

Cells



Combining aesthetics with shrewd science, Roger Tsien found a better way to look at cells—and helped to revolutionize several scientific disciplines.

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PHOTOGRAPHS BY JOE TORENO

INSIDE AN INSTITUTIONAL-GRAY scientific laboratory, it can be startling to find brilliant, paintlike fluorescent colors. But in one San Diego lab, bacterial colonies in bright hues of green, blue, magenta, yellow, and orange dot agar plates and linger in discarded microcentrifuge tubes. This eccentric biological palette is the work of HHMI investigator Roger Y. Tsien, and his “studio” is a pharmacology lab at the University of California, San Diego (UCSD).

“I like pretty colors,” Tsien says. His casual comment belies the fact that his artistic sensibilities—combined with shrewd scientific instincts—helped foment a revolution in cell biology and neurobiology.

Tsien is renowned for having created colorful dyes to track the movement of calcium within live cells—and without having to poke holes in them, the traditional way to do such tracking. Tsien also engineered the jellyfish green fluorescent protein (GFP) to glow more brightly in the visible part of the spectrum and created color variants in brightly fluorescent yellow, blue, cyan, yellowish green, orange, and red. These multicolored fluorescent proteins (FPs) aren’t just for making pretty pictures—although Tsien’s students and postdocs have been known to draw with them—but are more like a set of molecular

biologist’s crayons. They can be used to monitor gene expression or see biological processes inside living cells, and having more than one color available means that scientists can study more than one interacting process at a time.

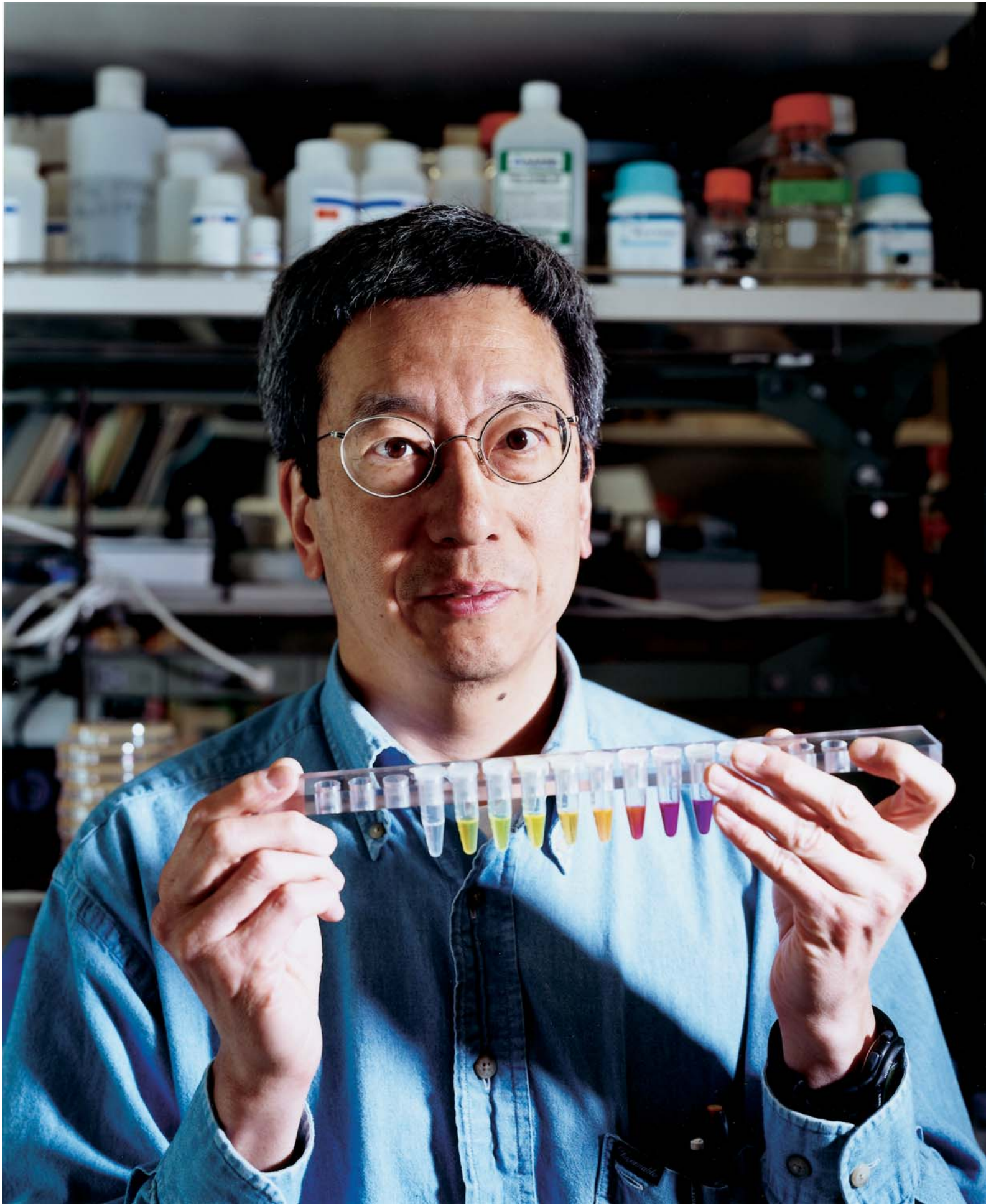
“GFP has revolutionized the fields of cell biology and neurobiology,” says Jennifer Lippincott-Schwartz, who heads the section on organelle biology at the National Institute of Child Health and Human Development at the National Institutes of Health. Tsien, she says, “stands out as probably the single person who most facilitated that revolution through his techniques, his insights, his contributions—in terms of creating reagents and showing how they can be used creatively to address important questions.”

