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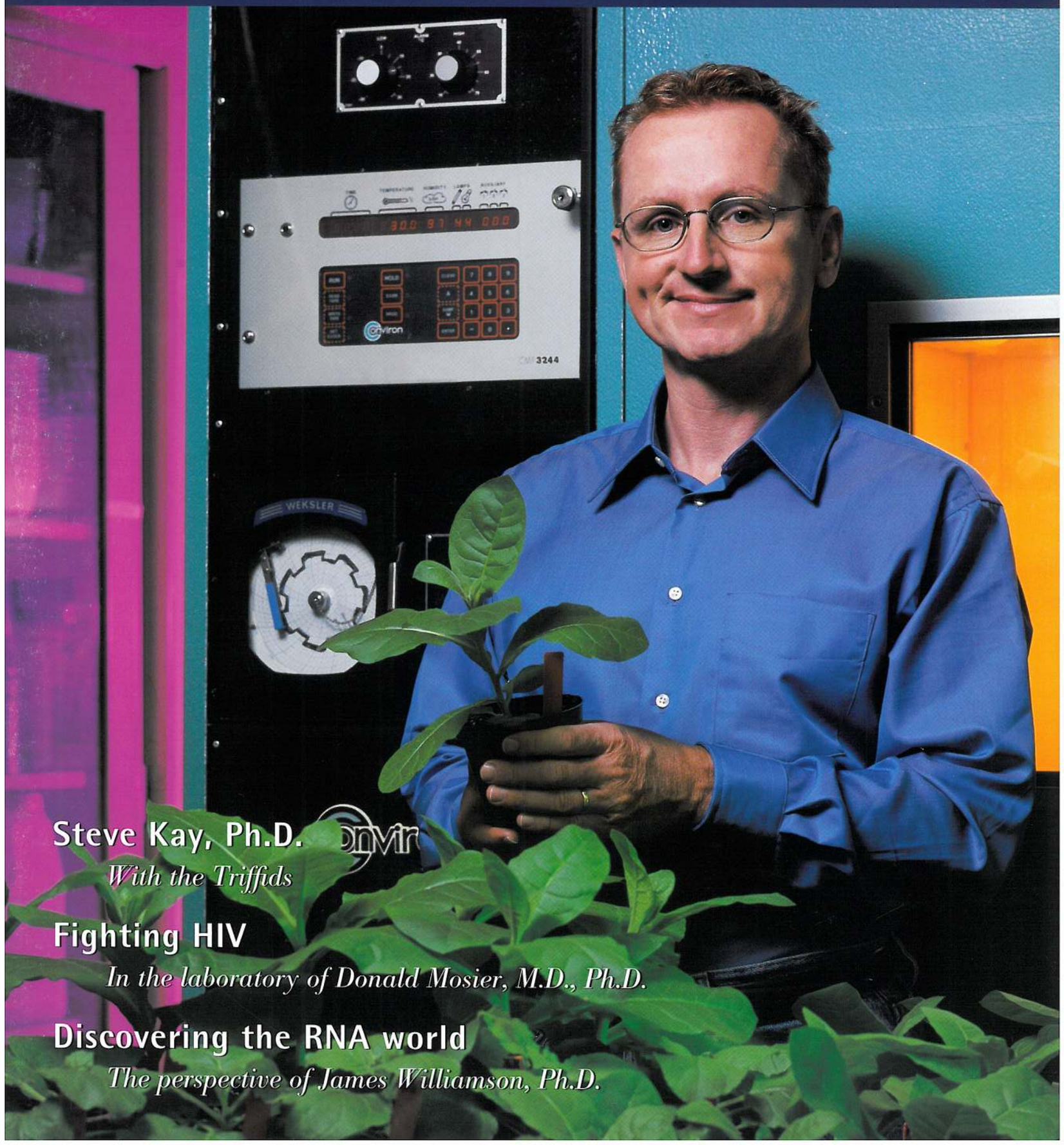
THE SCRIPPS RESEARCH INSTITUTE

Endeavor



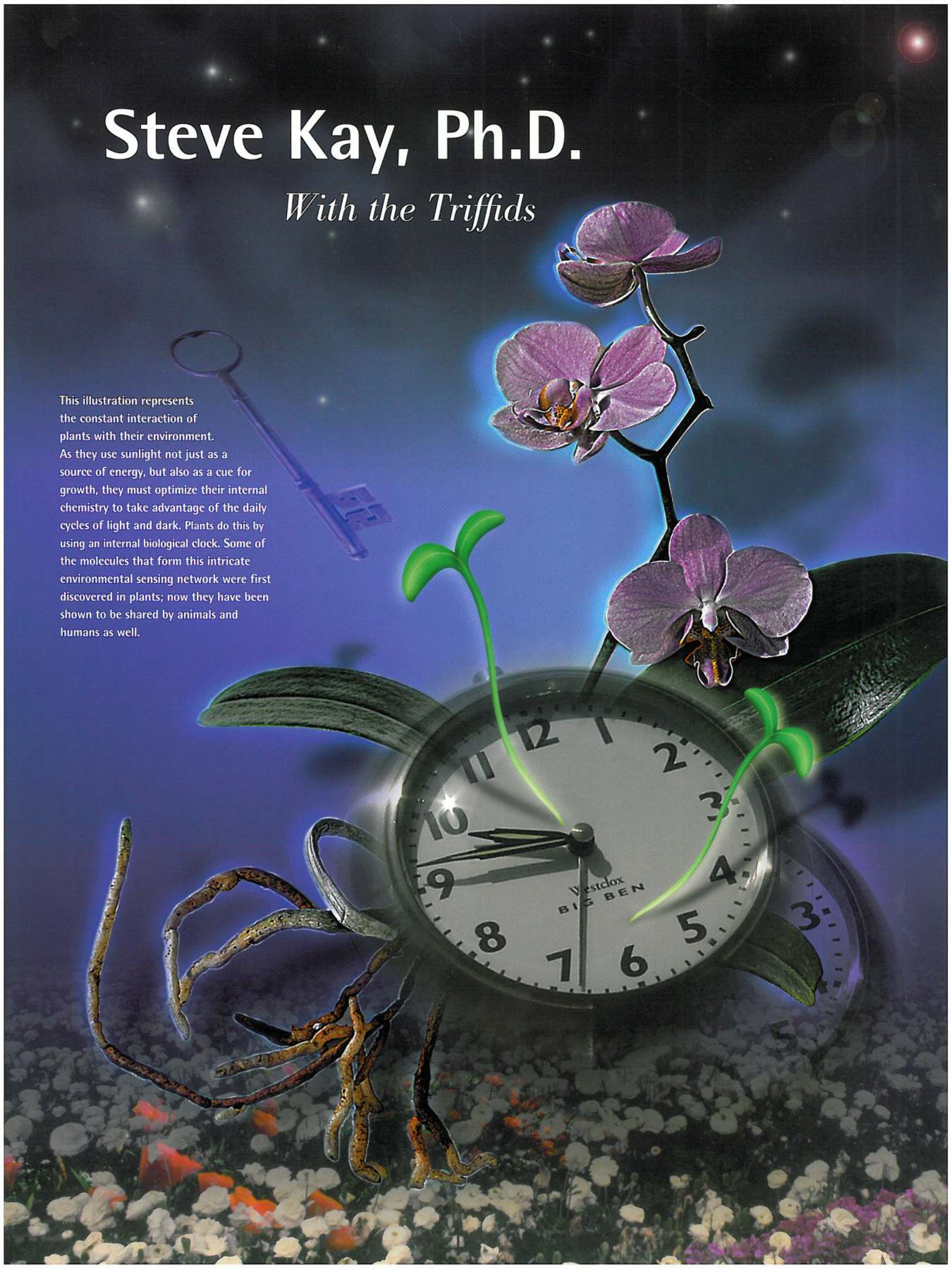
Steve Kay, Ph.D.
With the Triffids

Fighting HIV
In the laboratory of Donald Mosier, M.D., Ph.D.

Discovering the RNA world
The perspective of James Williamson, Ph.D.

Steve Kay, Ph.D.

With the Triffids



This illustration represents the constant interaction of plants with their environment. As they use sunlight not just as a source of energy, but also as a cue for growth, they must optimize their internal chemistry to take advantage of the daily cycles of light and dark. Plants do this by using an internal biological clock. Some of the molecules that form this intricate environmental sensing network were first discovered in plants; now they have been shown to be shared by animals and humans as well.

In The Day of the Triffids, a classic science fiction novel of the 1950s and one of Dr. Steve Kay's favorite books, huge carnivorous plants take over the earth. In his laboratory, they only glow in a circadian rhythm, although it's not hard to see the similarities between the plants in his studies and the man-eating triffids.

In both instances, the plants are a lot smarter than we know.

For Steve Kay, Ph.D., a professor in The Scripps Research Institute's Department of Cell Biology, the most fascinating aspect of plants (and other organisms) is that so many of them have internal biological clocks that control their activities. The petals of many flowers open at a certain time of day. Mice are only active nocturnally. People suffer from jet lag because of a dislocation in these internal timepieces, waking up in the middle of the night ready to start the day.

