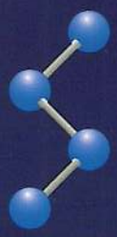
VOLUME SIX | NUMBER TWO



THE SCRIPPS RESEARCH INSTITUTE

Endeavor

Fall 2003

Chemical Biology Issue

From the origins of life to potential new approaches to cancer, vision loss, and Lou Gehrig's disease

Endeavor

VOLUME SIX | NUMBER TWO

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This issue of ***Endeavor*** magazine features work at The Scripps Research Institute in chemical biology — the burgeoning field of discovery at the intersection of chemistry and biology. As illustrated in the following articles, TSRI scientists pursuing an interdisciplinary approach have brought us new insights on topics from the origins of life to potential new approaches for medical problems such as cancer, vision loss, and Lou Gehrig's disease.

featured

also

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Chemical Turns Stem Cells into Neurons

A group of researchers from The Scripps Research Institute (TSRI) and the Genomics Institute of the Novartis Research Foundation (GNF) have identified a small chemical molecule that controls the fate of embryonic stem cells.

"We found molecules that can direct the embryonic stem cells to [become] neurons," says Sheng Ding, who recently completed his Ph.D. work at TSRI and is now an assistant professor at the institute.

Peter Schultz, Ph.D., TSRI professor of chemistry and Scripps Family Chair in TSRI's Skaggs Institute for Chemical Biology, adds, "This is an important step in our efforts to understand how to modulate stem cell proliferation and fate."

Reference: *PNAS*, 100, 7632-7637 (2003).

Scientists Identify a Protein Channel that Mediates the Body's Ability to Feel Frigid Temperatures

A team of scientists from TSRI and GNF have identified and isolated a novel protein that mediates the body's ability to sense cold through the skin. The group describes the "ion channel" protein, called ANKTM1, which is the first noxious (painful) cold receptor identified, and may be an important basic target for pain-modulating drugs.

Despite the fact that researchers at several other laboratories had previously identified receptors that sense hot temperatures, warm temperatures, and cool temperatures, the protein that detects cold temperatures had been conspicuously absent.

"This was one of the remaining puzzles," says TSRI Assistant Professor of Cell Biology Ardem Patapoutian, Ph.D., who led the effort with TSRI Research Associate Gina Story, Ph.D.

Reference: *Cell*, 112, 819-829 (2003).

News Flashes
New Researchers
Award Announcements
And More

At the Forefront

Researchers Solve Cholera Protein Structure — a Target for Vaccines and Antibiotics

Researchers from TSRI have solved structures of a bacterial protein called pilin, which is required for infection by pathogens that cause human diseases like meningitis, gonorrhea, diarrheal diseases, pneumonia, and cholera.

The group, led by TSRI Professor John Tainer, Ph.D., a member of the Skaggs Institute, reports two key structures of these pilins and discoveries about their assembly into fibrous "pili." This work directly focuses on two pathogens—*Pseudomonas aeruginosa*, which causes severe lung infections in cystic fibrosis patients, AIDS patients, and other immunocompromised individuals, and *Vibrio cholerae*, which causes cholera, a potentially fatal diarrheal disease that primarily afflicts people in developing countries.

Reference: *Mol. Cell*, 11, 1139-1150 (2003).

Floods in India's Orissa State during the summer of 2001 resulted in an outbreak of cholera. The state reported over 34,000 cases of diarrhea, including 33 deaths.

A New Type of Vaccine Against Nicotine Addiction

Scientists at TSRI have designed a new way to make vaccines against drugs of abuse that could become a valuable tool for treating addiction by helping the body clear the drug from the bloodstream.

The latest vaccine they created using this approach induces the body to clear nicotine.

"These new vaccines greatly suppress the reinforcing aspects of the drug," says TSRI principal investigator Kim D. Janda, Ph.D., who holds the Ely R. Callaway, Jr., Chair in Chemistry and is an investigator in TSRI's Skaggs Institute for Chemical Biology. "Blocking it before it gets to the brain—that's the key."

Once Janda, Research Associate Michael M. Meijler, Ph.D., and other members of the team had shown the vaccine's effectiveness in laboratory models, they went on to reformulate the vaccine for investigation for use in human trials. Eventually, this sort of vaccine could be given to people undergoing smoking cessation programs to aid in their recovery.

Reference: *J. Am. Chem. Soc.*, 125, 7164-7165 (2003).

According to the Centers for Disease Control and Prevention, tobacco use is the leading preventable cause of death in the United States.

Scientists Show that Rare Genetic Mutations Increase Susceptibility to Sepsis

A group of TSRI researchers has discovered rare genetic mutations in a subset of people who come down with a particular kind of severe sepsis, an acute and often deadly disease.

These rare mutations in a human gene called *TLR4* lend susceptibility to meningococcal sepsis, which strikes more than 2,500 people a year in the United States. "It's a very fast-moving, dramatic, and often fatal disease," says TSRI Immunology Professor Bruce Beutler, M.D., who led the research.

Besides demonstrating that the risk of severe sepsis increases with these mutations, which can be passed from parent to child, the study also suggests that it may be possible to protect people who are at risk. While not practical at the moment, eventually patients with mutations to their *TLR4* genes might be given prophylactic treatment, for instance, before they undergo surgery or travel where they are likely to be exposed to meningococcal bacteria.

Reference: *PNAS*, 100, 6075-6080 (2003).

"My research is typically focused on finding new ways to approach medical problems by controlling genes, developing new proteins that could be drugs, or working out new chemistries that could help produce new drugs."
— Carlos Barbas III, Ph.D.

Patchwork

Carlos Barbas III Pieces Together Diverse Research in Chemical Biology

A common thread runs through the various research projects of Professor Carlos Barbas III, although it may not be visible at first glance. Barbas, a 38-year-old Ph.D. who holds the Janet and W. Keith Kellogg II Chair in Molecular Biology at The Scripps Research Institute (TSRI) and is a member of TSRI's Skaggs Institute for Chemical Biology, readily admits that his research is more diverse than most. His lab's current menu of projects ranges from the creation of zinc finger proteins used for uncovering and activating genes to inventing new chemical processes in organic chemistry.

The thread that holds his seemingly disparate areas of research together is much like the sturdy, yet nearly invisible seams of an intricately crafted patchwork quilt, the result of the driving creativity of someone who, from the moment he thought seriously of becoming a scientist, wanted to create new drugs that would help patients.

And, as he first thought of becoming a scientist when he was around five years old, that thread has been growing for some time.

"My research is typically focused on finding new ways to approach medical problems by controlling genes, developing new proteins that could be drugs, or working out new chemistries that could help produce new drugs," Barbas says. "While I was classically trained as a chemist, I was also very interested in biology. But instead of switching from one field to another, I decided to maintain my ties to each."

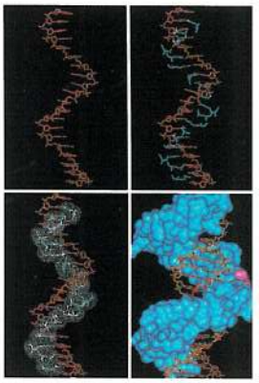
Barbas's quilt takes bits from each of these disciplines, selecting and arranging them to suit his own highly personal and remarkably productive approach to research.

"The way we do molecular biology is built on the methods organic chemists use," he says. "We approach problems by construction, by producing new molecules and proteins, and by inventing new procedures and technologies—some of which are now used in the pharmaceutical industry to create new drug candidates."

One of Barbas's first constructions employed a "phage display" approach to help identify and uncover the properties of proteins. A bacteriophage is a virus that infects only bacteria. In the process of phage display, new genetic material is inserted into the phage gene, producing an easily readable new protein displayed on the phage surface—in reality, amounting to accelerated and directed protein evolution. Barbas first applied this approach to developing human antibodies. The technology is now widely used, and a number of new drugs have found their way into the clinic faster than previously possible as a result. Later, Barbas and his colleagues used this approach to create new types of zinc finger proteins, commonly occurring proteins that bind to DNA. Barbas strung his novel proteins together to create "polydactyl" zinc finger proteins to not only bind specific genes but, more intriguingly, to actually turn any gene in the human genome on or off.

The fact that this type of directed protein evolution can be designed for specific disease targets makes it ideal in accelerating the development of therapeutics for a variety of diseases, including cancer and AIDS, two areas where Barbas's attention is currently focused.

"I wanted the freedom to explore and create across many different scientific areas—that's the main reason why I'm at TSRI and not working at a university or in the pharmaceutical industry," Barbas explains. "TSRI gives me that kind of freedom. I'm interested in the fundamental underpinnings of the world around us, basic research, but I look for ways to take new insights into nature's workings and apply them to human health. My dream has always been to have an impact on a significant disease."



The Barbas lab uses an approach to molecular biology built on the methods of organic chemistry. Above, views into four different stages in the design and construction of a gene switch.

AN EARLY START

That particular dream has held Barbas in its sway most of his life: "I would always tell my parents that I would become a brain surgeon, an astronaut, or a scientist. While I'm not quite past the due date for being an astronaut, I wanted to create something that would help more than one person at a time."