Tsien’s contributions haven’t gone without official notice. In January, he was awarded the prestigious Wolf Prize in Medicine for his “seminal contribution to the design and biological application of novel fluorescent and photolabile molecules to analyze and perturb cell signal transduction.”

“DOOMED BY HEREDITY”

Tsien has always appreciated color, not just for its scientific potential, but also in a sensual way. He is drawn more to Henri Matisse’s vivid colors, for instance, than to the brown, gray, and black tones of Georges Braque in his cubist phase. Imaging with pretty colors has always been close to his heart. “Your science should ideally feed the deeper parts of your personality, to pro-

Roger Tsien created dyes to track the movement of calcium within live cells.



vide some intrinsic pleasure to tide you over the inevitable periods of discouragement,” he says.

Born in New York City in 1952, Tsien grew up in Livingston, New Jersey. His family was chock full of engineers. Tsien’s father was a mechanical engineer. His mother’s brothers were engineering professors at the Massachusetts Institute of Technology. Tsien, who calls his own work *molecular* engineering, says, laughing, “I’m doomed by heredity to do this kind of work.”

His father’s cousin Tsien Hsue-Shen was a rocket scientist and professor at the California Institute of Technology until he was accused in 1950 of being a Communist. Put under house arrest for five years, he was finally deported to China, where he subsequently fathered that country’s ballistic-missile program.

Roger became interested in the chemistry of pretty colors as a youth—partly because his older brothers weren’t. “Younger siblings tend to try wild and crazy things because older siblings occupy certain ecological niches,” Roger says. His brother Richard (known as Dick)—seven years older and now a noted cellular physiologist at Stanford University School of Medicine (and member of HHMI’s scientific review board)—recalls that Roger had asthma as a child and was often obliged to stay indoors while his two older brothers were out doing sports. Roger studied and performed chemistry experiments in the basement, one time charring a table-tennis table in a gunpowder experiment gone awry.

But for the most part, he put the time to productive use. At age 16 he won first prize in the Westinghouse Science Talent Search with a project investigating how metals bind to thiocyanate. With a National Merit Scholarship, he attended Harvard College, graduating at age 20 with a degree in chemistry and physics.

