures and hormone levels. While these daily fluctuations help us make the transition from sleep to wake, they also make it more likely for events such as heart attacks or asthma attacks to occur at certain times of the day.

Professor Michael Young, head of the Laboratory of Genetics at The Rockefeller University, says he has been fascinated with nature's timekeeper since he was a junior →

By Urmila Ranadive

high school student. He earned his B.A. in biology in 1971 and in 1975 his Ph.D. in genetics at the University of Texas at Austin, where he noticed new work on the genetics of circadian rhythmicity. Several years later, after postdoctoral work in biochemistry at Stanford Medical School, he moved to RU and turned once again to this longtime interest, setting out to understand the molecular underpinnings of the biological clock.

By 1984, Young had cloned a gene named *period* (*per*) of the fruit fly *Drosophila* that appeared to affect circadian rhythms. A homolog, or descendant copy, of this gene has recently been isolated in humans by research teams at the University of Tokyo's Institute of Medical Science and Baylor College of Medicine.

"Based on this discovery, I'd say it's likely that the genes, proteins and mechanisms emerging from *Drosophila* are telling us how our own pacemakers work," says Young.

But in 1984, Young was still struggling to understand why *per* was important for rhythms. Young knew that the gene was needed for circadian rhythms because flies that expressed a *per* mutation in their brain's pacemaker cells had disrupted cycles of activity and rest. Colleagues at Brandeis University had shown that the mutations also blocked the cyclic synthesis of the *per*-coded PER protein. But it was unclear how, in normal flies, the gene made a product that was produced in a cyclical, rather than constant pattern.

In solving the puzzle, Young recalled a phenomenon involving biological oscillations of a very different kind. Two animal populations, lynxes and their primary prey, snowshoe hares, exist in highly synchronized, 10-year population cycles. For example, suppose the cycle begins when the lynx population has hit bottom: With few predators, the number of hares rises, supporting an ever larger lynx population to prey on the hares. As food supplies plummet, however, fewer lynx survive, and once again the hare population rebounds.

Although not an animal behaviorist, Young observed the interdependent interactions of the lynx and hare populations and saw a model for his molecular clock. Perhaps interdependence was the key: Was there a gene that influenced *per*, causing its levels to rhythmically rise and fall?

Looking for a second gene to work hand-in-hand with *per* involved screening tens of thousands of *Drosophila* for individuals with aberrant sleep/wake cycles. Adult fruit flies normally hatch from their pupal shells at dawn, so Young was on the lookout for flies emerging at any other time of day—those that probably had a mutation in a gene involved in the circadian rhythm. This process led to the identification, and eventually the cloning, of a gene called *timeless* (*tim*). Young's laboratory has since uncovered *tim*'s pivotal role in the molecular clock. In fact, the *per* and *tim* partnership is so close that their daily cycling cannot occur without their two proteins, PER and TIM, working in concert.

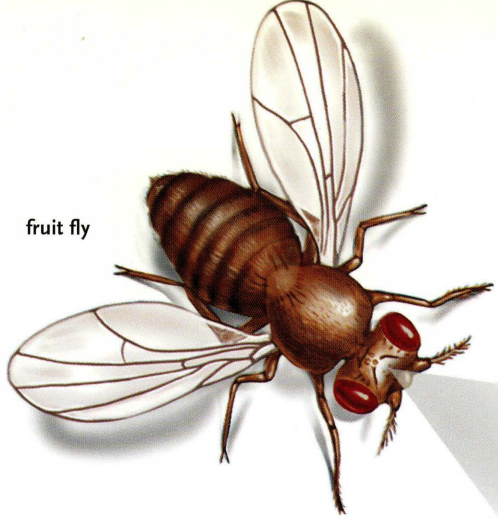
But when *tim* was first identified, Young had a lot of work ahead of him. He had yet to determine whether *tim* worked with *per*, and if so, how. He went into this research armed with a few key observations. First, the TIM protein was rhythmic. That is, TIM followed the same cycle as PER protein. Second, cycling of the PER protein did not occur in flies with a mutation of the *tim* gene. The key to the puzzle lay in a significant observation made by Leslie Vosshall, a doctoral student in Young's laboratory.

"Leslie's work was our first indication that there really was a close partnership between these two proteins, TIM and PER," says Young.

...the *per* and *tim* partnership is so close that their daily cycling cannot occur without their two proteins, PER and TIM, working in concert.

Vosshall noticed that although *tim* mutants—those that did not have the *tim* gene—made *per* RNA, she could not detect any PER protein. However, when she fused the PER protein to a very stable enzyme, she detected the fused PER protein. But it was produced in a constant pattern, without the daily fluctuations normally found in *Drosophila*. More intriguing was that in *tim* mutants, the fused PER protein was produced in the cytoplasm, but never appeared in the nucleus. In normal *Drosophila*, the PER protein cycles in and out of the nucleus.

Based on these and other observations, Young's laboratory worked out PER and TIM's interdependent relationship as follows: Normally, PER and TIM proteins are at their greatest concentration in the nucleus of the cell shortly before dawn. After hitting their peak concentrations, the proteins begin to disintegrate and leave the nucleus. When the cell perceives the declining concentration of the proteins, it signals the *per* and *tim* genes to begin making RNA that will produce more proteins. Although the RNA accumu-



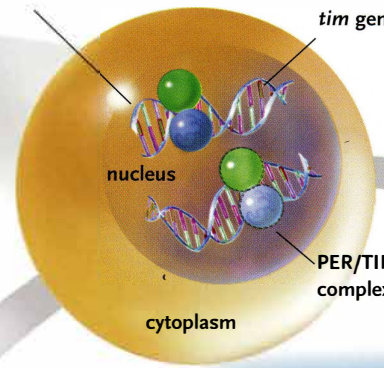
fruit fly

DAWN

- *No transcription of* per *or* tim *genes into RNA.*
- *Many PER/TIM protein complexes are in nucleus. Disintegration begins.*

per gene

tim gene

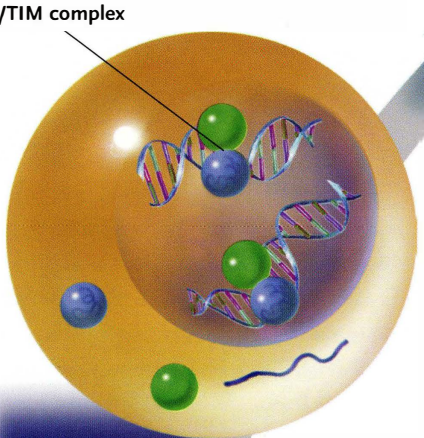


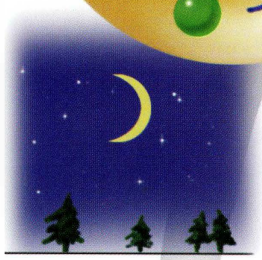
Cell from circadian pacemaker region of fly brain

NOON

- *PER/TIM protein complexes disappear from nucleus.*
- per *and* tim *genes begin transcribing RNA but no translation occurs.*

PER/TIM complex

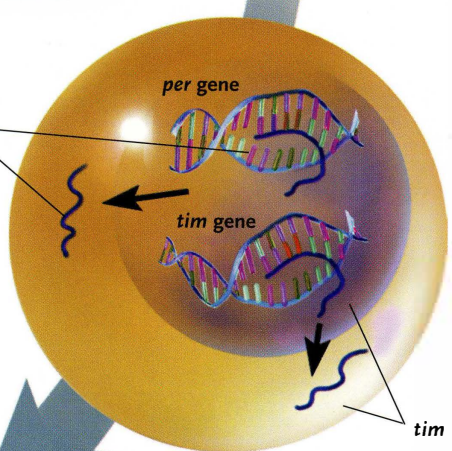




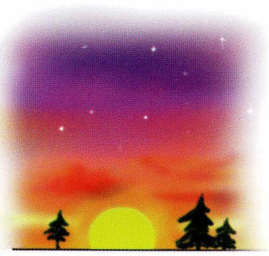
MIDNIGHT

- *As PER/TIM complexes accumulate in nucleus, cell stops producing* per *and* tim *RNA and blocks further accumulation of PER/TIM complexes.*

per RNA



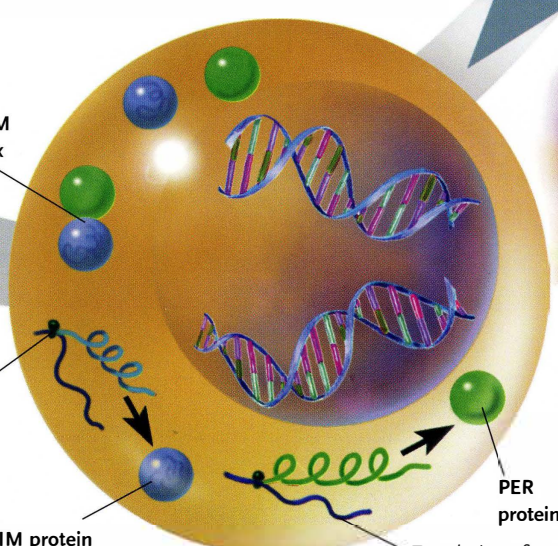
tim RNA



DUSK

- *Peak levels of* per *and* tim *RNA in cytoplasm produce high levels of PER and TIM proteins.*
- *Initial binding of PER and TIM proteins begins.*

PER/TIM complex



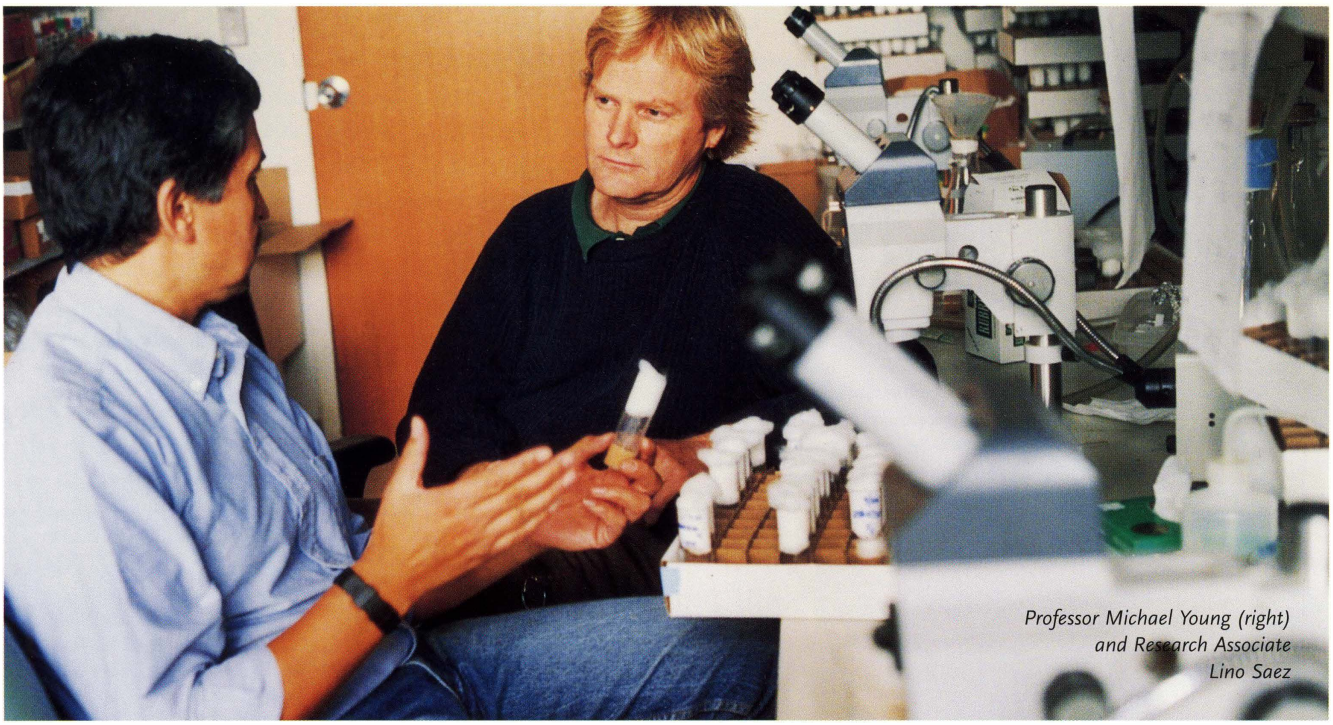
Translation of tim *RNA into TIM protein*

TIM protein

PER protein

Translation of per *RNA into PER protein*

ILLUSTRATION BY TERESE WINSLOW

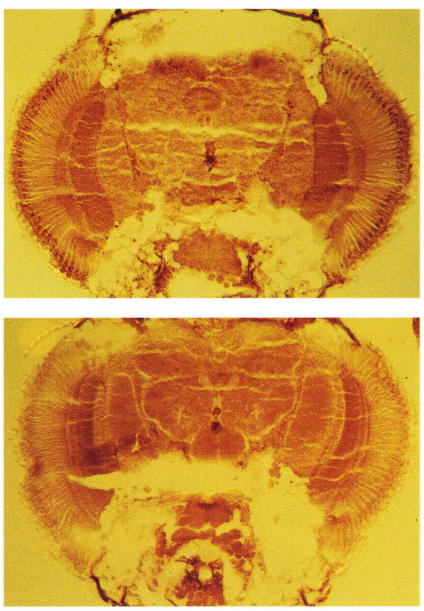


Professor Michael Young (right) and Research Associate Lino Saez

lates throughout the day, very little nuclear protein amasses during this time. Instead, PER and TIM abruptly appear in the nucleus shortly after dusk, long after RNA levels hit their peak. As the proteins build up inside the nucleus, they trigger a negative feedback loop telling the *per* and *tim* genes to stop making RNA. At dawn, the cycle begins anew with the disintegration of the accumulated proteins.