Barbas grew up in St. Petersburg, Florida and got an early start in his research career by starting college at the ▶

age of 16, basically because he wanted to learn more about chemistry. It worked out better than he anticipated because suddenly he had all the freedom he wanted, personal as well as academic, a significant improvement over his high school years where he was, "euphemistically, a smart but rebellious teenager."

Once in college, his rebellion took a decidedly scholastic bent. While he felt the pull of biology, he felt an even greater pull from physics and drifted into that science. By the time he finished, he was seriously thinking of going into nuclear chemistry. At that point, fate, or something quite like it, intervened.

He was on his way to the airport to catch a plane to Texas A&M for a graduate school interview when he missed the flight. When he rescheduled, he faced an entirely new slate of interviewers. One of them was Chi-Huey Wong, Ph.D., currently the Ernest W. Hahn Professor of Chemistry at TSRI's Skaggs Institute for Chemical Biology, and a world-renowned scholar in enzymatic organic synthesis and bio-organic chemistry. Barbas immediately dropped the physics idea and headed straight into organic chemistry for four years.

From Texas he moved east to Penn State for post-doctoral studies, attracted by the potential of collaboration with another researcher, Richard Lerner, M.D., now TSRI's president. Barbas stayed only six months. Wong was recruited by Scripps in 1989. The following year Barbas transferred to Scripps and has never left.

By the time Barbas arrived, he was already deep into the combinatorial power of molecular biology and chemistry as applied to the study of catalytic antibodies, which Barbas describes as a kind of molecular machine that can put molecules together or break them apart, a process needed to create new drug candidates.

"As part of my grad studies I went to MIT to learn molecular biology," he says, "and that lit a fire in me to expand my use of molecular biology, to see how it might be applied to chemistry and the development of therapeutic proteins."

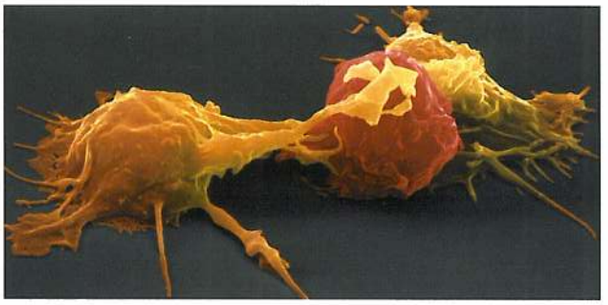
Together with Lerner, Barbas developed phage display for recombinant antibodies that started with the idea of creating catalytic antibodies outside of animal immunization, a classic, albeit costly and time consuming, method of producing antibodies for humanization.

In 1991, Barbas was promoted to assistant professor and began working with a small group of scientists to build on the technology that would eventually lead to the creation of antibodies that could be applied to a variety of diseases, and zinc finger proteins that could perform specific tasks when applied to the human genome.

A NEW APPROACH TO CATALYTIC ANTIBODIES

What was required was a new approach to catalytic antibodies. "At that point in the early 1990s, we began to take a novel approach to making protein catalysts," Barbas says. "At that time, there was no way to develop your own enzymes in the laboratory—that was something only catalytic antibodies would allow you to do."

Barbas and his colleagues went on to create the most efficient catalytic antibodies known, and with them, a potential new way to treat cancer. "We developed chemistries for new and approved cancer drugs that focused on their toxicity, one of the things that limit the use and duration of most chemotherapy drugs," he says. "We were able to add a new molecule to a drug that neutralized its toxicity. Then we used the catalytic antibody to help direct the drug to the target tumor, and then re-activate or unmask that toxicity at the tumor site."



Cancer attacked by killer cells: Colored Scanning Electron Micrograph (SEM) of two human Natural Killer (NK) cells attacking a cancer cell.

While this work offers a new approach to one of the longstanding problems of chemotherapy, it is not a magic bullet. The approach has been tested in animals with good results, and has been licensed for further study by the pharmaceutical industry. There are, however, certain difficulties. First, it requires a complex technology, and depends on a multidisciplinary approach that few labs have at their disposal. Second, any commercial treatment would involve multi-stage therapy, which is difficult to administer and monitor under even ideal circumstances.

But so far, the approach appears to be remarkably effective. In laboratory tests, Barbas found they could

treat animals with approximately 40 times the toxic dose of the drug without any harmful effects. By recasting the drug in a non-toxic mode, they were able to have an impact on tumor reduction. All tumors responded to treatment, and in seven out of eight the response was significant.

Coming on the heels of that development, Barbas recently published a paper on a simplified approach to the development of therapeutic antibodies based on catalytic antibodies, and founded his own company—called CovX Pharmaceuticals—to exploit its potential for new drug treatments.

"There are a vast number of drugs developed by the industry but only a small percentage of them make it as actual treatments," he says. "All drugs have certain inherent problems—their half-life and efficacy. To treat cancer effectively, you need a drug that stays at the tumor site for a substantial length of time. Because so many small molecule drugs are flushed from the body quickly, patients are given large amounts of the drug—and that brings with it unwanted side effects."

Barbas and his colleagues found a new cancer drug in development that showed significant tumor activity *in vitro* but inexplicably lost that activity *in vivo* in both animal and human studies.

"We were able to bind a catalytic antibody to the drug and the resulting complex was then directed to the tumor site," Barbas says.

But there's only so much one can do in a laboratory setting. The next step, of course, is to move the technology toward clinical development and human testing, a process that Barbas admits is very complex. And it's one of the things that attracted him to the idea of starting his own company.

He finds the psychology of drug development, the weighing and balancing of factors that affect a company's decision to pursue a specific drug candidate, captivating: "There is an entire science of drug development in the industry that is much different than it is in academia—testing on humans, for example, is far more complicated than testing on animals. I see my entrepreneurial activity as a way to extend our discoveries, providing me with the freedom to invent and discover new things."

This is Barbas's second foray as a biotech founder. A larger pharmaceutical company acquired the first company he co-founded several years ago.

UNLOCKING MYSTERIES

Barbas keeps adding to the patchwork of his scientific work, focusing on those areas that interest him, plowing deeper as he goes. His work with zinc finger proteins has already moved beyond the goal of uncovering the genetic switch to turn any gene off or on at will. In the last year, he has moved into a functional genomics mode, putting these switches into genes to see what happens. One area under study right now is the problem of drug resistance in cancer therapy, in which scientists are trying to uncover which genes and which cells develop resistance.

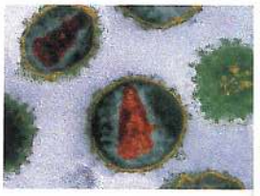
"That's what keeps me at TSRI, the freedom to think creatively, to follow my thoughts to where they might lead — and the opportunity to use what we learn to create new therapies."
— Carlos Barbas III, Ph.D.

He hopes to unlock many long-term mysteries with this technology. Viral infection, for example, and the ways different viruses enter cells: "In HIV, if a mouse is supplied with human receptor proteins, the virus doesn't proliferate the same way it does in people. From that, we know that other human proteins are involved in HIV proliferation.