A Marshall Scholarship then took him to the “other” Cambridge, in the United Kingdom, where he earned a Ph.D. in physiology and stayed on to complete a postdoctoral fellowship.

CHEMIST INTERLOPER

“Dick pointed out that neurobiology was the cardinal unsolved problem of all biology and perhaps philosophy,” Roger Tsien recalls. For Roger, certain mental lightbulbs began to flash when “I found out that chemistry could be applied to neurobiology.”

Dick recalls that Roger didn’t have an easy time in Cambridge: “His attempts at always doing things in a chemical way seemed a bit strange to them, but I think it was the beginnings of the realization that his chemical knowledge could be of great importance to biology.”

When life-science experiments require a new biological macromolecule, says Roger, biologists will just go out and make it. “But if it’s a chemical molecule you can’t order from a catalog, they usually figure ‘Well, forget about it. Let’s find another way of doing the experiment or scrap it altogether.’” The fear and loathing that most biologists have for chemistry, he says, “creates a niche for those of us who are willing to do it.”

As a graduate student, Roger started to develop a better indicator dye for intracellular calcium, which is an important messenger in numerous biological systems. It plays a critical role in neuronal regulation, muscle contraction, and fertilization, just to cite a few examples.

In those days, the only way to measure calcium inside a cell was to use

microelectrodes or inject through the cell’s membrane a luminescent calcium-binding protein called aequorin, which comes from jellyfish and glows when it binds calcium. But those techniques had several disadvantages, including having to work with big, sturdy cells, and only one at a time.

Tsien thought there ought to be a method for measuring calcium that is less damaging to cells. He developed organic dyes that twist their necks when they bind calcium. Such twisting drastically changes the dyes’ fluorescence or ability to re-emit light of a different color. Equally important, he found a temporary masking cloak to help the molecules sneak across a cell’s membrane, only binding and reporting calcium once they shed the cloak inside the cell—kind of like an army going over a wall in the middle of the night and then springing into action.

The practical result: no more injections, and the ability to work with all kinds of cells, including small cells. For the first time, scientists could study calcium easily inside a plethora of live cells. Tsien’s career—of using chem-

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istry to devise methods to study what biologists had only been able to study indirectly—thus began in earnest. Nevertheless, the British government decided not to pursue any patents, deciding that calcium inside cells was insufficiently important. The same agency had just turned down monoclonal antibodies, which subsequently earned other Cambridge scientists a Nobel Prize and launched a major segment of the biotechnology industry.

Tsien landed a job at the University of California, Berkeley, in 1981, where he spent the next eight years developing and applying better dyes for calcium and other ions. The University of California was eager for patents, so Tsien got his first in 1986, and now holds more than 60.

But by that time, Tsien wanted to “get out of the calcium box,” he says. “The problem with calcium is that it sounds too chemical, it’s a small inorganic ion, and famous biologists, especially at Berkeley, were very snooty about calcium.” They thought ions were insignificant compared to the really important biological molecules such as DNA, RNA, and proteins.

WORKING THE GOLDMINE

So Tsien turned to studying the next-most-important universal messenger inside cells—cyclic AMP. At that time, there was no means of imaging it in a live cell; the state of the art was to grind up cells in order to isolate the cyclic AMP contained within.

Tsien and his colleagues eventually figured out that they needed to hijack a protein that was a natural sensor for cyclic AMP—in particular, cyclic-AMP-dependent protein kinase (PKA). That realization led Tsien to move his lab in 1989 to San Diego in part to be near biochemist Susan S. Taylor, an HHMI investigator at UCSD who is an expert on PKA. By attaching a fluorescent dye to PKA, the Tsien and Taylor labs eventually made a protein that changed color when it bound cyclic AMP.



