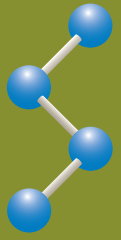


VOLUME EIGHT | NUMBER THREE



THE  
SCRIPPS  
RESEARCH  
INSTITUTE

# Endeavor

Fall 2005



## Childhood Diseases

Science suggests new approaches

# Endeavor

VOLUME EIGHT | NUMBER THREE

Fall 2005

This issue of *Endeavor* focuses on a topic close to many of our hearts—children, specifically ongoing investigations at Scripps Research that are suggesting new ways to bring devastating childhood diseases under control.

## featured

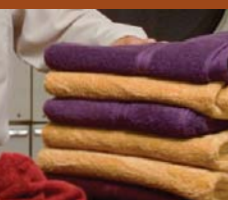


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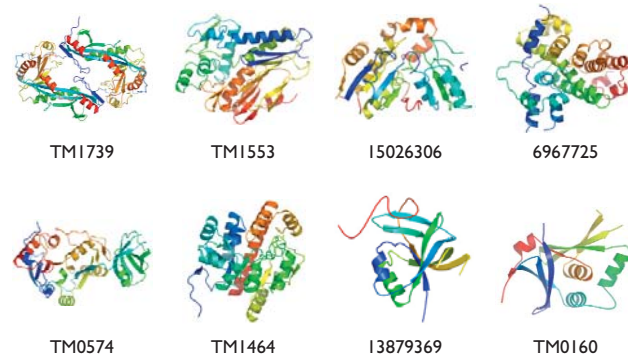
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## Center Awarded More than \$50 Million by NIH in Effort to Solve Protein Structures

A consortium of scientists at The Scripps Research Institute and several other California institutions has been awarded a \$52.7 million grant by the National Institute of General Medical Sciences (NIGMS), part of the National Institutes of Health. The grant is part of the second phase of a \$600 million national effort called the Protein Structure Initiative that ultimately seeks to find the three-dimensional shapes of all types of proteins. This structural information will help reveal the roles that proteins play in health and disease and will help point the way to designing new medicines.

The five-year grant will fund the Joint Center for Structural Genomics (JCSG), a multi-institutional consortium of laboratories in La Jolla and Palo Alto, California, which is based at Scripps Research and led by Professor Ian Wilson, D.Phil. The JCSG has been solving about 100 structures a year, and this output should now increase significantly.

As one of ten new research centers established nationwide under the Protein Structure Initiative, the JCSG will be one of only four centers that was selected to advance from the pilot stage to become a large-scale production center.



The Joint Center for Structural Genomics has been solving about 100 structures a year. With the new grant, this output will increase significantly.

# At the Forefront

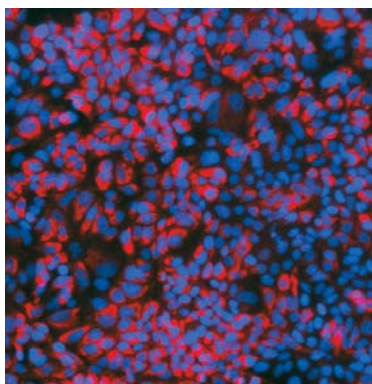
## Team Creates First Cell Culture System for Hepatitis C Virus, a New Tool for Vaccine and Drug Research

In a surprising first, a group of researchers led by scientists at Scripps Research has developed a way to create a robust infection of liver cells with one strain of hepatitis C virus *in vitro*. The researchers can grow the cells in the appropriate conditions, infect them with the hepatitis C virus, collect new virus particles as the virus replicates, and go on to infect more cells—essentially replicating exactly what the hepatitis C virus does in the liver of infected patients.

Developing a cell culture system is an important step, according to Scripps Research Professor Francis V. Chisari, M.D., “because it’s a great system to use to study the viral life cycle and to look for drugs that can block the virus in different stages of that life cycle.”

According to the U.S. Centers for Disease Control and Prevention (CDC), hepatitis C virus currently infects some 3.9 million Americans and more than 180 million people worldwide. An additional 30,000 or so people are infected each year in the United States. Hepatitis C virus is one of the most common causes of chronic liver disease in the United States and is the number one indication leading to liver transplantation.

Reference: PNAS, **102**, 9294-9299.



“The lifecycle of the [hepatitis C] virus is now completely open to us,” says Professor Francis V. Chisari, Ph.D. (Image of robust hepatitis C infection *in vitro* courtesy of Jin Zhong.)

## Scientists Describe Protein Used by Bacteria and Cancer Cells to Resist Drugs

Scientists at Scripps Research have solved the structure of a protein called MsbA, which is involved in resisting antibiotics and chemotherapy. Bacteria use these transporters to nullify antibiotics, and human cancer cells have similar membrane transporters on their surfaces that undermine the potency of chemotherapy drugs. The structures reveal molecular details that could be useful for improving cancer therapy and fighting antibiotic-resistant bacteria.

“We actually have very good drugs to fight cancer and to kill bacteria,” says Associate Professor Geoffrey Chang, Ph.D. “[But] they can’t always get in the cells to work.”

Reference: Science, **293**, 1793-1800 (2005).

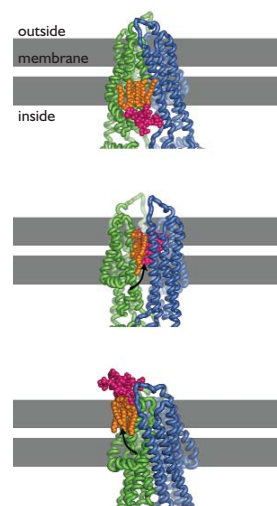
## Cooperation is Key—A New Way of Looking at MicroRNA

A group of scientists at Scripps Research and other institutions is reporting a discovery that sheds light on how genetic control can be exerted in living cells—an area of research fundamental to everything from the normal processes that govern the everyday life of human cells to the aberrant mechanisms that underlie many diseases, including cancer and septic shock.

The discovery concerns tiny fragments of RNA known as microRNA and their relationship to the genetic transcripts known as messenger RNA (mRNA).

“Most microRNA probably need the help of these other proteins and other molecules to target mRNA,” says Professor Jiahuai Han, Ph.D., describing the team’s results. “[This targeting] not only depends on their complementary sequence but on whether these proteins are around to stabilize them.”

Reference: Cell, **120**, 1-12 (2005).



The structures of MsbA that Geoffrey Chang, Ph.D., and Christopher Reyes, Ph.D., solved using high-resolution x-ray crystallography reveal molecular details that could be useful for improving cancer therapy and fighting antibiotic-resistant bacteria.