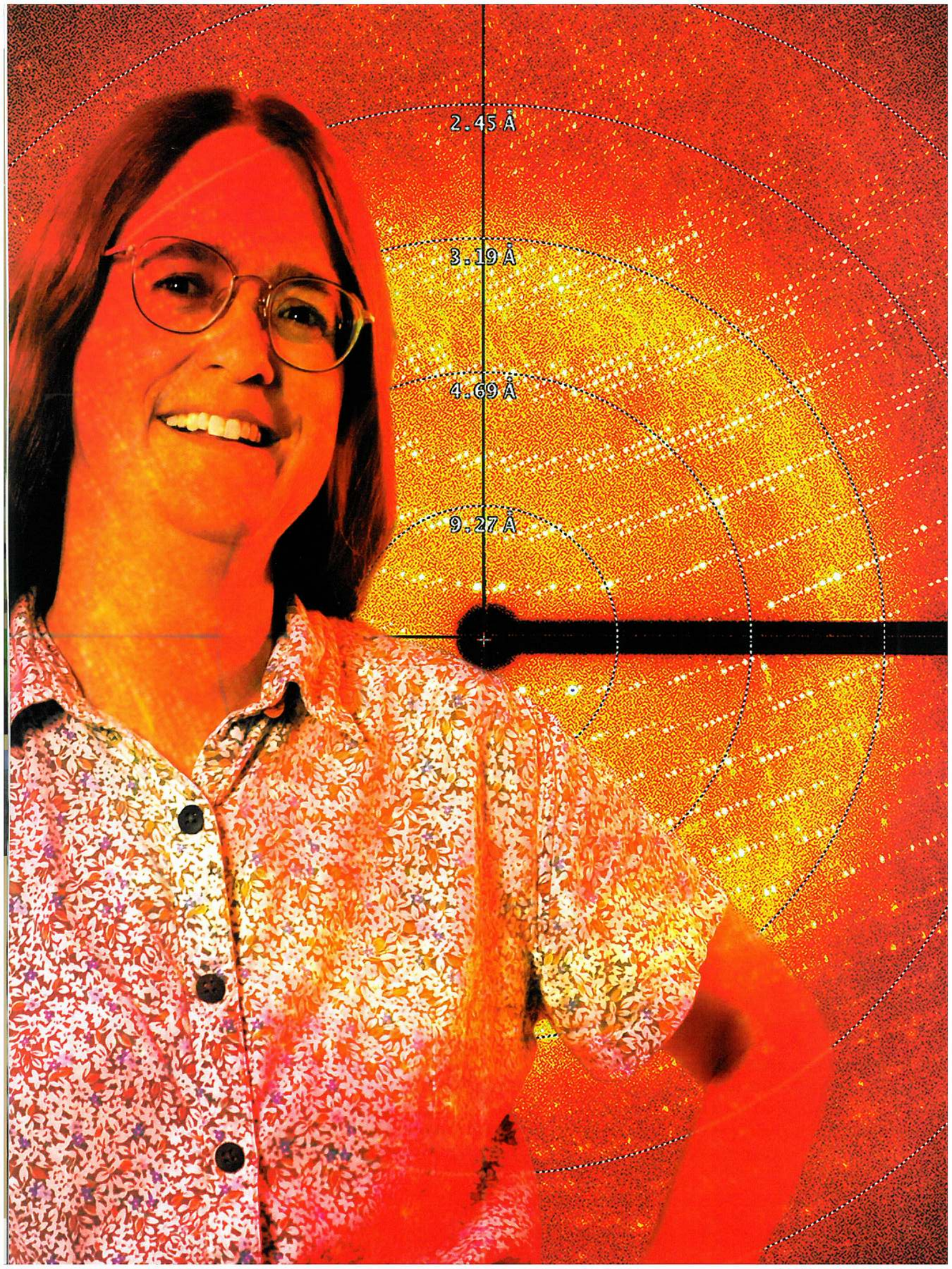
If we could identify those proteins, they might become new treatment targets. Targeting human proteins would help solve the problem of HIV resistance since our own proteins evolve on a much longer time scale than the viruses. Using our technology, we can move even faster."

As Barbas becomes more proficient at juggling the various aspects of his scientific life, it never enters his mind to simplify or focus on a single thing. Because in all these disparate projects, there is a cross-fertilization of information and ideas that sustains and nurtures his work. Right now, Carlos Barbas has a laboratory full of very smart people, 26 of them, who work together, elaborating on each other's ideas, adding to each other's designs, and building an impressive legacy of new technologies and new discoveries in the process.

"That's what keeps me at TSRI," Barbas says. "The freedom to think creatively, to follow my thoughts to where they might lead—and the opportunity to use what we learn to create new therapies." It's a patchwork approach that's already taken him, two years shy of 40, quite a distance. ■ *Eric Sauter*



HIV viruses: Colored transmission electron micrograph (TEM) of several human immunodeficiency viruses (HIV), which lead to AIDS.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"Studying proteins is never boring because there's something new to learn about all the time." — Libby Getzoff, Ph.D.

Beyond Conventional Wisdom

Libby Getzoff and Protein Crystallography

Even at The Scripps Research Institute (TSRI), a place known for its innovative approaches to scientific research, Elizabeth ("Libby") Getzoff, Ph.D., scores pretty high on the innovation meter. A professor in the Department of Molecular Biology, a chemist by training, and a crystallographer by choice, Getzoff shares some of her laboratory space and research with her husband and science partner, John Tainer, Ph.D., also a department professor. If this weren't enough, her activities are spread across TSRI with joint appointments in the Department of Immunology and The Skaggs Institute for Chemical Biology. Moreover, she has pushed the frontiers of crystallography by pursuing new crystallographic methodologies, taking her research equipment and experiments to synchrotron facilities across the United States and to facilities in Grenoble, France.

"I guess I just like to know how things work." — Libby Getzoff, Ph.D.

Her education was somewhat out of the ordinary as well. Raised in the suburbs of northern New Jersey, she received her undergraduate degree from Duke University in chemistry, the quintessential guy-science. She went against conventional wisdom once more, remaining at Duke for postgraduate work in biochemistry, and receiving her doctorate in 1982. At that point, she was focused on protein crystallography, perfecting her ability to interpret the diffraction patterns produced by x-rays as they passed through protein crystals, a field that even she admits was something of a scientific cul-de-sac when she and her husband first became interested in it.

She met her husband at Duke — they were in the same department and fascinated by the same kind of science: "When we got our Ph.D.'s, we both wanted to do the things we were excited about. At that time, there just weren't that many jobs for protein crystallographers, so we came to work as postdocs in molecular graphics at TSRI in 1983." Work with Professors David and Jane Richardson at Duke and then with Professor Arthur Olson at TSRI gave Libby Getzoff an in-depth appreciation for ways in which the visualization of molecular structures can define how they function in biology.

FORM AND FUNCTION

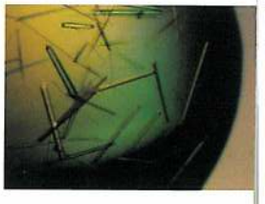
Getzoff's research choice remains unchanged—the experimental visualization of the most basic functions of proteins, those indefatigable workhorses of human metabolism. Expressed from genes and composed of linear chains of amino acids, proteins fold into intricate three-dimensional structures. Proteins regulate specific genetic actions, and act as catalysts for nearly all chemical reactions in the body. Our cells—even the extracellular environment in which they exist—are largely composed of proteins. Without proteins, we'd be useless puddles without structure or chemistry.

These days, Getzoff and her research team spend their time focused down on the most basic atomic level, a data-dense, chemistry-heavy scientific lair that she hopes will one day provide a detailed understanding of how proteins go about their indispensable tasks.

While what she searches for seems exotic, so do the technologies she uses: multi-wavelength anomalous diffraction; time-resolved Laue crystallography; femtosecond and nanosecond laser initiation; single-crystal spectroscopy; and computational and computer graphics analysis. In the most simple terms, all these methods help make snapshots of proteins at work for Getzoff and her colleagues to study and decipher.

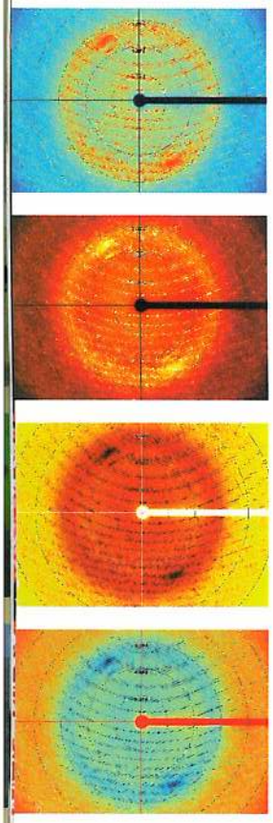
As she says about her 20-year career, "I guess I just like to know how things work."

A self-declared nerdy kid, she fell in love with math and science at a young age. By the time she reached high school, her career choice was pretty much a done deal: "I had some stellar high school teachers in chemistry, physics, and math who really made it all exciting and interesting." ▸



A family of crystals of green fluorescent protein growing in a suspended droplet, as viewed through the microscope.
By Timothy Wood

Color-coded images of the diffraction patterns for neuronal NOS produced by x-rays passing through the crystallized protein.
By Andrew Arvai



She carries that sense of enthusiasm with her today. "I like to think things over," she says. "In research, I get to do a lot of that. Studying proteins is never boring because there's something new to learn about all the time, some new technique or new biology. If you go on the web and type in 'nitric oxide synthase,' one of the molecules we study, you get tens of thousands of references. Of course, you have to sift through all those references to find the interesting and relevant ones. I live for stuff like that—discovering how proteins work and what is important for understanding their effects on human biology and disease."

Her love of lab work came early as well. Her last two years of undergraduate studies were spent working part-time in a Duke Medical School laboratory. She split her lab time between the chemistry and biochemistry departments, a crossover luxury allowed only for students at the time. That cross-disciplinary experience, commonplace at TSRI, was one of things that attracted her to the institute—that and the chance to use her skills as a crystallographer to study proteins.

"Today, everything moves closer to real time."
— Libby Getzoff, Ph.D.

PEBBLES INTO A POND

X-ray crystallography produces a simple outcome, an image, through a series of highly complicated processes. X-rays act like pebbles thrown into a pond, creating ripples and patterns as they pass through the protein crystal. From those patterns, Getzoff is able to discern the protein's three-dimensional fold and structure. Crystallization reorganizes the protein molecules into straight rows, which makes the process possible.

While the alignment of protein molecules remains unchanged, technology has made the science significantly easier. While you can grow hemoglobin crystals, for example, from a readily available substance like blood, most proteins exist in far smaller concentrations. When she started out, this fact made it extraordinarily difficult, if not impossible, to study certain types of proteins. But the technology of cloning has now rendered that issue less of a barrier, although still a challenge, giving Getzoff and her colleagues sufficient quantities of many of the rarer proteins.