This was a major advance over destroying cells. But the protein still had to be injected. “It was the dissatisfaction with having to make the protein in bacteria, put fluorescent dyes on it, purify it, and inject it back into cells—and then only into big cells—that led me to think we desperately needed a way to encode the fluorescent indicator by genetics,” says Tsien. Tsien realized that there was a need to have cells produce fluorescent markers directly, by molecular biology. From his work with organic calcium dyes, Tsien was familiar with the literature of the competing dye, the protein aequorin. He vaguely remembered a contaminant of aequorin, a naturally green fluorescent protein. “I typed ‘green fluorescent protein’ into MEDLINE and was amazed that somebody had just cloned it,” Tsien recalls. This was biologist Douglas Prasher, who cloned GFP in 1992.

Tsien telephoned Prasher, who was working at the Woods Hole Oceanographic Institute. Prasher offered to give Tsien the clone. He had run out of funding and wasn’t planning to work on GFP any more. “He was sit-

ting on a goldmine,” Tsien says, “but he had run out of steam and resources just short of the finish line.”

Prasher warned, however, that there wasn’t yet any evidence that any organisms other than jellyfish knew how to make GFP.

One other person, Martin Chalfie, at Columbia University, noticed the GFP clone at just about the same time. “I was the first to ask Prasher,” Tsien says, “but Marty was ready to start working first.” Tsien didn’t even have a molecular biologist in his lab and had to wait until a new colleague, Roger Heim, arrived from Switzerland.

Chalfie soon demonstrated that other organisms—in his case, *Escherichia coli* and *Caenorhabditis elegans*—could, in fact, make GFP just like jellyfish; the protein doesn’t need any special enzymes or cofactors to make it glow. The gene sequence for GFP can be inserted into an organism’s genome and butted up against the sequence for almost any protein a biologist wants to study. When the organism expresses that protein, it does so with a fluorescent tag attached to it—like a reindeer with a glowing nose. All the biologist has to do is follow the glow to find the protein.

Early GFP was difficult to see. Heim—following some suggestions from Tsien that Tsien calls “misguided”—modified the amino acid sequence of GFP to successfully improve its visibility. The lab eventually produced user-friendly fluorescent proteins of different colors ranging from blue to red. Most applications of fluorescent proteins now use versions pioneered by the Tsien lab.

The result of this work is simple in some respects, but its overall impact on science has been extraordinarily powerful: Researchers using FP tags can now see inside live cells with a light microscope and watch molecular processes in motion. And they can easily track where and when certain genes are expressed in cells or even in whole organisms.

CONSPIRACY DETECTION

Although Tsien was instrumental in helping to make GFP the incredibly useful molecular-biology tool it is today, he is conscious that nature made the protein in the first place and that he played what he considers the relatively minor role of tuning it up for research.

“In a way it’s like somebody who turns an obscure novel into a popular film,” Tsien says. “The basic idea didn’t come from us, but we maybe helped lots of people appreciate the stuff that wasn’t quite as easy to appreciate in its original form.”

Tsien’s modesty aside, GFP is a blockbuster. MEDLINE indexes only half a dozen papers on “green fluorescent protein” before 1992. And a search for papers with the acronym “GFP” in 1990 and 1991 turns up only nine papers—in which GFP stands for six different things, from “gonadal fat pads” to “Ghanaian traumatic fracture patients,” but not “green fluorescent protein.”

Prasher published the sequence of GFP in 1992, and Tsien reported his blue version two years later, in 1994—the same year that Chalfie published research using GFP as a marker for gene expression. Since then, more than

14,000 papers have been published that mention green fluorescent protein or its acronym GFP.

With GFP and other agents, Tsien's particular blend of biological insight and chemical and physical knowledge has allowed him to play a unique role. For example, he wasn't content merely to expand GFP's color palette. Like a skillful painter who achieves a new shade with a careful blending of hues, Tsien engineered two colors to illustrate what no single color could represent on its own.

If one protein is tagged with cyan GFP and another with yellow GFP, a researcher using a light microscope is able to follow the cyan and yellow fluorescence and learn whether the proteins have arrived in the same general vicinity. But to see whether the proteins are actually interacting with each other—which is far beyond the resolution of a light microscope—something much more sensitive is needed.