The pace of the clock is set by the time lag between the transcription of the genes' DNA to RNA and the subsequent nuclear appearance of PER and TIM. Young's laboratory has identified the key contributors to this lag. First, the researchers discovered that the PER and TIM proteins must join together in the cytoplasm in order to enter the cell's nucleus. Their second discovery hinged on two significant factors: First, Vosshall observed that the PER protein is rapidly broken down in the cytoplasm when it is not paired with TIM, and second, they identified a new gene, named *doubletime* (*dbt*), which regulates this process (as reported in *Cell*, July 10, 1998). The result is that PER protein levels are actively held down, even as RNA levels are rising.

MICHAEL YOUNG



The Drosophila *clock protein TIMELESS accumulates to high levels in nuclei of the eyes and certain cells of the brain at night (top), but is rapidly destroyed when the fly is briefly exposed to light (bottom).*

Add to this another complicating factor. The cytoplasm of the cell contains factors that also compete for the attention of PER. Newly made PER proteins have cytoplasmic localization domains (CLDs) that inhibit movement to the nucleus by holding them in the cytoplasm. It just so happens that PER's CLD is in the same area as its TIM binding site.

During the day, TIM is not present in high enough concentrations to successfully compete for PER's CLDs. This is because TIM is a light-sensitive protein. So while PER's CLDs hold it in the cytoplasm, allowing the unpaired proteins to be actively destroyed, TIM is being removed by light.

The scenario changes at dusk, when there is no light to destroy the TIM protein as it is being produced by its RNA. At this point, TIM accumulates in large enough quantity to capture PER before it is snatched by the CLDs and degraded in the cytoplasm. Thus bound, and only after this union, can the PER/TIM complex enter the nucleus.

"Requiring that the PER and TIM proteins be paired before entering

the nucleus is a good way of ensuring a lag between the time that the *tim* and *per* genes are transcribed to the time that the TIM and PER proteins can reenter the nucleus and suppress the transcription of their genes," explains Young. "So we find several regulatory steps that collaborate to give the *per* and *tim* genes a long period during which they are not inhibited by their protein products."

But it is light-sensitive TIM that determines the clock's period in a natural environment. TIM needs light—though not a full 12 hours—to maintain a 24-hour clock. The interval of light, which can reset the clock no matter where the body is in the circadian cycle, need only be long enough to completely degrade TIM and allow a sufficient buildup of RNA to restart protein production.

How do pacemaker cells detect light? In the fly, Young thinks, pacemaker cells in the brain have their own photoreceptors that detect light and influence the circadian cycle. The photoreceptor system is more complicated in mice and humans, who have more complex brain structures. Pacemaker cells in these two animals are located in the suprachiasmic nucleus (SCN), a conglomeration of neurons in the brain's hypothalamus. The sleep/wake cycle is probably controlled by PER and TIM cycling in these pacemaker cells, which are never directly exposed to light. But the proteins have also been found in the mammalian retina, which has its own clock. So communication between clocks in the eyes and brain may keep behavior in register with the environment.

A host of unanswered questions remain about how fluctuations of the PER and TIM proteins in pacemaker cells affect an animal's sleep/wake cycle. Young reasons that the cycling

MELATONIN MIRACLE

Books and store windows proclaim the melatonin miracle, the magic bullet for sleepless nights and jet lag. Does this natural wonder drug have a dark side? Assistant Professor **Philip Cole**, head of the Laboratory of Bioorganic Chemistry, wants to find out.

Cole first encountered melatonin during a clinical residency at Brigham and Women's Hospital in Boston. "At the time, a lot was being written about melatonin and a lot was being sold," recalls Cole. "I was surprised to find very little in the scientific literature about its function in people."

Scientists received a new tool to study melatonin nearly three years ago. A group of researchers at the U.S. National Institutes of Health, led by David Klein, cloned a gene that produces an enzyme that converts the neurotransmitter serotonin to N-acetylserotonin, the direct precursor to melatonin.

"This enzyme rises and falls with the circadian rhythm," says Cole. "In the evening, its level in the body increases to 100 times its daytime level, then decreases at daybreak, correlating precisely with melatonin production."

Melatonin production begins in the pineal gland, which is located near the third ventricle of the brain. Often called the "circadian pacemaker," the pineal gland weighs less than an ounce and contains small amounts of serotonin N-acetyltransferase, which makes it very difficult to work with. Indeed, although scientists have known about the enzyme since the early 1960s and Klein showed that the enzyme regulates melatonin's circadian cycle in 1970, nearly 25 years passed before researchers identified and cloned the gene.

With the cloning of the gene, Cole suspected that it would be possible to produce large quantities of the enzyme and design specific inhibitors to block melatonin production. "We could use these inhibitors to actually probe what melatonin does in animals and people," he says.

Unlike most parts of the brain, which are protected by the blood-brain barrier, the pineal gland is free of this barrier, making compounds more accessible and providing a better chance to penetrate and block the enzyme.

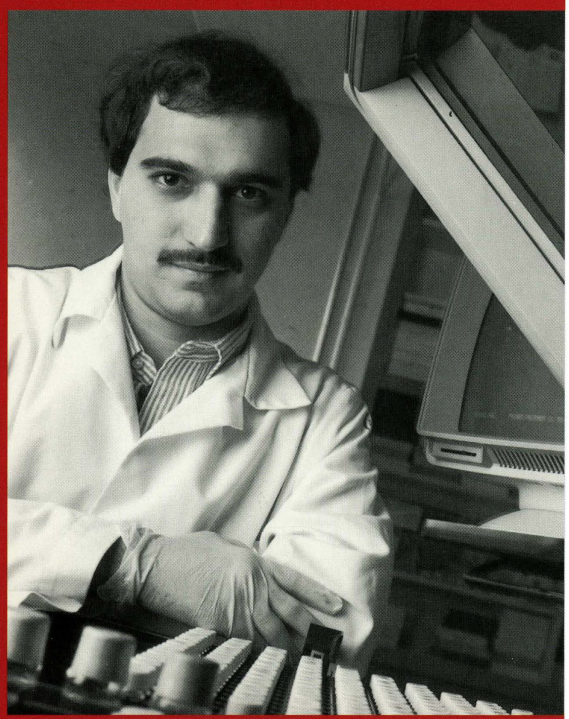
Cole and Postdoctoral Associate Ehab Khalil recently synthesized the first specific and potent inhibitor of serotonin N-acetyltransferase, reporting this work in the June 24, 1998 issue of the *Journal of the American Chemical Society*.

"There's reason to believe that compounds that block serotonin N-acetyltransferase may improve our understanding of circadian rhythms and might be therapeutic for a variety of conditions, such as depression or sleep disorders."—*Joseph Bonner*

CIRCADIAN CALL TO ARMS

The sleep/wake cycle is not the only circadian rhythm in the body. Levels of hormones, such as those that arise due to stress, follow a circadian rhythm as well, and may in turn influence the body's immune system, according to **Firdaus Dhabhar**, a research associate in the Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology.

Dhabhar studied the effects of stress on the immune system of rats for his doctoral work at Rockefeller. When subjected to mild stress, the rats produced a hormone called corticosterone, a molecular cousin of the hormone cortisol found in humans. Dhabhar found that as corticosterone levels increased, levels of immune cells in the blood decreased. Dhabhar showed that this immune cell redeployment is accompanied by a large increase in a skin immune response when the skin is immunologically challenged following mild stress.



Knowing that corticosterone levels also rise naturally at the beginning of an animal's active period, Dhabhar thought that a similar immune cell redistribution might occur. Dhabhar monitored the levels of immune cells in unstressed rats and found that these cells indeed traffic to other parts of the body in a circadian cycle.

Dhabhar hypothesizes that the rise in corticosterone and the accompanying redistribution of leukocytes act like a physiological wake-up call for the body. "The circadian corticosterone rhythm may increase immune surveillance just as it increases energy mobilization and prepares the body for the eventualities of the day," says Dhabhar. "We think that the organs where the leukocytes end up serve as potential battle stations if the body's defenses are breached."

Dhabhar suggests that these findings may have clinical applications, from standardizing the time of day when doctors administer skin tests to enhancing the accuracy of a blood test.

"Paying attention to both the circadian rhythm and the stress state of a patient could be important," says Dhabhar. "In a very simple way one could use this information to enhance the accuracy and validity of measures, such as tuberculin or allergy skin tests or diagnostic blood tests, or harness it to carefully time surgical or medical intervention, to maximize recovery and healing."—*Joseph Bonner*

of the *per* and *tim* genes will be echoed in the cycling of other genes in the cell. During the last few years, scientists have paid special attention to clock control genes (CCGs). The activity of these genes oscillates with the circadian rhythm, establishing cycles in the production of other hormones, transcription factors and intracellular components. The CCGs are influenced by, but do not themselves affect, the rhythm of the clock.

An example of a CCG is a gene that produces N-acetyl transferase, an enzyme required for melatonin synthesis. Because the transcription of this gene cycles up and down in concert with *per* and *tim* cycles in the pacemaker cells, melatonin is produced in cycles. Melatonin, a hormone that causes drowsiness, has a direct effect on the sleep/wake cycle (*see* Melatonin Miracle, *p.* 23).

"We imagine that as we build a bigger catalog of the products of these CCGs, we'll have a pretty good indication of the cascade of events that can drive the whole behavioral response to the biological clock," says Young.

The biological clock regulates more than just the sleep/wake cycle. All kinds of hormones are produced in rhythms, peaking at set times during a 24-hour period. The biological clock also controls the progression of events during a 24-hour interval, ensuring that event A occurs before event B, which precedes event C. In this way, an organism can anticipate the time of day both behaviorally and physiologically (*see* Circadian Call to Arms, *this page*).

Recent studies have demonstrated the existence of independent clocks throughout the body, indicating that time of day is important to all parts of the body, not just the brain. Steve Kay at The Scripps

Research Institute, for example, showed that leg, wing and antennae tissue from *Drosophila* will continue to rhythmically produce PER and TIM in the absence of any input from the fly's central nervous system. Other researchers have shown that in eye tissue removed from mice, the retina will continue to secrete melatonin with a 24-hour circadian rhythm.

"You've got cells all over the body that find it very important to pay attention to time of day," says Young, "important enough to keep track of time locally, instead of having to depend on a central integrating pacemaker like the one in the brain that controls the overall sleep/wake behavior."

Further evidence to support the importance of time-keeping can be found in nature, where activities controlled by the circadian rhythm extend from the turning of leaves in autumn to foraging and hibernation patterns of animals. These seasonal changes depend on an organism's measuring and comparing day length against its internal circadian oscillator.

Noting the use of biological clocks by nearly all organisms, scientists think that these clocks have been highly conserved throughout evolution. For example, a counterpart to the fruit fly's *per* gene has been found in mice. And the recently discovered homolog of the *per* gene in humans is further evidence that the components of the clock are highly conserved among species, making it likely that Young's *Drosophila* research will result in a better understanding of human circadian rhythms. His research may also provide a basis for therapies to treat afflictions that are influenced by circadian rhythms.

Timing medical treatments to the body's natural rhythm, for example, could become an important new therapeutic tool. Researchers have found that cancer patients, for instance, can be as much as 10 times more sensitive to a chemotherapeutic drug at one time of day than they are at another.

This sensitivity might result from the variable concentrations of other hormones and factors influenced by the circadian clock. Drug regimes are currently being created that specify time of delivery in addition to dosage and frequency.

Recent studies have demonstrated the existence of independent clocks throughout the body, indicating that time of day is important to all parts of the body, not just the brain.

Preventive measures could also be developed for asthma and heart attacks, which occur more frequently at specific times of the day. Treatments for jet lag and insomnia might also come from Young's research.