Computers and computer graphics have also greatly reduced the tedious and labor-intensive process of developing x-ray photographs of the crystals in the laboratory. "I remember when we started our lab it was a big deal to work with eight kilobytes of memory," she says. "Today, everything moves closer to real time. Plus, the Internet means we can send our data electronically as digitized images instead of waiting for films to be scanned in the lab."

And where once proteins were represented by stationary mechanical models held together with screws and manual labor like elaborate tinker toys, they are now displayed interactively in vivid, digitized color on a computer screen.

"The crystals themselves are often quite stunning," she says. "Some are colored, like the intense red of hemoglobin; all come in geometric shapes. One of the proteins we're studying, the photoactive yellow protein (PYP), allows a bacterium to sense light. It is a brilliant yellow because it specifically absorbs blue light out of the spectrum of sunlight, exciting an electron and turning the molecule yellow."

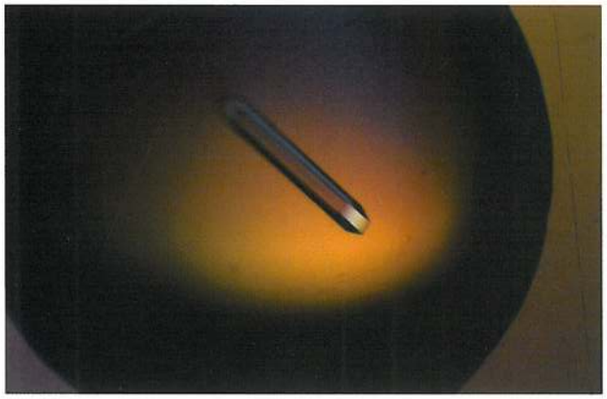
PROTEINS ON THE MENU

Getzoff is studying PYP precisely because of its interaction with light, and because this rather obscure protein is related to certain proteins found in the human eye. If we can understand how PYP interacts with light, Getzoff suggests, we can perhaps understand more fully how the eye functions. Working with PYP, her research team solved the mystery of the mechanism by which a slow-moving protein can conquer a trillion-fold difference in speed to catch a speeding photon.

Another one of the proteins on Getzoff's research menu has an even more obvious connection to evolving medical science. This protein is called superoxide dismutase (SOD), and is a powerful anti-oxidant that attacks free radicals in the human body. Free radicals are active and unstable molecules that cause organ or tissue damage in the same way rust eats away metal. If free radicals act like rust, then superoxide dismutase acts like Rust-Oleum.® Except when it doesn't, and that's when the trouble starts.

Working with her husband, Getzoff found that a number of patients with amyotrophic lateral sclerosis (ALS, a.k.a. Lou Gehrig's disease) had a genetic mutation that interfered with the protective function

of the SOD protein by distorting the protein's shape and allowing it to form amyloid-like fibers, similar to those formed in other neurodegenerative diseases.



The geometry of this beautiful single protein crystal reflects the underlying order of the aligned protein molecules. *By Lisa Craig*

"There are 153 amino acids in this superoxide dismutase protein," Getzoff points out. "If just one of some 90 of those is changed, you will have a propensity for the disease. As part of their basic function, all proteins fold in on themselves. The underlying genetic defect in ALS causes the SOD protein to unfold instead. These unfolded proteins clump together in aggregates in the neurons. When that happens, they no longer function—they cause damage. No one yet knows precisely how these unfolded aggregates damage neurons, nor how to prevent this process. However, understanding the basis for the disease at the molecular level provides fruitful directions for further study."

"The crystals themselves are often quite stunning. Some are colored, like the intense red of hemoglobin; all come in geometric shapes."
— Libby Getzoff, Ph.D.

In this collaboration, Getzoff and her husband use crystallography to try to determine the exact structures of these mutant protein aggregates. They've already discovered something interesting—some of the inactive proteins actually did fold correctly.

"Right now, we're tormenting these proteins in the lab to see what causes them to aggregate," she says, laughing wryly. "We expose them to heat and subject them to chemicals to test their stability." These experiments yield clues to how and why the mutant proteins lose their stability and assemble into filaments that may underlie the disease.

Another research subject is nitric oxide synthase or NOS. NOS is a protein involved in the regulation of cellular signaling. As Getzoff explains, NOS is critically important because it plays key roles in regulatory mechanisms throughout the human body. The work with NOS is aimed at finding inhibitors that distinguish among the three different human NOS enzymes—specific inhibitors that could, for example, prevent inflammatory disorders or migraines, without disturbing blood pressure.

A FAMILY AFFAIR

For Getzoff, this cooperative effort is a working arrangement that suits both her and Tainer.

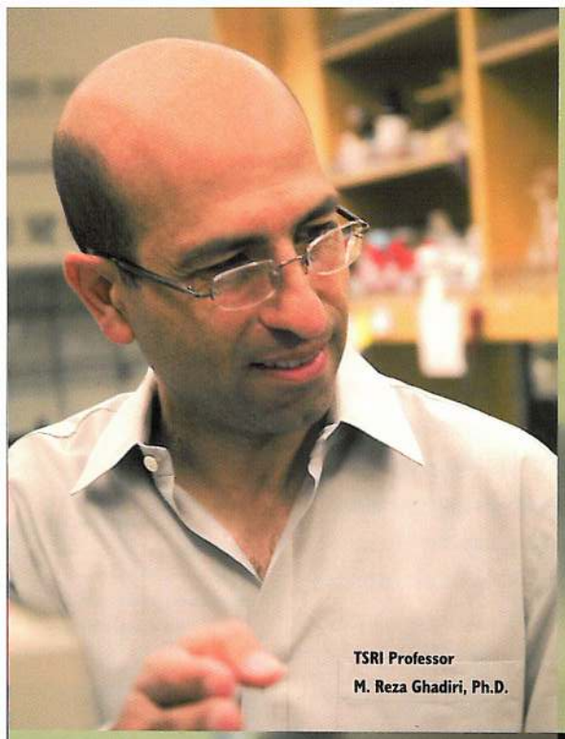
"It's great working with my husband," she says. "Scientists spend a lot of time in the lab, so if your spouse isn't a scientist, you don't get to see each other very much. Also, we both understand the concept of lab time—that's when you say you'll be home in half an hour, and suddenly its three hours later and you aren't there yet. Of course, the down side is that when something goes wrong, it happens to you both at exactly the same time."

Their cooperative spirit seems a perfect match for TSRI, where cooperation between people and disciplines is the norm rather than the exception. That much was clear to both of them from the very beginning.

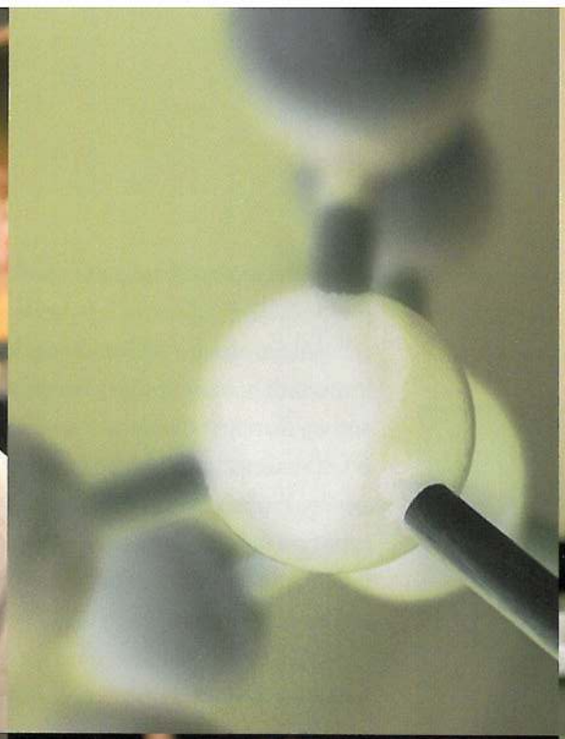
These days, when she isn't in the lab, Getzoff contributes to TSRI's interdisciplinary graduate program and co-directs the La Jolla Interfaces in Science training program. This program is sponsored by the Burroughs Wellcome Fund to provide fellowships that encourage graduate students and postdoctoral fellows trained in chemistry, mathematics, and engineering to apply their quantitative tools to the science of biology. Much of the rest of her time is spent with her seven-year-old daughter, who is already blazing her own path to science.

"She likes to do experiments in the sink," Getzoff says happily. "She grows sugar crystals and mixes soap and water together with spices just to see what happens."

