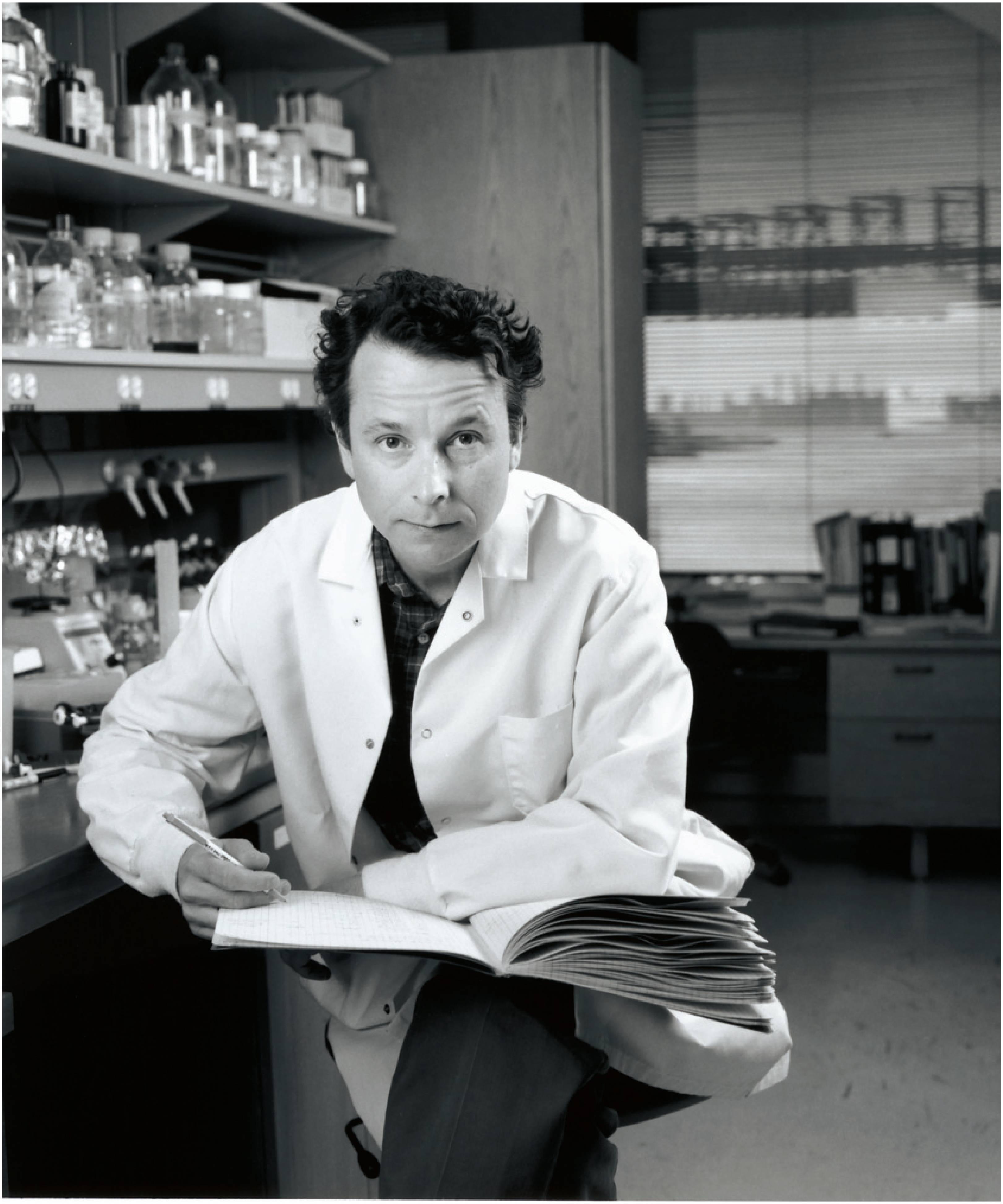


*His face etched with characteristic intensity, Roderick MacKinnon seeks answers to the ion channel's unsolved questions.*

ROBERT RATHE

• When Roderick MacKinnon abruptly switched research methods in midcareer, colleagues feared for his sanity. Vindication came in the form of a Nobel Prize. *By Richard Saltus*

# Courage and Convictions

NESTLED AMONG MAPLES AND OAKS just off a suburban Boston thoroughfare, Trout Pond is a small, serene getaway for lunch-hour wanderers, young lovers, and fishermen. “It’s a good place to come and get your head straight,” says an angler, waiting for a bite on a sunny fall morning.