But for now Young's interests will take him back to the PER/TIM protein complexes in the cell's nucleus. "For us, there are still important unsettled questions: How much of the *Drosophila* clock system is conserved in human pacemaker cells? How is the rate of the molecular cycle regulated with an accuracy of minutes? Can our discovery of the molecules forming this clock take us directly to the genes that affect behavior in a time-dependent fashion?" Young is sticking to fruit flies, looking to identify and understand the function of the remaining components of the molecular clock. ■

THE BODY'S CYCLE

TONY STONE IMAGES

1:00 a.m.
- Pregnant women are most likely to go into labor.
- Lymphocytes are at their peak.

2:00 a.m.
- Growth hormone levels are highest.

4:00 a.m.
- Asthma attacks are most likely.

6:00 a.m.
- Onset of menstruation is most likely.
- Plasma insulin is lowest.
- Blood pressure and heart rate begin to rise rapidly.
- Cortisol (stress hormone) levels begin to rise.
- Melatonin levels begin to fall.

7:00 a.m.
- Symptoms of allergic rhinitis (hay fever) are worst.

8:00 a.m.
- Calories are burned most readily.
- Risk for heart attack and stroke is highest.
- Symptoms for rheumatoid arthritis are worst.
- Lymphocytes are at their lowest daytime level.

noon
- Level of hemoglobin in blood peaks.

3 p.m.
- Grip strength, respiratory rate and reflex sensitivity highest; good time for athletes.

4 p.m.
- Body temperatures, pulse and blood pressure peak.

6 p.m.
- Urinary flow is highest.

9 p.m.
- Pain threshold is lowest.

11 p.m.
- Skin is most reactive; allergic response most likely.

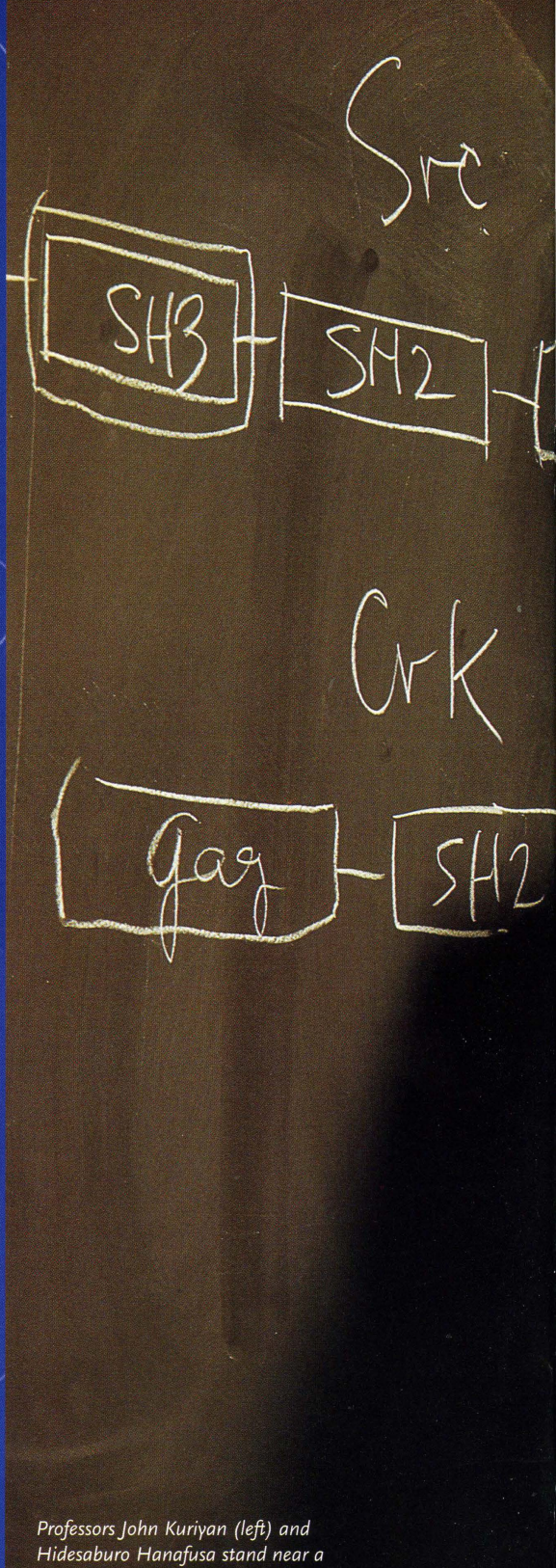
ADAPTED FROM *BUSINESS WEEK*, OCT. 2, 1995

Hunting of the Src

A Century of Cancer Research at Rockefeller

In 1989, John Kuriyan, a relative newcomer to The Rockefeller University, met with Professor Hidesaburo Hanafusa to discuss biological problems that might benefit from experimental approaches of the blossoming discipline of structural biology—a field in which the functions and activity of biologically important molecules are investigated by examining their three-dimensional structures. Hanafusa was working on understanding the origins of cancer, specifically a gene called *src*, known to cause sarcoma in chickens. The surprising feature about this gene was that it was a normal constituent of all cells, and only induced cancer when it reentered the cell in a mutant form as a stowaway aboard the genome →

By Neeraja Sankaran



Professors John Kuriyan (left) and Hidesaburo Hanafusa stand near a linear diagram of the Src protein that shows its component domains and the position of the key mutations.

MICHAEL DAMES

Peyton Rous (c. 1924) at the Rockefeller Institute for Medical Research

of an infecting virus. Hanafusa presented the following problem to Kuriyan: How did mutations in this single gene change the functioning of its protein product to induce a cell to start developing tumors?

Kuriyan, now Patrick E. and Beatrice M. Haggerty Professor, head of the Laboratory of Molecular Biophysics and a Howard Hughes Medical Institute investigator, has a vivid memory of Hanafusa introducing him to the Src problem by drawing a straight line on the blackboard and blocking out different regions, representing different parts of the molecule. Based on biochemical and genetic analyses, Hanafusa knew about mutations in different parts of the *src* gene that resulted in the "transformation" of normal cells to cancerous types, but he did not have a precise picture of how these mutations altered the normal activity of the Src protein.

One of the most significant breakthroughs toward answering this question came last year, when Kuriyan's group, as well as Stephen Harrison's laboratory at Harvard University, solved the crystal structures of two Src proteins. The achievement represents an important milestone, not only with respect to answering specific questions about Src activity, but also because of Src's leading role in the history of understanding the general mechanisms underlying cancer. It is a story with deep roots at Rockefeller, dating back to the turn of the century when Peyton Rous discovered the first tumor-inducing virus, thereby providing future cancer researchers with an indispensable tool for probing cell behavior and its breakdown.

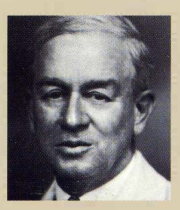
Rous came to RU—then the newly founded Rockefeller Institute for Medical Research—in 1909 on the invitation of the first director, Simon Flexner, to head a laboratory for cancer research. Within a few weeks of his arrival, a chicken breeder brought a chicken to Rous's laboratory. The bird had a large growth that he recognized as a sarcoma tumor. Knowing that fowl tumors had not yet been studied in any detail, Rous began a thorough analysis of its properties and pathologic effects.

Rous found that the tumors were transplantable over several generations and began to search for a causative agent within the tumor cells. Using new freeze-drying techniques developed by his colleague, James Murphy, Rous broke open the tumor cells, filtered the contents to ensure the complete removal of intact cells and injected this cell-free material into chickens to see whether they developed sarcomas. In a now classic paper entitled "A Sarcoma of the Fowl Transmissible by an Agent Separable from the Tumor Cells," published in *The Journal of Experimental Medicine* in 1911, Rous described the tumor agent for the first time and speculat-

Increasing the Magnification of Discovery

Technology led the way to discovery of the *src* gene.

1911



Peyton Rous

Discovers tumor agent, later called Rous Sarcoma Virus (RSV), that can transmit cancer in chickens.

1931

James Murphy

Proposes that RSV is a transmissible mutagen.

"The first tendency will be to regard the self-perpetuating agent active in this sarcoma of fowl as a minute parasitic organism."

—PEYTON ROUS, *THE JOURNAL OF EXPERIMENTAL MEDICINE*, 1911

ed on its identity.

"The first tendency will be to regard the self-perpetuating agent active in this sarcoma of fowl as a minute parasitic organism," he wrote, hinting at the possibility that the tumor agent was a virus. In 1966 Rous received a Nobel Prize for his discovery of the Rous Sarcoma Virus (RSV). Within the scientific community, however, there appears to have been little early acceptance for the idea that a virus could cause cancer. In 1931 Murphy published a paper in which he proposed that the tumor-inducing agent was perhaps a "transmissible mutagen," whose mode of activity was to induce permanent mutations in the host cells that resulted in cancer.

As is turned out, the two ideas were not mutually exclusive. Murphy's idea that mutations led to cancer was indeed correct. RSV, as scientists learned, served as one of the vehicles that introduced specific tumor-inducing mutations into the cell. Other mutagens such as chemicals and radiation could also cause mutations that initiated cancer. But neither of these agents produced consistent results with respect to the type of tumor that was formed. So, for a new generation of scientists, RSV served as the main vehicle for the molecular analysis of cancer. As tumor virologist Harry Rubin remarked in 1966, "...what else if not tumor virology can lead to an understanding of the molecular basis for the malignant behavior of cells?"

In 1958 Rubin and Howard Temin at the California Institute of Technology in Pasadena developed a method to assay RSV in tissue culture. This gave scientists a quantitative handle on the virus and allowed them to monitor changes induced by the virus directly at the cellular level, rather than having to inject a whole animal and wait for tumors to develop. It enabled scientists to map out the entire RSV genome and assign specific functions to specific genes. As a postdoc in Rubin's laboratory at the University of California, Berkeley, during the early 1960s, Hanafusa was an active participant in the analysis of mutants deficient in their ability to cause transformation—which eventually led to the identification of *src* as the single gene required for inducing cellular transformation.

At the outset, the discovery of *src* opened up more questions than it answered. How did it induce transformation? The gene was obviously not required for either viral replication or survival—so why did it exist at all? What function did the gene and its protein product serve?

In 1976 Michael Bishop and Harold Varmus at UC, San Francisco, found that a version of the viral *src* (called v-*src*) gene was present in normal uninfected cells. Hard on the

Photograph of a chicken (c. 1911) in which Rous induced a tumor using a filtered cell extract containing RSV.

ALL PHOTOS, P. 28-9: THE ROCKEFELLER UNIVERSITY ARCHIVE

1958

Harry Rubin and Howard Temin (Caltech)

Develop an assay for RSV in tissue culture which opens up genetic and biochemical analysis of tumor viruses

1960s

Identification of *src* gene as tumor-inducing component of RSV.

1976

D. Stehelin, M. Bishop and H. Varmus (UCSF)

Discover that a version of *src* exists in the DNA of normal, uninfected cells.

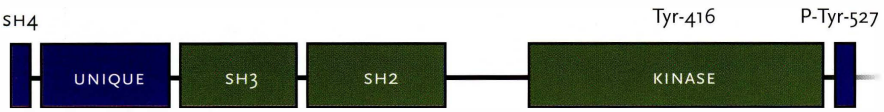
"...what else if not tumor virology can lead to an understanding of the molecular basis for the malignant behavior of cells?" —HARRY RUBIN, TUMOR VIROLOGIST, 1966

heels of this discovery, Hanafusa, who had moved to Rockefeller in 1973, furnished the genetic evidence that cellular *src* (c-*src*) sequences did in fact substitute for the transformation function missing in defective RSV mutants. To achieve this, he injected uninfected chickens with mutants of RSV that were known to have lost most of the DNA from their *src* gene and looked for the development of tumors.

"My prediction was that viruses containing partial deletions of the *src* sequence would undergo recombination with cellular sequences at a higher rate and thus be able to produce tumors," explains Hanafusa, who is now Leon Hess Professor and head of the Jeanette Warren Davidson Laboratory of Molecular Oncology.

Sure enough, the chickens injected with these mutants developed tumors that differed from the original sarcomas in two major respects: They appeared two months rather than a week after the injections and developed very far away from the site of inoculation of the virus. Upon examining the new tumor tissue, Hanafusa found he could recover viruses with complete *src* genes that were evidently derived from the host cell and not from the original sarcoma virus.

The discovery of c-*src* and v-*src* played a major part in reconciling the seemingly disparate theories of the viral and mutational origins of cancer in the following manner: The normal cell contains a gene with the potential for inducing cancer, but this gene—the protooncogene—can cause transformation only after it was mutated to form an oncogene. All known cancers can be traced to mutations in some normal protooncogene.



A schematic representation of the Src protein highlighting its principle domains and tyrosine phosphorylation sites. The green segments correspond to the protein fragment structure pictured on page 31.

Once scientists discovered the existence of cellular and viral *src* genes (and proteins), they naturally turned their attention to investigating the differences in the two forms that caused such a drastic change in their transformation capabilities. By 1987, Hanafusa and others had conducted a systematic comparison of the DNA and protein sequences of cellular and viral *src* and identified the major differences. They pinpointed the critical difference to a single amino acid in the tail region of the protein—a tyrosine present at position 527 of c-Src and missing from v-Src.

