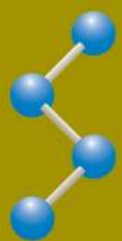


VOLUME SEVEN | NUMBER TWO



THE
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Endeavor

Fall 2004



In the Bloodstream

Pioneering investigations in modern biology

Endeavor

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In 1628, William Harvey described how the body's blood circulates. After moving through the heart and lungs, blood carries oxygen and nutrients to all tissues of the body before returning to the heart. About once every minute, the 10 to 15 pints of blood in the average human body completes a circuit through an incredible labyrinth of arteries, veins, and capillaries that if laid out end-to-end would nearly circle the planet four times.

Today, our knowledge of the bloodstream continues to evolve. The bloodstream is central to some of the most crucial issues in modern biology, and is intimately involved in the three leading causes of death in the United States—heart disease, cancer, and stroke. This issue of *Endeavor* features a few of the investigators at The Scripps Research Institute who focus on aspects of the bloodstream.

featured



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Scripps Research Breaks Ground in Florida

Florida Governor Jeb Bush joined Palm Beach County, Florida Atlantic University, and Scripps Research officials to break ground for Scripps Florida's temporary facilities on the Jupiter campus of Florida Atlantic University. These facilities, including lab and administrative space, are scheduled to open by the end of the year.

In the meantime, plans are being drawn up for permanent Scripps Florida facilities on 100 acres of undeveloped land in Palm Beach County west of I-95.

The research on the Florida campus, which will complement the science in the Scripps Research facilities in La Jolla, California, will focus on basic biomedical science, drug discovery, and technology development. The startup costs for Scripps Florida are being supported by a one-time appropriation of federal economic development funds by the State of Florida at the request of Governor Bush, and an economic package from Palm Beach County.



Florida Governor Jeb Bush (second from right) participated in a groundbreaking ceremony with (left to right) Scripps Research Professor Steve Kay; Florida Atlantic University (FAU) President Frank Brogan; Scripps Research Executive VP and General Counsel Douglas Bingham; and Palm Beach County Commission Chair Karen Marcus; among others.

Investigators Find Deafness Gene's Function

A group of scientists has discovered a key molecule that is part of the machinery that mediates the sense of hearing.

The collaborating scientists—affiliated with The Scripps Research Institute, the University of California, San Diego, The Oregon Hearing Research Center, and The Vollum Institute at Oregon Health & Science University—are reporting that a protein called cadherin 23 is part of a complex of proteins called “tip links” that are on hair cells in the inner ear. The tip link is believed to play a central role in the conversion of physical cues to electrochemical signals that results in hearing.

“In humans, there are mutations in [the gene] *cadherin 23* that cause deafness as well as Usher syndrome, the leading cause of deaf-blindness,” says Associate Professor Ulrich Mueller, Ph.D., a member of Scripps Research’s Institute for Childhood and Neglected Diseases.

Reference: *Nature*, **428**, 950-955 (2004).

A New Hypothesis About Autoimmunity—Is it Possible to be Too Clean?

Scientists at Scripps Research have found a connection between poor T cell survival in the body and the development of autoimmunity. On the basis of this connection, the scientists are proposing a new hypothesis about the cause of autoimmune diseases such as Type 1 diabetes and rheumatoid arthritis, in which components of a person’s immune system attack his/her own tissues.

“Autoimmunity has [traditionally] been considered a condition of too much stimulation,” says Professor Nora Sarvetnick, Ph.D. “What we are seeing is that it is a condition of too little stimulation.”

The hypothesis explains why childhood bacterial infections decrease the risk of developing autoimmune diseases and why autoimmunity has been rising in the last half century in populations with decreased exposure to pathogens. It also provides a new way of thinking about how to make autoimmune diseases more preventable—the key to decreasing the risk of autoimmunity may be to stimulate a person’s immune system by priming him or her with germs.

Reference: *Cell*, **117**, 265-277 (2004).

Scientists Create 22-Amino Acid Bacterium

A team of investigators at Scripps Research and its Skaggs Institute for Chemical Biology in La Jolla, California has modified a form of the bacterium *Escherichia coli* to use a 22-amino acid genetic code, rather than one based on the 20 amino acids found in nature.

“We have demonstrated the simultaneous incorporation of two unnatural amino acids into the same polypeptide,” says Professor Peter G. Schultz, Ph.D., who holds the Scripps Family Chair in Chemistry at Scripps Research. “Now that we know the genetic code is amenable to expansion to 22 amino acids, the next question is, how far can we take it?”

This latest result demonstrates that multiple unnatural amino acids can be added to the genetic code of a single modified organism. This proof-of-principle opens the door for making proteins within the context of living cells with three, four, or more additional amino acids at once.

Reference: *PNAS*, **101**, 7566-7571 (2004).



Scientists have expanded the genetic code beyond the common 20 found in nature. Here, a wallpaper is decorated with a gene sequence encoding 22 amino acids.



张东生教授

教授说：当时正是英雄，就是把毛主席的教训付和行动，就是要对英雄的重要性根本不大。题下，科学家对英雄的热衷，张选择了一段非一般的道路，当一九八三年中国开放学术交流的大门，张抓住机会，远赴美国休斯敦大学研究院深造。



Why Do Our Cells Betray Us?

DONG-ER ZHANG WORKS TOWARD BETTER LEUKEMIA TREATMENT

Growing up, Dong-Er Zhang had no science heroes. In her country, in fact, the whole concept of “hero” was narrowly defined.

“I was born and grew up in Beijing, the capital city of China,” says Zhang, Ph.D., associate professor in the Department of Molecular and Experimental Medicine at The Scripps Research Institute. “This was in the time of Mao, and we learned at an early age that the heroes of the revolution were communist philosophers and soldiers whose thoughts and actions embodied the teachings of Chairman Mao. Scientists were nowhere to be found in this script.”

But because she loved science, Zhang followed the unheroic path it offered, a road that led her out of China when the doors for scientific exchange flew open in 1982. She went to graduate school in 1983 at the University of Houston (“I was just lucky enough to arrive there at exactly the same time as Hurricane Alicia, which knocked out all the electricity in Houston for several 100-degree days in August”), to Harvard Medical School, and now, The Scripps Research Institute.

“Maybe because I didn’t grow up with any scientist models, I always thought of scientists as bigger than life. A scientist was somebody with amazing stature and a big, fancy imposing title,” Zhang says, her laugh a musical delight. “It wasn’t until a few years after I got my Ph.D. that I felt comfortable with that label. ‘So a scientist,’ I thought, ‘can, after all, be somebody just like me.’”