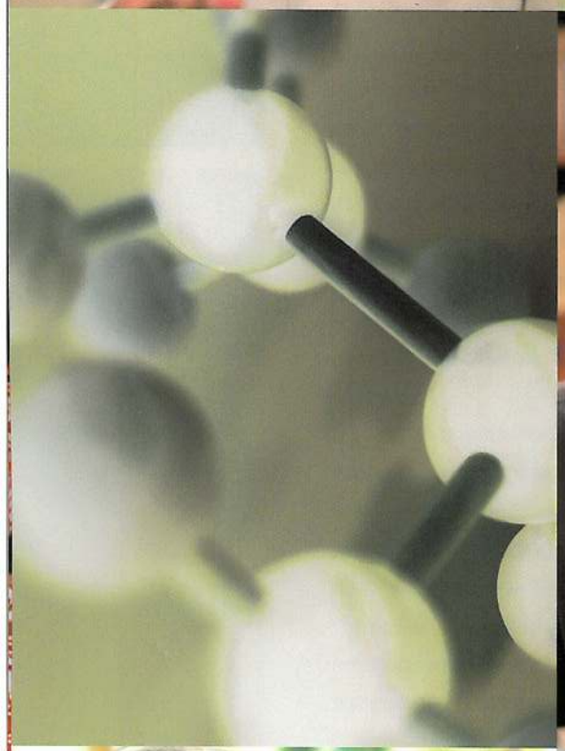
All in the family. ■ *Eric Sauter*

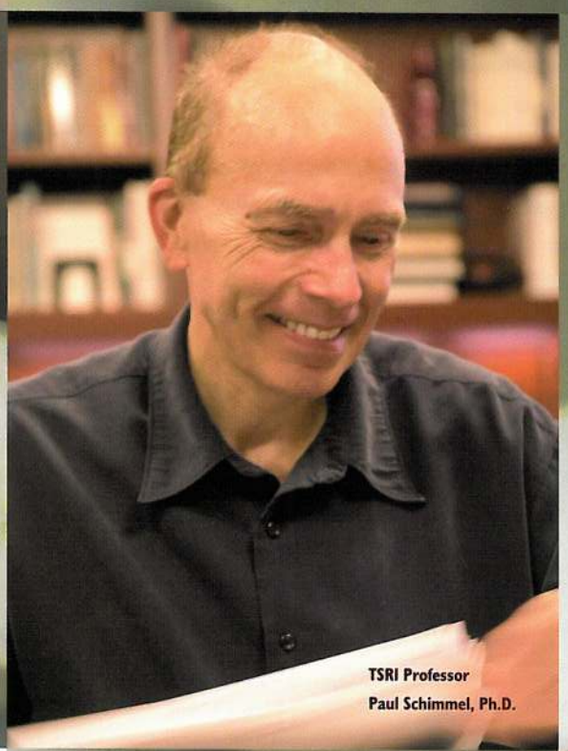


TSRI Professor
M. Reza Ghadiri, Ph.D.

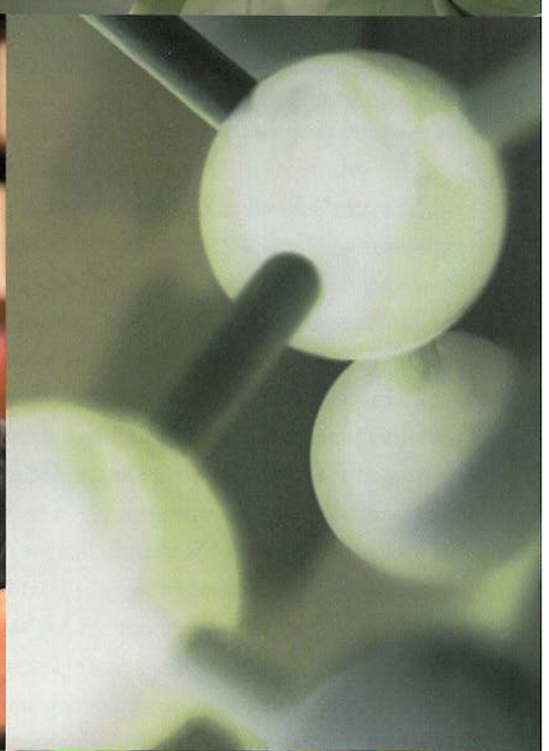


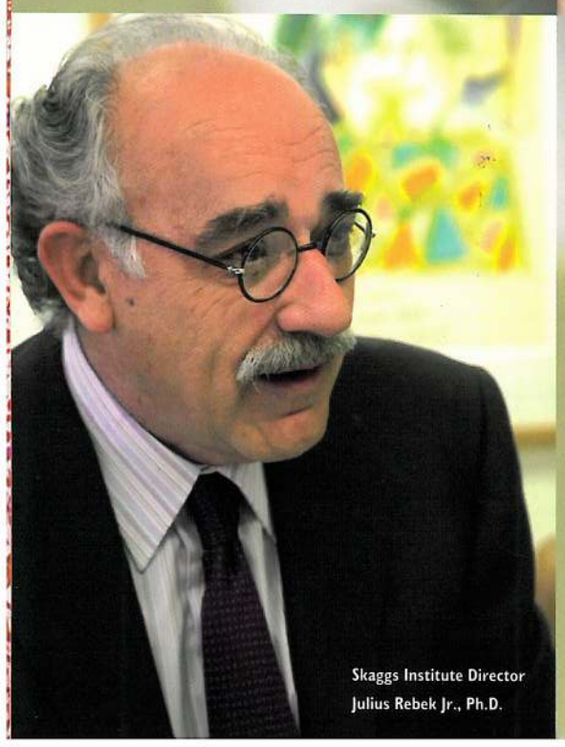
TSRI Professor
Gerald Joyce, M.D., Ph.D.



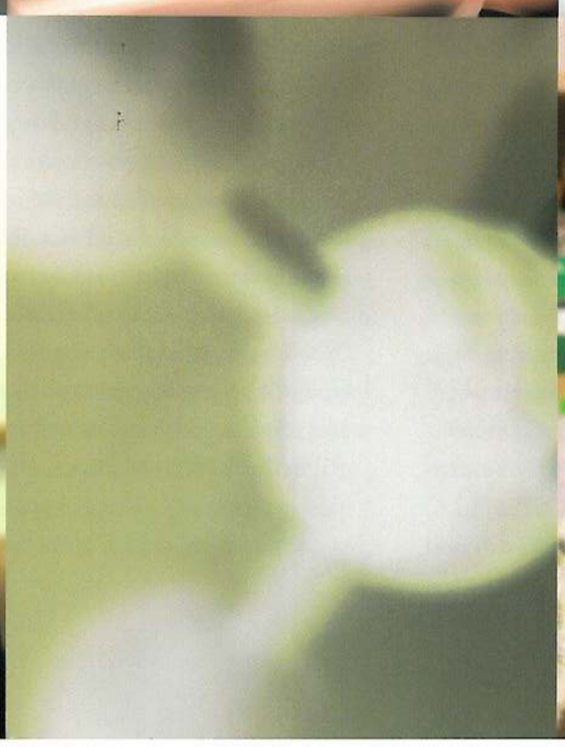


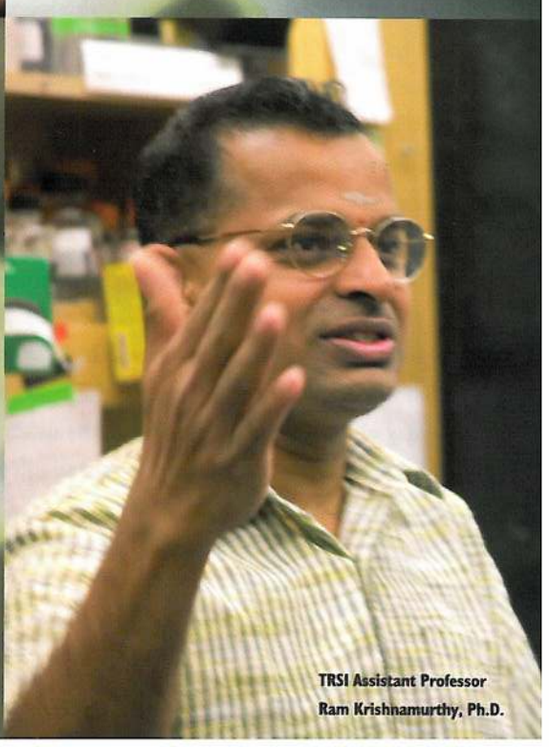
TSRI Professor
Paul Schimmel, Ph.D.





Skaggs Institute Director
Julius Rebek Jr., Ph.D.





TRSI Assistant Professor
Ram Krishnamurthy, Ph.D.

"The origin of life must be invented — it cannot be discovered."
— Albert Eschenmoser, Ph.D.

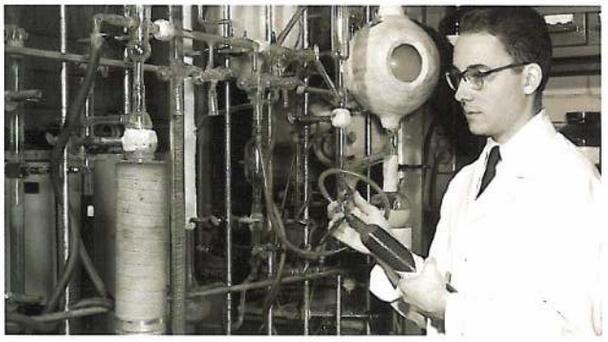
Origins of Life

Fifty Years Back, Several Billion Years Since

Celebrating the anniversary of a great discovery is like celebrating a birthday — it happens every year, but somehow there is special significance after 50, 100, or 200 years have passed.