But even this discovery failed to answer the crucial question of how the absence of a single amino acid changed the protein to a form that induced cellular transformation? Investigators looking into functional aspects of *src* had determined that the normal gene encoded a key component of the network of molecular signals that regulate growth and development patterns of a cell. Src functions as a kinase—a protein that transfers phosphate molecules to other cellular proteins and activates them—ultimately sending a message to the cell to start dividing. Kinases must be very tightly regulated or else cell division can run amok, resulting in cancer. In c-Src this regulation was found to be linked directly to Tyrosine 527: When this amino acid was phosphorylated, the protein appeared inhibited, while removal of the phosphate from this site resulted in an elevated activity.

This observation immediately suggested that v-Src induced transformation by permanently removing the internal brake from the cellular protein. Hanafusa obtained further evidence in support of this idea when he discovered a portion of the molecule called SH2 near the head of the Src protein, which has a special affinity for binding with phosphory-

1977

Hidesaburo Hanafusa (at Rockefeller)
Finds that non-tumorigenic RSV can become tumorigenic after picking up cellular *src* DNA.

1980s

Analysis of the differences between cellular and viral src DNA and protein sequences.

1990

Identification of regulatory regions (SH2 and SH3) in oncogenes
Hanafusa proposes model for Src regulation by interactions within protein.

"The whole enzyme appears to be functioning like a Rube Goldberg machine, employing many complex parts to perform a simple task." —JOHN KURIYAN

lated tyrosine molecules. The discovery had two exciting implications. First, it suggested that the SH2 region could bind to the phosphorylated Tyrosine 527, like a snake biting its own tail. An intramolecular contortion of this kind, Hanafusa imagined, would block Src's active site—its kinase domain—and thus prevent it from phosphorylating other proteins. The finding also indicated that the SH2 domain played the role of a molecular postman, who recognized specific intracellular addresses—the tyrosines—where the kinase delivered the phosphate molecules. When attached to the internal tyrosine, the postman could no longer reach the other addresses, which resulted in the suppression of Src activity.

This then was the model that Hanafusa presented to Kuriyan in 1989: A linear diagram of the Src protein with its component domains and the position of the key mutations, superimposed with biochemical information on the possible areas of interaction.

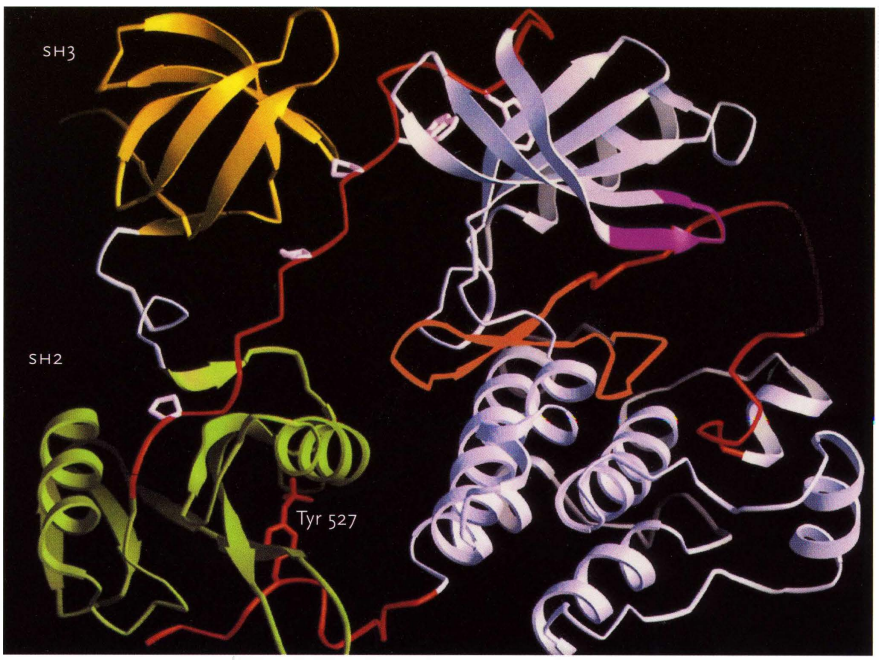
"The question that Saburo posed, very simply and directly, was 'How is Src regulated?'" recounts Kuriyan. "To proceed any further with understanding how it worked, one needed to know what it looked like in three dimensions."

The researchers had their first breakthrough when Kuriyan and his collaborators solved the crystal structure of a v-Src SH2 domain in 1992. The structure immediately clarified the way in which SH2 domains could recognize phosphotyrosine molecules and provided the first bit of support for Hanafusa's proposed mechanism of control.

Then last year, Kuriyan, along with postdocs Ismail Moarefi and Frank Sicheri, published the complete crystal structure of Hck—a Src-related molecule found in white blood cells—showing how the unmutated molecule looks in its regulated state. In the broadest sense, the structure confirms the validity of Hanafusa's conjectures that the Tyrosine 527 plays a key role in regulation by binding with SH2. In addition the structure reveals other, subtler details about interactions within the Src molecule, which contribute to the integrity of the overall structure.

"The whole enzyme appears to be functioning like a Rube Goldberg machine, employing many complex parts to perform a simple task," explains Kuriyan.

What next? "While our understanding of individual components such as Src and Hck is now quite advanced, there are hundreds of such proteins that are communicating with each other to regulate cell behavior," says Kuriyan. "The big challenges ahead lie in understanding how the cells integrate and sort through this multiparty system of communication." ■



JOHN KURIYAN

Three-dimensional crystal structure of the Src-family kinase Hck. The molecule is seen here in its inhibited form with the phosphotyrosine at position 527 bound to the SH2 domain. SH3 and kinase domains are also identified.

1992

Collaboration of several Rockefeller laboratories. Determination of the 3-D crystal structure of SH2 region of Src.

1997

John Kuriyan lab

Determination of 3-D crystal structure of Src-family protein Hck.

66th&York

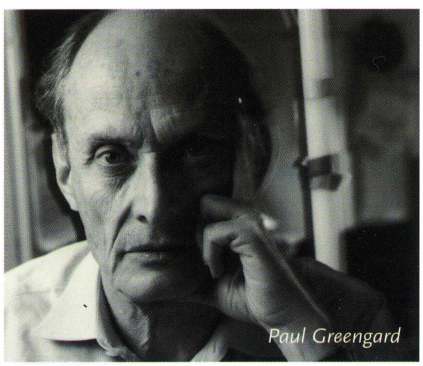
SCIENCE AND OTHER NEWS FROM THE ROCKEFELLER UNIVERSITY CAMPUS

Study shows estrogen may prevent onset of Alzheimer's

BY URMILA RANADIVE

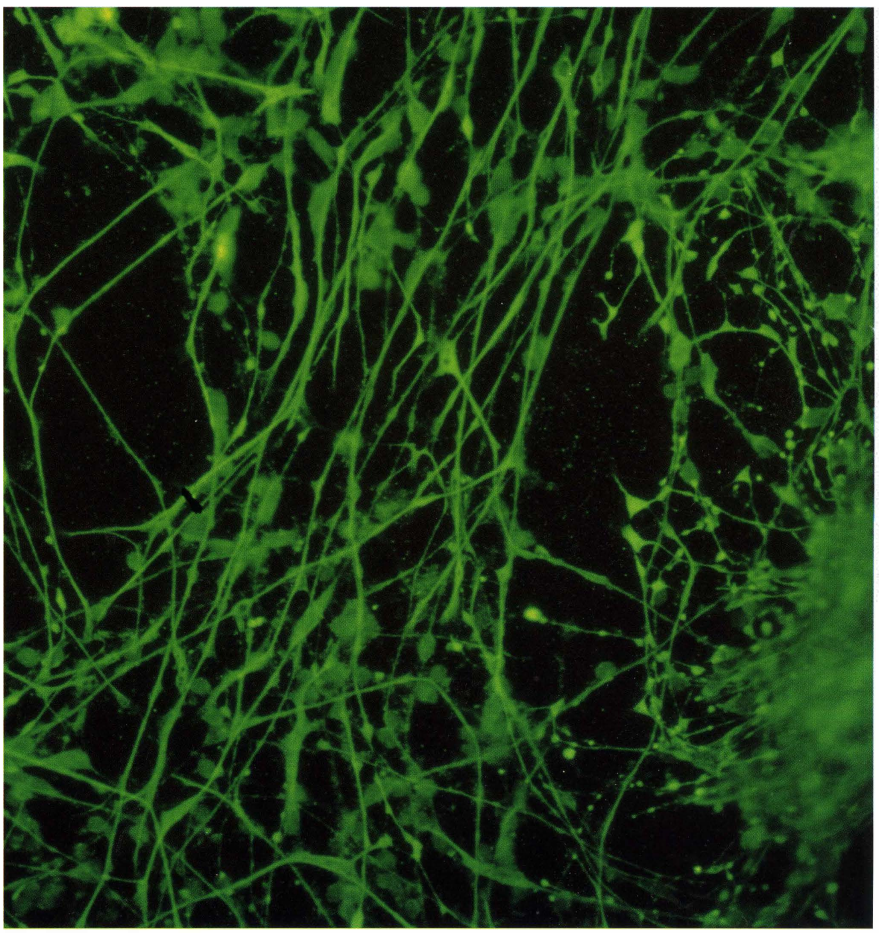
Alzheimer's disease, the devastating illness that leads an estimated 4,000,000 Americans through progressive and irreversible declines in mental function, occurs in postmenopausal women at a two-fold higher incidence than in males of the same age. During the last few years, retrospective epidemiological studies have shown that a history of estrogen replacement therapy in women after menopause was associated with a reduction, by about 50 percent, in the risk of developing Alzheimer's.

Alzheimer's disease is characterized by the buildup of plaques, which are primarily made up of β amyloid proteins, in the brain. These β amyloid plaques are widely regarded as the key to the disease.



Paul Greengard

In a study published in the April 1998 issue of *Nature Medicine*, a research group led by RU Professor Paul Greengard, along with members of RU Professor Brian Chait's laboratory and researchers at other institutions, demonstrated that exposure to estrogen correlates with a decrease in β amyloid protein production.

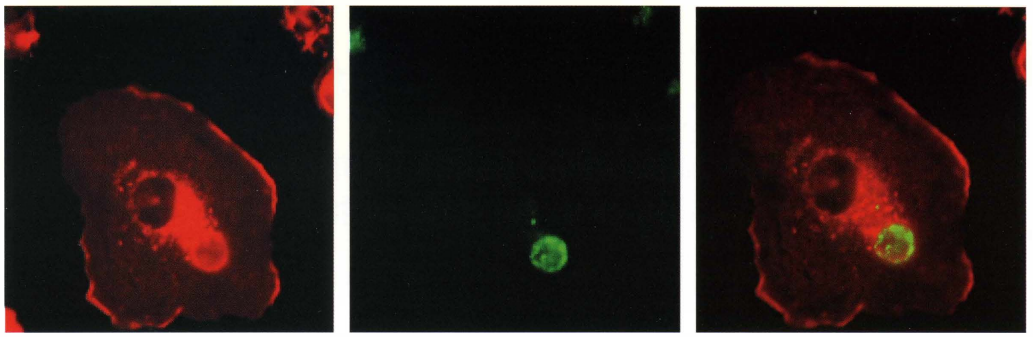


GREENGARD LAB IN COLLABORATION WITH GABRIEL FRIED OF THE KAROLINSKA INSTITUTE

Cortical neurons that were treated with estrogen (above) produced less plaque-forming, β amyloid protein than neurons that were not exposed to the hormone.

In making the link between estrogen and β amyloid, the Greengard lab's study provides the first molecular basis for understanding why estrogen therapy works. It also supports the continued use of estrogen replacement therapy, which is already widely employed to treat and prevent osteoporosis in postmenopausal women and which has also been found to protect healthy, older women against heart attacks.

Greengard's study also showed that estrogen need only be replaced at premenopausal levels and that the longer a woman is treated with the hormone, the greater the degree of protection against Alzheimer's disease.



NINA BHARDWAJ

Immature dendritic cells (far left) engulfing influenza-infected monocytes (middle).

17β-estradiol, the form of estrogen used by Greengard and his colleagues in this study, is one of a family of molecules that includes the male sex hormones. In fact, testosterone is a precursor of estrogen and is the major source of estrogen in men.

Greengard and his colleagues plan to test other compounds, including testosterone and estrogen analogs, for their effectiveness, since estrogen therapy itself has been associated with increased risk of developing blood clots and certain types of cancer.

Now that the researchers have established that estrogen reduces the production of β amyloid, the next step is to understand how.

The Greengard laboratory's strategy is to look inside the cell to see where the β amyloid forms—before and after treatment with estrogen—to see where estrogen might intercede in the metabolic pathways.