Although only 44, Zhang has already made her mark in the scientific world. She has authored or co-authored 67 journal articles on the molecular pathology of cancer development, progression and treatment. She has secured over a million dollars in funding from the National Institutes of Health. Her passport has stayed busy as Zhang has flown around the world to give more than 50 invited lectures—in the United States, Italy, France, England, Germany, Japan, China, and Korea. And most recently, she was honored by the Leukemia & Lymphoma Society as one of five Stohlman Scholars for her original research on leukemia, lymphoma and myeloma.

Zhang seems unfazed by her impressive record, saying that even though “you do feel good when people ask you to come give a talk,” her vita is basically just evidence that she’s doing her job. She prefers to talk about her current research, which she carries out with the support of 12 postdocs. The focus of this work? Investigations of blood cells that go wrong and eventually cause acute myeloid leukemia, a rapidly progressing disease characterized by the accumulation of immature, functionless cells in the bone marrow and blood.

CELLS GONE BAD

Acute myeloid leukemia begins with an accumulation of blast cells, the earliest marrow cells that can be identified, which block normal blood-cell development. As a result, red cells, white cells and platelets are not produced in sufficient numbers. The Leukemia & Lymphoma Society estimates there are 10,000 new cases of myeloid leukemia every year in the United States, and reports that approximately 22,000 people died from leukemia last year in this country.

“I always thought of scientists as bigger than life. A scientist was somebody with amazing stature and a big, fancy imposing title. It wasn’t until a few years after I got my Ph.D. that I felt comfortable with that label. ‘So a scientist,’ I thought, ‘can, after all, be somebody just like me.’” –Dong-Er Zhang, Ph.D.

Zhang explains that during normal blood-cell growth in the marrow, stem cells develop into precursor cells—a series of developing marrow cells in each blood-cell lineage. Precursor cells then become immature blood cells (red cells or white cells of various types) through a process called “differentiation.” These young cells further develop into fully functional blood cells. Then, somewhat analogous to young birds finally leaving the nest, these cells leave the marrow and enter the bloodstream.

“I’ve put a lot of effort into studying how stem cells become precursor cells and then become myeloid cells,” says Zhang. “How does the normal process get off track? We know it simply stops →

somewhere, so a cell is stuck—as a precursor or immature cell, for example—in its process of development. The problem is, these cells may keep proliferating without any function. These are deadly leukemia cells.”

To try to get to the bottom of this lethal proliferation without differentiation, Zhang’s lab is studying the regulation of gene expression. Particular genes are expressed in blood cells, and Zhang has been working to find out how this gene expression is regulated. “Our hypothesis is if we can understand the regulation of this gene expression, we can learn how cell differentiation happens.”

Gene expression, she explains, is regulated by transcription factors—proteins that bind to DNA during the initial stages of gene transcription. Because of her lab’s earlier discovery that a gene called *AML1* is involved in the regulation of myeloid-lineage specific genes, and other labs’ work that indicates *AML1* is involved in chromosome translocations identified in leukemia patients, she focused on *AML1* as an important transcription factor that might provide some valuable clues to how myeloid cells form.

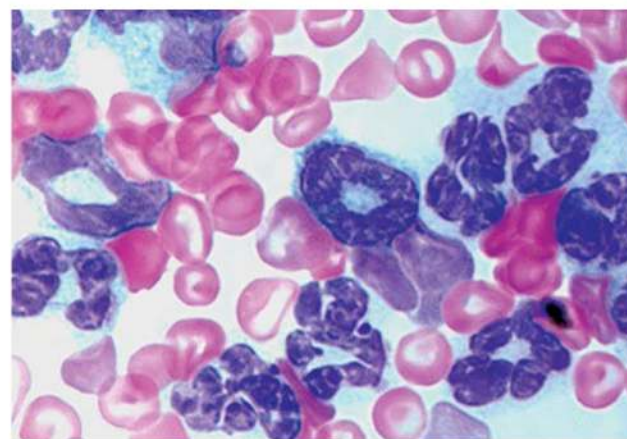
Crucial to her plan at this point was a finding from another research team that, in 12 percent of acute myeloid leukemia patients, *AML1* was fused to a gene called *ETO* to encode a protein called *ALM1-ETO*. Zhang wondered if this fusion protein might be involved in the development of leukemia cells.

To study the effect of *AML1-ETO* on blood-cell development, Zhang and her team used mice in which the *AML1* gene was replaced by *AML1-ETO*. The researchers used what’s called the “knock-in” technique to add this fusion gene to the mouse’s genetic structure, a corollary approach to the more widely used “knock-out” technique in which a gene is deleted from a mouse. The knock-in results were dramatic: normal blood-cell development was blocked, and the embryos were unable to survive.

able to see the definitive, negative effect of *AML1-ETO*, and we now know that this fusion protein is a critical factor in blocking normal blood-cell development.”

A NOVEL GENE THAT MAY LEAD TO BETTER CANCER TREATMENT

The experiment, however, contributed to further scientific understanding. Zhang’s team decided to clone several genes from the host knock-in mice, genes that are highly expressed in knock-in mice but not in normal mice. One of these genes was *UBP43*,



To try to get to the bottom of the lethal cell proliferation without differentiation that occurs in leukemia, Associate Professor Dong-Er Zhang is studying the regulation of gene expression. Particular genes are expressed in blood cells, and the lab has been working to find out how this gene expression is regulated.

and it was instrumental in providing a happy surprise for the Zhang team. “We found that this gene codes for a protease, an enzyme that breaks the conjugation between ISG15 and ISG15-conjugated proteins. ISG15 was identified more than two decades ago and is highly expressed in most cell types when infected with viruses or bacteria,” Zhang explains. She adds that this protein was originally identified as an interferon-stimulated gene whose expression is greatly induced with cancer treatments using interferon.

To try to understand the importance and function of *UBP43*, this time Zhang and her team knocked out the gene in the mice they used. “When we knocked out *UBP43*, we saw dramatic results. The mice showed a hypersensitivity to interferon stimulation,” Zhang says. She adds that the fact *UBP43*-deficient cells become hypersensitive is a significant finding. “This has clear applications for cancer treatment. If you use interferon to treat cancer

“How does the normal process get off track? We know it simply stops somewhere, so a cell is stuck in its process of development. The problem is, these cells may keep proliferating without any function. These are deadly leukemia cells.”