# Shaping the Growing Mind

SHELLEY HALPAIN UNCOVERS THE FORCES BEHIND FRAGILE X SYNDROME

In the photographs of neurons on Shelley Halpain's Web site, the cells appear to be gangly, colorful creatures, their spectral blue nuclei sprouting fluorescent green tendrils speckled with candy-apple-red dots. They may be neurons, but they look like some alien life form. In a ten-second video of a neural cell, the tendrils quiver and shake, their filaments undulating like dense fiery tongues. This is your brain; this is your brain on fire. This is cellular biology in CinemaScope.

Shelley Halpain, the scientist responsible for this cinematic oeuvre of neuron formation and growth, has for decades been taken by the seemingly paradoxical idea that you can study the human mind by studying its cells. As an undergraduate, she was interested in human behavior but thought psychology a bit nebulous. She wanted to get down to the nuts and bolts. Nearly a quarter century after graduating from the University of California (UC) at Irvine, that is precisely what she's doing—drilling down into the basics of the brain, studying the fundamental mechanisms of neuron development.

## INTO THE BIOLOGICAL MICROCOSM

Born in Missouri, but raised in California from the age of five, Halpain first wanted a job to “to save the planet, especially the animals and plants in the national park system.” While UC Irvine was strong in ecology, she quickly realized that no such job existed and turned toward medicine. Students were encouraged to gain lab experience and, as a result, she came under the influence of Professor Gary Lynch, whose dynamic instructional style turned her attention to the biological microcosm, in particular neurons, the cells of the brain.

The nuts and bolts of studying the brain through its cellular structure appealed to her love of details and her innate curiosity about how things work. (It is a trait she likes to think of as genetic: “My five-year-old daughter takes after me, she has a great curiosity about everything,” she notes.) Her focus on the details of neuron development has had an impact on how she goes about research as well. For example, her lab takes full creative advantage of the latest microscope technology for their assays.

As a post-graduate, Halpain became fascinated with the sheer beauty of cells observed through a microscope. As a result, she eventually studied microscopy at the Woods Hole Oceanography Institute, an organization famous for this approach. When she put together her own laboratory, she helped design and build her own microscopy system.

Halpain left Irvine for graduate studies at New York's Rockefeller University, making the geographic and cultural leap to the East Coast in 1981. Not knowing a soul, her first year was hard, but she stuck it out (“I have a very stubborn streak”). She not only stuck it out, she spent the next 10 years at Rockefeller, first completing her Ph.D. with Professor Bruce McEwen in 1986, then moving on to postdoctoral work in the laboratory of Professor Paul Greengard, a 2001 Nobel laureate in Physiology of Medicine.

After that, she accepted an assistant professorship in neuroscience at the University of Virginia Health Sciences Center, where she started work on the function and regulation of cytoskeletal proteins, the basic molecular components of neurons. Halpain came to The Scripps Research Institute's Department of Cell Biology in 1996 and was named an associate professor in 1999. She is also a member of the Institute for Childhood and Neglected Diseases.

*“The types of things possible at Scripps, not just the physical resources but the collegial resources, don't exist anywhere else. There's no barrier to collaboration.” —Shelley Halpain, Ph.D.*

The widespread use of the microscope in Halpain's lab is also the result of a sea change in technology, the ability to examine—to actually look at—brain functions in real time. For decades, conditions like mental retardation were considered intractable diseases because no one could see what was actually going on.

“People didn't even talk about higher brain function in terms of cell biology,” Halpain says. “We certainly didn't dare suggest that structural changes in neurons were at the root of mental →

retardation or autism. With a tumor or trauma, you can see the damage. In patients who suffer from retardation, their brains look perfectly normal. But there's something more subtle going on—at the synapse level. Using a microscope, you can see that the shape of the synapse is clearly distorted in these patients. My lab's expertise is understanding the molecular formation of the synapse and what goes wrong when the synapse is destabilized.”

### A NEURON IS BORN

The other aspect of her lab, at the other end of the spectrum, is morphogenesis, the study of the initial molecular events that shape the neurons themselves. In terms of development, neurons are terminally differentiated, wholly committed to their ultimate fate. They are postmitotic, meaning they don't divide again and have limited repair and regeneration capabilities.

Once a neuron becomes postmitotic, it does change its shape, shifting from a round cell to an elongated shape that sends out neurites, tendrils or filaments that form the basic structure of the delicate communications network that exists inside everyone's head. These tendrils also change; some become axons, others become dendrites.

Axons look like tentacles; dendrites look like spiky trees. They also provide separate but similar functions. Simply put, dendrites bring information to the cell, while axons send information out of the cell. That information, in the form of neurotransmitters, flows from one neuron to another neuron across the synapse, the small gap separating neurons.

Halpain and her colleagues are trying to understand the process by which the neuron generates its first small tendrils. And while there is a great deal of information about how axons move throughout the brain, not much is known about the initial transformation process, called neurite initiation. One reason for that lack is the sheer rapidity of the change.

“It's difficult to study neurite initiation *in vitro*,” she says, “because the transformation happens so quickly. Once the neuron is born, it almost immediately sends out these tendrils. It starts as a random process, then the brain selects the neurites that form the right connections—the ones that the brain needs to connect the motor cortex to the spinal cord, for example. At every stage of development the brain overproduces its components, then prunes back. It overproduces neurons, but eventually kills the ones

that aren't needed; it overproduces axons and also synapses, then removes the ones that don't form strong connections in a circuit. The basic idea is to stabilize and strengthen the most useful synapses. Neurologists have a kind of nursery rhyme about the process—neurons that fire together, wire together; neurons that are not in sync, fail to link.

### SPEAKING IN ION FLOW

This basic wiring process is called neuroplasticity, a kind of pop art name for what is essentially a neuronal policy of slash and burn. Halpain sees it as the most flexible and most effective way for the brain to maximize its potential, reaching out everywhere and then refining these pathways through a rigorous selection process. Neuroplasticity is central to how the brain develops, as well as how it processes and stores information. It is the brain's ability to change circuitry that gives it the power to respond to new information. The capacity for neuroplasticity is enormous in newborns and slows down as people reach adulthood, but never stops. In fact, the act of mentally challenging your circuitry throughout your lifetime helps keep it in top condition, like aerobics for the brain.