"If we are successful in elucidating the intracellular β amyloid production location and the molecular apparatus that is responsible for the reduction of the β amyloid, this will provide new targets for drugs that will be effective in treating the disease," says Greengard, who directs the university's Zachary and Elizabeth M. Fisher Center for Research on Alzheimer's Disease.

Funding sources for this research included the National Institutes of Health, the Alzheimer's Association and the Fisher Foundation.

Rockefeller researchers develop method to fight tumors using dendritic cells

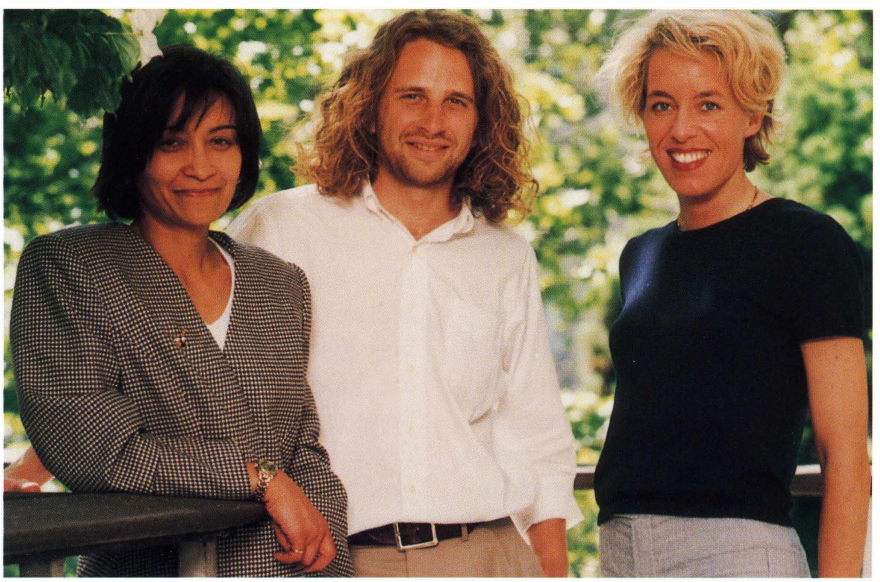
BY JOSEPH BONNER

Dendritic cells patrol the body's borders, guarding against outside invaders by capturing, processing and presenting antigens to the body's T cells. Dendritic cells also capture mutated pieces of the body's own tissue from tumors as well as normal self-tissue. Most importantly, they are able to initiate immune responses to either fight or tolerate these molecules.

Recently, a team of researchers from the Laboratory of Cellular Physiology and Immunology, led by Assistant Professor Nina Bhardwaj, found a new way to harness the dendritic cell's antigen-presenting ability to fight human tumors.

These findings—published in the March 5 *Nature* by Bhardwaj and her coauthors, Biomedical Fellow Matthew Albert and Postdoctoral Fellow Birthe Sauter—widen the possibility of using dendritic cells to treat human tumors, viruses and infections.

Using the influenza virus as a model system—because the flu vaccine induces only antibodies and not killer T cells—the researchers found they could take dendritic cells, infect them with influenza virus and elicit killer T cells in culture very efficiently from normal individuals. The cytolytic killer T cell, also known as the CD8+ cell,



Assistant Professor Nina Bhardwaj (left) has been working with Biomedical Fellow Matthew Albert and Postdoctoral Fellow Birthe Sauter to harness the dendritic cell's antigen-presenting ability to fight tumors.

plays a key role in eradicating viruses, such as HIV, and in tumor immunity. Autoreactive killer cells (cells capable of recognizing the body's own cells or antigens) must in turn be tolerized, or turned off, to prevent autoimmune disease.

During the course of these studies, the researchers found that if they took other antigen-presenting cells, like macrophages, and infected them with influenza, the cells die from the infection.

In fact, the cells undergo a process called apoptosis, a type of cell suicide or programmed death. Because they die in culture, the apoptotic cells do not get a chance to efficiently present antigens to T cells.

Further experiments showed that an influenza-infected apoptotic macrophage, cocultured with an uninfected dendritic cell and T cells from someone who had been previously exposed to influenza, allowed the dendritic cell to acquire the influenza antigens and trigger killer T cells.

"Our hope is to be able to take blood donations from a patient and grow dendritic cells from precursor cells in the blood," says Albert. "We can then charge or pulse the dendritic cells with apoptotic cells and reinject them into the patient to induce activated T cells.

"The discovery of this new pathway," he continues, "allows the dendritic cell's natural machinery to decide which pieces of the protein are useful to an individual's immune system."

Funding for this work was provided in part by the National Institute of Allergy and Infectious Diseases, part of the federal government's National Institutes of Health (NIH), and by the NIH Medical Scientist Training Program.

THE HYDROGENOSOME AT 25:

New hypothesis points to organelle's role in evolution of eukaryotic cells

A new hypothesis coauthored by Associate Professor Miklós Müller and published in the March 5 *Nature* may change the way evolutionary biologists look at the origins of eukaryotic cells.

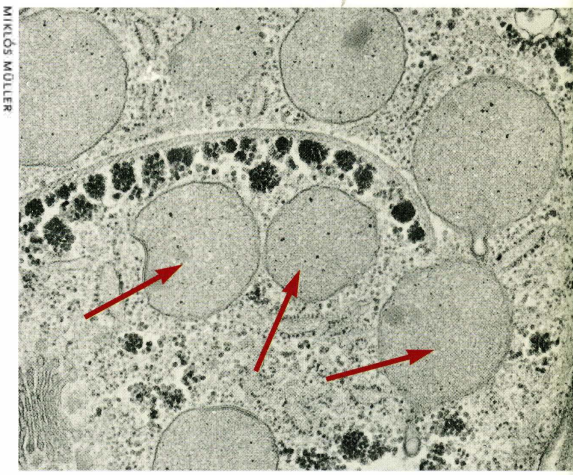
The classic theory of eukaryotic evolution contends that eukaryotes descended from proto-eukaryotes, single-celled organisms that already possessed a nucleus to hold their genetic endowment. These ancestral organisms contained inner membranes and a cytoskeleton that enabled them to eat and digest other anaerobic prokaryotes, but contained no mitochondria.

Sometimes the engulfed bacteria was assimilated and became an endosymbiont—an internal partner in a mutually beneficial relationship—assisting the host in respiration and receiving nourishment and physical protection in return. The endosymbiont became the mitochondrion, the cell's energy-producing powerhouse.

With their "hydrogen hypothesis," Müller and his coauthor, William Martin, a plant biologist from the Institute for Genetics at the Technical University in Braunschweig, Germany, suggest that eukaryotes arose through the need to share the machinery of energy metabolism for survival and not by an evolutionary roll of the dice. According to Müller and Martin, the first host was a methanogen, a single-celled organism that produces methane by consuming hydrogen and carbon dioxide. The endosymbiont derived from a bacterium that produced hydrogen as waste, an ancestor of the mitochondrion and a cell organelle called the hydrogenosome.

Hydrogenosomes, discovered at Rockefeller in 1973 by Müller and Donald Lindmark, are found in single-celled organisms that lack mitochondria, so-called amitochondriates. Living free or in multicellular hosts, these microscopic entities live in inhospitable environments—digestive and urinary tracts, mud, sulfur springs—and produce hydrogen as waste.

MIKLÓS MÜLLER



An electron micrograph of a cell taken from a parasite called a trichomonad reveals an organelle called the hydrogenosome (arrows).

The researchers suggest that the methanogen and the bacterium that provided its "fuel of life" met in a hydrogen-rich environment. The pair, separated from the hydrogen source by an unknown means, survived by gradually integrating and becoming structurally and functionally dependent on each other.

"The future symbiont possessed hydrogenosomal function and the host used the hydrogen and carbon dioxide produced by it," explains Müller. "This is the link that forged integration."

Funding for this work was provided by the National Institutes of Health.—J.B.

RU researchers elucidate stress response in plants

BY MARGUERITE LAMB

Professor Nam-Hai Chua (left) and coauthors and Postdoctoral Fellows Yan Wu and Eric Maréchal.

How do plants respond to stresses brought on by drought? Last December, Nam-Hai Chua, Andrew W. Mellon Professor and head of RU's Laboratory of Plant Molecular Biology, and members of his laboratory announced in the journal *Science* that they had come one step closer to elucidating this vital process, having uncovered an important piece of the plant stress-response puzzle.

Scientists have long known that a plant's ability to endure environmental challenges depends on stress hormones such as abscisic acid (ABA), which signals the release of intracellular calcium, in turn activating a variety of stress-response genes.

Usually, a number of molecules—including receptors, proteins and messengers—will fill key positions along the signal-transduction pathway, passing the message one to the next. But until recently, the molecular bucket brigade linking ABA to calcium to ABA-responsive stress genes had remained largely a mystery.

That mystery began to be unraveled last winter, when Chua and his RU team reported that it had definitively—and for the very first time—located a second messenger molecule called cyclic ADP-ribose (cADPR) in the ABA signaling pathway.

Through a series of tomato experiments, postdoctoral fellow Yan Wu, along with her coauthors and fellow Chua lab postdocs Randy Foster and Eric Maréchal, demonstrated cADPR's role in ABA signal transduction.

The researchers began by injecting the stems of tomato seedlings with either of two ABA-sensitive stress genes. They used a third, light-activated gene as a control. Prior to injection, all three genes had been attached to a reporter gene, which gives off a telltale blue hue when activated with a substrate, allowing researchers to monitor its activity, as well as the activity of the genes to which it is linked.

The researchers demonstrated that ABA and cADPR, introduced individually into the altered plants, can induce expression of the stress-responsive genes. Yet inhibitors of either cADPR or calcium will stop stress-gene expression, even in the presence of ABA.

Next, to show that cADPR is present and active in plants, Maréchal, along with coauthor and former postdoctoral fellow Jennifer Kuzma, attached one of the stress-response genes to *luciferase*, the gene that makes fireflies glow, and transferred this gene into a tiny plant called *Arabidopsis thaliana*. The researchers then introduced ABA and monitored the plant's tissue for a revealing glow.

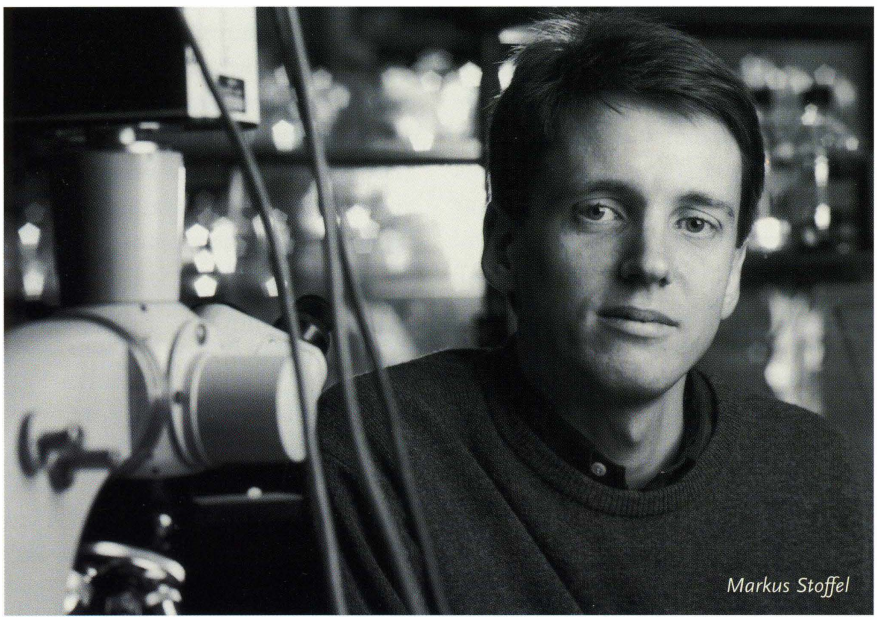
Gene expression peaked after four to eight hours of exposure to ABA. And, as the researchers had suspected, activation of this gene was preceded by a rise in the levels of cADPR, manifested by a rush of calcium ions.

The team's findings hint at intriguing possibilities for the world of agriculture and beyond, since cADPR is believed to be an active messenger in a variety of cell systems, including those of humans.

Scientists have long known that a plant's ability to endure environmental challenges depends on stress hormones such as abscisic acid, which signals the release of intracellular calcium and activates a variety of stress-response genes.

Glucose metabolism defect identified in rare form of Type 2 diabetes

In December 1996, Assistant Professor Markus Stoffel, in collaboration with researchers at the Howard Hughes Medical Institute at the University of Chicago and the University of Michigan Medical Center, mapped MODY1, a particularly rare but severe form of Type 2 diabetes, to a gene called HNF4α. Less than a year later, Stoffel and a colleague in the Laboratory of Molecular Cell Biology identified the mechanism of this defect, which impairs the pathway that breaks down blood sugar and provides the main signal for insulin secretion in the pancreas. This work, reported in the Nov. 25, 1997 *Proceedings of the National Academy of Sciences*, provides the first insight into the molecular mechanism of this disease and opens new avenues for developing better therapies to treat more common forms of late-onset diabetes.