—Dong-Er Zhang, Ph.D.

“Actually, our goal was to develop a leukemia mouse, so in one way we were disappointed by this result,” Zhang says. “On the other hand, we were

and can modulate this sensitivity, that's obviously a good thing. Some patients are very sensitive to interferon, and some are extremely resistant. So we've opened a new avenue of study that we believe can lead to clinically related applications."

Zhang adds that this research isn't just about cancer: interferon is also used to treat bacterial infection, viral infection, autoimmune disease, and a host of other human problems that might be addressed as a result of her findings.

THE JOYS (AND FRUSTRATIONS) OF DOING SCIENCE

One of the many challenges of doing science, Zhang says, is figuring out how to turn a major disappointment into a major discovery. It's all part of the scientific research process, she says, something she clearly loves to talk about.

"When I think about starting a project, I have two important prerequisites: it has to be scientifically interesting and it has to be clinically related." Zhang says that once she decides to do a project, she takes a good look at what other scientists have discovered and what she has done, using that confluence of information as a starting point.

"Then I make a hypothesis. In the case of *AML1-ETO*, my hypothesis was that it was important for leukemia development." The problem then is how to demonstrate this gene's importance. "At that point it's crucial to design an experiment to lead you to the answers you're seeking. And of course there's the very real possibility that you may fail. But

even if you do," Zhang adds, laughing, "science advances because you've found a dead end that, now, no one else will have to run up against again."

Doing science, she says, requires both flexibility and stubbornness. "I think the trickiest part is knowing when to change gears and follow some other path, to be flexible enough to do that. It's not hard to decide when to start a project, but it's very hard to decide when to stop a project," she says, evoking an image of a car running downhill without brakes.

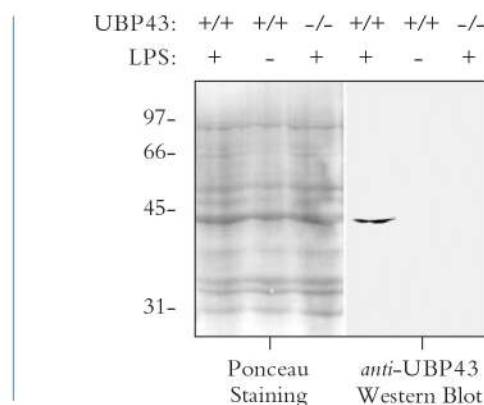
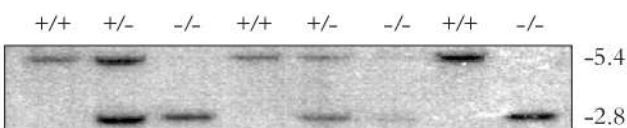
"Finding the answer is the greatest joy, but there's no time to celebrate. As soon as you finish one project, there's another one standing at the door wanting in."

Zhang says she very much enjoys her work at Scripps Research, despite the 11-hour days she puts in, and it's clear she's held in high esteem by her colleagues. "Dong-Er has a natural instinct for what is important in science and what is doable, combined with a superb scientific mind. She also is a terrific colleague," says Peter K. Vogt, Ph.D., professor in Scripps Research's Department of Molecular and Experimental Medicine.

"Dong-Er is one of the rising young scientific stars in our department," says Ernest Beutler, M.D., professor and chair of the Scripps Research Department of Molecular and Experimental Medicine. "She has devised some very important new systems that allow us to learn in mice how human leukemia develops. Ultimately, such understanding will lead to better treatment of this disease."

•Jeff Worley

UBP43 KNOCKOUT MICE



The lab's research has shown UBP43-deficient cells become hypersensitive to interferon stimulation. "When we knocked out UBP43, we saw dramatic results," says investigator Dong-Er Zhang.



Going Where Science Takes You:

DAVE LOSKUTOFF FOLLOWS A SURPRISING PATH TO UNDERSTANDING BLOOD CLOTTING, HEART DISEASE, OBESITY, AND CANCER



Scripps Research Professor David Loskutoff was a sports kid in college before he discovered a new passion: science.

Even though he got his undergraduate degree in biological sciences from the University of California at Berkeley in 1965, Dave Loskutoff didn't start out wanting to do science. Growing up near Candlestick Park in San Francisco, the former chairman of The Scripps Research Institute's Department of Vascular Biology, and now a professor in the Department of Cell Biology, was a sports kid. His physical inclination was a big part of his undergraduate days in Berkeley, where he was captain of the school's gymnastics team (he was a trampolinist).

"I didn't do a lot of thinking about science back then," he says. "I really didn't have a clue as to what I wanted to be." Until he was introduced to the laboratory of Dave Hogness, a Stanford biochemist. Like the flick of a switch, Loskutoff's interest in science was permanently turned on by what he found. The object of his studies at that time was bacteriophages—strange-looking viruses that infect bacteria.

"It was simply astounding what you could learn about things you couldn't even see," he says, recalling his own sense of astonishment, seemingly still fresh after almost 40 years. "It sparked my imagination."

Loskutoff followed his newfound passion to graduate school at the University of Colorado at Boulder, where he continued to work on bacteriophages, receiving his Ph.D. in molecular biology in 1972. By that time, he had decided he wanted

to work on more complex problems in higher organisms, so he moved to Ed Reich's laboratory at The Rockefeller University in New York City.

Supported by a fellowship from the Damon Runyon Cancer Research Foundation, Loskutoff began to explore the contribution of proteases to the abnormal behavior of malignant cells.

PROTEINS THAT CHEW OTHER PROTEINS

Loskutoff calls proteases "proteins that chew other proteins," and it's a gruesome but nearly perfect description of what they do. Water-soluble proteins that act like catalytic enzymes, proteases are prodigious workhorses that help split other proteins into smaller peptide fractions and amino acids by a process known as proteolysis. For example, metastatic cancer cells use proteases to chew through the surrounding cell matrices when they begin their deadly migration. Many viruses have to be processed to multiply; proteases cut them into precise pieces that reassemble into infectious agents. Blood clotting is the result of an especially complex protease cascade, and defects in these proteases may lead to hemophilia.

"It was simply astounding what you could learn about things you couldn't even see. It sparked my imagination." —David Loskutoff, Ph.D.

By 1975, Loskutoff had developed a clearer understanding of how proteases worked in abnormal cells. But his thinking was already shifting away from oncology toward the role of proteases in normal physiology. He also thought a change of scene would do him good.