It was in that spirit that biophysicist Roderick MacKinnon came to the pond one day in 1995 to walk and talk with his longtime scientific mentor as he wrestled with a pivotal career decision. MacKinnon, who at the time ran a lab at Harvard Medical School, studies ion channels—tiny doughnut-shaped pores that penetrate the membrane that surrounds living cells. They permit ions—charged atoms of potassium, sodium, chloride, and calcium—to flow across cell membranes, thereby generating electrical signals. Ion channels are fundamental to health and to normal function of the human body; their impulses create the sparks of the brain and nervous system, allowing us to walk, talk, fall in love, and, for example, cast a fishing line with accuracy. Conversely, they may malfunction, and can then cause a variety of diseases.

Building on decades of clever observations by their predecessors, MacKinnon and others had been inching toward a deeper understanding of how the pores performed their feats of exquisite discrimination among ions and responsiveness to minute changes in their environment—enabling the cell membrane to suddenly become permeable, but only to highly specific types of ions.

But now, though the genes behind the channel proteins had been cloned, which gave scientists new traction on the problem, channel aficionados were still struggling. HHMI investigator Christopher Miller, a Brandeis University biochemist and the mentor who circled the pond with MacKinnon that day, explains: “The field had been on a roll, but now its researchers were hitting a wall. The tools were too blunt to tackle the fine details, and people were afraid that the bonanza of discoveries was slowing down.”

So he lent an empathetic ear as MacKinnon described a bold though risky strategy he believed might break the impasse. The younger scientist would abandon the biophysical and biochemical methods that had advanced the field thus far, give up his thriving lab and his secure Harvard professorship, and move to the Rockefeller University in New York City. There, he would bet his scientific stake on x-ray crystallography, a powerful visualization technology for determining the three-dimensional arrangements of atoms in the channel proteins. Such a map would make clear for the first time how a pore’s filters and gates worked. But many of his colleagues were highly skeptical. For one thing, MacKinnon was practically a novice in x-ray crystallography. While at Harvard, he had gotten a leg up through voracious reading, taking a graduate course, and picking the brains of colleagues in the lab of HHMI investigator Stephen C. Harrison, a structural biologist at Harvard Medical School. Still, he was a long way from being a master of the method.

Then there was the personal risk. Miller feared that MacKinnon had been seized with a death wish. "I thought he would torpedo this wonderful career that was going so well," says Miller, who remembers the dialogue as "pretty intense." Finally, he says, "I told him he was out of his mind."

## • GOING FOR BROKE

Miller argued forcefully that MacKinnon should keep half of his Harvard lab while pursuing the x-ray path, instead of leaping into an all-or-nothing situation. But that was not MacKinnon's way. "My problem is that I'm kind of passionate about things, and I go for broke when I make a decision to do something," he says. As for the crash course in x-ray methods the new venture would require, "I really love learning new things and doing them myself."

Not that he wasn't a bit worried. "I could imagine people saying, 'Whatever happened to that MacKinnon guy? He got this harebrained idea and that was the last we ever heard from him.'" Nevertheless, against Miller's advice, he assented to the energetic recruiting of Rockefeller's then-president, Torsten N. Wiesel, and left Harvard in 1996.

"Perhaps I could have done this work at Harvard," MacKinnon reflects. "But I felt a change of environment would help, and Rockefeller struck me as a good place to concentrate on a long-term problem."

MacKinnon and his small x-ray crystallography lab hit the ground running. By winning an appointment in 1997 as an HHMI investigator—he was one of 70 selected in an intense national competition that drew 370 applicants—MacKinnon gained secure funding that "absolutely" helped his lab move even faster. And then, to the surprise of the doubters, MacKinnon's gamble not only paid off, but paid off quickly. In April 1998, his team reported in two landmark papers in *Science* that they had obtained the first high-resolution three-dimensional atomic structure of a potassium ion channel, prompting one researcher to call it "a dream come true for biophysicists."