Markus Stoffel

Diabetes affects the way sugar is taken up and metabolized by cells. In the pancreas, β cells secrete insulin, the hormone that promotes absorption of glucose and other nutrients by cells. When glucose increases in the bloodstream—for example, after eating—a molecule in the β cell called the glucose transporter-2 takes up the sugar. The pancreatic β cells then sense the glucose concentration, break glucose down and provide a signal for insulin production and secretion. As glucose increases in the blood, insulin secretion increases.

There are two major forms of diabetes. Type 1 diabetes occurs when the body's immune system destroys β cells. Type 2 diabetes, the more common type accounting for more than 90 percent of cases, is caused by ineffective insulin secretion or improper insulin action on target tissues such as muscle, leading to impaired glucose uptake from the blood and increased levels of blood glucose.

Genes are some of the most important risk factors for Type 2 diabetes, although environment also plays a role in the disease. Most common forms of diabetes are polygenic, meaning that more than one gene is involved in the disease, making it difficult to identify the genes responsible. But in about 1 to 3 percent of cases, inheritance follows a classic autosomal dominant pattern: Anyone in a family who has one copy of the defective gene is likely to develop hyperglycemia, or increased levels of blood glucose. Known as maturity onset-diabetes of the young (MODY), this form of diabetes usually develops before age 25. Scientists have found four diabetes genes, each linked to a different form of MODY.

In the new study, Stoffel and co-author Stephen A. Duncan, formerly an assistant professor in Professor James E. Darnell Jr.'s laboratory, showed that the defect in HNF4α that causes MODY1 is a loss of function mutation, meaning that the disease develops due to the inactivation of this gene.

"We have also shown that there is gene-dosage effect—if the gene's activity is decreased, there is a corresponding reduction in a related transcription factor called HNF1α, which is important for insulin production," explains Stoffel.

Both HNF1α and HNF4α belong to a class of proteins called transcription factors, molecules that switch other genes on or off. In the early 1990s, Darnell's laboratory identified HNF4 and showed that it regulates gene expression in the liver, kidney and intestine.

Stoffel and Duncan developed a novel technique to study the effect of HNF4α on glucose transport and metabolism. Using embryonic stem (ES) cells—cells found in early stages of the embryo that can theoretically turn into any tissue in the body—the researchers produced clumps of about 2,000 cells that contain the visceral endoderm and

later develop into the yolk sac. Genes that are expressed in the liver, like HNF4α, are also expressed in the visceral endoderm. The yolk sac, which is the main tissue that produces insulin during development, acts like a gut, providing nutrients to the embryo. This feature makes the visceral endoderm a good model to study pancreatic β cells, explains Duncan.

"The visceral endoderm provides a physiological system for the genetic dissection of metabolic pathways," says Stoffel.

Most common forms of diabetes are polygenic, meaning that more than one gene is involved in the disease, making it difficult to identify the genes responsible.

When HNF4 was removed from the visceral endoderm, the scientists found a decrease in activity of several genes that act at different stages of the insulin-secretion signaling and insulin-production pathway.

These new findings suggest that drugs designed to target HNF4α activity could lead to improved treatments for Type 2 diabetes.

"Activating HNF4α would lead to an increased expression of the glucose transporter-2 and the enzymes of glucose metabolism, increasing the rate of glucose metabolism and increasing insulin production and secretion," says Stoffel.

Stoffel is an Irma Hirschl Scholar, a Pew Scholar and Robert and Harriet Heilbrunn Professor. Duncan is a Naomi Judd American Liver Scholar and an Alexandrine and Alexander Sinsheimer Scholar. This work was supported by the American Diabetes Association.—J.B.

New research challenges 30 years of dogma on treating lupus and other autoimmune diseases

For the more than 500,000 Americans with systemic lupus erythematosus, the only therapies for this devastating autoimmune disease are ineffective and nonspecific antiinflammatory and immune-system suppressing drugs. But research from the laboratory of Professor Jeffrey V. Ravetch, reported in the Feb. 13 *Science*, overturns a 30-year-old dogma and may change the way doctors treat patients with lupus.

The disease causes severe inflammation and kidney disease. Lupus develops when the immune system attacks the body's own tissues by producing autoantibodies directed against its own cells. The new research identifies a critical link between autoantibodies and inflammation and suggests novel ways of uncoupling this connection.

"These studies show that preventing the activation of antibody receptors by autoantibodies is an effective way to treat autoimmune diseases like lupus," says Ravetch, who is the Theresa and Eugene M. Lang Professor and head of the Leonard Wagner Laboratory of Molecular Genetics and Immunology.

The traditional dogma on how autoantibodies caused disease in lupus is based on a component of the immune system called the complement system, which scientists thought triggered inflammation.

But research from the Ravetch lab and elsewhere during the last few years has pointed to an alternative pathway in which scientists think that Fc receptors, antibody-binding molecules that are crucial to both triggering an immune response and to turning off the response once the threat has been eliminated, play an important role.

In the new research, Ravetch and

Jeffrey Ravetch

his coauthors, Research Associate Raphael Clynes and graduate researcher Calin Dumitru, bred Fc receptor-deficient mice to a strain of mice that spontaneously develop a disease closely matching human lupus. The researchers found that 82 percent of mice without the Fc receptor were alive after nine months, as compared to less than 20 percent of lupus mice with an intact Fc receptor.

Ravetch and his colleagues found evidence of immune complexes and a complement protein called C3 in the kidneys of both strains of mice, but the Fc receptor-deficient mice showed no evidence of inflammatory disease.

"These results indicate that Fc gamma receptors are required for the initiation of the inflammatory cascade and complement activation is not sufficient," says Ravetch. "These findings argue for the development of new therapeutic strategies for the treatment of lupus based on blocking Fc receptors."

This work was supported by the National Institute of Allergy and Infectious Diseases and the National Institute of Diabetes and Digestive and Kidney Diseases, both part of the federal government's National Institutes of Health. —J.B.

Mutated gene causes death of nerves in brain

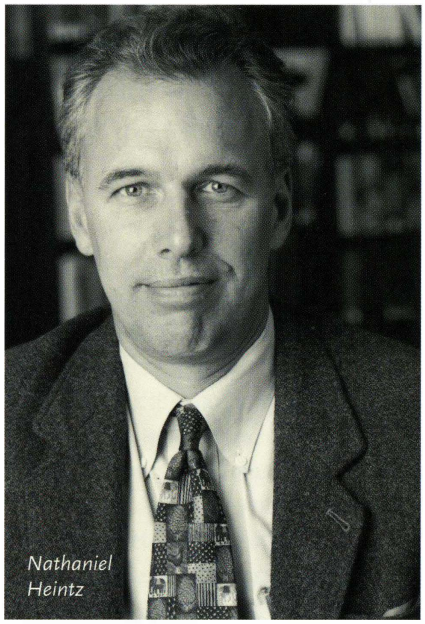
BY MARION E. GLICK

A gene responsible for the degeneration and death of certain nerve cells in the brain has been cloned by investigators from the laboratory of RU Professor Nathaniel Heintz and The Johns Hopkins School of Medicine.

The gene carries instructions to make a receptor for neurotransmitters, which nerve cells use to communicate, and its isolation may be useful for further studies of such diseases as Alzheimer's and Parkinson's. The discovery, resulting from mouse studies, marks the first time scientists have identified and directly linked a mutant gene in the glutamate receptor family to the death of brain cells. Because of the mutation, the resulting faulty receptor acts as if a neurotransmitter always is present—even when none of the chemical is there. This false detection causes the nerve cells to die.

"The mutations in the δ2 glutamate receptor gene may play a role in changing the metabolism of the adult nerve cells to reactivate a program of cell death that normally occurs only during natal development. If we can reveal more about this process and understand it, it may be possible to slow down or stop the process and preserve the neuron," explains Heintz, head of the Laboratory of Molecular Biology and an investigator at the Howard Hughes Medical Institute.

During fetal development, programmed cell death is used to sculpt the final number of cells in the mature brain. About twice as many cells begin the process of developing into neurons than are needed in an adult brain. Consequently, many of the cells activate a biochemical program to commit suicide, known as an apoptotic death, because they receive certain chemical signals.


Nathaniel Heintz

"We think that the surveillance mechanisms that monitor the normal metabolism of neurons are much like those monitoring the cell-division cycle in other types of cells," says Heintz. "In neurodegenerative diseases, these mechanisms may activate the apoptotic cell death pathway as a normal response to the severe dysfunction of neurons. Our discovery of the δ2 glutamate receptor gene mutation helps us to understand how this gene functions in normal neurons, but the $64,000 question remains: 'How does its altered function trigger cell death?'" The study appeared in the Aug. 21, 1997 *Nature*.

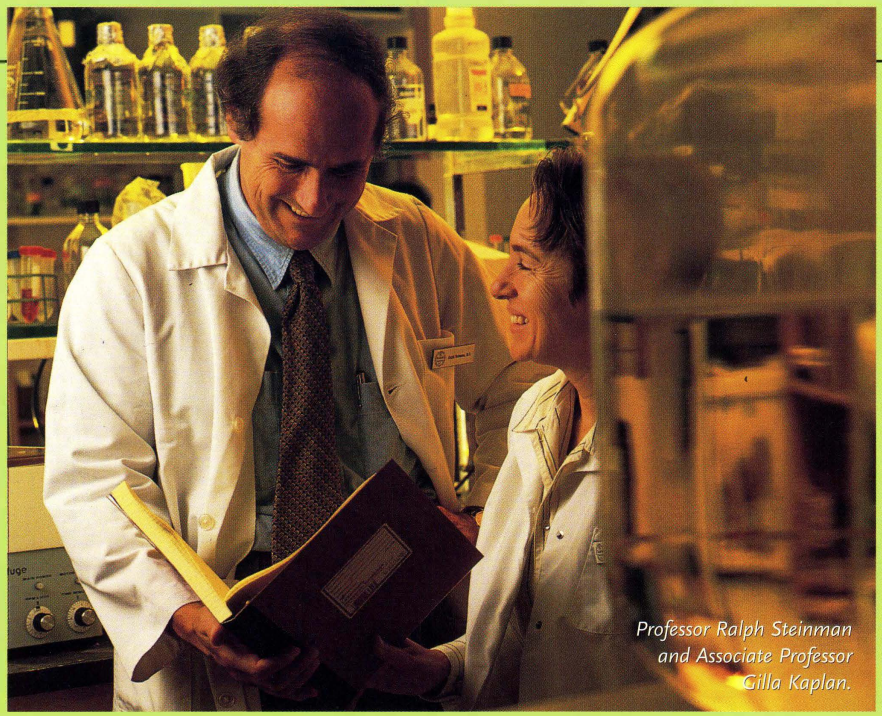
The Howard Hughes Medical Institute and the National Institute of General Medical Sciences, part of the federal government's National Institutes of Health, funded this research, with support from the National Institute of Mental Health, the McKnight Foundation, the Derelbiss Fund and the National Alliance for Research on Schizophrenia and Depression.

Support

University launches center for immunology studies

In January, the university launched the Christopher H. Browne Center for Immunology and Immune Diseases. "This center will allow us to create an integrated program that capitalizes on the talents of a diverse group of investigators, using the newest scientific methods to study the immune system's complexities in ever-increasing detail," says Torsten N. Wiesel, M.D., president of the university. "We are enormously grateful to Chris Browne for helping us to launch an initiative that will have a major impact on this vital area of biomedicine."

The new center will be directed by Ralph M. Steinman, M.D., Henry G. Kunkel Professor and head of the Laboratory of Cellular Physiology and Immunology. Other members of the center include Michel C. Nussenzweig, M.D., Ph.D., professor and head of the Laboratory of Molecular Immunology and an associate investigator with the Howard Hughes Medical Institute (HHMI); Jeffrey V. Ravetch, M.D., Ph.D., Theresa and Eugene M. Lang Professor and head of the Leonard Wagner Laboratory of Molecular Genetics and Immunology; Yongwon Choi, associate professor, head of the Laboratory of Immunology and an assistant investigator with HHMI; and David D. Ho, M.D., professor at Rockefeller and



Professor Ralph Steinman and Associate Professor Gilla Kaplan.

scientific director of the Aaron Diamond AIDS Research Center (ADARC) for the City of New York. ADARC affiliated with the university in 1996.

Plans for the Christopher H. Browne Center include creation of new laboratories at Rockefeller focusing on key areas of immunology research. Scientists working in the center will also have access to essential core resources, including a facility to study genetically altered mouse models that can advance understanding of gene function and disease states. Other shared resources will house state-of-the-art instruments for cell and tissue analysis.