What makes all these biological clocks tick is a question that has puzzled Kay for years and one that came his way almost by accident. As an assistant professor at the Rockefeller University in New York, he worked on a project to discover the plant genes responsible for measuring the direction and duration of light. Plants are actually quite good at measuring light, according to Kay, particularly red light, which has a slightly longer wave length. By measuring sunlight, plants make basic decisions about their lives such as when to germinate and flower.

The experiment was moved out of the laboratory into the more natural environment of the greenhouse. At that point, Kay remembers, the data suddenly turned confusing. Kay and his research partner worked different schedules; in the morning, Kay would find that the plant's gene expression was extremely high, while his colleague, who worked in the afternoon, found his gene sample was low. It soon became clear that these genes were being regulated by the plant's internal biological clock. Like all true clocks, the gene expression continued whether the plant was in continual light or darkness.

"Because of that experience, I discovered that biological clocks are fantastically complex," Kay says. "This isn't about a linear pathway for one particular enzyme but the study of nature's own time pieces. All these important questions arise. Why do we have them? Are plant and animal timepieces the same? Are they related by evolution? How do they affect our behavior?"

THE CASE FOR ARABIDOPSIS

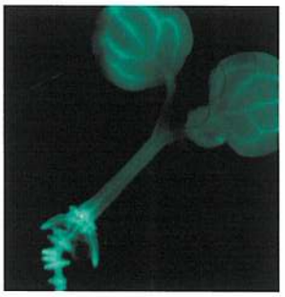
His first step, Kay explains, was to find a better way to identify the components. So, he helped invent a new technology, an innovative bit of cross-species genetic transference. After first manipulating the DNA of Arabidopsis, a member of the mustard family, Kay inserted a firefly gene, one that controls light production. Fireflies create light when an enzyme called luciferase breaks down molecules of luciferin (the name derives from the Old English word for match or firelight, a lucifer). Kay attached the firefly luciferase gene to the genetic segment of the Arabidopsis plant that is essential for the process of photosynthesis each morning.

What makes all these biological clocks tick is a question that has puzzled Kay for years...

When Kay sprayed the mustard plants with luciferin, he discovered that they glowed brightly all day but stopped at night in complete harmony with their circadian rhythm. Kay had found a new way to visually measure the effects of the circadian rhythm in plants.

Perhaps the most unique part of the experiment was the choice of Arabidopsis itself. Capable of being grown in very small areas, Arabidopsis has a small genome as well, only 20,000 genes. But its genetic structure is representative of the world's major crop plants including

On The Cover: Steve Kay, Ph.D., Professor, Department of Cell Biology.



Bioluminescence from a transgenic Arabidopsis thaliana seedling carrying the firefly luciferase gene. Note the strong expression of the transgene in the vascular tissues of the young seedling. The seedling was imaged using a highly sensitive video camera.

soybeans, corn, and wheat. So, anything discovered by manipulating the biological clocks of Arabidopsis would also be applicable to the world's food crops.

"Plants measure the length of the day so they can respond more effectively to their environment," Kay says. "The study of modern botany looks at the plant world and sees just how dynamic it really is. If you watch the way some desert plants use their clock to gain maximum light, they turn east before the sun comes up and they continue turning even after the sun goes down. Plant biological clocks are a very spooky phenomenon but one with enormous potential in agriculture."

One of these phenomena, known as photoperiodism, also controls the reproductive cycle. If you could manipulate how crop plants use day lengths to control flowering, you could develop a new generation of rapid cycling crops that could be harvested two or three times per season instead of only once. Some plants are more susceptible to pathogens at certain times of day than at others. Photoperiodism is also true for animals. To make certain that mating occurs in the summer when food is plentiful, for example, the maturity of the testes of the common hamster is controlled by day length. At the other extreme, sheep need short days for optimum breeding.

The ultimate goal of his work, Kay comments, is to understand the workings of the biological clock and then turn that knowledge into practical applications. This is not so surprising when you consider the fact that he is the grandson of a fisherman — a lobsterman, to be exact, one of the most practical-minded occupations in the world, especially when it's practiced in the turbulent waters of the English Channel.

LIFE IN THE TIDE POOLS

Kay grew up on the Channel Island of Jersey, one of a cluster of English islands closer to France than to their sovereign. The island of Jersey is situated on the outer edges of the French Gulf of Saint-Malo where the tide comes rushing back into the bay at tremendous and often deadly speeds. It was a treacherous, even isolated marine environment, Kay remembers, but one that held more mysteries and more excitement than a young boy could ever hope to find.

"We would go into rock pools and find these amazing creatures, eight-foot-long rays that had been cut off from the ocean," he says. "Because I was exposed to this seafaring environment, I developed a serious interest in biology at a young age. I also helped my grandfather with his lobster fishing. We sailed all over, sometimes far offshore."

As a teenager, Kay was urged to get a medical degree but went into biochemistry instead. Toward the end of his degree, he and a Welsh scientist set out to find the answer to the question that has puzzled mankind for so many centuries: Why are plants yellow in the dark and green in the light? Thanks to their work, we now know that the protochlorophyll reductase enzyme is the responsible party.

In 1985, Kay moved to the Rockefeller University in New York City where he stayed until 1992. From there he was offered the opportunity to join the Center for Biological Timing at the University of Virginia. In 1996, he joined The Scripps Research Institute along with his wife Shelley Halpain, Ph.D., Assistant Professor, Department of Cell Biology.

Bioluminescence from a transgenic Arabidopsis thaliana seedling carrying the firefly luciferase gene. The transgene is active throughout the young seedling.

ENTER DROSOPHILA, THE HUMBLE FRUIT FLY

Although Kay's first love has always been plants, his study of biological clocks was clearly leading him further into other branches of the evolutionary tree.

"I was introduced to an animal biologist who had the same questions about circadian rhythms in animals that I had for plants," Kay explains. "In animals and in humans there are a lot of pathologies associated with circadian rhythms. Up to 40 percent of Americans suffer from insomnia, and certain types of depression — seasonal depression, for instance, is probably due to malfunctioning clocks. Biological clocks also change with age, so we can gain insight from them into how we might better manage our aging population."

There is also a direct clock application in disease treatment. In the human body, many epithelial cells divide on a 24-hour rhythm, and these types of cells suffer the brunt of chemotherapy side-effects. Coupling that with the knowledge that the biological rhythms of tumors are different than epithelial cells, a field called chronotherapy has emerged that suggests delivering anticancer drugs at certain times of day may help reduce side effects.

"What we found is that the brain is still important but it's clearly not the only place where there is clock activity..."

Kay's fruit fly adventure began, strangely enough, in a dimly lit bar in Geneva, Switzerland, when he was introduced to Jeff Hall, a scientist from Brandeis University whom Kay calls an eccentric genius. There, Hall convinced Kay to help him make fruit flies glow in the dark, the same way he had with Arabidopsis.

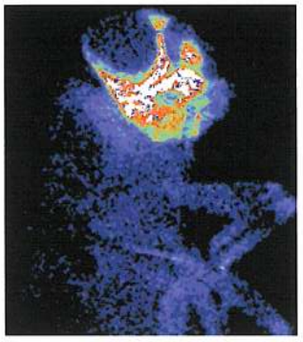
As with the mustard plant, the firefly luciferase gene is attached to the fruit fly's biological clock gene — called *per* for period. By feeding the mutated flies luciferin and monitoring the *per* gene expression, they could watch Drosophila grow brighter and dimmer as the creatures worked their way through the rhythms of their daily lives. Kay and Hall were astounded by what they found.

THE FRUIT FLY, IT TURNED OUT, IS COVERED WITH CLOCKS, ONE FOR NEARLY EVERY BODY PART

"It was an enormous surprise," Kay says. " We discovered that the wing cells have clocks, so do the leg cells, the abdomen, and the testes. Most animal research had focused on the brain as the center of the biological clock. What we found is that the brain is still important but it's clearly not the only place where there is clock activity or even the most important."

Mice quickly followed the fruit fly as subjects and others have discovered the same phenomenon — clocks all over the place. "The main point is that local clocks are responsible for local processes," he says. "That's important for things like chronotherapy. You want to be able to look at cellular clocks, not only the brain clock, to determine the effectiveness of it."

One problem facing plants and humans is the fact that the earth tilts on its axis, changing the length of the day during the year. As a result, our clocks need to adjust themselves to match these changes. As it turns out, light can shift our clocks, moving the hands of our clocks to accommodate shorter or longer periods of light. Understanding exactly how light changes our internal clocks, Kay believes, will help us better understand things like jet lag and seasonal depression.