Only five years later came the ultimate vindication of MacKinnon's out-of-the-box creativity and persistence in the face of high risk: He shared the 2003 Nobel Prize for Chemistry with Peter C. Agre of the Johns Hopkins University School of Medicine (and a member of HHMI's Scientific Review Board), who discovered water channels in cells.

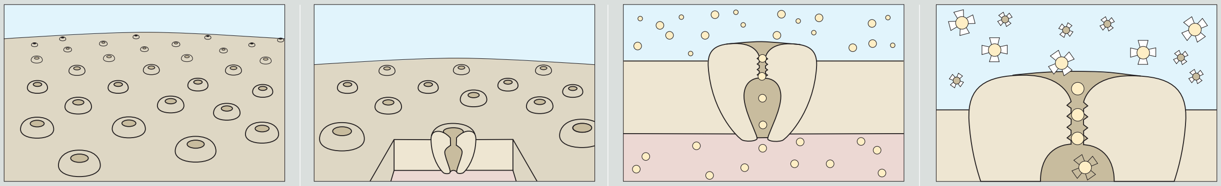
*"I could imagine people saying, 'Whatever happened to that MacKinnon guy? He got this harebrained idea and that was the last we ever heard from him.'"*

That MacKinnon was awarded the prize so soon after his initial breakthrough underscores the importance of the field and the decisive effects of his findings. "Roderick MacKinnon's successful exploitation of x-ray crystallography signals the dawning of a new era in the study of ion channels and the diseases associated with them," says Rockefeller University President Paul Nurse. "His total dedication led to the spectacular achievement of the first four high-resolution structures of ion-selective channels—a notable advance."

All who have been associated with MacKinnon are impressed with his probing intellect and lucid descriptions of his findings. The Nobel "is appropriate recognition for the beauty of MacKinnon's science and the clarity with which he expresses the biological phenomenon," said HHMI President Thomas R. Cech. "MacKinnon's work is exquisite, but he also makes the data come alive with very thoughtful and clear explanations."

MacKinnon's Nobel serves as a touchstone for those who feel that academic science fosters a play-it-safe mentality at the expense of creative risk-taking. Harrison, the Harvard structural biologist, sees the Nobel as a fitting tribute to MacKinnon, who, he says, "is one of those outstanding and independent scientists whose ambitions are driven not by career considerations but by the inner need to discover something." As an HHMI investigator himself, Harrison says the Hughes support "encourages you