A principal goal of the center is to promote new clinical research on an expanded range of immune-related conditions, particularly autoimmune diseases and cancer, with the ultimate goal of developing more effective therapies. The Rockefeller University Hospital, the largest clinical research center supported by the U.S. National Institutes of Health, will play a major role in this endeavor. Currently, more than 25 clinical protocols focusing on immunology are under way at the hospital, including studies of the drug thalidomide, which has proven effective in alleviating the wasting associated with AIDS and tuberculosis. *—J.B.*

"This center will allow us to create an integrated program that capitalizes on the talents of a diverse group of investigators, using the newest scientific methods to study the immune system's complexities in ever-increasing detail."

Kreek lab receives $12.3 million grant from NIDA

The National Institute on Drug Abuse (NIDA) at NIH has awarded $12.3 million to The Rockefeller University in continuing support of a NIDA-NIH Research Center established here in 1987, under the direction of RU Professor Mary Jeanne Kreek, head of the Laboratory of the Biology of Addictive Diseases.

"We are absolutely thrilled to receive this renewal grant," says Kreek, who credits the center's "talented team of scientists for working tirelessly over the last two years" to produce the successful proposal.

"This was an extremely competitive process, but we received outstanding reviews across the board." The center, says Kreek, is dedicated to identifying biological correlates of addictions to heroin, cocaine and alcohol—alone and in combination with one another—while also studying other factors that might affect treatment outcomes.

"Effective treatments must be based on a fundamental understanding of the molecular neurobiological basis of each specific addictive disease," she maintains, "as well as of the effects of drugs of abuse and any inherent and environmental factors that may increase vulnerability prior to exposure to such drugs."

Over the next five years, the NIDA grant will support the center's core resources and six projects to be undertaken by scientists in the Kreek laboratory.—*M.L.*

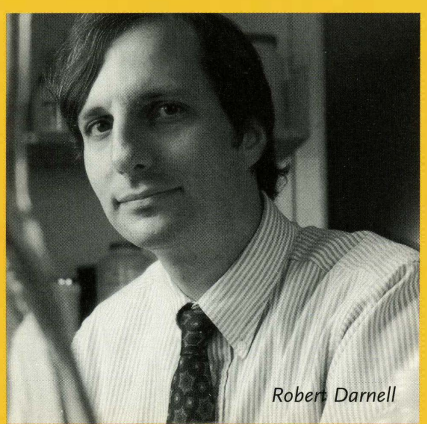
People

HONORS

Professors **Jan Breslow** and **David D. Ho** were elected to the Institute of Medicine of the U.S. National Academy of Sciences. Breslow is head of the Laboratory of Biochemical Genetics and Metabolism. Ho is scientific director of the Aaron Diamond AIDS Research Center for the City of New York.

A paper on Chagas' disease, coauthored by Abby Rockefeller Mauzé Professor **Joel E. Cohen**, head of the Laboratory of Populations, was awarded the Fred L. Soper Prize by the Pan American Health and Education Foundation.

Associate Professor **Robert Darnell**, head of the Laboratory of Neuro-Oncology, was named the ninth recipient of the Derek Denny-Brown Young Neurological Scholar Award by the American Neurological Association. The award goes annually to "a newly elected member of the association who is deemed to have achieved a significant stature in neurological research and whose promise of continuing major contributions to the field of neurology is anticipated."


Robert Darnell

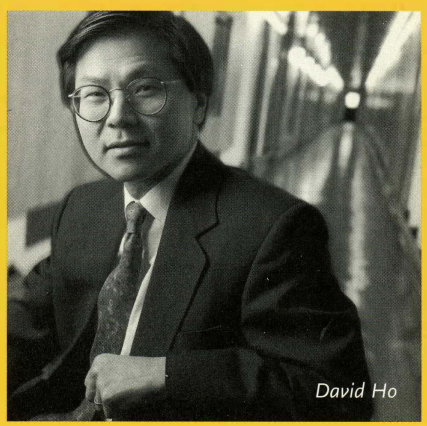
Paul Greengard, Vincent Astor Professor and head of the Laboratory of Molecular and Cellular Neuroscience, received the 1997 Charles A. Dana Award for Pioneering Achievements in Health, an accolade that recognizes "outstanding accomplishments that hold great potential for fostering positive change in health." He shared the award with Trustee **Eric R. Kandel**, university professor at Columbia University.

Alumnus **Scott M. Grundy** ('68) was the winner of the 1997 Bristol-Myers Squibb/Mead Johnson Award for Distinguished Achievement in Nutrition Research.

Leon Hess Professor **Hidesaburo Hanafusa**, head of the Jeanette Warren Davidson Laboratory of Molecular Oncology, was elected a fellow of the American Academy of Microbiology, the only honorific leadership group devoted entirely to microbiologists and the science of microbiology.

Professor **Mary E. Hatten**, head of the Laboratory of Developmental Neurobiology, delivered the keynote address at commencement at her alma mater, Hollins College in Virginia, where she was presented with an honorary doctorate.

The Rockefeller University's Aaron Diamond AIDS Research Center (ADARC) was awarded a $500,000 Bristol-Myers Squibb Unrestricted


David Ho

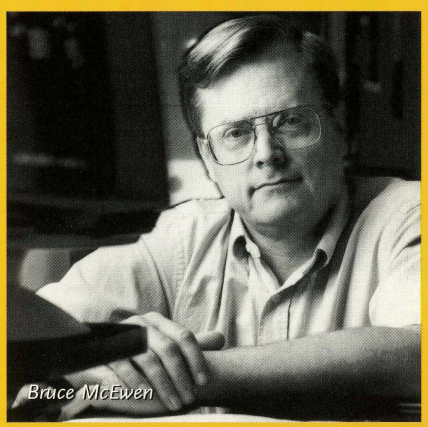
Infectious Disease Research Grant to help support studies on the dynamics of HIV replication. Professor and ADARC Scientific Director **David Ho** will supervise the grant. Ho, who was honored by the New York Academy of Medicine at its Fourth Annual Gala Dinner, gave commencement addresses at Swathmore College and at the Massachusetts Institute of Technology, where President Clinton also spoke.

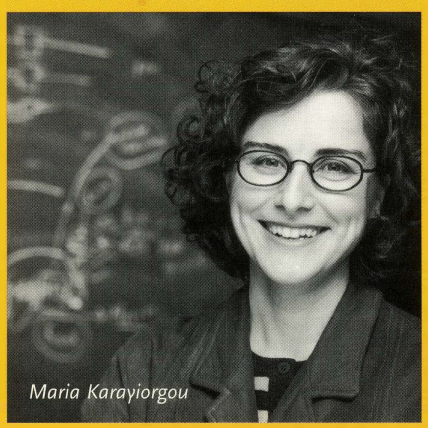
Sherman Fairchild Professor **Attallah Kappas**, head of the Laboratory of Pharmacology, was appointed to a six-year term as a member of the Council of the State University of New York (SUNY) Health Sciences Center in Brooklyn, N.Y. The council serves as the governing body of the center, which includes its medical school and graduate education and hospital facilities and is the largest health science center in the SUNY system.

Professor and President Emeritus **Joshua Lederberg**, head of the Laboratory of Molecular Genetics and Informatics, received an honorary Doctor of Military Medicine

degree from the Uniformed Services University of the Health Sciences.

Alumnus **Robert Mackel** ('78), adjunct faculty member in the Asanuma laboratory, has been named president of the Council for Higher Education in Luxembourg. Charged with advising the government on policy decisions affecting higher education and scientific research, the council is comprised of leaders from academia, business and industry.


Bruce McEwen


Maria Karayiorgou

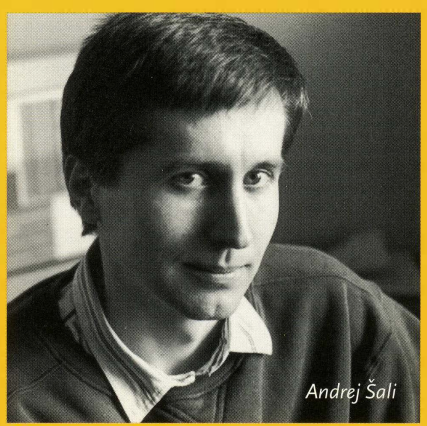
The National Alliance for Research on Schizophrenia and Depression (NARSAD) awarded Professor **Bruce McEwen**, head of the Harold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, a 1998 Distinguished Investigator grant and named Assistant Professor **Maria Karayiorgou**, head of the Laboratory of Human Neurogenetics, and **Barbara Porton**, postdoctoral associate in the Laboratory of Molecular and Cellular Neuroscience, recipients of 1998 Young Investigator awards.

Bruce Merrifield, John D. Rockefeller Jr. Professor Emeritus and head of the Laboratory of Biochemistry, and university Trustee **Ronald C. Breslow**, professor of chemistry at Columbia University, were among the top 75 "distinguished contributors to the chemical enterprise" named by *Chemical & Engineering News*, the news magazine of the American Chemical Society.

Assistant Professor **Peter Mombaerts**, head of the Laboratory of Vertebrate Developmental Neurogenetics, was named a Guggenheim Fellow by the John Simon Guggenheim Memorial Foundation. The fellowships are awarded to individuals "who have demonstrated exceptional capacity for productive scholarship or exceptional creative ability in the arts." He also received a Scholar Award from the Rita Allen Foundation and was awarded a 1997 Presidential Early Career Award for Scientists and Engineers (PECASE), one of only 11 scientists to be granted the award by President Clinton. Mombaerts is the second Rockefeller faculty member to receive the award. Associate Professor **Ali Hemmati-Brivanlou**, head of the Laboratory of Molecular Vertebrate Embryology, received a PECASE in 1996.

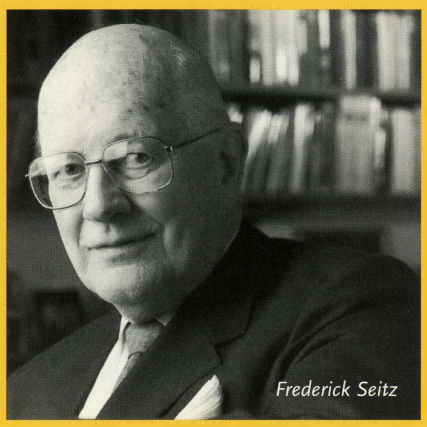
Professor **Michael O'Donnell**, head of the Laboratory of DNA Replication and an HHMI investigator, received a Method to Extend Research in Time (MERIT) Award from the National Institute of General Medical Sciences at NIH in support of his study "Biochemical Mechanism of DNA Polymerase III Holoenzyme."

Melissa Pope, assistant professor in the Laboratory of Cellular Physiology and Immunology, and **Leonidas Stamatatos**, assistant professor and staff scientist at the Aaron Diamond AIDS Research Center, were named winners of the American Foundation for AIDS Research Award for their innovative research on HIV vaccines.


Andrej Šali

Andrej Šali, assistant professor and head of the Laboratory of Molecular Biophysics, was awarded a 1998 Sloan Research Fellowship by the Alfred P. Sloan Foundation.

President Emeritus **Frederick Seitz** was awarded the Joseph Henry Medal by the Smithsonian Institution. The medal recognizes


Frederick Seitz

Seitz's "exemplary contributions to the Smithsonian Institution."

Henry Kunkel Professor **Ralph Steinman**, head of the Laboratory of Cellular Physiology and Immunology, received the Cancer Research Institute's 1998 William B. Coley Award for Distinguished Research in Basic and Tumor Immunology.

Rong Wang, assistant professor in the Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, received a $150,000 grant from the Alzheimer's Association to investigate the role of metabolism of amyloid beta-protein in Alzheimer's disease.

President **Torsten Wiesel** is the recipient of The New York Academy of Medicine's 1998 John Stearns Award for Lifetime Achievement in Medicine. Presented at the academy's 1998 Spring Stated Meeting, the award recognizes Wiesel's "pioneering studies of the mammalian visual cortex, [which] have significantly shaped current understanding of brain structure, function and development."

Alumnus **Cecil Cheung-Ching Yip** ('63) received a McMaster Award for Science for contributions to insulin research.

FACULTY

Markus Stoffel, assistant professor and head of the Laboratory of Metabolic Diseases, was appointed the first Robert and Harriet Heilbrunn Professor. The professorship provides support for a member of the junior faculty conducting research on diabetes.

PROMOTIONS

TO PROFESSOR:

Steve DiNardo, head of the Laboratory of Developmental and Molecular Genetics.

Titia de Lange, head of the Laboratory of Cell Biology and Genetics.

TO ASSOCIATE PROFESSOR:

Yongwon Choi, head of the Laboratory of Immunology.

Robert B. Darnell, head of the Laboratory of Molecular Neuro-Oncology.

Seth Darst, head of the Laboratory of Molecular Biophysics.

Ali Hemmati-Brivanlou, head of the Laboratory of Molecular Vertebrate Embryology.

David S. Thaler, member of the Laboratory of Molecular Genetics and Informatics.