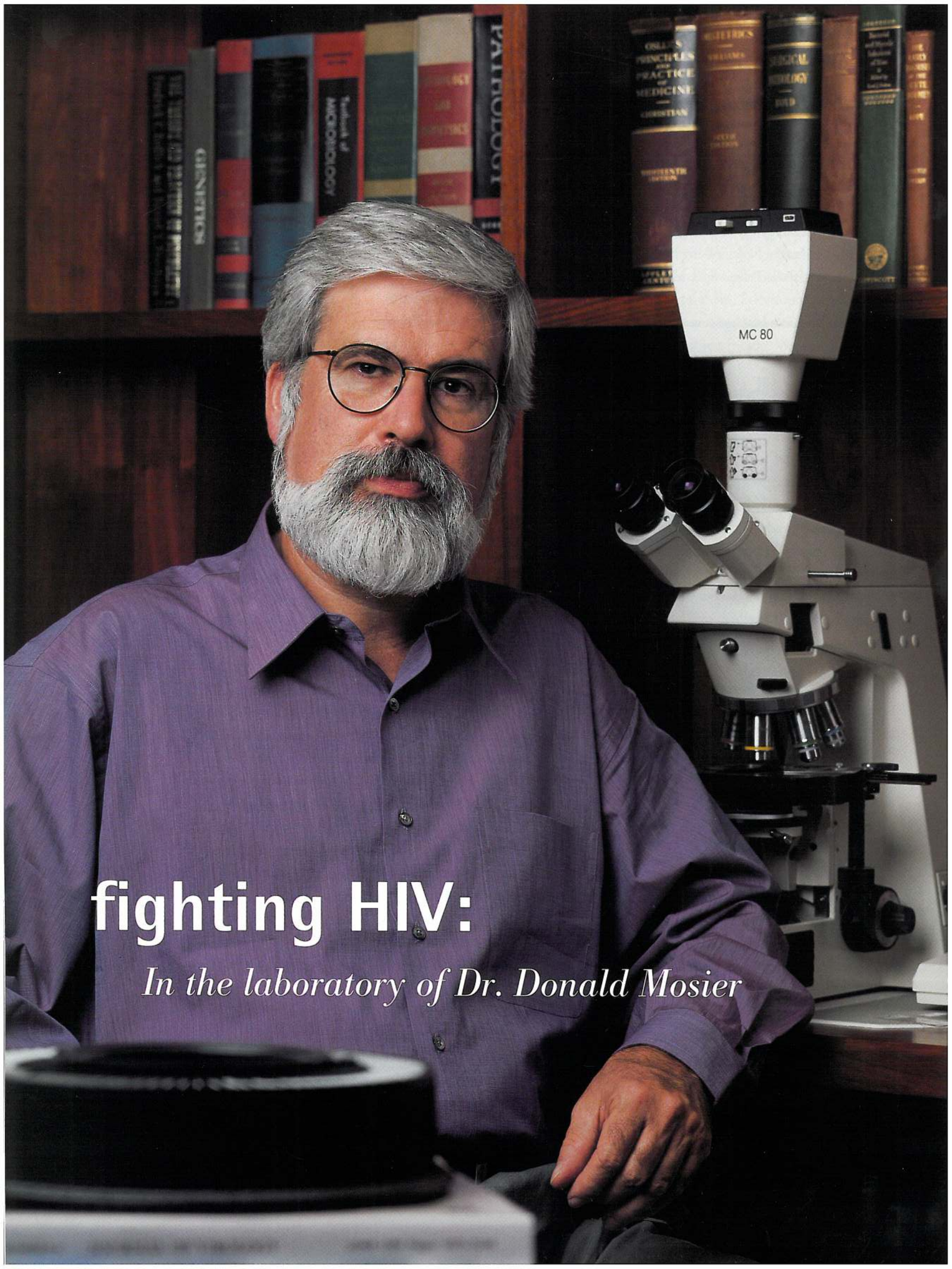
Bioluminescence from a transgenic fruit fly carrying the firefly luciferase gene. The transgene is active in the eyes of the fly.

"We already know some of the molecules that make up the clock," Kay explains, "and we're beginning to understand some of the molecules that respond to light signals. In the next phase, we're going to initiate studies into human genetics and take advantage of the plant and animal models we've created. The clock genes that we and others have found in mice are the same ones found in humans."

Kay and collaborators from the VA Medical Center in San Diego and the Scripps Clinic have identified several patients with serious sleep disorders as well as families in the San Diego area who have shown familial traits for the same disorders. First they will look for abnormalities in the clock genes and then try to develop genetic tests to identify them.

"A great many diseases have circadian components," he says. "In some forms of epilepsy, the occurrence of seizures is circadian. It may also play a role in bipolar disease. If we can show which types of these diseases are due to a malfunction of the circadian system, we can give physicians a new diagnostic tool to help their patients."

People take widely different paths to become scientists. Steve Kay seems to have taken a mostly circuitous one, from the tide pools of the English Channel to the blue-green waters of the Sea of Cortez, from plant to human and back again. Perhaps his most tantalizing discovery is that the line between plant life and human existence is a great deal finer than we ever imagined. ■



fighting HIV:

In the laboratory of Dr. Donald Mosier

Scientific research follows a pattern of minute incremental advances interspersed with breakthrough discoveries that determine the course of events for years to come. In the era of increasing specialization in biological research, an investigator is lucky to be involved in even one key discovery that greatly advances understanding in any field.

Donald Mosier, M.D., Ph.D., Professor in the Department of Immunology, has been present at numerous important events in the AIDS and cancer research field throughout his 35-year career.

He got interested in science in high school and began taking summer jobs working in laboratories, first at the University of Louisville, then at the University of Indiana at Bloomington. After high school, he studied biology at the University of Chicago, eventually receiving a medical degree from the Pritzker School of Medicine.

"Early on I liked science, especially the experimental aspect. Having already worked a lot in labs, I was pretty disappointed with medical school — the competitive environment, and the emphasis on didactic facts rather than formulating questions. Anatomy was not my favorite subject."

This lingering dissatisfaction led him back to what was truly his favorite subject, laboratory research. He took a leave of absence from medical school to finish his Ph.D., also from the University of Chicago, working in the nascent field of immunology.

Mosier's fate has been intertwined with mice from the beginning of his career. In fact, mouse spleen cells brought him to La Jolla for the first time.

He explained, "I had been working in a basement lab in Chicago with no luck trying to replicate a newly published technique for making antibody responses from mouse spleen cells in tissue culture. I convinced my advisors to send me to The Scripps Research Institute to talk to the people who had developed the test, researchers Bob Mishell and Richard Dutton, so I could find out what I was doing wrong. I stayed two weeks, learned how to do the culture, and everything worked great."

The knowledge gained during that visit further propelled his research. He earned his Ph.D. based on experiments done using the technique. This same technique led to his first published paper.

THE EARLY DAYS OF IMMUNOLOGY

As a graduate student he worked on the mysteries of cellular immunology. At that time, immunology was in its early days. One fundamental question concerned how many cells were required to generate an antibody-forming cell. In his first research paper, Mosier reported that the answer was three, an antigen-presenting cell, a helper T-cell, and a B-cell. This discovery has stood the test of time and formed the basis for much research to follow. This was the first of the numerous key discoveries in which Mosier participated.

"I was lucky to have the right technique and to be in the right place on the ground floor of these discoveries."

"I was lucky to have the right technique and to be in the right place on the ground floor of these discoveries," he notes with characteristic modesty.

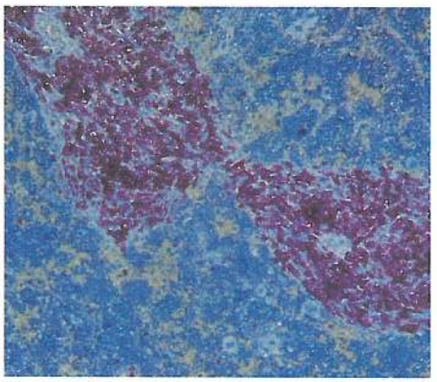
After serving his medical internship in 1971-72 in the Department of Pathology, Children's Hospital Medical Center, Boston, Mosier moved to the National Institutes of Health. He spent seven years there, rising from postdoctoral fellow to senior investigator. During his tenure, he got more interested in virology, particularly in animal retroviruses.

Mosier then spent six years as research physician at

Donald E. Mosier, Ph.D., Professor, Department of Immunology.

the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia. During that time, the first reports began to trickle in of a mysterious illness that rapidly destroyed the immune systems of healthy young gay men. This was the beginning of the AIDS epidemic.

Science made rapid progress in isolating HIV, the virus that causes AIDS. But the questions of how HIV infects cells and disables the immune system, and how this infectious process can be blocked, continue to challenge researchers 15 years later.



A thin section of a hu-PBL-SCID mouse lymph node magnified 400 times to show individual cells. Human lymphocytes are stained green and human cells infected with HIV-1 are stained black. Mouse red blood cells are pale orange. Infected cells were detected by annealing a labeled complementary RNA probe to HIV-1 RNA.

"When the AIDS epidemic began, there was no useful animal model of AIDS. When you are trying to model a disease like AIDS it is important to have an animal model, one step removed from human," he said. At that time, a mutant mouse with virtually no immune system, the SCID (severe combined immunodeficiency disease) mouse was discovered at Fox Chase by a colleague, Dr. Mel Bosma.