"I think when you're trained in a specific area of research you need to take that work in a new direction when you leave," he says. "I was in contact with Tom Edgington at Scripps Research, and he invited me to come out and look around. We shared an interest in endothelial cells—they had just been grown in cell culture for the first time—so we decided to collaborate." →

In late 1975, Loskutoff was named a research associate in the Department of Molecular Biology at what was then known as the Scripps Clinic and Research Foundation. He began work on endothelial cells, which make up the inner layer of blood vessel walls, the only cells in the tissues that contact the blood. As such, they form a protective barrier between the blood and the underlying tissue that guarantees continued blood flow. If this barrier is compromised or damaged, the exposed tissue activates the protease cascade; as a result, the blood clots explosively at the site of the damage, preventing excessive blood loss.

BALANCING ACT

The process of blood clotting is an elegant if inherently dangerous physiological balancing act: if a blood clot is prevented from forming, you keep right on bleeding; however, if a blood clot doesn't get dissolved, you run the risk of thrombosis (as in heart attack, stroke, etc.). Loskutoff wondered whether endothelial cells produced plasminogen activators that might contribute to the blood clot removal process.

"We were able to show that the endothelial cells do make plasminogen activators," Loskutoff says, "but in the back of my mind, I thought they might also produce inhibitors of plasminogen activators. We just had to find them."

Protease inhibitors are compounds that interfere with the ability of enzymes to chew up other proteins. Some protease inhibitors can prevent a virus from making copies of itself, one reason that so many have been pursued as treatments for HIV infection. In the early 1980s, Loskutoff discovered a new protease inhibitor which he called plasminogen activator inhibitor-1 (PAI-1). It turned out that PAI-1 was the physiological inhibitor of plasminogen activation in the body, and an important regulator of plasmin formation.

"That's one of the good things about The Scripps Research Institute... You come up with a project and the grants to support it, and you can go wherever it leads—learn new things, even change direction if dictated by the work. You go where the science takes you." —David Loskutoff, Ph.D.

Loskutoff's discovery of PAI-1 changed his entire scientific career, and he followed this new inhibitor gladly and intently. In fact, he's spent most of the rest of his career pursuing it.

"The key thing to remember," Loskutoff says, "is that proteases can be dangerous and have to be

controlled. Plasmin, if not inhibited, can quickly chew up all of the clotting proteins in the blood—in other words, if you are cut, you cannot stop the bleeding. Bacterial plasminogen activators like streptokinase had been used clinically to dissolve clots in heart attack and stroke patients, but the patients often developed severe bleeding problems because large amounts of uncontrolled plasmin were formed in the circulation. The situation improved dramatically with the discovery of the body's natural clot busting protease, tissue-type plasminogen activator (tPA). PAI-1 is the major inhibitor of tPA, and we discovered that here at Scripps Research. We also realized that if you have too much of this inhibitor you are in danger of tipping the balance in the other direction towards pathological thrombosis."

THE LABORATORY BECKONS

Loskutoff's early work on tPA and PAI-1 was based entirely on biochemical and cell culture studies. Stimulated by these and related discoveries, other researchers, those with a more clinical bent, began applying these observations to the development of advanced therapies like the use of recombinant tPA as a new and highly specific clot buster. During this time, Loskutoff was asked to chair the Department of Vascular Biology (now part of the Department of Cell Biology), a position he left in 2002 after nine years. The weight of holding what was essentially two full time jobs simply became too much.

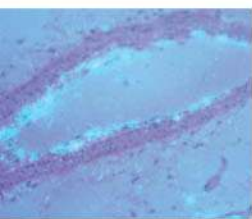
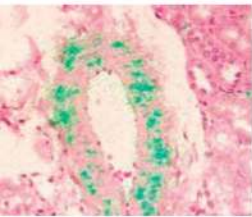
And there were new observations that beckoned to him from his own laboratory.

"That's one of the good things about The Scripps Research Institute," he says. "You're totally self-sufficient. You come up with a project and the grants to support it, and you can go wherever it leads—learn new things, even change direction if dictated by the work. You go where the science takes you."

And so, for the last ten years, Loskutoff has been working with mouse models to see what PAI-1 does *in vivo*.

"It was a natural progression to move into *in vivo* studies," he says. "With the availability of a variety of new and highly sophisticated technologies, you can actually determine how observations made in the test tube are related to real life—at least in mice."

Loskutoff made the shift to *in vivo* studies in the early 1990s. It was not a commitment made lightly. It involved a number of difficult and time-consuming changes in laboratory reagents and techniques, but he believes it to be one of the best decisions he's made

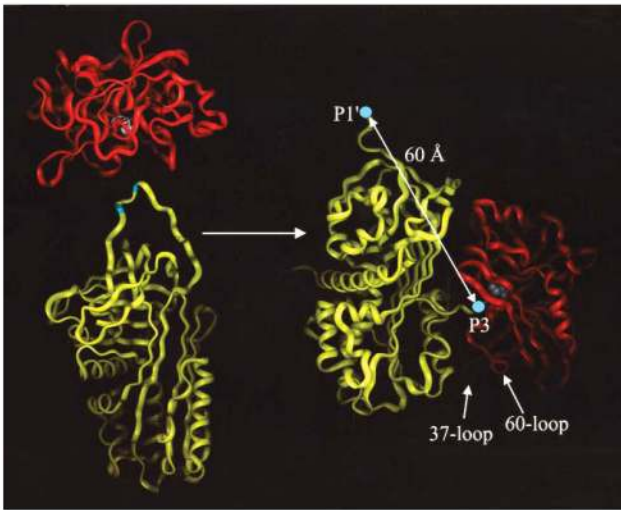


Dave Loskutoff's discovery of PAI-1 changed his entire scientific career, and he's spent much of the rest of his career pursuing research on the inhibitor. These photos identify PIA gene expression in a blood vessel wall (shown in green, top; blue, bottom).

as a scientist: “It was kind of fun because you make unexpected observations when you change systems, you develop new concepts, new ideas. All in all, it was extremely interesting and very productive.”

For Loskutoff, the larger picture is his growing understanding of the role of PAI-1 in a variety of disease states.

“The most exciting projects in the lab now have implications for obesity and cancer,” he says. “We got interested in obesity not only because it’s a major health problem in Westernized societies, but also because a number of clinical studies had shown that obese people have high levels of PAI-1 in their blood. Thus, there was a potential link between the elevated risk for cardiovascular disease in obesity and the high PAI-1 levels. We wondered whether obese mice also had high levels of PAI-1 and if they did, could we use them to identify the cells that produce it, and the factors that regulate it.”