ADMINISTRATION

The board of trustees presented the David Rockefeller Award for Extraordinary Service to The Rockefeller University to university Trustee **John C. Whitehead** and **David J.L. Luck**, the late Alfred E. Mirsky Professor and vice president for academic affairs, who died May


Titia de Lange


Yongwon Choi

23, 1998 (*see* In Memoriam, *next page*). The award recognizes an individual from the RU community who exhibits an enthusiasm for RU's scientists and their research, dedication to furthering the university's mission and strengthening the institution, and encourages others to support the biomedical sciences for the benefit of humankind. It was established and first presented in June 1995 to David Rockefeller, in honor of his 55 years of distinguished service on the board of trustees.

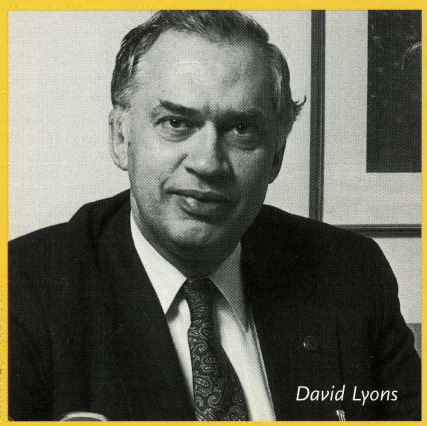
Life Trustees **Brooke Astor** and **David Rockefeller** were each awarded a Medal of Freedom, the nation's highest civilian honor, by President Clinton.

RU Trustee and Adjunct Professor **Alexander G. Bearn** has been named executive officer of the American Philosophical Society, the oldest U.S. learned society, founded

by Benjamin Franklin in 1743 to "promote useful knowledge" in science and the humanities.

Science Outreach Program Director **Bonnie Kaiser** was elected director-at-large of the American Chemical Society's New York Section for 1998.

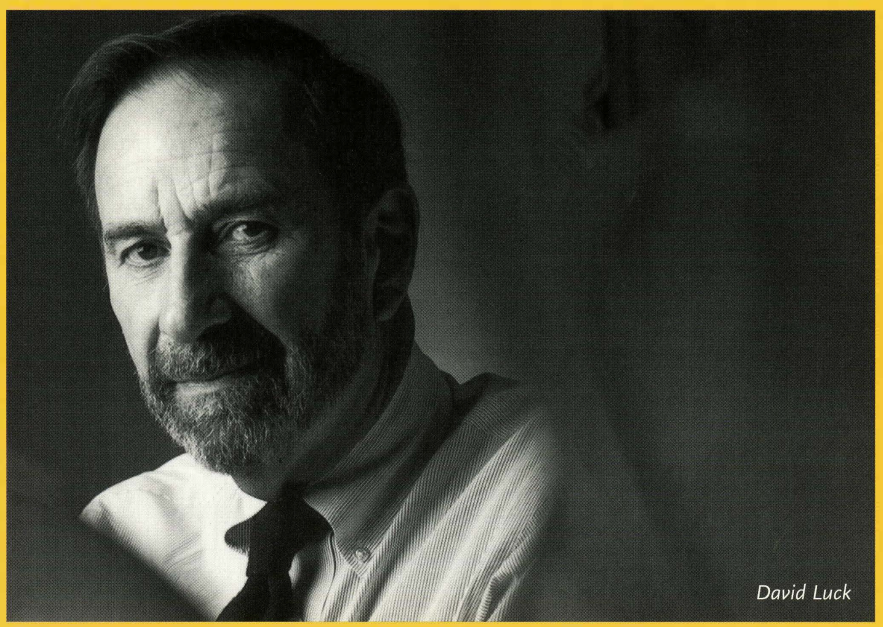
David J. Lyons, senior advisor to the president and retired vice president for business and finance and treasurer at RU, received the National Association of College and University Business Officers 1997 Distinguished Business Officer Award.


INGBERT GRÜTTNER

David Lyons

Research Associate **Marguerite Mangin** is now associate dean of graduate studies. She was previously assistant dean. Associate Professor **Thomas P. Sakmar**, head of the Laboratory of Molecular Biology and Biochemistry and an associate investigator with HHMI, has been named associate dean of graduate studies, with special responsibility for Rockefeller's participation in the Tri-institutional M.D.-Ph.D. Program.

Kenneth W. Rose, assistant to the director of the Rockefeller Archive Center, has been awarded a Fulbright grant by the United States Information Agency to teach American history and American studies at Ege University in Izmir, Turkey.



David Luck

IN MEMORIAM

DAVID J.L. LUCK

The Rockefeller University community is mourning the loss of David J.L. Luck, Alfred E. Mirsky Professor and vice president for academic affairs, who died Saturday, May 23, at New York Hospital. He was 69 years old. The cause of death was lymphoma.

Luck was a medically trained researcher with a keen interest in basic science. After earning a medical degree from Harvard Medical School and clinical training at Massachusetts General Hospital (MGH), Luck came to Rockefeller as a graduate fellow in 1958, where he joined the cell biology laboratory of Nobel laureate George E. Palade. His early research focused on how growing cells form mitochondria, structures contained in the body of the cell that produce metabolic energy. In 1962, Luck and Rockefeller biochemist Edward Reich, working with the simple mold *Neurospora*, were among the first to discover that mitochondria contain their own DNA, distinct from the DNA found in the cell nucleus. He established the existence within mitochondria of a class of ribosomes—RNA-protein complexes that are the sites for protein synthesis—that can serve as a means for converting the genetic information of this unique DNA into protein. In the late 1970s, Luck showed that one ribosomal protein, a component of the small subunit of the ribosome, was made within mitochondria and regulated the entire assembly process of protein synthesis.

Luck's later research focused on microtubules, the dynamic skeletal structures that assist cells in movement, communication with other cells and division. Using the single-celled aquatic alga *Chlamydomonas* as a model organism, Luck and his associates were able to identify and study the function of some of the more than 200 accessory proteins that are present in flagella and cilia. His investigations of microtubules in *Chlamydomonas* provided a methodological framework for negotiating the far more complex microtubular systems in human cells and toward establishing possible links between microtubular failure and lung, reproductive or neurological

disorders, or in the cell-division defects in cancer.

Luck received a medical degree from Harvard Medical School in 1953. Except for two years of medical service with the United States Air Force, he was on the staff of MGH between 1953 and 1958 as intern, assistant resident, physician and resident physician.

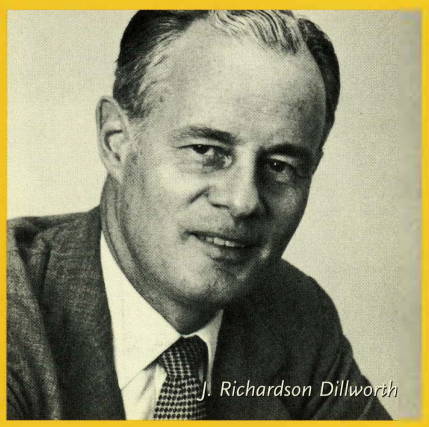
Luck received his doctoral degree from Rockefeller in 1962 and became a research associate. He was appointed assistant professor in 1964, associate professor in 1966 and professor in 1968. In 1985 he was named the university's first Alfred E. Mirsky Professor, and in 1994, vice president for academic affairs.

Luck was elected to membership in the U.S. National Academy of Sciences in 1984 and was a fellow of the American Association for the Advancement of Science.

He was a member of the Medical Advisory Board of the Howard Hughes Medical Institute (HHMI) and served on the Scientific Review Boards of HHMI (1989 to 1993) and the Massachusetts General Hospital (1989 to 1992).

J. RICHARDSON DILWORTH

Trustee Emeritus J. Richardson Dilworth died of complications from heart surgery on Dec. 29, 1997, at Princeton Hospital in Princeton, N.J. He was 81. A noted philanthropist and financier, Dilworth served RU as a trustee from 1960 to 1991. As treasurer and chairman of the university's finance and investment committee, he helped to ensure the financial health of the institution and oversaw a fourfold growth of its endowment. Dilworth played a key role in the development of housing for students and faculty, as well as the construction of new laboratory buildings that

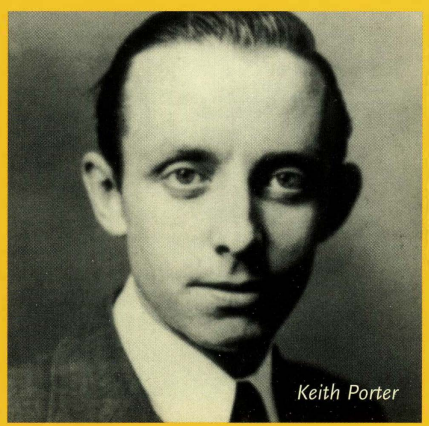


J. Richardson Dillworth

transformed the south end of campus into a modern biomedical research complex. In 1992 Dilworth was named a trustee emeritus and the following year he was awarded an honorary doctor of science degree in recognition of his outstanding service to The Rockefeller University community. At the time of his death, he was on the board of directors of AEA Incorporated, a private investment company he helped to found.

KEITH R. PORTER

Keith R. Porter, a former member and professor of the Rockefeller Institute for Medical Research who is widely recognized as a founding father of modern cell biology, died May 2, 1997. Porter joined Rockefeller as a research assistant in 1939. In 1953, he became the head of the Laboratory of Cytology, later renamed the Laboratory of Cell



Keith Porter

THE ROCKEFELLER UNIVERSITY ARCHIVE

Biology. While at RU, Porter cocreated the first electron micrograph of an intact cell, along with Albert Claude and Ernest Fullam. Later, Porter identified the cell's endoplasmic reticulum, elucidated the structure of collagen fibers, cilia and microtubules, and defined the microtrabecular lattice. His innovative methodology for the use of the electron microscope, design of the Porter-Blum microtome and techniques for tissue culturing opened up the field of cell biology by allowing much greater insight into the structure and function of cells.

In 1955, Porter founded the *Journal of Cell Biology*, which is published by the RU Press. He founded the Tissue Culture Association in 1946, and in 1961 he cofounded and became the first president of the American Society for Cell Biology. Porter was a member of the National Academy of Sciences and received the National Medal of Science. After leaving RU, Porter established laboratories of cell biology at Harvard University, the University of Colorado and the University of Maryland. He was a professor emeritus at the University of Pennsylvania when he died.

NANCY DICKERSON WHITEHEAD

RU Council member Nancy Dickerson Whitehead died at age 70 on Oct. 18, 1997, of stroke complications at New York Hospital. The wife of RU Trustee John Whitehead, she also served on the boards of Covenant House, the Hospital for Special Surgery and the New York Public Library and was a member of the Central Park Conservancy.

For continually updated information about RU, visit our web page: http://www.rockefeller.edu/.

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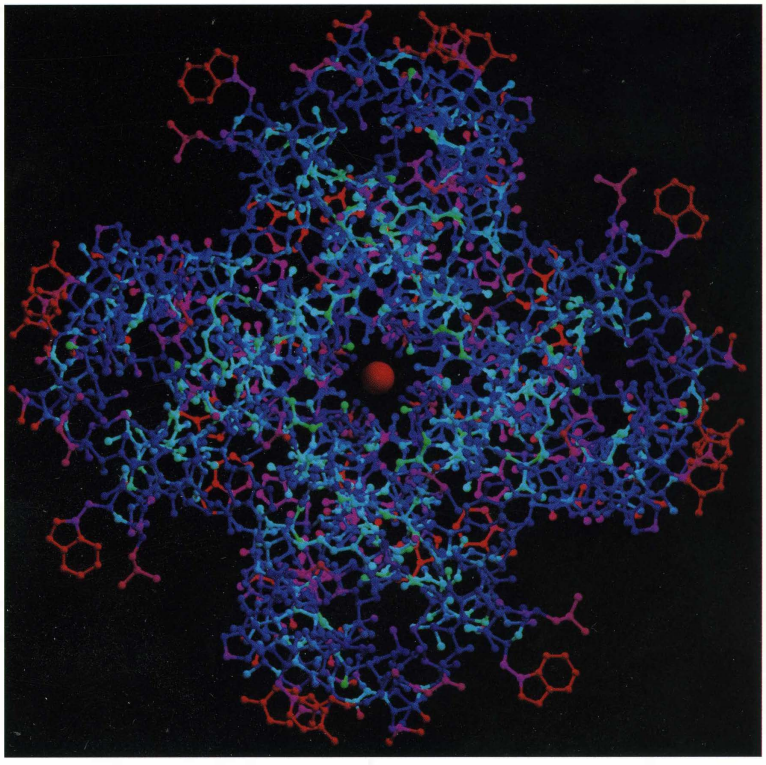

A ball-and-stick model of a potassium ion channel from the bacterium Streptomyces lividans *shows a potassium ion (red sphere) inside the pore. The protein is comprised of four identical subunits that join together like the staves of a barrel around the central pore.*
RODERICK MACKINNON