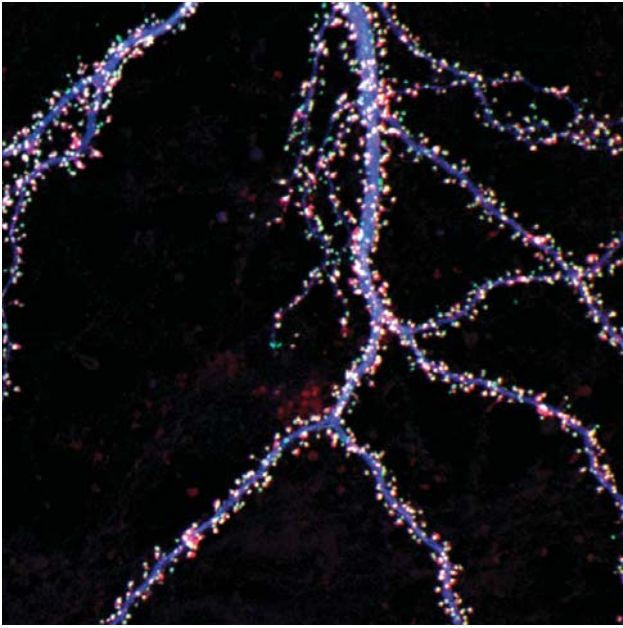
If that capacity to change circuitry is damaged or distorted, the brain misfires. One result of this synaptic misfiring is autism, a form of developmental retardation. “It could be that autistic children have too much plasticity and respond in an excessive fashion,” says Halpain. “We think that autistic children may have an ineffective ability to prune away unnecessary circuits. We're trying to identify the key molecules involved in building and regulating synapses at the most fundamental level. We believe that it may lead to therapeutic targets in complex disorders like autism.”

The actual study of these circuits involves growing neurons in a culture dish, where they are induced to express fluorescently tagged molecules so that their growth becomes visible. All of this was virtually impossible until about 10 years ago. This is still a very young science.

The neurons in these culture dishes speak in ion flows, which Halpain and her colleagues measure. The flux of ions tells the scientists which channels are opening and closing. Sodium carries synaptic firing potential, while calcium carries important information for neuronal functions. In its simplest and stripped down form, it is the same language that activates the human brain.



This time-lapse sequence shows a young neuron extending its neurite outward from the cell body. The growth cone at the tip is filled with actin filaments (red), and the neurite is filled with parallel arrays of microtubules (green) that push forward into the core of the growth cone. “Pioneer” microtubules that succeed in reaching the tip of the growth cone help steer the growth cone's direction as the cell seeks out connections with other neurons in the developing brain. Total elapsed time = 9 min. (Images courtesy Dr. Leif Dehmelt, Halpain laboratory, Scripps Research.)



This image shows a portion of a dendritic arbor of a brain cell grown in culture by the Halpain laboratory. The hundreds of tiny dots are the sites of synaptic connections formed with numerous other neurons in the culture dish. (Image courtesy Barbara Calabrese, Ph.D., Halpain laboratory, Scripps Research.)

Of course, cells in a culture dish release their neurotransmitters in a network that has no organization. Essentially, they are talking gibberish to each other. If that happens in the human brain, something has gone terribly wrong—the way it goes wrong in Fragile X syndrome.

Fragile X, the most common cause of genetically inherited mental impairment, can cause everything from mild learning disabilities to severe cognitive damage, including autism. The upcoming Fragile X project—which she will work on with her husband, Scripps Research Professor Steve Kay, director of the Institute for Childhood and Neglected Diseases—is aimed at discovering how molecular change in the synapse composition can end in retardation.

Fragile X is caused by a genetic mutation that results in the lack of a single protein. The missing protein can effect the development of synapses to the degree that the shape of synapses is distinctly abnormal. Using a mouse model, Halpain hopes to characterize the compositional changes in the proteins that make up the abnormal synapse in Fragile X and to identify therapeutic targets for treating the disease.

“Until recently we didn’t have the tools to study this at the molecular level,” she says. “Now, because of the genomic revolution, we can identify the protein. To be able to pinpoint a mutant protein as part of a mental disorder is revolutionary.”

#### FINDING COMMON GROUND

This project also represents the first time she and her husband have worked together. It’s a new world for both of them.

“We’ve been fortunate to coordinate our careers over the years,” she says, “but because Steve started out studying circadian rhythm in plants, we didn’t have much common ground. Now that Steve has moved into neurobiology, I’ve recruited his interests in mental health and childhood disorders. It’s a two-way street. [While I know about neurons,] he’s an expert in mouse models that can be used to explore human diseases. So far we’re both really enjoying working together on a project.”

Coming to Scripps Research has allowed Halpain to become engaged in research that she admits would never have happened anywhere else. “The types of things possible at Scripps, not just the physical resources but the collegial resources, don’t exist anywhere else,” she notes. “There’s no barrier to collaboration. If it feels like a natural fit, it can be easily done.”

It is here that the science of neurons and synapses, the new microscope technology, the fact that Halpain is the mother of a highly inquisitive five-year-old daughter, her recent scientific collaboration with her husband—all the various nuts and bolts of her life—more or less coalesce around a new awareness of her work.

*“It could be that autistic children have too much [neuronal] plasticity and respond in an excessive fashion.” —Shelley Halpain, Ph.D.*

“I’m here doing science at one of the top research institutes in the world and I’m a parent,” she says. “As a mother, watching your child’s development unfold before your eyes heightens your curiosity about what’s going on in those synapses that allows these leaps in motor skills and understanding.”

For Shelley Halpain, being both a scientist and a mother has clear benefits. “Although it’s incredibly challenging, doing both is possible if you want it and are willing to work at it,” she says. “I hope to encourage young women interested in science and to dispel the myth that research is not compatible with other interests. This path provides a rich life if you love science and still want to feel like a whole person.”

•Eric Sauter





# Folding and Misfolding

WILLIAM BALCH OFFERS A NEW PERSPECTIVE ON CYSTIC FIBROSIS

About 1,000 people, mostly children under the age of three, are newly diagnosed with cystic fibrosis every year, making it one of the most common genetic diseases in the United States. For these patients, cystic fibrosis is a debilitating and ultimately fatal disorder that impedes, to varying degrees, the most basic functions of life—the ability to breathe, digest food, and reproduce.

Many scientific advances have been made—especially since 1989, when the cystic fibrosis gene was discovered—in understanding the mechanisms that go awry, and today scientists are working on a number of fronts to address the disease.

Professor William Balch, Ph.D., a member of the Department of Cell Biology and the Institute for Childhood and Neglected Diseases at The Scripps Research Institute, is gearing his work toward finding ways to overcome the protein defect at the heart of cystic fibrosis, with the goal of new drugs to treat some of the 30,000 people in the United States with the condition.