In the early 1980s, Professor David Loskutoff discovered a new protease inhibitor, which he called plasminogen activator inhibitor-1 (PAI-1). This illustration shows the interaction between PAI-1 (in green) and a protease (in red), in which a covalent bond forms between the two molecules. In the process, PAI-1 is cleaved and consumed, giving rise to the term “suicide inhibitor.”

UNEXPECTED LINKS

Loskutoff and his colleagues assembled several different types of obese mice—six strains in all—and began testing. What they found was that they all had high circulating levels of PAI-1, and that adipocytes—the cells that store fat in the body—were producing large amounts of it. The truly odd thing, however, was that the obese mice, with the same high levels of PAI-1 as obese humans, did not share an increased cardiovascular risk.

Loskutoff knew from other studies that his laboratory mice were obese because they were unable to produce leptin, a hormone that binds to brain receptors and tells the mouse when to stop eating. Without leptin, Loskutoff’s mice kept on eating—incessantly.

“The most exciting projects in the lab now have implications for obesity and cancer.” –David Loskutoff, Ph.D.

“We followed our noses on leptin,” Loskutoff says, “and we now have data to show that if you administer leptin to a mouse, that mouse develops atherosclerosis much faster, accelerating the risk for thrombosis.” Some researchers believe that reduced sensitivity to leptin in obese patients may actually cause the body to produce even more of the hormone, adding to their elevated risk.

Another new project in the lab may have implications for cancer. The idea that proteases are linked with cancer was something that Loskutoff understood from his early work at Rockefeller. But within the past few years, it has also become apparent that cancer patients with high levels of PAI-1 have a decreased chance of survival. This unexpected link between high levels of PAI-1 in breast and uterine cancers and a poor prognosis seemed to go against all that he’d learned. Shouldn’t PAI-1, by inhibiting cellular proteases, actually help prevent metastases?

What Loskutoff found was that when PAI-1 was given to tumor cells *in vitro*, the cells detached from the surrounding matrices, a sudden and unexpected event. Although the underlying mechanism of this detachment isn’t fully understood, Loskutoff’s work suggests that the PAI-1 binds to the cell, causing cell adhesion receptors (integrins) to let go of the extracellular matrix to which they are attached. The cells in tumors that produce high levels of PAI-1 may also be less firmly attached, and this could be a contributing factor to the patients’ poor prognosis. This discovery may eventually help point the way toward better treatment.

From Loskutoff’s perspective, this is just one more fascinating aspect of what has become his life’s work.

“I’m now interested in how PAI-1 could do something like this. Since it doesn’t seem to do so by inhibiting a protease, it must be doing something else entirely. It’s a bizarre little observation. We’ll see where it leads.”

•Eric Sauter



Grandfather Riedesel

**"I JUST WANNA KNOW ABOUT
THE ROOMS BEHIND YOUR MINDS."**
-Jimi Hendrix, "Up From the Skies" from Axis: Bold as Love (1967)

Hitting the Charts:

GREGORY DEL ZOPPO SETS HIS SIGHTS ON IMPROVING OUTCOME FOR STROKE PATIENTS

Scripps Research Associate Professor Gregory del Zoppo grew up in Seattle, Washington in the 1950s and early 1960s around the same time as guitar legend Jimi Hendrix. The two were only a few years apart in age, just a few miles apart in address, but light years apart in career paths.

While Hendrix was experimenting with sound and music, moving to New York and then London in the late 1960s, and forming his band Experience, del Zoppo was living in Seattle, experimenting with molecules and reactions, and graduating *magna cum laude* in the Class of '70 with an honors degree in chemistry from the University of Washington. Whereas Hendrix made three classic albums, del Zoppo obtained three degrees, including one in molecular biology and neurophysiology from Caltech and an M.D. from the University of Washington.

And whereas Hendrix combined the hard sounds of bluesy rock with the sophisticated phrasing of jazz, del Zoppo combined the practice of clinical medicine with the discipline of laboratory science.

In 1982, after completing his internship, residency, and fellowship requirements to become a licensed physician, del Zoppo went to London for two stints at the Institute of Neurology. He then came to La Jolla to divide his time between Scripps Clinic and Scripps Research.

It was during this time that del Zoppo began to actively pursue a goal he had since childhood—finding ways to improve the clinical outcome of stroke.

Pursuit of this goal led del Zoppo and his colleagues to design and conduct the first international clinical trials for the acute intervention of stroke, which took place in La Jolla and Aachen, West Germany from 1984 to 1986. These trials proved successful and showed that treating stroke soon after its onset resulted in a significant improvement in clinical outcome.

“[The treatment] increases the number of patients who do very well,” says del Zoppo. “We think parts of the brain that would normally die are salvaged.”

While the success did not bring del Zoppo nearly as much fame as his fellow Seattle native Hendrix once enjoyed, it did make his name well known within the community of doctors who treat stroke and scientists who study it.

Del Zoppo’s resolve to find better treatments for stroke was reinforced by one particular memory of his grandparents—two German immigrants who settled in the Pacific Northwest in the 1920s. They were lovely people, del Zoppo recalls. He also remembers how his grandfather had trouble walking around his farm.

“[The treatment] increases the number of patients who do very well. We think parts of the brain that would normally die are salvaged.” —Gregory del Zoppo, Ph.D.

“I remember [that] part of his body was lame,” says del Zoppo. “I couldn’t understand why—what had caused that?”

Later, of course, he learned that his grandfather had suffered a stroke. And as del Zoppo grew up and attended school, he wondered over and over, how he could help someone like his grandfather to fully recover.

TREATING STROKE

When del Zoppo began medical school in 1973, there was no treatment for patients who showed up in the emergency room in the middle of a stroke. Doctors could treat a patient who had survived a stroke to reduce the chances of a second stroke. Later, they could preemptively treat a patient who was at risk of having a stroke, and they could help stroke patients recover through physical therapy programs. But nobody could treat a stroke while it was happening.