EVALUATING HOW HIV INFECTS CELLS

Because SCID mice lack a normal immune system, it was possible to graft human cells into them and challenge them with various viruses. Mosier was involved in the very early stages of SCID mouse research, participating in experiments in grafting human immune cells into the mice. Success with these experiments led to an animal model suitable for evaluating the infection of human immune cells by HIV.

SCID mice were in short supply, so they tried to find a way to monitor human cell engraftment in the mice in a nondestructive way. Mosier and colleague Darcy Wilson injected their own white blood cells into the mice. They then took blood samples from the mice, using a method called ELISA, to look for human immunoglobulins. Two weeks later, the ELISA test lit up, indicating that the experiment had worked.

"We were all excited. It looked like the experiment had worked on the first try. We started doing follow-up experiments to confirm the result and were ready to write it up for publication. But then the first mice started dying, " he recalled.

Dismayed, the researchers performed autopsies on the mice and found that tumors, human B cell tumors, had killed the mice. Mosier guessed correctly that another human virus, Epstein-Barr Virus, might be involved. Almost everyone is infected with EBV, the virus that causes mononucleosis, but the healthy human immune system easily keeps it in check. However, in patients with depressed immune responses, such as those undergoing transplantation, EBV can cause a form of cancer, lymphoma, that can be fatal.

"It turns out that not everybody gives these tumors to SCID mice very well. Darcy and I both did. After we started using blood cells from donors who had been infected with EBV earlier in life, the problem was solved. It was wholly unexpected, that what turned out to be a model designed to look at HIV infection has also had a second application, the study of how EBV causes lymphomas. Ironically, EBV-induced lymphomas are becoming more common complications of later stages of AIDS."

Mosier guessed correctly that another human virus, Epstein-Barr Virus, might be involved.

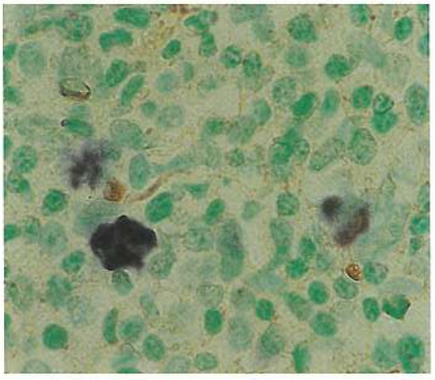
As quickly as possible, Mosier, who had now moved to La Jolla, and colleagues built a containment facility for HIV research using the new mouse model. Sure enough, when the animals were injected with HIV, they quickly became ill with AIDS.

"At last we had a reasonable small animal model for AIDS. It allowed us to study HIV infection, and to determine the consequences of the infection in terms of what the human cells were doing. "

The creation of the animal model led to much of the progress that has been seen in understanding and treating HIV today. As in humans, humanized SCID mice infected with HIV also showed a sudden depletion

in CD4 T-cells after HIV infection. The researchers learned that different variants of HIV preferred to infect different elements of the human immune system. While the standard HIV isolate used in most AIDS research grows well in T-cells but not in macrophages, they observed that other variants preferred to invade macrophages in addition to T-cells.

This research led Mosier and others to a recent discovery in AIDS research, that the different variants of HIV used different cellular co-receptors for entry. While some HIV invaded T-cells by a receptor called CXCR4, the variant that preferred macrophages used a different receptor, CCR5. Moreover, Mosier and others found that it was the envelope gene of the virus that determines which co-receptor HIV uses to enter cells. Indeed, this genetic difference is the major determinant of how fast it causes CD4 T-cell depletion — the key to its destruction of the immune system. A difference of a single amino acid within the 480 amino acid long envelope gene determined the target cells for virus infection.



A section of a hu-PBL-SCID mouse spleen, magnified 100 times. Mouse spleen cells are stained blue and human cells are stained red. The human lymphocytes have filled the space normally occupied by mouse lymphocytes, which are missing because the SCID mutation prevents their formation. [SCID=severe combined immunodeficiency]

DETERMINING THE SPREAD OF THE VIRUS

These findings are important because they help explain how the entry of HIV into the human immune system determines the spread of that virus. The finding that these receptors are what the virus uses for entry opened a whole new avenue of possible therapeutic options. If a drug could be developed that could somehow block the receptors, HIV would have nothing to latch onto.

The CCR5 receptor's normal role is to bind one of three chemokines: MIP-1 alpha, MIP-1 beta, and RANTES. Chemokines are chains of amino acids that signal cell migration and form an early part of the immune response.

Early work with the RANTES chemokine showed it to be fairly effective at blocking infection by the virus that preferred the CCR5 receptor. Different labs created modified RANTES compounds, hoping to find one that was particularly good at blocking the receptor. One of these, AOP-RANTES, appeared to work well in the test tube. However, when given to humanized SCID mice, it did reduce viral load, but did not prevent mice from getting infected. Another more potent RANTES analog, NNY-RANTES, produced by former TSRI scientist Stephen Kent, worked even better in the test tube, and was able to block infection in 60 percent of mice.

The finding that these receptors are what the virus uses for entry opened a whole new avenue of possible therapeutic options.

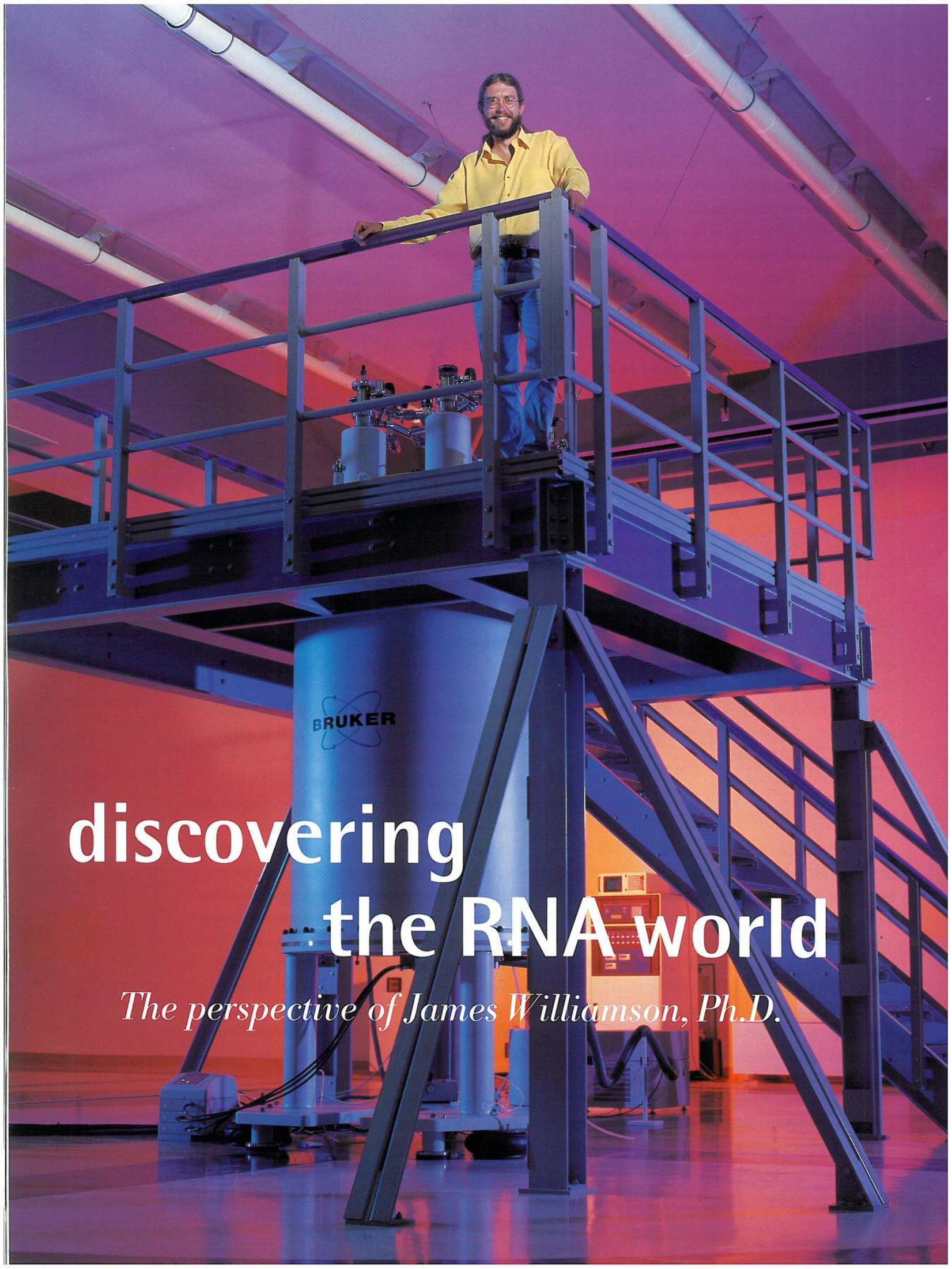
"We thought this was good news and looked pretty promising. However, the stuff was in short supply. In two mice experiments we used 30 mg, half of the world's supply."