The year 2003 is crowded with such milestones. It has been 200 years since Lewis and Clark began their expedition; 100 years since Orville and Wilbur Wright flew their prototype powered airplane for a few seconds above the windy dunes of Kitty Hawk, North Carolina; and 50 years since James Watson and Francis Crick described the anti-parallel, double-helical structure of DNA in the journal *Nature*.

Make room for one more celebration: the 50th anniversary of the publication in the journal *Science* of a paper entitled "A Production of Amino Acids Under Possible Primitive Earth Conditions." This paper by Stanley L. Miller described an experiment he devised with Harold C. Urey that tested an idea on the origins of life put forth three decades earlier.



Stanley Miller (above) conducted a seminal experiment he devised with Harold C. Urey that launched a new approach to exploring the questions of the origins of life.

Russian scientist A.I. Oparin had hypothesized that on the early Earth, the elements carbon, hydrogen, oxygen, and nitrogen, minerals, metals, and violent early-Earth energy sources like lightning, solar radiation, comet impacts, and volcanic vents conspired to create organic compounds, like amino acids and nucleotides — the building blocks of life — which led to organic life. The Miller and Urey experiment, as it is now known, gave experimental underpinnings to Oparin's ideas.

In the experiment, Miller boiled H_2O, CH_4, H_2, and NH_3 gases in a glass apparatus containing a pair of tungsten electrodes. These subjected the chemicals to an electric discharge in a reducing environment that was meant to simulate the atmosphere on the early Earth. Below the discharge chamber in the apparatus was a condenser through which the heated discharge passed. A tap at the bottom of this condenser allowed Miller to collect any molecules that formed. When he analyzed the molecules he had collected, Miller found that he had made amino acids — alanine, glycine, and a few others — besides lots of unknown organic compounds.

"That experiment really changed what the discipline was," says TSRI Professor Gerald Joyce, M.D., Ph.D., one of several TSRI investigators who study questions related to the origins of life. Recently he took part in a series of public lectures celebrating 50 years of prebiotic chemistry given at the University of California, San Diego, where Miller is professor emeritus. "Until 1953, [the origins of life] was not an experimental science."

Now it is — and, after 50 years, there is an enormous amount still to be learned. And, it turns out, some of this knowledge may have practical applications to the health problems of today.

"It is intrinsically difficult to comment on a process so far back in the past," says TSRI Professor Albert Eschenmoser, Ph.D. He adds, with cautious optimism, "You may not solve the problem, but you should not adhere to the principle according to which the problem will not be solved."

THE DIFFICULTY OF THE QUESTION

Without experimental approaches, the question of the origins of life is something of a historian's nightmare. Even if we start with the earliest fossils, we can only reach back so far in time. As simple as the oldest fossilized bacterial life forms are, they were almost certainly too complex to be the first life.

"We're not going to see a fossil of the early planet," says Joyce. ▸

Imagine trying to write the history of transportation if all you had for sources were a collection of preserved automobiles, the oldest of which was a vintage Model T Ford. Certainly the Model T is primitive by today's standards, but it doesn't necessarily reveal anything about the early internal combustion vehicles that came before it.

"The only thing you can do [to ask about the origins of life]," says TSRI Professor M. Reza Ghadiri, Ph.D., "is determine what is chemically feasible and what is not chemically feasible."

Chemical feasibility is a good starting point because, rather than focusing on the impossible-to-answer question of how life did form, it poses the possible-to-answer question of how does life form.

"It's pretty clear that there was a time when life was based on RNA. Not just because it's feasible that RNA can be a gene and an enzyme and can evolve, but because we really think it happened historically."
— Gerald Joyce, M.D., Ph.D.

At TSRI's Skaggs Institute for Chemical Biology, several faculty members do research in the field, including Eschenmoser, Ghadiri, and Joyce, as well as Ram Krishnamurthy, Ph.D., an assistant professor in the Department of Chemistry, Julius Rebek Jr., Ph.D., who is director of the Skaggs Institute, and Skaggs investigator Paul Schimmel, Ph.D., who is the Ernest and Jean Hahn Professor and Chair of Molecular Biology and Chemistry.

These scientists use what they discover and already know about chemistry and biology to narrow the possibilities of how chemical structures and reactions that could have existed on early Earth might have given rise to living systems.

THE RNA WORLD

Research on the origins of life in the last generation has suggested the notion of an ancient RNA world — one in which RNA genes stored genetic information (something done by DNA today), carried out the chemistry necessary for life, and formed the essential physical structures of life (something done primarily by proteins today).

"It's pretty clear that there was a time when life was based on RNA," says Joyce. "Not just because it's feasible that RNA can be a gene and an enzyme and can evolve, but because we really think it happened historically."

One of the world's leading experts on RNA, Joyce has been working to understand the RNA world by simulating parts of it in real time. He employs a technique where he evolves nucleotide enzymes in the test

Five Billion Years: A Brief History

The sun and the solar system form from the gas and dust of a collapsing nebula around 4.57 billion years ago, an approximation astronomers base on elemental isotope deposits in the solar system's oldest meteorites.

The center of this collapsing cloud condenses into a star, and the outlying material begins to rotate around this star as a spinning disc. Millions of years go by, and the dust, frozen gases and other particles in this disc collide and condense into larger objects. About a hundred million years after the star — our sun — is born, enough of this material has condensed to make an early Earth.

The celebration, however, is short-lived. About 4 billion years ago, an object the size of Mars smashes into the planet. Half of the Earth's mantle vaporizes in the ensuing inferno, but when things cool and condense, the moon is in a stable orbit. The Earth continues to cool and water condenses into liquid form.

Then — some time in the next few hundred million years — life emerges.

The earliest fossils scientists have found are stromatolites — large clumps of cyanobacteria that grew in abundance in the ancient world over 3.5 billion years ago in what is now western Australia. These most likely evolved from some simpler life forms because, like all modern life, cyanobacteria are highly sophisticated living organisms — with cell walls, complex metabolism, and DNA genes. The question of the origins of life is: what came before the stromatolites?

tube by subjecting pools of mutant nucleotide enzymes to changing selective pressures and amplifying the ones that are able to "adapt."

Joyce used this technique to make the world's first DNA enzymes a few years ago. These single-stranded DNA enzymes, says Joyce, are not that hard to make and they can do pretty much whatever an RNA enzyme can do. Joyce was also able to evolve an RNA enzyme using only three nucleotides (instead of the four that are used in nature). He has even been able to evolve an enzyme using only two nucleotides.

His overall goal is to use test tube evolution to create molecules that can evolve by themselves — catalyze their own formation, handle information, and perhaps even undergo evolution.

"That to me is crossing the boundary from non-historical events (chemistry) to historical events (biology, life)," says Joyce.

THE CODE BEFORE THE CODE

Also at TSRI, Schimmel has pioneered the idea that one of the clues to the emergence of the modern DNA–RNA–protein biological world from the ancient RNA world lies in a class of modern RNA enzymes called aminoacyl transfer RNA (tRNA) synthetases.

These tRNAs as we know them today are involved in protein synthesis, where amino acids are strung together in the order assigned by an organism's genetic code. The synthetases' job is to help match the correct genetic code word, or codon, with the correct amino acid, which in turn attaches to a "universal" motif at the end of the tRNA. The universality of this motif may indicate something about its evolutionary origins.

"Perhaps," says Schimmel, "there was a second genetic code, a code before the genetic code."

Schimmel calls this an operational RNA code that would have related sequences of RNA to amino acids, allowing RNA enzymes to grab amino acids and "borrow" their chemical structures. Amino acids have the ability to catalyze a larger range of reactions than nucleotides, and perhaps RNA life forms evolved ways to use amino acids and their catalytic abilities.

"Perhaps there was a second genetic code, a code before the genetic code."
— Paul Schimmel, Ph.D.

This tendency could also have led to the formation of proteins, says Schimmel. A few years ago, he showed that the simple docking together of two nucleic acids loaded with amino acids led to the formation of a peptide bond — the basic link between amino acids in a protein chain. ▶

The earliest fossils yet discovered are of stromatolites — large clumps of cyanobacteria that grew over 3.5 billion years ago. Stromatolite reefs still flourish in western Australia today.

If the age of the solar system were represented as one year instead of 4.57 billion, then the Earth would have cooled enough for the oldest rocks we know of to form by February 14, the stromatolites would appear on March 22, and most of the really interesting stuff, biologically speaking, would happen in the fall and winter.

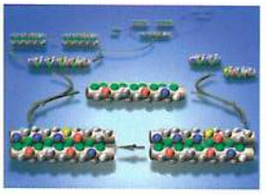
The Cambrian explosion, when the ancestors of most modern invertebrates appeared on the scene some 570 million years ago, would be in mid-November. Earth's oldest mammals would appear in mid-December. The dinosaurs would go extinct the day after Christmas, the earliest humans would show up on the scene a few minutes before the New Year, and the Miller–Urey experiment would be conducted about a half-second before midnight.