“CF holds high interest for me not only because of its devastating effect on the quality of life for children with the disease,” Balch says, “but also because it is an area where my scientific focus leads me to believe that I can significantly contribute to generating new therapeutic insight.”

## IT'S ALL IN THE FOLDING

Although cystic fibrosis is a complex disease that affects many of the body's organs, its mechanism of action everywhere in the body is essentially the same—a defective gene causes certain tissues to produce abnormally thick, sticky secretions. In the lungs, mucus clogs the airways, setting up conditions for life-threatening bacterial infections. In other tissues such as the pancreas, these thick secretions prevent digestive enzymes from reaching the intestine, leading to malabsorption of nutrients; in the liver, they block ducts, which can lead to permanent liver damage; and in the reproductive organs, the thick secretions block the sperm ducts and the ovaries, rendering most men (and some women) sterile.

Balch and his colleagues at Scripps Research are aiming to describe the fundamental mechanisms behind cystic fibrosis to enable therapies to address the root causes of the disease. And protein folding appears to be key.

For over a decade, Balch and his colleagues have been studying the roles of both protein folding and the cellular export machinery that transports “cargo”—proteins—through the cellular secretory pathway. “We have always been focused on understanding the basis of misfolding disease,” Balch says, adding that Scripps Research is a place that encourages this type of innovative research. “What we have here is a combined ability to move quickly on a problem, state-of-the-art technologies, and a faculty mind-set that stresses innovation. All of this helps to move the science here in unanticipated directions. These are usually the avenues that lead to breakthroughs.”

*“CF holds high interest for me not only because of its devastating effect on the quality of life for children with the disease, but also because it is an area where my scientific focus leads me to believe that I can significantly contribute to generating new therapeutic insight.”* —William Balch, Ph.D.

## FRESH IDEAS

When one thinks of cystic fibrosis, diseases such as Alzheimer's and Mad Cow don't usually spring to mind. But scientists have found that these apparently unrelated diseases share certain characteristics with cystic fibrosis. In fact, all three are so-called “protein-misfolding” diseases.

Proteins, the fundamental components of all living cells, are made using cellular “machines” called ribosomes that string together amino acids into long, linear chains. A disease such as cystic fibrosis can occur if the end product of this stringing-together process is a misfolded protein that cannot function properly because of its abnormal configuration.

The protein implicated in cystic fibrosis is a mutant form of cystic fibrosis transmembrane conductance regulator (CFTR), an enormous molecule made up of about 1,500 amino acids that spans the membrane →

surface of epithelial cells multiple times. Normally, CFTR acts as a chloride channel and must be present on the cell's surface to regulate the balance of certain ions and water between the inside and outside of the cell. More than 1,000 different mutations have been detected in cystic fibrosis, causing different levels of defects and different levels of disease severity. The most common cystic fibrosis gene mutation (which occurs in greater than 90 percent of patients), delta ( $\Delta$ )F508, has a single amino acid missing. This leads to the production of a misfolded CFTR protein that prevents it from being transported to the cell surface by the cell's secretory pathway.

The secretory pathway starts with a specialized folding compartment called the endoplasmic reticulum, or ER. When CFTR is folded properly, the ER packages and prepares it for transport to the cell surface via vesicle containers. The  $\Delta$ F508 mutation, however, prevents CFTR from entering that pathway. The misfolded CFTR gets stuck in the ER, which is designed in such a way that it degrades molecules that remain trapped in the compartment for too long.

"The irony is that  $\Delta$ F508 CFTR does have chloride channel function. Most people with cystic fibrosis produce  $\Delta$ F508 in normal amounts. It simply can't be moved out of their cells," Balch says.

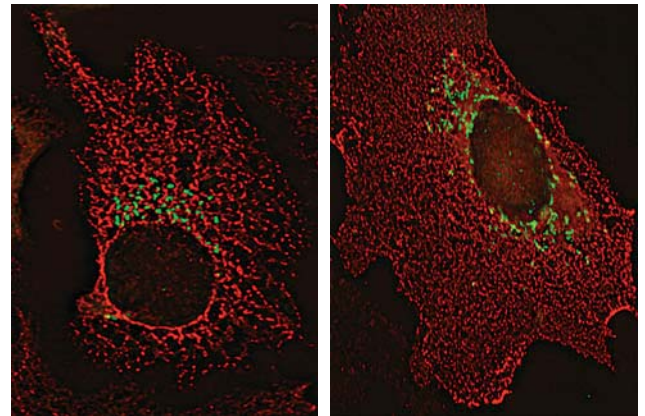
If CFTR never makes it to the cell surface, the balance of salt, chloride, and water is thrown out of whack and the surface is not properly hydrated. Without proper hydration of the lung and other tissues, secretions become thick and sticky. Lung failure is the principal culprit in most cystic fibrosis patients.

*"The irony is that  $\Delta$ F508 CFTR does have chloride channel function. Most people with cystic fibrosis produce  $\Delta$ F508 in normal amounts. It simply can't be moved out of their cells." –William Balch, Ph.D.*

"A normal lung is always bathed in a wonderful, well-hydrated coating of mucus, with is constantly swept clean by cilia," says Balch. "In a CF lung, the lack of adequate hydration causes mucus to become so packed down on the lung surface that the cilia are trapped in a sticky matrix and can't function."

The poorly hydrated mucus-covered lung surface becomes an ideal breeding ground for *Pseudomonas aeruginosa*, a normally harmless bacteria found in soil and water. This common, opportunistic

bug has found a niche in the CF lung, where it wreaks havoc. The lungs of every child with the disease eventually become colonized with *Pseudomonas*, causing repeated infections that scar the lungs and ultimately prove fatal.



Cells expressing  $\Delta$ F508 CFTR lack surface expression (left) when compared to cells expressing wild-type CFTR (right).

#### FIXING THE FOLD

In recent years, much cystic fibrosis research has focused on finding ways to keep the mutant CFTR from being broken down by the cell. The thinking has been that if CFTR could be kept from being put into the "trash," it might eventually leak to the surface despite a defective export pathway. But Balch and others have found that it is not useful to simply prevent degradation because the ER is finely tuned to activate a destruct-mode if it detects a build-up of misfolded CFTR.

"The ER compartment is actually very sensitive to unfolded proteins, so if it starts to accumulate these aberrant proteins, a new signaling pathway is created called the 'unfolded protein response,'" Balch says. "The cell first tries to rescue the fold. If it cannot, it starts creating inflammatory pathways and begins to recognize itself for self-destruction. This triggers apoptosis, or cell death. At this point, there's not even a cell around anymore to produce the CFTR."