THE VIRUS' MUTATIONS POSE PROBLEMS

Any number of promising treatments for AIDS have been stymied by the ability of the virus to mutate, changing its protein coat to avoid efforts to stop it from infecting cells. Subsequent experiments with NNY-RANTES showed that the HIV virus quickly mutated its way around the receptor-blocker.

Countless labs are now working on ways to block these chemokine receptors in an effort to slow or stop the HIV infection process. Mosier presented two papers at this year's International AIDS Conference in Geneva involving a synthetic molecule that may block one of the receptors. The next step will be to make a cocktail of chemicals that block all of the co-receptors targeted by HIV as it tries to infect a host.

When asked about ongoing efforts to develop an AIDS vaccine, Mosier reflects, "I like to be realistic. There is optimism, pessimism and somewhere in between is realism. However, if you keep doing research and searching for new cures and new approaches, you are obviously an optimist at some level." ■



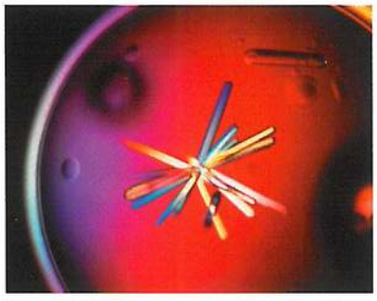
discovering the RNA world

The perspective of James Williamson, Ph.D.

Two of the most interesting discoveries in biology over the past two decades have been the discovery of HIV, the retrovirus that causes AIDS; and the discovery of the important role that RNA plays in many biological systems. These two discoveries intersect in the laboratory of James Williamson, Ph.D.

James Williamson, Ph.D., a professor in the Department of Molecular Biology and The Skaggs Institute for Chemical Biology, is conducting pioneering work unraveling the three-dimensional structure and function of RNA. Much of his research involves looking at particular pieces of RNA involved in the replication of HIV. Williamson and colleagues seek answers to questions about the possible molecular shapes RNA can take, how these structures relate to RNA-protein interactions and the mechanics of how large RNA molecules fold into complex structures. To understand how Williamson became involved in these key areas of biological research, it helps to go back, way back.

Born in Massachusetts, as the only child of a Methodist minister, "Jamie" Williamson spent his early years moving from one small New England town to the next, until the family eventually settled near Groton, Connecticut, where his father ministered to the Naval community. Jamie learned piano, bassoon, trombone and voice. Music became a guiding interest in his life, which it remains to this day.



AN EARLY IMPACT ON HIS LIFE

Something else happened when he was only three years old that was to have an unexpectedly important impact on his life:

"I vividly remember being in a supermarket, and in the toy section I saw a little tiny chemistry set with test tubes and neat colors. I asked for it and Mom brought it home and I started playing with it. It had some of the standard chemistry set tricks, changing the color or pH of one thing or another. I got a bigger chemistry set when I was seven and a really big one when I was nine. Then my Dad built me a bench and I ended up with a chemistry laboratory in the basement."

During his days as a graduate student, the dogma of DNA was overthrown.

Williamson read all of the chemistry books he could find and began to conduct more elaborate experiments. Eventually he even built a cloud chamber, an elaborate device used to detect elementary particles and other ionizing radiation. While the cloud chamber "never quite worked," Williamson was well on his way to a lifelong interest in chemistry, and the tools of the trade.

After high school, he attended a small liberal arts college, Mt Union College in Ohio. At this point his interest in chemistry began to dominate his studies, although he continued to participate in music and theatre. After receiving a BS in chemistry, he moved to the fast lane for graduate school, Stanford University.

"I had received a good basic education, but when I got to graduate school I was a bit behind my peers. The first year was tough. In the undergraduate setting you do labs that have already been done by thousands of students before you. The answers are known in advance. The problem with research is that it never works the first time. In fact, there is nothing more sobering than looking at real data and trying to figure out what it really means."

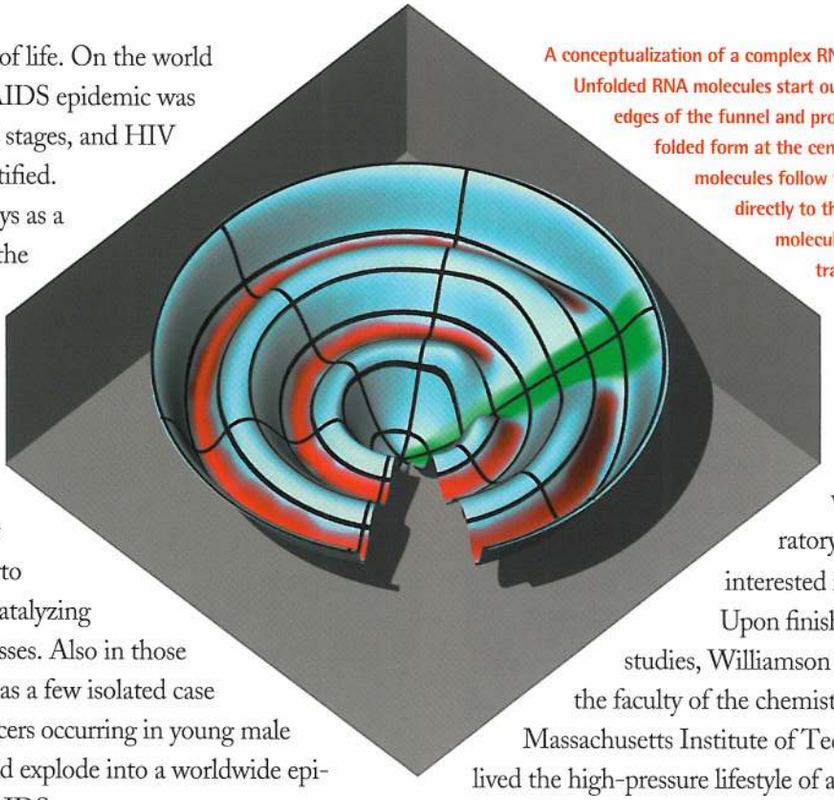
When he arrived at Stanford in 1981, the existing scientific dogma emphasized the importance of DNA, assigning RNA a secondary, dependent role in the

Facing Page: James R. Williamson, Ph.D., Professor, Department of Molecular Biology, atop a Bruker Instruments 800 MHz Nuclear Magnetic Resonance Spectrometer.

A cluster of crystals of a ribosomal RNA-protein complex viewed through a microscope under polarized light. The crystals are approximately 0.5 mm long by 0.05 mm wide. The different colors arise due to an effect called birefringence, which is characteristic of RNA containing crystals. These crystals will be used in X-ray diffraction experiments to solve the three-dimensional structure of this RNA-protein complex.

molecular process of life. On the world health stage, the AIDS epidemic was only in the earliest stages, and HIV had not been identified.

During his days as a graduate student, the dogma of DNA was overthrown in favor of a new view known as the RNA world, when researchers demonstrated that RNA had a hitherto unknown role in catalyzing essential life processes. Also in those years, what began as a few isolated case reports of rare cancers occurring in young male homosexuals would explode into a worldwide epidemic known as AIDS.



A conceptualization of a complex RNA folding landscape. Unfolded RNA molecules start out at high energy at the edges of the funnel and proceed downward to the folded form at the center of the funnel. Some molecules follow the fast track (green) directly to the folded state, but most molecules get stuck in kinetic traps that are represented as moats on the folding funnel (red).

Facing Page:
The structure of the yeast L30 ribosomal protein in complex with mRNA as determined by NMR. The L30 protein (blue) interacts with a novel RNA structural motif (orange). Formation of this complex blocks translation of an mRNA, and this is the first example of such a regulatory complex. The structure reveals a new principle of RNA-protein recognition, and sheds light on the mechanism of translation regulation by RNA-protein interactions.

Williamson eventually found his metier in the field of NMR (nuclear magnetic resonance) spectroscopy, an area that allowed him to design new kinds of experiments to unravel the three-dimensional structure of organic molecules, while at the same time building new tools to do these projects.

"All the chemists here are really interested in looking toward biology to apply their craft."

By the mid-1980s a buzz was developing in the field of RNA research. A researcher named Thomas Cech discovered that RNA was more than a helper molecule; it played a very active role in guiding the molecular processes of biology. Williamson landed a postdoctoral position at the University of Colorado in Boulder in the laboratory of Tom Cech, who would receive the Nobel Prize in 1989, while Williamson was a postdoc. He found himself among like-minded souls. At Stanford he had been the only person in his department doing what he was doing. In Boulder, he worked with a laboratory full of people interested in RNA.

Upon finishing his postdoctoral studies, Williamson was invited to join the faculty of the chemistry department at Massachusetts Institute of Technology. Here he lived the high-pressure lifestyle of a junior faculty member, teaching classes and conducting research with little time left for anything else. At MIT he figured out how to label RNA with stable isotopes C13 and N15 for NMR studies. This has since become the standard technique used around the world. Williamson's lab also solved the three-dimensional structure of the first RNA-ligand complex, and solved the three dimensional structure of two RNAs that were important for HIV replication.