And, less than one tenth of one second to midnight, TSRI Professor Albert Eschenmoser would say over the phone from Switzerland:

"The origin of life must be invented — it cannot be discovered."

■ *Jason Socrates Bardi*

Professor Reza Ghadiri has developed complex networks of interacting molecules that can self-replicate and carry out other catalysis. This figure illustrates the process of peptide self-replication.



"It's plausible that you could have had tRNA-like molecules in an early RNA world that would be loaded with amino acids by the action of another RNA enzyme and these 'loaded' RNAs would eventually condense together to make peptides," says Schimmel. Such RNA/protein life forms might have a selective advantage over just plain RNA life forms and eventually outcompete them for the same resources.

Schimmel also has suggested that early proteins may have been rough proteins. "Not the precise entities we know today," he says. An RNA code that roughly coded for the amino acid valine, for instance, might have picked up valine or any one of five or six chemically similar amino acids.

From this rough protein world, a few might have emerged that were "pretty perfect" in Schimmel's words. These would have been more efficient than others, giving them an evolutionary advantage and leading to their selection over the rough proteins.

THE PRE-RNA WORLD

Another area of investigation at TSRI seeks to examine the question of why nature selected the building blocks it did to make RNA — the bases, phosphodiester linkers, and the sugar ribose. Why ribose? Why a pentose and not a hexose? And if ribose, why ribofuranose, and not ribopyranose?

"There exists a variety of informational oligonucleotide families composed of sugar building blocks other than ribose that may have chemical potentials similar to those of RNA," says Eschenmoser.

Eschenmoser is interested in conceiving, synthesizing, and studying such alternative nucleic acid structures, focusing on those that — according to chemical reasoning — could have assembled themselves under natural conditions.

Through chemical synthesis, Eschenmoser and his collaborators created an RNA-like molecule, called TNA, with an alternative type of sugar called a threose. Threoses are four carbon sugars similar in structure to ribose except that they have no 5' carboxy linker to stitch them together with phosphates. They can base pair with each other and with the ribose-based nucleic acids as well.

"TNA speaks the same language as RNA," says Eschenmoser. Because of this common tongue, Eschenmoser says, TNA is able to cross-pair with RNA, which in evolutionary terms means that they could transmit information back and forth and act as catalysts, one for the other. Chemically, the structure of TNA is simpler than that of RNA. Therefore, there are scientists who think that TNA could have set the stage for the appearance of RNA.

COMPLEX NETWORK BEHAVIOR

Ghadiri's lab at TSRI focuses not on the particular molecules involved in the origins of life, but on how complex network behaviors among systems of molecules could lead to the emergence of life.

Complex network behavior is important for life because it is the basis for things like information processing, higher orders of organization, and the emergence of new characteristics, attributes, and functions.

"We're interested in [knowing] whether molecular species have the feasibility of doing this," says Ghadiri.

Ghadiri has developed complex networks of interacting molecules that can self-replicate and carry out other catalysis. Ghadiri creates mini-ecosystems with short proteins, mixes them with other short proteins and molecules, and looks for new forms and functions among competing molecular species. Properties that cannot be carried out by any one molecular component emerge due to the combined efforts of all the molecular species exhibiting complex network behavior.

"Instead of competing with each other for a common resource, [molecular species] join hands and catalyze each other's formation."
— Reza Ghadiri, Ph.D.

Ghadiri has been experimenting for a few years on such systems, with a rational and experimental approach to studying this complexity and modeling this emergence in his laboratory. He has created mini-ecosystems of helical polypeptides with various 15 to 17 amino acid sequences, looking for new forms and functions among competing molecular species. He has, in recent years, found emerging behaviors.

"That is astonishing," says Ghadiri. "Based on [our] understanding, polypeptides can self-replicate, form complex networks, error correct, and form mutual systems. They have all sorts of emergent properties."

Symbiosis is an example of an emergent phenomenon

he has observed, in which the efforts of the molecular species combine to create a property not otherwise possible.

"Instead of competing with each other for a common resource, they join hands and catalyze each other's formation," he says.

OTHER FORMS OF REPLICATION

Rebek has raised another exciting possibility by demonstrating that the familiar molecules of life like nucleic acids or proteins are not needed to carry out some of life's chemical reactions. Several years ago, in fact, Rebek demonstrated emergent life-like properties such as self-replication and autocatalysis in chemical (rather than biological) molecules.

"What I did was to show that just about anything would do as long as the two things were complementary and when you put them together they were self-complementary," says Rebek.

Since self-replication is one of the fundamental requirements of life, this work is relevant because it suggests that it may be possible — on Earth or perhaps elsewhere in the universe — to set the stage for the emergence of life based on chemistries that we would not even consider to be biological.

"Autocatalysis is one bottom line, but it's not enough," says Rebek. "You could have a bunch of replicating molecules, but if you didn't have some of these other things, like a continued source of pieces that they are made from, you couldn't call it living."

"Autocatalysis is one bottom line, but it's not enough. You could have a bunch of replicating molecules, but if you didn't have some of these other things, like a continued source of pieces that they are made from, you couldn't call it living."
— Julius Rebek, Ph.D.

REINVENTING LIFE

The child-like dream demonstration of any researcher studying the origins of life would be to generalize the Miller-Urey experiment on a grand scale. Even without such a grand solution, however, the work of these investigators and others has helped to clarify some of the questions about the origins of life. And, as is often the case in basic science, their research has created interesting scientific and medical applications as by-products.

For instance, Ghadiri used his technology to create a class of biological polymers known as cyclic peptide nanotubes, which stack inside the cell membranes of bacteria, and poke holes in their membranes, killing the cells. These "nanotube" stacks have demonstrated strong bactericidal activity both in the test tube and in living tissue against a number of deadly pathogens including multi-drug-resistant *Staphylococcus aureus*, one of the most common hospital-acquired infections.

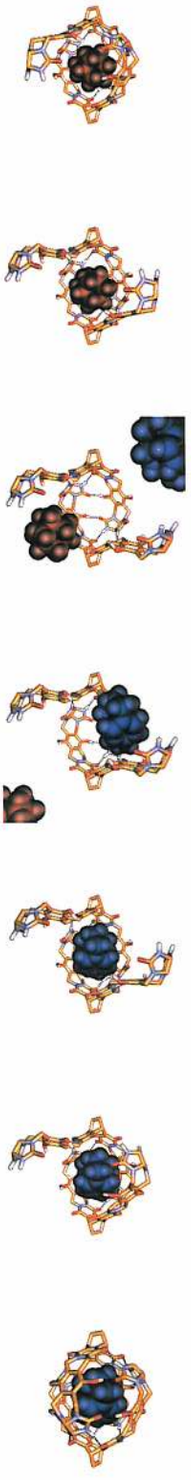
One of the DNA enzymes Joyce evolved, called 10-23, has great therapeutic potential because it chops up RNA molecules with high efficiency. These RNA molecules produce proteins — and the DNA enzymes therefore reduce the expression of those proteins. These DNA enzymes have shown promise for the treatment of leukemia by inhibiting certain proteins in bone marrow cells of patients with leukemia.

Rebek has been applying his expertise to the development of a nanotechnology known as nanocapsules. Rebek's nanocapsules are like two identical half eggs that can close around a reactant molecule, sequestering it. With these nanocapsules, Rebek demonstrated that he could achieve chemical amplification without the presence of an autocatalyst, which is a product of the reaction that acts as a catalyst for more product. Chemical amplification resembles a chain reaction (one molecule makes two, two makes four, four becomes eight, eight becomes 16, 32, 64, 128...). These findings show a different way of controlling reactivity — a way of turning a reaction on and off.

And while doing work related to what was previously described, Schimmel recently discovered molecules that have the potential to address a range of human ailments, from blindness to cancer.

These molecules have the ability to regulate angiogenesis, a process that is implicated in cancer tumor growth and in age-related macular degeneration and diabetic retinopathy — two of the leading causes of vision loss in the United States. These diseases afflict tens of millions of Americans and cause catastrophic vision loss in many. There is currently no effective treatment for the vast majority of these patients, but Schimmel is pursuing the application of his newly discovered molecules to address this suffering.