Balch and his team have taken a different tack—rather than concentrating on how to keep mutant CFTR from being degraded, they have tried to understand how normally folded proteins are exported from the cell by the ER. From past studies with other protein molecules, they have discovered "exit codes"—regions of amino acids that define and dock into a binding pocket on the ER export

machinery and signal the ER: “I’m folded and ready to go out.”

“Through searches of gene databases we discovered that the normal CFTR channel has a similar exit code,” Balch says. “So we asked ourselves: if we remove the exit code, would that cause the protein to become stuck in the ER? The answer was, ‘yes.’ And then we asked: if we remove the exit code will it no longer see the machinery it needs to leave the ER? Again the answer was, ‘yes.’” Intriguingly, Balch also found that  $\Delta F508$  cannot see the same machinery.

Thus, it seems that the mutant CFTR loses the ability to have its exit code “read” by the ER transport machinery. By studying the exit code of the normal CFTR, and the machinery that recognizes that exit code, Balch and his colleagues were able to learn how CFTR engages the ER and why the  $\Delta F508$  mutant form does not—the loss of the amino acid phenylalanine at position 508 in the polypeptide chain is likely to cause a change in the protein fold that disrupts this interaction.

“For the first time,” Balch says, “we understand what it takes to get CFTR out of the ER. By understanding this step, we think we can contribute to better understanding the biochemical basis for this disease, which, in turn, will help us find a cure.”

These findings were published in the October 2004 issue of the *Journal of Cell Biology*.

#### **COLLABORATING TO FIND A DRUG**

The next step is to identify where in the pathway CFTR’s folding is defective and to find ways of targeting the defect to correct the fold. The goal of Balch and others is to identify potential “targets” that affect the folding pathway defective in CFTR. Once targets are located, small molecules—drugs—could either bind to CFTR and modify it in such a way that it obtains a more normal fold, or interact with the folding machinery itself to restore the structure of the protein. Once a normal fold is achieved, the correct cascade of events can occur—CFTR will recognize the export machinery, it will be brought to the surface and achieve function.

“By targeting the primary defect, you in essence create a cure for the disease,” Balch says.

Working with other scientists such as Professor John Yates at Scripps Research, Balch is using mass spectroscopy techniques in the search for potential

targets that assist protein folding and export. “We actually have work that’s quite advanced that has helped us focus in on key proteins that seem to be important in the folding pathway,” Balch says.

Recently, the compound curcumin, an ingredient in the common spice turmeric, generated a great deal of excitement in the field, because it seemed to be effective in mobilizing  $\Delta F508$  to the surface. But so far curcumin has turned out to be only effective in certain mouse models and remains to prove effective in CF.

Meanwhile, biotech companies like Vertex in La Jolla, California, near Scripps Research, are working diligently on developing “first-generation” drugs, so called because they have not yet been tested for toxicity or in clinical trials. These companies are using the technique of high-throughput screening to evaluate the potential of millions of compounds. When a small molecule is found that seems to have some efficacy in correcting protein folding and ER export, it is considered a “chemical chaperone” that holds promise for future testing. These chemical chaperones will provide the basis for drug development.

*“By targeting the primary defect, you in essence create a cure for the disease.”*  
—William Balch, Ph.D.

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#### **“ONCE YOU SEE THESE KIDS...”**

With the cost of development of a new drug now estimated in the hundreds of millions, many of the major pharmaceutical companies are reluctant to become involved in the search for drugs for diseases like cystic fibrosis that affect relatively few people. But with direct support from the Cystic Fibrosis Foundation and from government funding agencies, more biotech companies have become involved in developing first-generation drugs. They now need to test whether these drugs can actually correct CFTR’s folding problem in human clinical trials.

“We at Scripps can work very effectively with these companies,” says Balch, himself the father of two teenagers. “The bottom line is that we need to figure out how to engage the mutant protein with the ER export machinery in a way that would most benefit the patient. That’s what this problem is all about. Once you see kids with CF struggling with this disease, as a scientist, you want to figure out a way to help.”

•Anna Sobkowski

ELIZABETH WINZELER AS...

# DR. DECODER!

FIRST  
ISSUE!

1  
NOV.

BACK OFF,  
ANOPHELINE  
NEMESIS!

FIGHTING  
MALARIA  
ISN'T KID'S  
STUFF!

HA!  
YOU CAN'T  
GET RID OF  
ME THAT  
EASILY!

VERSUS THE  
MOSQUITO MENACE!



# The Trouble with Malaria

ELIZABETH WINZELER BATTLES AN ELUSIVE KILLER



Scripps Research Associate Professor Elizabeth Winzeler applies cutting-edge technologies to the search for new drugs and vaccines.

To some of his contemporaries, Oliver Cromwell was the hero of the English Civil War; to others, he was a traitorous usurper. Either way, Cromwell's rise was one of the most remarkable in history.

He was a farmer who became a member of parliament and then joined the rebellion in 1642. Even though he had no formal military training, he became a brilliant military leader, helping to rout King Charles I and his royalist forces, and ultimately putting the king to death. After the war ended, Cromwell became a ruthless politician, capping his remarkable rise by dismissing the "rump" parliament that ruled England for a few years after King Charles was executed and installing himself as the country's leader. He was eventually made Lord Protector of England, Scotland, and Ireland—king in all except title.

He might have remained in power for a long time, too, except that he died a rather unremarkable death from fever on September 3, 1658, probably as a result of malaria. Cromwell's case, in fact, is a lesson in the mighty leveling power of a few humble parasites. Unlike the rump parliament, malaria was not so easy to dismiss.

Neither is malaria merely of historical interest, though in England, the United States, and most other parts of the industrialized world, it would certainly seem so.

In the United States, for instance, malaria was once endemic but was essentially eradicated decades ago through the widespread use of insecticides to control mosquito populations. Today the risk of contracting malaria within the United States is exceedingly rare, and most of the 1,200 or so cases of the disease that are diagnosed in the United States each year are imports from other countries—typically travelers who are infected in a foreign country where malaria is still

endemic and then travel back to the United States where the symptoms of their disease manifest.

But this burden of disease is nothing compared to the hundreds of millions of people in the world each year who continue to contract malaria—many of them in poor rural areas with limited access to healthcare.

In fact, some 40 percent of the world's population lives in areas where malaria is endemic. These are usually wet or marshy areas that are home to *Anophele* mosquitoes, which can carry the microscopic *Plasmodium* parasites and transmit them to people. Domestic transmission of malaria does still happen occasionally, since the type of mosquitoes that transmit malaria from person to person still exists in certain parts of the United States, including Florida and California. In fact, in 1988, San Diego County witnessed the largest U.S. outbreak of malaria in the last half century (30 cases).