THE MOVE TO TSRI

While he had no complaints about his career situation at that time, the position was very demanding, and logistically difficult. He and his wife, Martha Fedor , Ph.D., were both working constantly, he at MIT and she at the University of Massachusetts Medical School, commuting hours each day in opposite directions. Both jumped at the opportunity to move to La Jolla when offered positions at The Scripps Research Institute. The two now have more time to spend together, both as spouses and as colleagues.

"This is a truly amazing place. There is something very exciting going on here. At MIT, I was in a chemistry department. Although I had a strong background as a chemist, my research was taking on a more biological direction. I went from a situation where I had one crystal-

lographer colleague and one computational colleague to a situation here where I have five x-ray colleagues, five NMR colleagues, and five computational colleagues, along with a broad range of biology colleagues. Moreover, all the chemists here are really interested in looking toward biology to apply their craft."

The folding pathways of large, highly structured RNA molecules are largely unexplored. Williamson utilizes nuclear magnetic resonance spectroscopy to unravel the three-dimensional structure of RNA. This research has already produced important progress in how RNA can best be visualized using these techniques. "My goal is to study nucleic acid structure using a wide variety of techniques and approaches. Not only do we have a good idea of what the general problem is, what we really like to do in my program is to invent methods that allow us to do new experiments. We are problem driven, and do whatever it takes to attack these areas that interest us," says Williamson.

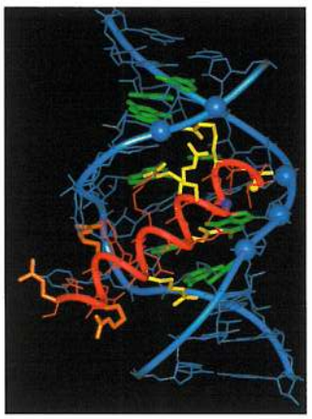
THE IMPORTANCE OF PROTEIN FOLDING

In the grand scheme of things, RNA is a large, chain-like molecule. Most of the time, the structurally complicated molecule folds itself up into a very tiny package. Understanding this folding and unfolding process is considered a key to elucidating a large number of biological mysteries including the origin of life, replication of viruses and more complex organisms, and the interaction between drugs and microbes. This latter aspect is important for addressing the increasingly worrisome problem of treatment-resistant microorganisms.

The RNA folding problem has two parts. The first question is what is the structure of the RNA molecule from end to end. The other question is how does this process of folding and unfolding take place.

Williamsons' lab is working on both of these interwoven problems simultaneously.

"This folding and unfolding of RNA is an important part of how the molecule functions. We have been investigating how this process can be monitored. We have managed to develop imaging methods that tell us what parts of the RNA molecule are folded or unfolded at any given time. This work led to the discovery of one of the first complete folding mechanisms of a large RNA molecule," Williamson explains.



The three-dimensional structure of the HIV Rev protein in complex with the Rev Response Element (RRE) RNA as determined by NMR. The Rev protein forms an alpha helix (red) that is inserted into the major groove of the RRE (blue). Numerous side chains make specific contacts to the bases of the RRE to mediate specific recognition. Formation of the Rev-RRE complex is critical for HIV replication, and knowledge of this structure may assist in developing new HIV therapeutic strategies that block this interaction.

THE INTEGRATION OF BIOLOGY AND CHEMISTRY

His RNA research highlights a sea change in the field of biological research. Until fairly recently, researchers from the fields of biology and chemistry worked in separate camps. Each spoke its own language, and communication between researchers in the different specialties was not all that it should be. Now the situation is changing rapidly. Chemists find they cannot work without reference to molecular biology, and biologists find they can benefit from the viewpoint of chemical researchers.

Understanding this folding and unfolding process is considered a key to elucidating a large number of biological mysteries including the origin of life, replication of viruses and more complex organisms, and the interaction between drugs and microbes.

He continues, "I think the Scripps Research Institute is on the leading edge in this regard, because we have an excellent collection of forward-looking biologists and chemists. This is a truly amazing place. I can interact with a wide range of biology and chemistry colleagues. There is a tremendous synergy among researchers here." ■

New Study by TSRI Scientists Sheds Light on Viral Clearance in Acute Hepatitis B Infection

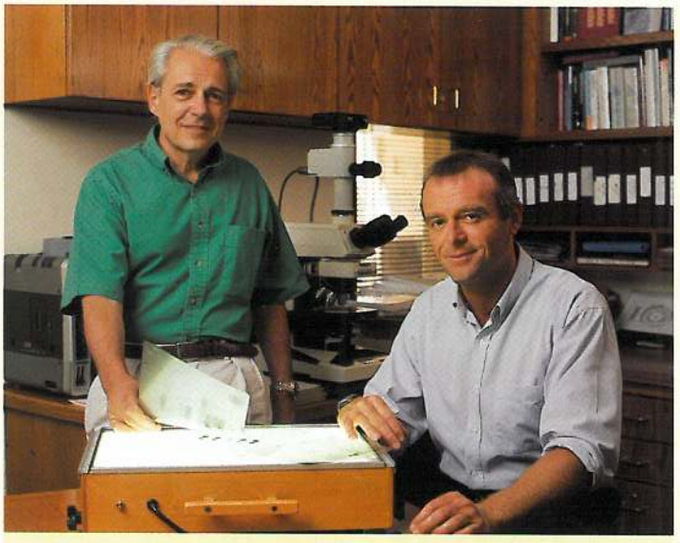
A study published in a recent issue of *Science* by Drs. Luca G. Guidotti and Francis V. Chisari demonstrates a new paradigm in viral immunology, namely that the immune system can cure viral infections without destroying the infected cells. Until now, scientists believed that viral clearance was due to the destruction of infected cells by cytotoxic T cells. But the article, "Viral Clearance Without Destruction of Infected Cells During Acute HBV Infection," demonstrates that nondestructive antiviral mechanisms can contribute to viral clearance by eliminating a virus from inside the cell without killing it.

"This work provides fundamental new insights into the immunological mechanisms of infection control."

According to Dr. Chisari, Professor, Department of Molecular and Experimental Medicine, and Director, General Clinical Research Center, "The immune system has evolved a defense mechanism that allows it to cure certain viral infections by instructing the infected cells to stop producing virus and to accelerate viral elimination. This appears to be a survival strategy to control infections of vital organs that would be destroyed if the only way to control the infection was to kill all of the infected cells."

Hepatitis B is one of the most common, serious infectious diseases in the world. More than 350 million people worldwide are chronic carriers of the virus and it has infected 1-1.25 million Americans. The leading cause of liver cancer, the World Health Organization estimates that the infection leads to more than one million deaths every year. Each year approximately 300,000 people will become infected with the Hepatitis B virus. Although there is a safe and effective vaccine for the prevention of HBV, it is of no value to those already infected. While treatments are currently available for the infection, they have considerable limitations in terms of toxicity and long-term benefits.

In this study, the authors demonstrate that the DNA of the Hepatitis B virus disappears from the liver and the blood of acutely infected animals long before the onset of disease and the peak of T cell infiltration, suggesting that nondestructive antiviral mechanisms, triggered by inflammatory cytokines secreted by the first wave of inflammatory cells that enter the liver, contribute to clearance of the virus. According to Dr. Guidotti, these cytokines — proteins that are secreted by cells of the immune system — attach to receptors on infected cells and trigger them to purge



Francis V. Chisari, M.D., Professor, and Luca Guidotti, D.V.M., Assistant Professor, Department of Molecular and Experimental Medicine.

themselves of the virus. This significant decrease in viral load reduces the number of cells that must be killed, thereby preserving the function of the organ while the virus is removed.

The scientific basis for the current study is the result of work conducted by Guidotti and Chisari over the past five years using a transgenic mouse model. In these experiments HBV replication was completely eliminated by inflammatory cytokines under conditions in which there was no injury to the liver.

Chisari commented, "If we can harness the curative function of the immune response in patients with chronic HBV, it may be possible to cure these patients. No treatments are available today either to trigger the production of cytokines or to deliver them to the livers of infected patients in sufficient quantities or in enough time for elimination. This work provides fundamental new insights into the immunological mechanisms of infection control."

The study was funded by the National Institutes of Health. ■

Dr. Wong's Group Develops Computer Program to Aid in the Synthesis of Oligosaccharides

Oligosaccharides — biomolecules made up of chains of sugar groups — play an important role in the body, from the ability of immune cells to recognize their targets to the spread of cancer cells, for example. Synthesizing them, however, has been a complicated and painstaking endeavor.

Now, Chi-Huey Wong, Ph.D., Professor, The Skaggs Institute for Chemical Biology and Department of Chemistry, has devised a computer program for creating a range of oligosaccharides in one simple reaction, a "one-pot" operation. Scientists believe that speeding up the process of synthesizing these sugar chains can help them delineate their precise roles.

"This is the first time that a personal computer has been used in a systematic manner to guide the synthesis of carbohydrates by researchers who may or may not know that much about carbohydrate synthesis," according to Wong. "We simplified the synthesis of carbohydrates by developing a program called OptiMer. You can create a library of oligosaccharides one-by-one, as individual compounds, or you can synthesize a specific target for study," he continued. Wong notes that while the technique initially will be used to facilitate the reactions used in oligosaccharide synthesis to link sugar groups together, it may be applicable to other types of organic reactions as well.