In the early 1980s, when he was just unpacking his bags in La Jolla, del Zoppo was looking at the causes of stroke and attempts to treat it, and he realized that much of the work in the field was focused on the brain and on protecting the neurons. But he and a few of his colleagues had the radical idea that the way to treat stroke was to focus on the bloodstream. →

His first trial testing this idea used the naturally occurring human enzyme urokinase to treat stroke patients within a few hours of their having a stroke. Urokinase, a plasminogen activator belonging to a class of enzymes known as serine proteases, is part of the body's natural mechanism for removing blood clots from arteries. They cleave a circulating inactive blood protein called plasminogen to make active plasmin—another protease. Plasmin degrades fibrin, the sticky protein that gloms together with platelets in the bloodstream to form a blood clot.

Plasminogen activators are so effective in removing clots that cardiologists at the time were beginning to study another “clot busting” agent, tissue plasminogen activator (tPA), for treating heart attack victims who had blood clots in their coronary arteries. Many strokes are caused by blood clots that form in the carotid arteries, which feed directly into the brain, and del Zoppo and his colleagues reasoned that clinical improvement would result if doctors could break up these blood clots by directly delivering urokinase.

This was something of a radical suggestion at the time because use of these agents involves a risk of intracerebral hemorrhage, i.e. bleeding in the brain. Clot busters can significantly increase the risk of a hemorrhage. Early trials treating stroke with agents like t-PA had actually made the stroke worse.

However, many of the patients in these earlier studies were treated late—a day or more after the stroke. Del Zoppo's trials demonstrated that stroke could be treated acutely without harming the patient. In fact, the data demonstrated that treating the patient within the first few hours after the onset of stroke was of great benefit. Many other clinical trials by colleagues followed, and they proved that t-PA, if administered quickly after the onset of stroke, is invaluable for treating the condition.

In the 1980s, the National Institutes of Health sponsored two separate placebo-controlled trials to determine whether intravenous delivery of clot busting agents is clinically relevant. In 1995, the NIH-sponsored trials were published, proving that t-PA delivered in the acute phase was beneficial. It is now standard care for ischemic stroke patients.

A few years later, del Zoppo made another pioneering contribution to the treatment of stroke by conducting the first placebo-controlled clinical trials on the local delivery of clot busting agents like t-PA.

VASCULAR RESPONSES IN STROKE

Del Zoppo has made significant contributions in the laboratory as well as the clinic.

For years, doctors focused on the ischemic injury to neurons during stroke, and for logical reasons—a stroke deprives the brain of oxygen-rich blood, the lack of oxygen causes neurons to die, and the loss of neurons leads to all the health problems associated with stroke recovery. There is no question that the blood clots and lack of oxygen are the major insult to the neurons.

But that is not the whole picture.

More of the brain is injured than those areas directly affected by the initial blood clot. The microvasculature, all the tiny blood vessels (including the capillaries) in the brain, respond almost as fast as the neurons to the stoppage of blood with their own program of events. And a lot of the injury from an ischemic stroke seems to come from how the cells lining the blood vessel where the clot occurs respond.



While much work in the field of stroke was focused on the brain and on protecting the neurons, del Zoppo and a few of his colleagues had the radical idea that the way to treat stroke was to focus on the bloodstream. Here, a blood vessel is shown surrounded by cathepsin B-stained cells.

“Stroke is a vascular disorder,” says del Zoppo. “We’re trying to develop ways to reduce the ischemic injury by [focusing on] the microvasculature.”

One of the major problems for the microvasculature during ischemic stroke is that often the protective barriers separating the blood from the brain tissue are broken down. Breakdown of the barriers occurs in the ischemic area and leads to hemorrhaging in the brain, which enhances the injury. Some 65 percent of stroke victims experience some sort of hemorrhaging, says del Zoppo.

The blood–brain barrier is found mostly in the blood vessels of the brain. These vessels are lined with endothelial cells, which are linked together

with tight junctions that prevent the plasma of the blood from leading into the brain. Also present in the brain's vessel walls are a less abundant cell type called the astrocytes—named for their star-like appearance. They form the outside of the blood vessels and support neurons. Between the endothelial and astrocytic cells is a mesh of proteins with carbohydrates (proteoglycans), called the extracellular matrix, which prevents blood cells from leaking out—similar to how barricades define lanes and channel traffic around construction sites.

During ischemic stroke, endothelial cells detach from each other and the astrocytes, and the extracellular matrix breaks down. Components of blood, including red cells, leak in the tissue. Other blood cells that produce inflammatory chemicals enter the brain and wreak havoc there, injuring tissue and killing brain cells (including neurons).

In the clinic, del Zoppo has designed several clinical trials to look at the effect of blocking the adhesion of granulocytes and other immune cells to endothelial cells—to see if agents that block this adhesion can reduce injury to stroke patients.

In the laboratory, del Zoppo is asking more fundamentally if some treatment designed to stop the bleeding might reduce injury as well.

A few years ago, he and his colleagues showed that a protease called matrix metalloprotease (MMP-2) and a related protease called MMP-9 were significantly increased within the first half hour to hour of the onset of ischemic stroke in the same area where the neuronal damage and bleeding occur, respectively. They showed that these proteases, if transferred to normal tissue, could degrade the matrix, and this led del Zoppo and his colleagues to propose that MMP-2 and MMP-9 are largely responsible for the degradation of the matrix and are related to neuronal injury.

This is important because it means that the MMPs and other matrix proteases are potential players in the injury process, and, as such, are potential targets for therapy. The scientists are testing MMP inhibitors to see if they prevent the degradation of the matrix in laboratory models.

If this succeeds, then eventually a carefully controlled human trial might answer the question of whether MMP inhibitors could preserve brain function.

“It works experimentally,” says del Zoppo. “It [may be] a little more complicated in patients.”

•Jason Socrates Bardi

What Happens During Stroke

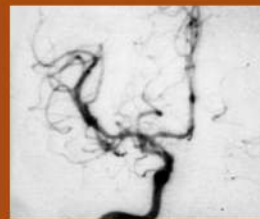
Stroke is a vascular disease involving the arteries that feed blood to the brain. A stroke occurs when this blood flow is interrupted, cutting off part of the brain from oxygen. Starved of oxygen, that part of the brain is stunned, and neurons in the area will undergo an excitatory spike of activity followed by silence and death.

Those neurons that don't die immediately are plunged into a Hamlet-like “to be or not to be” drama, where cells decide whether they can survive or whether they're so damaged that they should self-destruct. Even when blood flow is restored after a stroke, neurons may continue dying for several hours or days, and the damage to a person's brain can grow worse. But, overall, restoring blood flow early (within hours) is a good thing.