Tsien uses the analogy of electronic monitoring bracelets for criminals. If, say, the spatial resolution of the bracelets was half a mile, a monitor could tell whether two criminals were in the same vicinity but not whether they might be conspiring. However, if their bracelets interacted with each other in a special way when the criminals got to within a few feet of each other, then a signal could be sent back to headquarters.

"Zing! Conspiracy!" says Tsien.

That special signaling is exactly what happens when the two different colors of GFP overlap. In a quantum-mechanical handoff of energy, one GFP absorbs light of a certain wavelength and transfers the energy to the other GFP, which emits light of a different wavelength. So, for instance, light that would ordinarily cause a GFP to glow cyan makes it glow yellow instead. Anything in the biochemistry of a cell that changes the distance between the GFPs, or their relative orientation, sends a conspiracy signal back to headquarters.

Tsien says one good thing about prizes—such as his Wolf Prize—is that it allows the honoree to tie a bow around an old research area and have the confidence to move into a new field.

Viols with a variety of fluorescent proteins.



Tsien does acknowledge that FPs sometimes have their downsides. "There are times when GFP is too big, and it really does mess up the protein you put it on," he says. "Sometimes you have to switch to something completely different." To that end, he says, "we have several alternatives to GFPs, including tags that are much smaller."

Apart from fine-tuning FPs, though, Tsien also has his eye on some new research directions.

NEXT TARGET: CANCER

The chemical technique by which two differently colored fluorescent proteins emit that special signal when they are in close proximity to each other is called fluorescence resonance energy transfer (FRET). An early application of FRET that Tsien explored was monitoring the action of enzymes, called proteases, that cleave proteins. In a short sequence of amino acids, he bound two FPs together so that they showed the FRET signal. When the amino acid chain was cleaved by a protease, the signal went away. Nearly any biochemical signal that can shift one FP relative to another can now be monitored inside living cells.

Aspects of this work appear to have given Tsien new ideas to dream about. Cancer cells, unlike normal cells, have protein cleavers on their surfaces. These proteases help cancer cells break down connective tissue that would otherwise prevent them from escaping and metastasizing.

Tao Jiang, an HHMI research associate in Tsien's lab, has built peptide molecules basically shaped like horseshoes. One end of the "U" likes to enter cells and carries a payload—such as an imaging molecule or a chemotherapeutic agent. The other end covers up the first, preventing the payload from sticking to or entering cells. That is, until a cancer cell's protease cleaves the



bottom of the U, unleashing the sticky half with the payload to enter the nearest cell.

"When you have really sticky tape, it usually comes with nonstick backing paper so that you can handle it," Tsien says. "It doesn't stick down until you peel the halves apart." Similarly, the peptide carrying the radioactive isotope or the cancer drug won't stick to normal tissue but only to cancerous tissue. "The dream is that in the morning, we would put a tiny tracer dose into the cancer patient" and

then look to see where it stuck. "If you see a good image, then in the afternoon you come in with a bigger dose" of the toxic agent that's designed to kill the cancer.

Far in the future, Tsien imagines, doctors might use as a payload a sensitizing agent that leaves cancer cells susceptible to X-rays or neutrons. Healthy tissue, however, which lacks the sensitizing agent, is left intact even though it's exposed to the beam.

In this pursuit, Tsien is conscious of his limitations—for one thing, he has not yet done clinical experiments in his lab. "Obviously, cancer has defeated an awful lot of researchers," he says. "I'm very conscious that there's an enormous failure rate, but one still has to try."

Tsien says one good thing about prizes—such as his Wolf Prize—is that it allows the honoree to tie a bow around an old research area and have the confidence to move into a new field. "It's very hard to give up an area in which you are one of the world's experts and try something where you are like a graduate student again," he says. "But we're having a try, it's fun, it's new, and we'll see." **11**