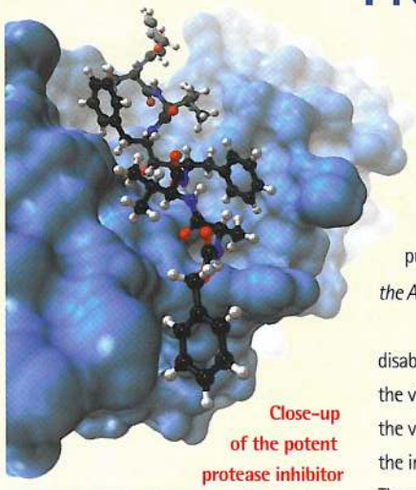
"It is very important work," says Samuel Danishefsky, Ph.D., an organic chemist at the Sloan-Kettering Institute for Cancer Research and Columbia University. And Carolyn Bertozzi, Ph.D., a chemist at the University of California, Berkeley, commented, "We spend 95 percent of our time making the compounds. This new work could help us turn that around" and spend 95 percent of the time on the biology, she says.

While it is relatively straightforward to synthesize peptides–short protein chains whose building blocks are linked with the same bond–oligosaccharides are more complex and can form a multiplicity of three-dimensional shapes. With OptiMer, all of the requisite reagents and carbohydrate building blocks necessary to create the desired oligosaccharide are preselected.

Then they are mixed in the same vessel and react to form the desired product. By using different patterns of protecting groups to adjust the reactivity of the building blocks, they can be joined together in the correct order.

TSRI has applied for a patent on the technology and Wong believes it will be commercialized. ■

Researchers, Led by Chi-Huey Wong, Develop Protease Inhibitors Against HIV



Close-up of the potent protease inhibitor TL-3 binding to FIV protease (in blue).

A group of TSRI scientists, led by Chi-Huey Wong, Ph.D., has developed a new protease inhibitor that is effective against mutating strains of the AIDS virus that are resistant to current drugs. The work was published recently in *The Journal of the American Chemical Society.*

Most therapies against the HIV virus disable it by latching onto an enzyme that the virus needs to multiply. However, the virus' ability to quickly mutate renders the inhibitors ineffective within weeks. The most successful treatment to date attempts to overwhelm HIV with two or three of these drugs in a combination therapy, but even this approach eventually becomes ineffective.

But, Wong now thinks he knows how HIV adapts readily to these treatments. Over time HIV proteases apparently change structure so that the inhibitors can no longer bind tightly. He commented, "We have studied the mutation pattern of HIV protease from patients who take the existing drugs and found that the enzyme often rejects the drug by reducing the size of the drug binding site."

The researchers looked at the corresponding binding site on HIV protease inhibitors and discovered that most of them have large chemical structures that interact with the constricted areas in drug-resistant proteases. By redesigning the drugs, they have given them a smaller chemical group at the critical binding site that increased their effectiveness against both HIV protease and its drug-resistant mutants. "More important," adds Wong, "no resistant mutants were detected in cell culture after one year. The new drug may last longer as the chance for development of drug resistance is lower."

The same chemical also may become the first treatment for feline AIDS, a major threat to the world's cat population. ■

Crippled Version of HIV Used to Transfer Genes to Human Blood Stem Cells

In a significant gene therapy advance, a team of scientists from TSRI and The Salk Institute has used a crippled version of the AIDS virus to deliver genes to human hematopoietic stem cells, a class of cells capable of reconstituting blood and bone marrow, and transplanted the treated cells back into mice.

The transferred cells survive and differentiate in the mice for at least five months, a significant portion of the life span of a mouse. The scientists believe that this will open the door to many applications, including thalassemias, sickle-cell anemias, hemophilias and certain cancers. Stem cells are capable of becoming any type of cell in the blood, from immune system cells to oxygen-ferrying red blood cells.

This study represents a major advance in the scientist' ability to manipulate the genetic content of blood cells.

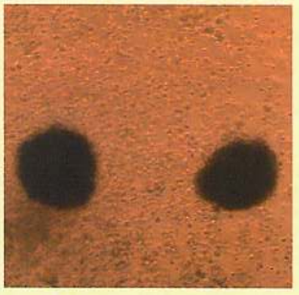
UNIQUE ABILITIES OF HIV-BASED VECTOR

Previously available gene therapy vectors, usually based on retroviruses, have proven successful at delivering genes to mouse hematopoietic stem cells, but not to those from humans or other large animals. Researchers suggest that this is probably because the human stem cells are at rest; that is, they are not actively dividing. There is often a problem in scaling up gene therapy procedures from mice to large animals. The HIV-based vector, however, is unique in its ability to penetrate the nucleus of non-dividing cells. This ability will allow investigators to add genes to virtually any cell type in the body.

Genes have been successfully inserted into stem cell precursors, but only in the presence of cytokines, hormone-like substances that can interfere with the developmental program of the stem cells. Therefore, this study represents a major advance in the scientists' ability to manipulate the genetic content of blood cells. TSRI's research group on the study includes Drs. Hiroyuki Miyoshi, Kent Smith, Donald E. Mosier, and Bruce E. Torbett. Their colleagues at The Salk Institute include Drs. Inder Verma, Frege Gage, and Didier Trono.

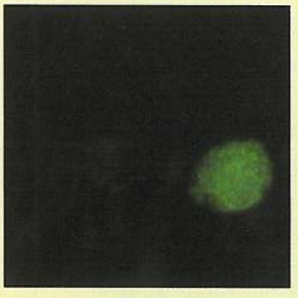
The work was supported by the National Institutes of Health, the March of Dimes, the H.N. and Frances C. Berger Foundation, and the Uehara Memorial Foundation. ■

BFU-E



Bright Field

This is a picture of human cells, called blast forming cells-erythroid, "BFU-E," that will make red blood cells. Each of the two colonies of cells in the left panel came from human hematopoietic stem cells, cells that have the ability to produce all the blood cells in the body. The stem cells were given a "green fluorescence gene" (from a jellyfish) using a crippled version of HIV and then allowed to develop into red blood cells. All blood cells developing from stem cells containing the GFP gene as part of its genetic makeup (DNA) also will contain the GFP gene. The GFP gene makes a protein that, when viewed under a fluorescent light, glows green. As can be seen under a microscope the cells in the left panel show the characteristic reddish color of cells about to become red blood cells and the right panel shows that one of the two BFU-E colonies viewed under a fluorescent light was glowing green, demonstrating that one colony of cells had the GFP gene. Scientists believe that this new "gene delivery" system will be useful in gene replacement of many different diseases in cells that are quiescent and are not dividing - such as hematopoietic stem cells, muscle and brain cells. ■



Fluorescence

New Laboratory Building for Department of Molecular and Experimental Medicine Opens in Spring

The 70,000-square-foot first phase of the laboratory building housing the Department of Molecular and Experimental Medicine opened in March and TSRI scientists will fully occupy the three-phase project in April, 2000. The 5.5 acre, $43-million project in the Torrey Pines Science Park will contain 110,000 square feet when completed. The site's developer is Lankford and Associates.

The structure has been designed to "hug" the site terrain; berming has been employed to lessen the visual impact of the three-story facility, which has been oriented to take advantage of views of contiguous dedicated open space. The building interiors have been designed to provide natural ambient light in offices and laboratories.

The development team includes Hensel Phelps Construction Co., McGraw-Baldwin Architects, Robert Davis Architects, The Advanced Technology Group of Carrier Johnson and Wallace Roberts & Todd, landscape architects.

Chairman of the Department of Molecular and Experimental Medicine is Ernest Beutler, M.D. The majority of studies in the department seek to understand the life processes that, when disturbed, lead to disease. The scope of research is so broad that the scientific programs overlap with those of virtually every department at TSRI.

The new site of the Department of Molecular and Experimental Medicine.

Scientists in each division are engaged in clinical trials that directly involve patients, usually in the General Clinical Research Center, which is supported by the National Institutes of Health. The Division of Biomathematics is involved in every clinical study that is underway. Scientists in the Division of Arthritis Research are studying cartilage from patients with different kinds of arthritis in an attempt to better understand how the death of cartilage cells leads to joint disease. Much of the work conducted in the Division of Experimental Hemostasis and Thrombosis includes understanding and interdicting the interaction of blood platelets with the lining of blood vessels, studying the vascular endothelium, and investigating the treatment of strokes. Researchers in the Division of Research Rheumatology study the pathogenesis of a variety of autoimmune diseases. Investigations are underway in patients with allergic rhinitis, asthma, and hereditary angioneurotic edema to gain understanding of the factors that cause and modulate these disorders. Members of the Division of Hematology have initiated a large-scale clinical trial, in conjunction with physicians at Kaiser Permanente in San Diego, to study the effect of mutations of the gene that causes hereditary hemochromatosis, or iron-storage disease. Researchers in the Division of Biochemistry have devised a simple storage method to allow storage not only of blood granulocytes but also of stem cells in bone marrow.