Scripps Research Associate Professor Gregory del Zoppo sees the effect of strokes first-hand in the clinic. Patients experience sudden numbness or weakness, especially on one side of the body; they may suffer sudden confusion or trouble speaking or understanding speech; they may have sudden trouble seeing in one or both eyes, sudden trouble walking, dizziness, or loss of balance or coordination.

Stroke is a major medical problem in the United States, where it is the third leading cause of death. The U.S. Centers for Disease Control and Prevention estimates that about 700,000 people suffer a stroke each year and about a fifth of them die.

Those people who survive have an increased chance of having another stroke within the first year. And this situation is worsened by the fact that stroke is the leading cause of serious, long-term disability, including paralysis, cognitive deficits, speech problems, depression, and other disabilities. The lives of stroke survivors and their families are often deeply impacted by these lingering effects.



A stroke occurs when an artery that feeds blood to the brain becomes blocked (top). Plasminogen activators can dissolve the block and allow blood to flow into the arteries downstream (bottom).

“Explore. Dream. Discover.”



Raymond A. Dwek, professor, director of the Glycobiology Institute, and head of the Department of Biochemistry at Oxford University, gives the keynote address at Scripps Research's 12th commencement.

Distinguished scientist Raymond A. Dwek, D.Phil., professor, director of the Glycobiology Institute, and head of the Department of Biochemistry at Oxford University, received an honorary degree from The Scripps Research Institute's Kellogg School of Science and Technology in the institute's 12th commencement on May 21. As part of the ceremony, Dwek shared his words of advice and insight into the current state of science with the 31 graduating students and their many supporters in the audience. Excerpts from his remarks follow.

This is indeed a very special day for you all as you complete your graduation for your Ph.D. degree. However you should not think of this occasion as the end of your education. As Winston Churchill said in another context, “This is not the end. It is not even the beginning of the end. But it is perhaps, the end of the beginning.”

You have graduated from one of the great institutes in the world and, as well as being a great privilege, this carries with it great responsibility.

We do science because we love it and because we are passionate about it. As we head into the third millennium, one of the greatest impacts that scientific research will have on our lives, and also one of the greatest challenges for us, will come from the ever-increasing amounts of scientific information that are being generated.

TRUTH, SCIENCE, AND THE SEARCH FOR MEANING

As individual scientists, we can know and research only a small part of this huge body of information so if we are to make coherent sense of the world we live in we need to join together with others from all walks of life as we search for truth and meaning.

There is a story told in the Talmud of a debate that took place in heaven about whether mankind should be created. The vote was split right down the middle; two in favour and two against. Love and Righteousness were in favour; Truth and Peace were against.

The tie was broken by God who hurled Truth to the ground smashing it into thousands and thousands of jagged pieces. Now the vote was 2:1 in favour and so mankind was created.

“Why do you break your emblem Truth?” the angels asked. And the answer came, “Because Truth

will spring from the earth.” From then on Truth was dispersed, split into fragments like a jigsaw puzzle. For us the analogy is clear. While one person might find a piece, it holds little meaning until we join with others who have also painstakingly found different pieces of the puzzle. Only then, slowly and deliberately, can we fit the pieces of truth together to make sense of things.

Scientists have an absolutely key role to play in mankind's efforts to reforge Truth...In Science, we still do not have all the pieces. We will probably never have all the pieces. But, as we search for the truth there are a number of consequences. All of us live in the darkness of preconceived ideas, prejudices, and inherited assumptions. Into this darkness scientific discovery comes as a penetrating light. And this light makes us question our prejudices or preconceived ideas. Scientific knowledge sometimes runs ahead of our legal, ethical, and moral framework. It is essential that we learn to work with those with expertise in other disciplines to ensure that our research ultimately benefits human kind and human civilization and enables us to live in harmony with the rest of creation.



Raymond Dwek receives an honorary degree from the Scripps Research Kellogg School of Science and Technology.

OXFORD, SCRIPPS RESEARCH, AND THE QUEEN

As you heard, I come from Oxford University—a unique and historic institution. As the oldest English-speaking university in the world, it can lay claim to nine centuries of continuous existence...Yet

strangely enough, although the university's first overseas student, Emo of Friesland, arrived in 1190, Oxford has never had a joint degree course with any other university or institution—until April 2004. That's when the University Council voted to create the first-ever joint Ph.D. with The Scripps Research Institute. Given the Byzantine number of bylaws, regulations and statutes that the university has acquired over the last nine centuries, this innovation was a Herculean labor—it even brought the Queen and the Privy Council into play! So [Scripps Research] and its standards of excellence are being discussed at the highest circles of government in the UK—another testimony to the leadership of President Lerner, a great visionary, an outstanding and pioneering scientist, and wonderful colleague and friend.

Of course, while a joint degree is new, there has long been a fruitful academic connection between the United States and Oxford, exemplified by the famous Rhodes Scholarships. And today, the new Scripps Oxford Ph.D./D.Phil. scholarships—funded by the generosity of the Skaggs family—bring a new dimension to this tradition of transatlantic partnership. Such partnerships are also vital in creating multinational understanding in these turbulent times.

THE CUTTING EDGE OF SCIENCE

There are many exciting problems to be tackled at the cutting edge of science today. Physicists are attempting to rationalize the world of small particles, cosmologists are looking for the missing matter in the universe, engineers are exploring applications of nanotechnology that will change the way we use materials, biomedicine is exploring how we can correct genetically transmitted diseases, while ecologists and geologists are discovering more about earthquake zones, the deep ocean and the atmosphere.

Over the next 10 to 15 years single DNA molecule sequencing will lead to the ability to sequence a human genome in about a day. This will potentially serve as the basis for determining a probabilistic health history for each individual, something that clearly has major implications for pharmaceutical companies, lawyers, and financial institutions.

The human genome project also provides us with information that demonstrates that our individual existences result from the most amazing combination of events and circumstances. Such an understanding, drawn from our scientific endeavor, allows us to perceive

life as a gift rather than as a right. Understanding that life is a gift enables us to come to terms with our awareness of the finite nature of life and the power that the fear of losing it can have over us. The desire for instant gratification that arises from this fear prompts jealousy and destructive competition, and if we are not careful, allows us to justify laying hold of the lives of other people or other species to our own advantage. Experiencing life as a gift enables us to transform the life that we have been given into one that we are ready to use for the well being of others.



A record number of students, 31 in all, graduated from the Kellogg School in 2004.