*"Malaria is still one of the leading causes of infectious disease in the world. There is currently no effective vaccine."* —Elizabeth Winzeler, Ph.D.

Furthermore, malaria is becoming a more severe problem because certain strains have developed resistance to the drugs commonly used to treat it.

"Malaria is still one of the leading causes of infectious disease in the world," says Scripps Research Institute Associate Professor Elizabeth Winzeler, Ph.D., who is applying cutting-edge technologies to the search for new drugs and vaccines for malaria. "There is currently no effective vaccine."

## MALARIA—A CHILDHOOD DISEASE?

Winzeler specializes in malaria research as a member of Scripps Research's Institute for Childhood and →

## The Malaria Cycle



- 1 An infected mosquito, usually of the *Anopheles gambiae* species, bites a person, transmitting microscopic *Plasmodium* parasites in its salivary gland into the human body when it pierces the skin.



- 2 Once inside the human victim, the parasite transforms into a “trophozoite,” which grows and multiplies, infecting cells inside the liver. The newly infected victim does not yet feel sick.



- 3 After a period of a few days to several weeks, the parasites leave the liver and enter the bloodstream as “merozoites,” the form of the parasite that infects red blood cells.



- 4 The infected red blood cells eventually burst, freeing merozoites to attack other red blood cells, releasing *Plasmodium* toxins into the blood, and causing the person to feel sick, often with fever, chills, and anemia.

*continued on page 14*

Neglected Diseases, and this sometimes raises an interesting question: Why is malaria considered a childhood disease if it strikes adults as well as children?

What many people don't know is that malaria inflicts a disproportionate burden on the young. The disease kills more than a million children a year worldwide, it is one of the top five causes of child mortality in many countries in the developing world, and one in ten children who die in the developing world are killed by malaria. Despite the death from the illness of such high-profile adults as Cromwell, the U.S. Centers for Disease Control and Prevention and the World Health Organization (WHO) consider malaria a childhood disease.

Malaria strikes children more severely than it does adults, and children with the disease generally have a much higher number of malaria parasites in their blood. Children also show increased numbers of complications such as anemia, and many children become so sick they fall into comas. Brain damage and death are the disease's frequent end-stages in these severe cases. About three-quarters of the people in the world who die from malaria each year are children.

The reason why malaria affects adults less severely is that adults who live in countries where malaria is widespread are repeatedly exposed to the *Plasmodium* parasite, perhaps even several times a year, and they acquire some resistance to it. “By the time most people reach adulthood, they are semi-immune,” says Winzeler. “They [sometimes even] stop showing symptoms of the disease.”

Nonetheless, this acquired immunity is not perfect. People can still show mild to severe symptoms any time they contract malaria, and they lose their partial immunity if they leave the area. This is because *Plasmodium* parasites have evolved a tricky way of altering their antigens—those pieces of protein or lipid that the immune system recognizes. Normally, once a person's immune system has been “primed” by the antigens characteristic of a pathogen, it will make a rapid and vigorous response to that pathogen if that person is later exposed again. Vaccines work because of this response.

The difficulty with malaria is that when it shifts its antigens, it masks the pathogens from immune recognition and makes the immunity developed the previous year ineffective. “The malaria infection that you are seeing this rainy season may not be with the same parasite that you saw last season,” says Winzeler.

A few years ago, an international consortium involving researchers from the United States and the United Kingdom solved the DNA genome of *Plasmodium falciparum*—a major six-year, \$17.9-million effort. Now Winzeler is trying to determine what the genes and the proteins they encode actually do—work that could accelerate the process of drug and vaccine development.

In addition to support from the National Institutes of Health, Winzeler’s research is supported by a gift to the institute from Tom Friedkin and a grant from the Ellison Medical Research Foundation. She also recently received a distinguished young investigator award of \$1 million from the W. M. Keck Foundation, which is given to only five outstanding junior faculty members in the United States each year.

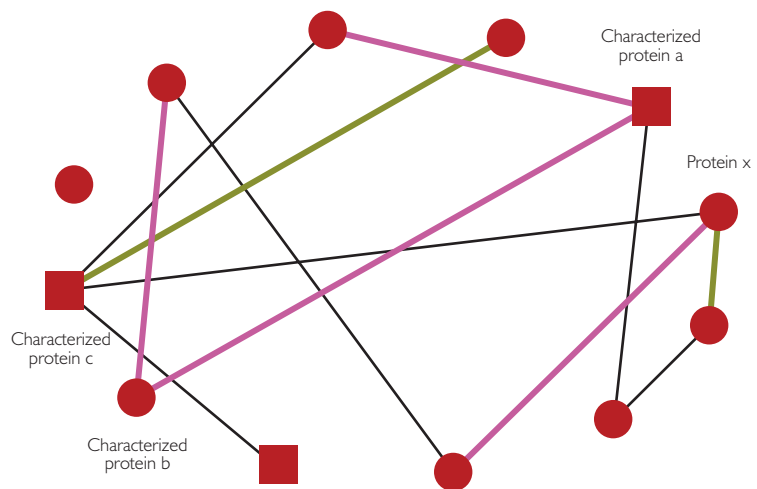
Winzeler uses a “systems biology” approach, gaining as much information as possible about the malaria parasite so that she and her colleagues can assemble a virtual knowledge base. Their goal is eventually to be able to describe the potential functions of all the proteins in the parasite.

She is the first to admit that this goal is a long way off. But she says that this systems biology approach offers hope because traditional approaches have proven to be very slow since the malaria parasite is extremely difficult to work with. This is most apparent, she says, when you compare what we know about the parasite to what we know about baker’s yeast—a favorite experimental organism that Winzeler and many other biologists use to study the function of specific genes.

“We know a huge amount about baker’s yeast, which is completely benign, and almost nothing about *Plasmodium falciparum*, which is a major killer,” Winzeler says.

#### SEARCHING FOR THE MYSTERIOUS SIXTY PERCENT

There are about 5,500 genes in the *Plasmodium falciparum* genome, the most deadly of the four known *Plasmodium* parasites and the one that Winzeler works on. Scientists know through direct experimental evidence the functions of only a small fraction of the proteins these genes encode—a few hundred at most. Add to these another 1,500 or so genes whose functions are roughly indicated by sequences similar to known genes from other organisms (genes similar in sequence tend to function similarly, even in diverse organisms).



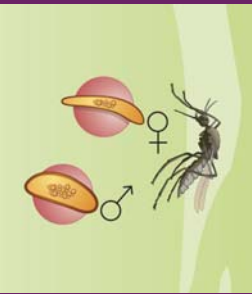
A gene chip measures activity patterns for some malaria genes during different lifecycle stages.