## What Is a Potassium Ion Channel?



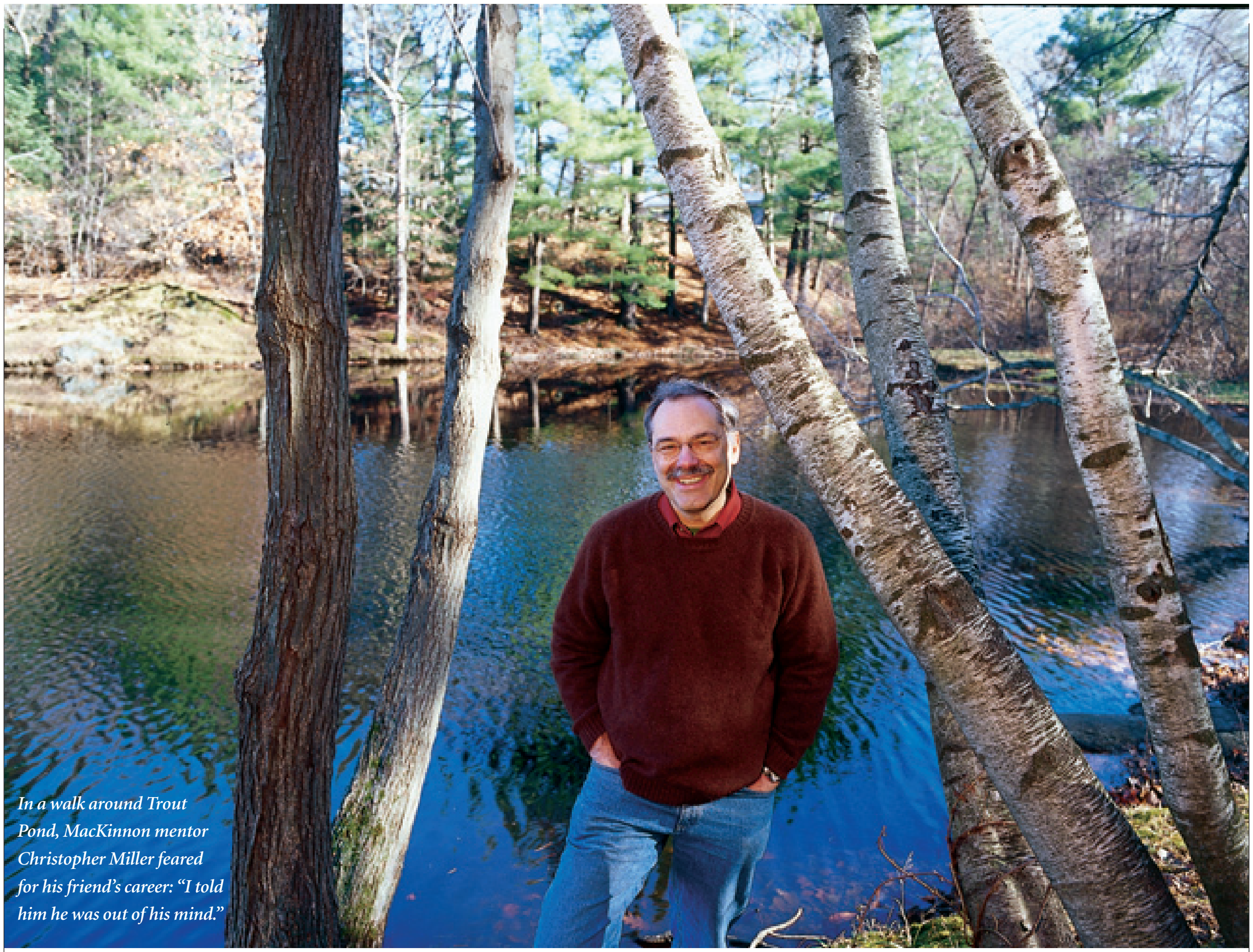
*Specialized proteins called ion channels move electrical signals across a cell surface, turning a thought into an action.*

*Each type of channel has its own particular configuration. The type shown above is specific to a potassium ion.*

*Ion channels work according to the power of diffusion. But they must be selective and diffuse only those ions that move the signal.*

*Because of the selectivity filter within the channel, only potassium ions readily move through, while smaller sodium ions do not.*

ADAPTED FROM A DIAGRAM BY THE ROCKEFELLER UNIVERSITY/OFFICE OF COMMUNICATIONS AND PUBLIC AFFAIRS



*In a walk around Trout Pond, MacKinnon mentor Christopher Miller feared for his friend's career: "I told him he was out of his mind."*

ASIA KEPKA

to take risks" to a much greater extent than does government funding. "It's no wonder [HHMI] has managed to capture scientists who are doing some of the most exciting work in their fields."

## • EXPLORING THE UNKNOWN

MacKinnon, now 47, was born in Burlington, Massachusetts, a northern suburb of Boston, and was part of a family of seven children. From a young age he delighted in the marvels of nature and was captivated by the prospects that opened to him when, in a high school summer science program, he was allowed to take a microscope home. "I loved watching the tiniest things swim and move," he says.

MacKinnon, who retains the compact build of the gymnast that he was in high school, believes the sport has helped his scientific career as well; he specifically credits his coach with instilling discipline and the lesson that hard work yields big payoffs. A deep vertical furrow between his eyebrows has been etched by years of fierce concentration—by all accounts, one of MacKinnon's foremost traits; another is his voracious curiosity. Encountering ion channels for the first time as Miller's student at Brandeis, he recalls, "They intrigued me. I didn't know much about them. I liked physical science, and they were carrying an electric current, and that appealed to me."