EMBRACE YOUR PASSIONS

I would like to leave you with a message from Nelson Mandela that may help you on your future journey.

“Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light not our darkness that most frightens us.” And this next bit applies even more to this class of 2004. “Don’t question your right to excel. Your playing small doesn’t serve the world. There is nothing enlightened about shrinking so that other people will not feel insecure around you. And as we let our own light shine, we unconsciously give people permission to do the same. And as we let our light shine, we unconsciously give people permission to do the same...”

Be thankful for the life you are building. Honor it by doing the things you are most afraid of and embrace your passions as you discover them. Mark Twain once stated, “Twenty years from now you will be more disappointed by the things you didn’t do than by the ones you did. So throw off the bowlines. Sail away from the safe harbour. Catch the trade winds in your sails. Explore. Dream. Discover.” ●

In Memoriam



Bernard Babior was a renowned physician and biochemist.

Bernard M. Babior, M.D., Ph.D. (1935–2004)

Bernard M. Babior, M.D., Ph.D., professor and head of the Division of Biochemistry at The Scripps Research Institute and staff physician at Scripps Clinic, died in San Diego, California on June 29, 2004, after a long battle with prostate cancer.

Babior was noted for his groundbreaking insights into human biochemistry, particularly as they pertained to the body's defenses against infection. He was one of those rare individuals who was highly respected and considered as "one of our own" both by members of the medical profession and professional biochemists.

"Bernie contributed so much during his long tenure at the institute..."

—Richard A. Lerner, M.D.

Bernard Babior was born in Los Angeles on November 10, 1935. He received his M.D. degree at the University of California at San Francisco in 1959. After interning at Peter Bent Brigham Hospital in Boston, he joined the laboratory of Nobel laureate-to-be Konrad Bloch at Harvard University and was awarded a Ph.D. degree in 1965. He received further training at The National Institutes of Health, then served on the faculty of Harvard University and Tufts University before moving to Scripps Research in 1986.

Early in his career, while studying a vitamin B12-dependent enzyme, he recognized that free radicals,

very unstable and difficult-to-measure molecules, might play an important role in biological processes. He showed that highly reactive oxygen derivatives were weapons that white cells use to kill invading bacteria. This revolutionary concept, initially slow to be adopted, is now recognized as one of the important mechanisms that enable humans and lower life forms to exist without being destroyed by invading microbes.

Babior and others also showed that the very weapons that the body makes to protect itself against microbial invasion can also play an important role in a variety of common diseases, including arthritis, arteriosclerosis, and Alzheimer's disease. Treatments that are now being devised for these disorders are based on Babior's insights concerning basic biochemical mechanisms.

Scripps Research Institute President Richard A. Lerner, M.D., expressed his sorrow at the loss, stating, "Bernie contributed so much during his long tenure at the institute, not only by the invaluable research that has enriched science worldwide, but also through his humanity, his sense of serving those with whom he came in contact. The Scripps community will miss him dearly."

•Ernest Beutler, M.D.

THE BABIOR LECTURESHIP

Friends and colleagues of distinguished scientist Bernard M. Babior, M.D., Ph.D., are establishing a lectureship in his memory.

Contributions can be sent to:

The Babior Lectureship Fund
c/o Ernest Beutler, M.D.
Chair, Department of Molecular and
Experimental Medicine
The Scripps Research Institute
10550 N. Torrey Pines Road, MEM-215
La Jolla, CA 92037

*Please make checks payable to the
"TSRI Babior Lectureship."*

Behind The Scenes:

PRIVATE SUPPORT UNDERPINS SUCCESSFUL SCIENCE



By the time he celebrated his 100th birthday, Arnold Beckman had given more than \$270 million to support scientific research.

Arnold O. Beckman, Ph.D. (1900–2004)

The Scripps Research Institute remembers Arnold O. Beckman, Ph.D., a leading scientist, inventor, philanthropist, and business and civic leader, who died on May 18 at the age of 104.

The Arnold and Mabel Beckman Foundation, which issues grants on the behalf of Beckman and his wife, provided major funding toward the Arnold and

Mabel Beckman Center for Chemical Sciences, a building that opened in 1986 on the Scripps Research campus. Today, the Beckman Center houses more than 400 scientists in fields such as molecular design, chemical synthesis, and bioorganic chemistry.

In 1935, Beckman founded National Technical Laboratories, later renamed Beckman Instruments, to manufacture an inexpensive pH meter he had invented for use in citrus juicing plants.

“When you’re faced with the necessity to do something, that’s a stimulus to invention,” Beckman once said. “If [my classmate] hadn’t come in with his lemon juice problem, chances are I never in the world would have thought about making a pH meter.”

A forerunner of modern electrochemical instrumentation, this scientific instrument simplified and expedited acidity and alkalinity measurements. It quickly became a ubiquitous tool in the laboratory, earning Beckman a place in the National Inventors Hall of Fame. The pH meter also provided the foundation for the firm that was to grow to become one of the world’s leading suppliers of scientific instruments and related products.

Calendar

SEPTEMBER 2004

14 Donor luncheon featuring Jorge Nieva, M.D., on “Cancer and Breast Cancer Research.”

OCTOBER 2004

15 The “Tuxes for Tia” event at the San Francisco Ritz-Carlton benefits the work of Raymond Stevens, Ph.D., in PKU.

NOVEMBER 2004

* A donor luncheon features Ernest Beutler, M.D., as speaker.

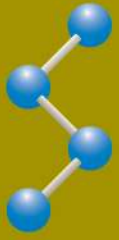
* Donor event at the Indian Wells Country Club featuring Steve Mayfield, Ph.D.

*Date of event to be determined.

If you are interested in attending any of the above events, please contact the Scripps Research Development Office, 858.784.9367.

The institute also hosts two lecture series—“Second Cup of Coffee,” a science education series designed for lifelong learners, and “State of the Science Symposium,” for biotech leaders and business partners.

For more information on these events, contact Marcia Gravette, Institute Relations, 858.784.2915.



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*A publication of
The Scripps Research Institute*

*Office of Communications—TPC20
10550 North Torrey Pines Road
La Jolla, California 92037*

NON-PROFIT
U.S. POSTAGE
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PERMIT 751
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Publisher:
Keith McKown

Editor:
Mika Ono Benedyk

Writers:
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Design:
Greenhaus

Production:
Greenhaus
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Kevin Fung

Cover Illustration:
Nicholas Wilton

Portrait Photography:
Jonathan Woodward

Printing:
Precision Litho