However, about 60 percent of the genes in the *Plasmodium falciparum* genome are only hypothetical. They look like genes, and in some cases they are expressed like genes, but they have no homologues (comparable sequences) in other organisms. “We have no idea what they do,” says Winzeler.

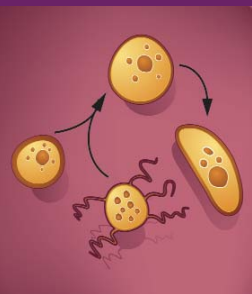
This mysterious majority is what drives Winzeler’s research, and in her research she seeks to identify the proteins that are most likely involved in the different pathways and stages of the parasite’s lifecycle. This is no simple task. *Plasmodium* is an intracellular parasite that has a number of life stages. Each stage grows in a different setting, from inside red blood cells to inside the gut of a mosquito, and it is difficult to obtain sufficient quantities of parasites from some of the stages. Some of Winzeler’s colleagues, for instance, had to laboriously dissect parasites from the salivary glands of mosquitoes to collect samples from that life stage of the parasite.

*“If we can find small molecules that bind to these DNA sequence motifs and block activation of sexual development, we can block the transmission of the disease from one person to the next.”* –Elizabeth Winzeler, Ph.D.

Luckily, Winzeler has been able to work with researchers at the Genomics Institute of the Novartis Research Foundation, who collaborated with her to create a malaria-specific “gene chip” with probes specific for the entire genome of the malaria pathogen. Gene chips are basically glass or silicon wafers onto which are deposited short fragments of DNA. In this case, the chip contained →



5 Some of the merozoites in the bloodstream undergo a complicated transformation into another form of the parasite called “gametocytes,” in which the parasite exists as male and female. Gametocytes are the only form of the parasites that are fit for transmission because they are the only form of the parasite that can survive in the gut of the mosquito.



6 If a mosquito bites an ill person in this stage of the disease, it will become a carrier of the disease. Once inside the gut of the mosquito, the male and female gametocytes mate to form “zygotes,” which then rapidly transform into another form of the parasite called “ookinetes.”



7 Within about a week, the ookinetes migrate through the lining of the mosquito stomach and form into “oocysts.”



8 The oocysts eventually enlarge, bulge, and finally rupture, releasing thousands of tiny mobile “sporozoites,” which migrate to the salivary glands of the mosquito. Now the parasite is ready to infect another victim.

over 260,000 nucleotide sequences from the *Plasmodium falciparum* genome.

An infected red blood cell will contain lots of molecules from both the red blood cell itself and from the parasite. Included in this mixture will be RNA messages expressed by different genes in the *Plasmodium* parasite. These messages can be fluorescently labeled and washed over the surface of the gene chip, where they will find their complementary DNA sequences on the chip. By measuring the amount of fluorescence at each block on the array, Winzeler and her colleagues can tell if a particular gene is turned on at a particular time in the parasite’s lifecycle.

“Having this type of technology and the genome sequenced allows us to look at the genome in a whole new way,” says Winzeler. “If we understand malaria better, we may have better ideas of how to attack it with the human immune system, which may lead to the development of an effective vaccine.”

#### TOWARDS A NEW VACCINE

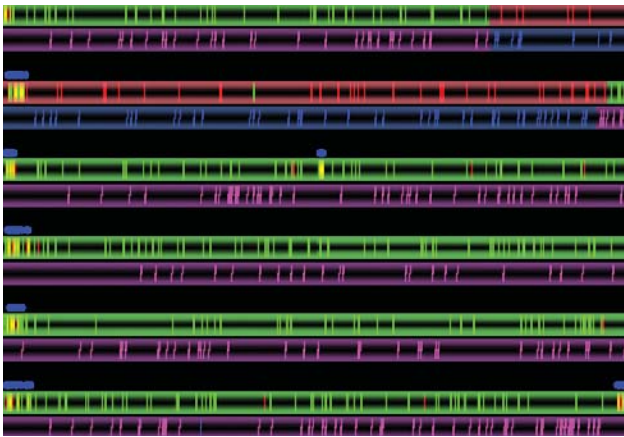
A few years ago, Winzeler and her colleagues used this tool to construct and publish a comprehensive global profile of genes in the malaria parasite, finding potential functional roles for more than half of the previously uncharacterized genes in the genome. Now they want to go even further and systematically understand the genes and processes that drive the parasite’s lifecycle.

Recently, Winzeler and her colleagues have identified some of the DNA sequence motifs that may be responsible for controlling the development of the parasite during the time it’s in its sexual cycle, a stage which is necessary for the transmission of the disease from one person to another. “Even though the asexual parasites must somehow change into male and female parasites for the disease to spread from person to person, we have no idea how this process occurs—parasites do not carry sex chromosomes like most higher organisms,” says Winzeler.

Still, she adds, “If we can find small molecules that bind to these DNA sequence motifs and block activation of sexual development, we can block the transmission of the disease from one person to the next.”

Winzeler and her colleagues are also using their chips to study the genetics of the parasite, focusing on the genetic diversity of parasite populations. In this endeavor, Winzeler collaborates with Dyann Wirth





Gene chips, which can be used to discover and type new genetic markers on the parasite's chromosome, may enable scientists to better predict patterns of drug resistance.

of Harvard University, who operates a field station in the West African nation of Senegal. “Our goal is to obtain enough patient samples that we can look at the genomic DNA [of the parasites] and identify the genetic signatures in the strains that are particularly virulent or resistant to drugs versus those that are not,” says Winzeler.

“If you start doing longitudinal studies after you introduce a new drug,” she adds, “you might be able to identify the drug targets or the mechanisms of resistance. If you can start finding the mutations that are associated with drug resistance, then that tells you how to treat patients in the field. Using the correct drug regimens for the different infections will certainly save lives.”

Finally, they are looking at the genes involved in interacting with the host immune system, identified on the assumption that these types of genes would be changing. “We have identified several hundred genes that appear to be evolving at very high rates,” says Winzeler. “Many of these genes are unique to the parasite and may be the genes that the parasite uses to escape recognition by the immune system.”

The problem with making a malaria vaccine is that many of the vaccine candidates are based on recombinant forms of antigens expressed by the parasite during the time it resides inside red blood cells. But targeting the parasites within red blood cells is exceedingly difficult, since red blood cells are less likely to be scrutinized by the immune system.