After graduating from Brandeis in biochemistry, MacKinnon entered medical school at Tufts University, also near Boston. When he obtained his M.D., he went on to complete a residency in internal medicine at Beth Israel Hospital in Boston, where colleagues say he was an excellent physician. But MacKinnon felt too strongly the pull of exploring fundamental scientific problems. So in 1986, he returned to Brandeis as a postdoctoral fellow, where Miller immediately set him to work studying ion channel function. His first assignment: Find out how a toxin extracted from scorpion venom inhibits potassium channel signaling. In short order, MacKinnon determined that the toxin molecules sat down on the opening of the pore and blocked it, and he adopted the toxin as a tool to further study channel structure.

## • QUICK ON THE DRAW

The concept that electrical currents in living tissues are generated chemically by the movement of ions across cell membranes—and not by electrons in motion, as in wires—dates as far back as 1890. The existence of narrow ion channels was first proposed early in the 20th century, but it was not until the 1950s that Alan L. Hodgkin and Andrew F. Huxley developed the first detailed model of the nerve impulse based on ionic flow across the membrane. Their studies of ion transport in a squid giant axon, a cable-like nerve cell, earned them a Nobel Prize in Physiology or Medicine. Later research revealed that potassium ions move through the membrane in single file.

An electrochemical signal, or "action potential," passing along a nerve cell is generated by an exchange of ions through the cell membrane. When the nerve is at rest (not transmitting a signal), there is a greater proportion of sodium atoms outside the nerve cell and potassium ions inside it, and the inside of the neuron is negative relative to the outside. When the nerve is stimulated, the positively charged sodium ions rush into the cell, converting the negative charge to a positive one: This is called "depolarization." Shortly afterward, the potassium ions start flowing out through their channels, causing the cell to return to its polarized, negatively charged state. It is the dynamic of polarization-depolarization-polarization that creates the nerve signal.

In the 1960s and 1970s, biophysicists began to create a picture of the channels as having a funnel-shaped opening on the outside of the cell membrane, leading to a narrow channel within the membrane that projects into the interior of the cell. But researchers were still far from understanding how the channels perform their tasks so selectively. Even though a potassium ion is larger than a sodium ion, a potassium channel can allow its ions to flow through at lightning speed while excluding sodium ions almost without error. Like a doorman at a trendy nightspot waving the "desirable" patrons in while turning away the unannointed, the body has very good reasons for making such distinctions. Says Miller: "If you didn't have the selectivity filter, you could never make an electrical signal." In addition, the pores are quick on the draw, opening and closing in milliseconds in response to a variety of signals, including minute changes in voltage across the membrane.

Through the 1980s and 1990s, ion channel researchers gained much ground. For example, they developed techniques of electrophysiology, in which tiny electrodes record the voltages across a single channel while researchers manipulate variables and observe consequences. Another technique exploited with great success by MacKinnon while still at Harvard was mutating different proteins in the pore machinery and getting clues to their locations by the effects they caused. But still, no one had actually seen the components of the pores at the level of their atoms.

So MacKinnon, with his 1996 move, took up the challenge virtually alone; only one postdoc, Declan A. Doyle, came to Rockefeller with him. And, says MacKinnon, his wife, Alice, volunteered to run the laboratory out of pity.

There, MacKinnon learned the methods of x-ray crystallography and began trying to visualize proteins after using detergents to extract them from the cell membranes. "You want to keep the proteins happy, and then identify conditions under which they will crystallize," he says. By this time, several other labs had joined the quest, according to Miller—including his own at Brandeis.

But few, if any, other scientists worked with MacKinnon's relentless enthusiasm. Says Harrison at Harvard: "He's the kind of guy who gets up in the morning and if the day doesn't offer him the opportunity to get closer to knowing how a voltage-gated channel works, he's not going to be happy."

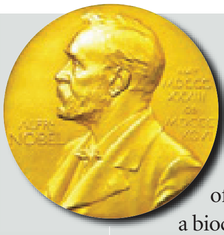
## • MYSTERIES REVEALED

Not long after he set up shop at Rockefeller, a scientific surprise came to light that would give MacKinnon a badly needed boost. Contrary to expectations, researchers studying the newly obtained DNA sequence of a bacterium discovered that it, too, contained ion channels. The finding would help the crystallography project immensely; it would be far easier to purify channels in large quantities from bacteria than from higher organisms. So MacKinnon began working with the potassium channel of a soil bacterium, *Streptomyces lividans*, using a common lab workhorse bacterium, *Escherichia coli*, to express the protein.