The various types of hepatitis, one of the world's major killers, have been under intensive study in the Division of Experimental Pathology, and Phases 1 and 2 of a clinical trial of a new type of hepatitis vaccine have been successfully completed. Those divisions whose work does not deal directly with patients are nonetheless engaged in research projects that will most certainly have an impact on patients in the future. The emergence of antibiotic-resistant bacteria has been an increasing health problem, and the Division of Cellular Biology is working with industry to develop a new and more effective class of antibiotics. Oncogenes, mutated regulatory genes, are thought to be the basis of most forms of cancer. The Division of Oncovirology has been systematically isolating such genes from animal tumors. Those that are identified can then be sought in human tumors, and the genes that play an important role in tumorigenesis can be used both for diagnosis and as targets for treatment. ■

Skaggs Clinical Scholars Program is Established

With a contribution of $2 million from the Skaggs family, TSRI has established the Skaggs Clinical Scholars Program in an effort to more closely integrate clinical and basic research within the Scripps organization. Ernest Beutler, M.D., Chairman, Department of Molecular and Experimental Medicine, has been named chairman of the program's steering committee.

According to Richard A. Lerner, M.D., "It is our intention that collaborative efforts will lead to new ideas, re-focus basic research discoveries on human conditions and instill a new sense of camaraderie between clinicians and basic researchers in the shared task of applying research to alleviate human disease."

The new program identifies research-oriented clinicians and funds meritorious collaborative research projects between the clinical scholar and an appropriate TSRI scientist. The broader goal of the program is to expand the body of knowledge related to human disease and to develop effective therapeutic interventions.

An initial group of Skaggs Scholars has been selected. The initial funding levels have been set at a maximum of $150,000 in direct costs and will include salary for research time of the clinical investigator. Grants are awarded in a manner similar to that of the NIH peer review system.

The first group of clinicians to be designated as Skaggs Clinical Scholars are as follows:

Faith H. Barnett, M.D., Ph.D.,
Division of Neurosurgery, Scripps Clinic

Sandra C. Christiansen, M.D.,
Division of Allergy, Scripps Clinic

Clifford W. Colwell, Jr., M.D.,
Division of Orthopaedic Surgery, Scripps Clinic

Hubert T. Greenway, Jr., M.D.,
Division of Dermatology, Scripps Clinic

James R. Mason, M.D.,
Division of Hematology/ Oncology, Scripps Clinic

David A. Mathison, M.D.,
Division of Allergy, Scripps Clinic

John G. McHutchinson, M.D.,
Division of Gastroenterology, Scripps Clinic

Robert J. Russo, M.D., Ph.D.,
Division of Cardiovascular Diseases, Scripps Clinic

Alan Saven, M.D.,
Division of Hematology/ Oncology, Scripps Clinic

Jack C. Sipe, M.D.,
Division of Neurology, Scripps Clinic

Richard A. Smith, M.D.,
Medical Staff, Scripps Memorial Hospital

Donald D. Stevenson, M.D.,
Division of Allergy, Scripps Clinic

Paul S. Teirstein, M.D.,
Division of Cardiovascular Diseases, Scripps Clinic

Prabhakar Tripuraneni, M.D.,
Division of Radiation Oncology, Scripps Clinic

TRSI's Graduate Program Ranks Among the Country's Top Ph.D. Programs

TSRI's Graduate Program was recently ranked by *U.S. News and World Report* as one of the most outstanding in the country, based on the results of a survey sent to department heads and directors of graduate studies at universities throughout the country. The results were published in a special publication, *Best Graduate Schools 2000.* It ranked in eighth place in the "Top 10 Ph.D. Programs in Chemistry," tied with Columbia University, and tied for tenth place in the "Top 10 Ph.D. Programs in the Biological Sciences," with The Rockefeller University. When the programs were further categorized by specialties within a scientific discipline, TSRI ranked first tied with California Institute of Technology in bioorganic chemistry, and was in seventh place in organic chemistry.

The Graduate Program was established in 1989, when TSRI combined its strength in the integration of such disciplines as cell and molecular biology, structure and chemistry, to develop the Graduate Program in Macromolecular and Cellular Structure and Chemistry. In addition, in an effort to draw upon the capabilities of the then newly assembled chemistry faculty with an outstanding record of achievement in contemporary areas of chemical, biological and structural research, a doctoral program in chemistry was established three years later. With a current enrollment of approximately 130 students, these programs provide an exceptional training opportunity in a unique learning environment for a select group of outstanding and intellectually diverse students.Having graduated its first student in 1993, TSRI's Graduate Program has now conferred doctoral degrees on more than 80 students, who have gone on to promising scientific careers in academia, industry and government. ■

Peter G. Schultz, Ph.D., Joins TSRI Staff

Peter G. Schultz, Ph.D., a former professor of chemistry at University of California, Berkeley, principal investigator at Lawrence Berkeley National Laboratory and investigator with the Howard Hughes Medical Institute, has joined the staff of The Scripps Research Institute as a professor in the Department of Chemistry and the Skaggs Institute for Chemical Biology. He also has been appointed Director of Novartis' Institute for Functional Genomics, a discovery-focused research institute funded by the Novartis Foundation.

Peter G. Schultz, Ph.D.

A member of the National Academy of Sciences, Schultz holds B.S. and Ph.D. degrees from the California Institute of Technology, and completed a postdoctoral fellowship at the Massachusetts Institute of Technology. He began his academic career at the University of California, Berkeley, in 1985.

According to TSRI President Richard A. Lerner, M.D., "Peter Schultz is a very important addition to our faculty. His prodigious talents are well known in the scientific community and we look forward to many more contributions to the body of scientific knowledge based on his work and insights. He is one of the country's most creative and dynamic young scientists."

Schultz's research interests combine the tools and principles of chemistry with the molecules and processes of living cells to create molecules with new properties and functions found neither in nature nor the test tube. By studying the structure and function of the resulting molecules, he is providing new insights into the molecular mechanisms of complex biological and chemical systems.

His scientific inquiries have shown that the combinatorial diversity of the immune response can be chemically reprogrammed to generate selective enzyme-like catalysts, work for which he and Dr. Lerner have been recognized with such prestigious honors as the Wolf Prize and the California Scientist of the Year Award. They have developed antibodies to catalyze a wide array of chemical and biological reactions; in a number of cases rates comparable to those of naturally occurring enzymes have been achieved. Most recently, Schultz has focused on in vitro evolution methods that involve the development of novel chemical screens and selections for identifying mutants with enhanced function. His work also is providing insights into the mechanisms and evolution of catalytic function in nature. Extending molecular diversity to material science, Schultz recently developed a new technology for the parallel synthesis, processing and screening of large libraries of solid state inorganic and organic materials and devices — electronic, magnetic, optical and catalytic — for new properties. In addition, he has developed a general biosynthetic method to site-specifically incorporate unnatural amino acids into proteins. This new technology not only allows scientists for the first time to carry out detailed physical organic studies on proteins, it also may provide a new generation of proteins with properties not restricted by the naturally occurring twenty amino acids.

Schultz has been recognized for his work with numerous honors and awards, including the Alan T. Waterman Award, National Science Foundation; Arthur C. Cope Award, American Chemical Society; American Chemical Society Award in Pure Chemistry; membership in the American Academy of Arts and Sciences; Eli Lilly Award in Biological Chemistry, American Chemical Society; DuPont Merck Young Investigator Award, The Protein Society; Wolf Prize in Chemistry; and California Scientist of the Year. ■

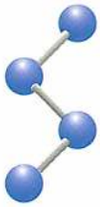
TSRI Researchers Win Prestigious Scientific Awards

A number of TSRI scientists are the recipients of prestigious scientific awards for a variety of significant scientific achievements.

Bernard M. Babior, M.D., Ph.D., *Professor, and Head, Division of Biochemistry, Department of Molecular and Experimental Medicine,* has been elected to membership in the National Academy of Sciences. Members are elected in recognition of their distinguished and continuing achievements in original research; election to the Academy is considered one of the highest honors that can be accorded a scientist. The Academy membership comprises approximately 1,900 members and 300 foreign associates, of whom more than 160 have won Nobel prizes.

Francis V. Chisari, M.D., *Professor, and Head, Division of Experimental Pathology, Department of Molecular and Experimental Medicine,* has received the 1999 Rous-Whipple Award from the American Society of Investigative Pathology. This award, for experimental pathologists over age 50 who have had a distinguished research career and are continuing to contribute to the field, is one of the most prestigious honors in the discipline of experimental pathology. Previous Rous-Whipple awardees from TSRI are Drs. Michael B.A Oldstone and Thomas Edgington.

Richard A. Lerner, M.D., *TSRI President,* has been selected as a recipient of the Cancer Research Institute's 1999 William B. Coley Award for Distinguished Research in Basic and Tumor Immunology. He is being honored for the creation of new forms of antibody molecules for use in the laboratory and clinic. He shares the award with Dr. Greg Winter. In addition, Dr. Lerner has been awarded the 1999 Windaus-Medal for his contributions in natural product chemistry.



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