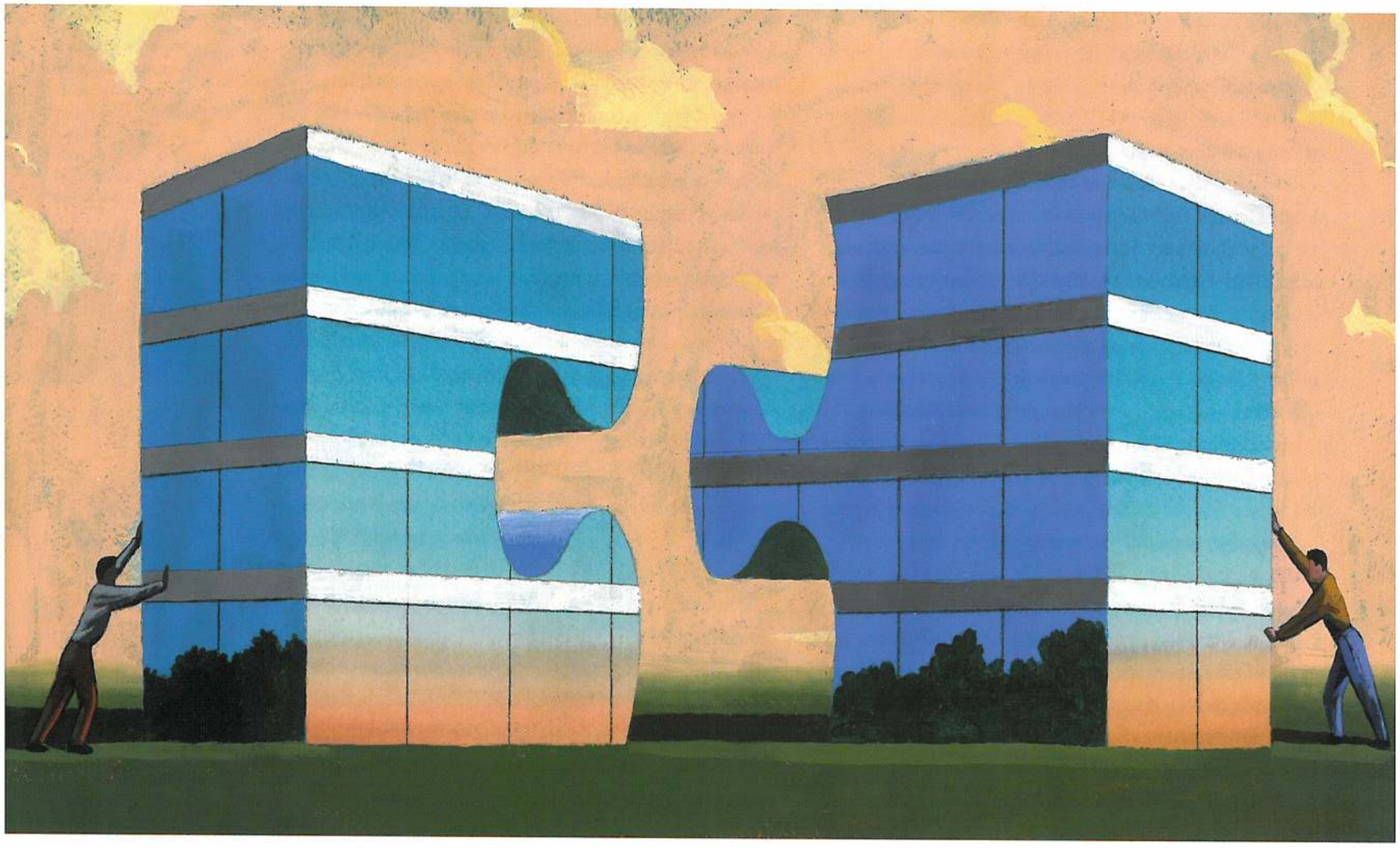
■ *Jason Socrates Bardi*



Skaggs Institute Director Julius Rebek, Jr., has demonstrated emergent life-like properties in chemical (rather than biological) molecules. The above sequence shows flaps opening in a chemical structure, allowing the departure of a residing molecule, adamantane, and the substitution of an incoming molecule, paracyclophane.

Behind the Scenes

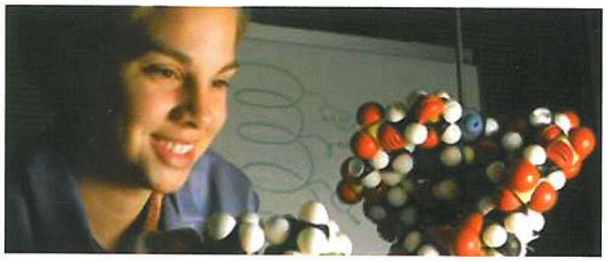
Public and Private Support Fuels TSRI's Research in Chemical Biology



The research in chemical biology at TSRI would not be possible without crucial public and private funding. Major support for these endeavors has been provided through:

The Skaggs Institute for Research

In 1996, The Skaggs Institute for Chemical Biology was established at TSRI, thanks to a gift of more than $100 million to The Skaggs Institute for Research from Aline W. and L.S. Skaggs. At the time, the Skaggs family headed American Stores Inc., a multi-billion dollar retail drug and grocery chain later acquired by Albertsons. Scientific members of TSRI's Skaggs Institute also hold appointments in various departments at TSRI. These scientists have broad expertise in areas including the structure of biological macromolecules, chemical and antibody catalysis, synthetic and combinatorial chemistry, molecular recognition, and molecular modeling methods. Skaggs Institute faculty include 13 members of the National Academy of Sciences and three Nobel laureates.



Graduate student Shannon Biros conducts research at The Skaggs Institute for Chemical Biology at TSRI. The Skaggs Institute was created with a gift of more than $100 million to The Skaggs Institute for Research from Aline W. and L.S. Skaggs.

The National Institutes of Health

Research in chemical biology at TSRI is supported every year by public funding through the National Institutes of Health (NIH). The NIH — comprised of 27 separates institutes and centers — is the federal focal point for health research and seeks to promote new knowledge that will lead to better health for all. In addition to supporting the research of scientists in universities, medical schools, hospitals, and research institutions, the NIH conducts research in its own laboratories, helps in the training of research investigators, and fosters communication of medical and health sciences information.

The Arnold and Mabel Beckman Foundation

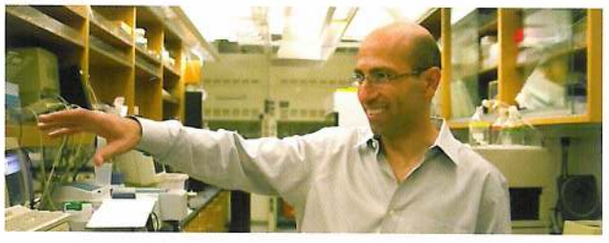
Arnold and Mabel Beckman have made numerous significant contributions to TSRI over the years. Most notably, in 1992, the Arnold and Mabel Beckman Foundation — which was established by the Beckmans to support basic scientific research — gave $5 million to TSRI to support the construction of the Arnold and Mabel Beckman Center for Chemical Sciences. Arnold Beckman is the founder and former chairman of Beckman Instruments, Inc., a major manufacturer of laboratory analytical instruments, related chemical products, control systems, and precision electronic components. He is the inventor of the glass electrode pH meter and the D.U. ultraviolet spectrophotometer.

The W. M. Keck Foundation

The W. M. Keck Foundation contributed funds for the construction of the Arnold and Mabel Beckman Center for Chemical Sciences and the recruitment to TSRI of prominent chemists in the field of chemical biology. The Keck Foundation was established in 1954 by the late William Myron Keck, founder of The Superior Oil Company. The foundation's grant-making is focused primarily on the areas of medical research, science, and engineering.

National Aeronautics and Space Administration (NASA)

The National Aeronautics and Space Administration (NASA) has supported many studies in chemical biology at TSRI. For instance, NASA's Specialized Center for Research and Training (NSCORT) in exobiology provided a million dollars a year over the last decade for undergraduates, graduate students, and postdoctoral fellows in several groups around La Jolla, including those at TSRI. NASA also funds research at TSRI through its astrobiology institutes. One of these, headed by TSRI Professor Reza Ghadiri, looks at chemical and biological perspectives of reproducing molecular systems.



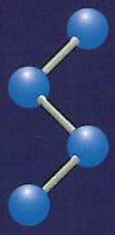
The National Aeronautics and Space Administration (NASA) has supported many studies in chemical biology at TSRI.

In addition, support for research in chemical biology at TSRI has been provided through named chairs. Named chairs held by investigators in the field of chemical biology include the Janet and W. Keith Kellogg II Chair in Molecular Biology; Ely R. Callaway, Jr., Chair in Chemistry; Lita Annenberg Hazen Professorship in Chemistry; Cecil H. and Ida M. Green Chair in Chemistry; Darlene Shiley Chair in Chemistry; Ernest and Jean Hahn Professor and Chair of Molecular Biology and Chemistry; Scripps Family Chair; W.M. Keck Professor in Chemistry; Ernest W. Hahn Professorship and Chair in Chemistry; and Cecil H. and Ida M. Green Investigatorship in Medical Research.

Numerous other individuals and organizations have also provided critical support, enabling TSRI to assume international prominence in the field.

For information on making a contribution to TSRI, contact:
Denise M. Scalzo · TSRI Development Office
10550 North Torrey Pines Road, TPC2 · La Jolla, CA 92037
(800) 788-4931 or (858) 784-2339

The Arnold and Mabel Beckman Foundation made possible the construction of the Beckman Center for Chemical Sciences on the TSRI campus.



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