Perhaps a more effective way is to target the form of the malaria pathogen that the body first encounters—the sporozoites injected by the bite of a

mosquito. In fact, this does work. Sporozoites from the salivary glands of mosquitoes that are irradiated (to kill the pathogens) and then injected into humans have shown to be very protective against malaria. “It’s actually better than the type of protection you would get from living in a malaria-endemic area,” says Winzeler.

Unfortunately, this strategy is of limited use because large quantities of mosquito salivary glands are needed to vaccinate one person. So Winzeler and her colleagues are identifying the genes that are active in parasites recovered from mosquito salivary glands. This is a first step toward trying to determine which of the 5,300 genes are likely to be protecting people from infection in the sporozoite challenge.

“If you could actually identify the key protein that is immunodominant,” says Winzeler, “that might allow you to begin designing a vaccine that would be effective at preventing the disease.”

•Jason Socrates Bardi

#### MALARIA: A CHILDHOOD DISEASE

Why does the World Health Organization consider malaria—which also afflicts adults—a childhood disease? Consider these facts:

- Malaria kills more than a million children a year worldwide.
- Malaria is one of the top five causes of child mortality in many countries in the developing world.
- One in ten children who die in the developing world are killed by malaria.
- Malaria strikes children more severely than it does adults, and children are at increased risk for complications, such as anemia and coma.
- In areas of intense transmission, young children may have as many as six episodes of malaria each year.
- About three-quarters of the people in the world who die from malaria each year are children.

# Behind the Scenes



## Getting Acquainted with Scripps Research

- 1 More than 500 people attended an open meeting and reception to introduce four Scripps Florida scientists to guests at The Society of Four Arts in Palm Beach. Shown here is Garrison duP. Lickle, managing director of Lehman Brothers, Palm Beach, which sponsored the April 15 event.
- 2 In celebration of the first annual gathering of The Scripps Legacy Society on April 21, Scripps Research planned giving donors, board members, staff members, and Kellogg Graduate School of Science and Technology students enjoyed cocktails and dinner at the Rancho Valencia Resort in Rancho Santa Fe. The Scripps Legacy Society is composed of individuals who have included Scripps Research as a beneficiary in their estate plans. Pictured at the event are planned giving donors Joyce and Martin Nash; also, planned giving donor Mary Soares and Jo Winsor.
- 3 Fifty scientists and supporters of Scripps Florida were guests at a get-acquainted reception May 12, sponsored by Trustee Alexander W. Dreyfoos. Pictured here are Jana Hermann, business development officer for Siemens Corp. (a sponsor of the 2005 Scripps/Oxford International Biotechnology Conference to be held November 13 to 15 at The Breakers in Palm Beach); Harry W. Orf, Ph.D., vice president, scientific operations for Scripps Florida; and Maria Acosta, M.D., regional medical liaison for Amgen (also a conference sponsor). Also pictured are Donny Strosberg, Ph.D., professor of infectology at Scripps Florida; his wife Eliane Strosberg, an author; Judy Goodman, a public issues and government affairs attorney in West Palm Beach; and Thomas Schroeder, Ph.D., a staff scientist in medicinal chemistry at Scripps Florida.
- 4 The Second Cup of Coffee series featured a presentation on June 7 by Scripps Research Assistant Professor Dianne McKay, M.D., "Organ Transplantation: The Gift of Life." Pictured at the event, held at the La Jolla Beach and Tennis Club, are Jeanette Foushee, president of Achievement Rewards for College Students (ARCS), which provides funding for Scripps Research graduate students, and Jeffery W. Kelly, Ph.D., vice president for academic affairs and dean of graduate studies at Scripps Research.

## Marjorie Fink: Investing in the Future

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Marjorie Fink looks forward, not back.

A Palm Beach resident with an English degree from the University of Wisconsin and a merchandising background from New York, she has a restless energy, coupled with relentless curiosity, that attracted her to The Scripps Research Institute when plans were announced to build the campus in Florida.

“My husband, Rod, died of non-smoker’s lung cancer. The experience of that devastating disease added to my general interest in science and prepared me to ask questions and understand the answers I received when I began to look into Scripps Research,” says Marjorie. “What I heard when I met the scientists in Florida, and what I saw when I toured the labs in California, convinced me to make a commitment to Scripps Research sooner rather than later.”

## Katja Van Herle: Promoting Better Health Through Education

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Katja Van Herle, Scripps Research’s director of medical education, believes in both medical research and public education. An internist and endocrinologist, Van Herle recently formed the Center for Excellence in Medicine to advance these convictions.

The Center for Excellence in Medicine works in collaboration with Scripps Research scientists to educate patients and families on the most current research in a specified area and the new potential treatment strategies that may help them. It also assists all kinds of patients, focusing on cases that haven’t been solved and suggesting specialists for difficult diseases.

Van Herle gives presentations to donors and community groups and serves as special advisor to The Scripps Council of 100, those philanthropists who contribute \$100,000 annually or make a single contribution of

The result of Marjorie’s rapid immersion in Scripps Research was a gift by bequest, created quietly with her attorneys even before Scripps Florida opened its first lab, followed a few months later by a \$1 million gift—one of five \$1 million contributions made to Scripps Florida in its first year.

Adds Marjorie, “After seeing evidence of so much work in so many areas of science being started in Florida—and already underway in California—I decided not to restrict my gift to any particular area of science, but to make an investment in the future by allowing Scripps Research to put the money for research where it is most needed.”

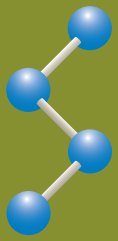
But Marjorie hasn’t rested. Since writing her bequest and making her \$1 million gift, she became an active charter member of The Scripps Council of 100, the group of philanthropists who contribute \$100,000 or more a year to Scripps Research, attend semi-annual meetings with scientists and trustees, and represent Scripps Research to donors and decision makers nationwide.

\$1 million or more to Scripps Research. In this capacity, Van Herle speaks to the council about diseases of particular interest to its members and offers one-on-one counseling.

“Scripps Research is one of the largest and greatest science centers in the world and I feel so fortunate to have the opportunity to translate what happens on the research side to the clinical side for our patients, Scripps Research donors, and friends,” says Van Herle, who received both her M.D. and M.S. in Public Health from the University of California, Los Angeles (UCLA) and served as chief resident of internal medicine at the busy UCLA Medical Center.

Because of her work at UCLA, Van Herle has a special interest in diabetes, the disease that engendered the Scripps Metabolic Clinic—forerunner of Scripps Clinic and The Scripps Research Institute—in 1924. Van Herle hopes to raise both awareness and funds from American businesses to help stop the rapid spread of preventable, obesity-related diabetes among children and teenagers.





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