The effort was slow and tedious, but MacKinnon and his small team patiently tried one set of conditions after another in pursuit of success. One strategy that advanced the crystallization efforts was the replacement of potassium atoms in the proteins with heavier analogs, rubidium and cesium, which are more electron dense than potassium but still go through potassium channels. On New Year's Day of 1998

# HHMI and the Nobel Prize

HHMI investigators have won Nobel Prizes in four of the past five years. In addition to Roderick MacKinnon, six other current HHMI investigators have won the Prize:



■ **Günter Blobel**, at the Rockefeller University, won the 1999 Nobel Prize in Physiology or Medicine for his discovery that certain proteins have intrinsic signals that govern their transport and localization in the cell.

■ **Johann Deisenhofer**, at the University of Texas Southwestern Medical Center at Dallas, shared the 1988 Nobel Prize in Chemistry for work that used x-ray crystallography to describe the structure of a protein involved in photosynthesis.

■ **H. Robert Horvitz**, at the Massachusetts Institute of Technology, shared the 2002 Nobel Prize in Physiology or Medicine for discoveries that identified key genes regulating organ development and programmed cell death.

■ **Eric R. Kandel**, at Columbia University, shared the 2000 Nobel Prize in Physiology or Medicine for discoveries concerning signal transduction in the nervous system.

■ **Susumu Tonegawa**, at the Massachusetts Institute of Technology, won the 1987 Nobel Prize in Physiology or Medicine for his discovery of the genetic principle for generation of antibody diversity.

■ **Eric Wieschaus**, at Princeton University, shared the 1995 Nobel Prize in Physiology or Medicine for work to discover and classify 15 genes of key importance in determining the body plan and the formation of body segments of the fruit fly *Drosophila melanogaster*.

In addition, Institute President **Thomas R. Cech** shared the 1989 Nobel Prize in Chemistry for the discovery that RNA in living cells is not only a molecule of heredity but also can function as a biocatalyst.

HHMI investigator **Daniel Nathans**, now deceased, shared the 1978 Nobel Prize in Physiology or Medicine for the discovery of restriction enzymes and their application to problems of molecular genetics.

Two HHMI investigator alumni are also Nobel laureates. **Edwin G. Krebs**, professor emeritus at the University of Washington, shared the 1992 Nobel Prize in Physiology or Medicine for discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism. **Stanley B. Prusiner**, at the University of California, San Francisco, won the 1997 Nobel Prize in Physiology or Medicine for his discovery of prions, a new biological principle of infection.

*To the surprise of the doubters, MacKinnon's gamble not only paid off, but paid off quickly.*

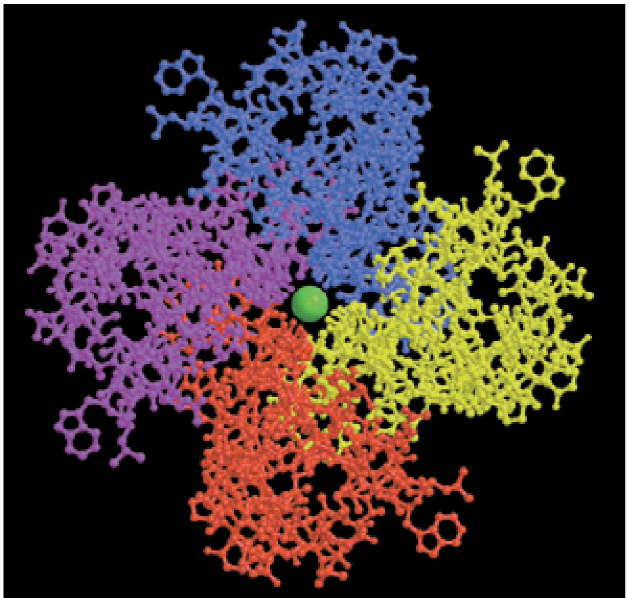
came the "aha" moment. "I could see the ions queued up inside the pore; I was just staring at three ions in a channel."

In April 1998, just over two years after they began working with *S. lividans*, MacKinnon and his team publicly revealed their capture of the holy grail. Their two papers in *Science* described the three-dimensional crystal structure of the *S. lividans* potassium channel and also showed that this bacterial channel was very similar to potassium channels in humans.

Once they determined the atomic layout of the channel, MacKinnon saw that it is shaped like a conical coffee filter. At the membrane's outer surface, the channel is wide, and tapers as it passes through the membrane to a narrow outlet into the cell's cytoplasm. The selectivity filter, held in the wide portion of the channel, has a smaller diameter than the channel itself. The pore is composed of four identical subunits, with atoms of carbonyl oxygen all pointing toward the channel's center.

This structural image helped clarify a long-standing mystery: How do the water-loving potassium ions slip through the narrow channel, which forces the ions to shed their customary retinue of water molecules? MacKinnon recognized that the filter proteins temporarily offer atoms of carbonyl oxygen as an escort—he calls them "surrogate water"—with four of the atoms above each ion and four below. When the potassium ions have passed through the filter, they release their escorting oxygens and pick up another set of their usual water molecules inside the cell. The x-ray data enabled MacKinnon to explain, for the first time, how the selectivity filter so accurately blocks sodium ions while admitting potassium ions. Sodium ions are smaller, but they cannot pass through the channel "because the selectivity filter, which is held in a very precise conformation, is more tuned for the larger potassium ion." At a press conference on Nobel day, he told reporters that "the sodium ion doesn't really fit into the 'cages' of oxygen that surround the potassium ions—it's too small." So the long-mysterious selectivity "is a matter of the detailed chemistry of the ion channel," he said.

In the years following the 1998 *Science* papers, MacKinnon was on a roll, publishing important papers describing additional structure-function discoveries. He obtained crystal structures of pores both in their open and closed configurations, suggesting a mechanism for channel "gating," or precisely regulating ion flow. (Channel gating is the mechanism by which channels open their gates in response to the appropriate stimulus—the stimulus can be either a ligand or membrane voltage.) He discovered the molecular basis for another regulatory operation, the mechanism by which an inactivation particle blocks the flow of ions through the pore by binding to the pore, called "ball-and-chain inactivation." And most recently, he took a significant step toward understanding one of the field's most stubborn mysteries: the mechanism by which a voltage-gated channel can sense very small changes in voltage across the membrane. It appears that the voltage change is the stimulus for the chemical signals that enable channel gating.



*This image shows the four-fold axis of the KcsA potassium channel from the bacterium* Streptomyces lividans. *Each of the channel's four identical subunits is a different color. The center of the channel holds a potassium ion, which is shown in green.*

RODERICK MACKINNON

## • WALKING THE WALK

Even as he prepared to go to Stockholm to accept his award, MacKinnon continued his research at an intense pace. He says he wants to dig deeper into the ion channel questions that remain unanswered. Yet, he admits to have begun thinking of the next big problem to attack—and in all likelihood, it will not be ion channels. "It makes me nervous," he said a few days after the Nobel Prize announcement, "when people say you get less creative as you get older, or you have less energy. I'm hoping that if you change what you do, so you are naive again, you'll remain youthful."

MacKinnon won't share his thoughts just yet about which scientific Mount Everest he intends to climb. His only immediate plan, he said at a press conference on the day the award was announced, was to shop for "a nice sea kayak."

En route to winning the Nobel, MacKinnon was selected for an Albert Lasker Basic Medical Research Award in 1999. At that time, the Lasker Foundation arranged to have him interviewed by Christopher Miller, who had strenuously played devil's advocate that day in 1995 at Trout Pond. The chastened Miller of four years later now referred to MacKinnon's career gamble as "almost a mythic, heroic change into crystallography."

Moreover, he's willing not only to talk the talk but to walk the walk. Miller spent several months in 2003 on sabbatical in his former student's lab at Rockefeller, where he worked on, of all things, x-ray crystallography. "I had a lot to learn from him," Miller explains.

Did he feel awkward—the mentor now turned student? "Not at all," responds Miller. "You don't have ego problems with your children." H