THE ROCKEFELLER UNIVERSITY scientist

STORIES OF DISCOVERIES THAT TRANSFORM SCIENCE

FRIDAY, JULY 16, 2004

Why chemotherapy fails

A single molecule may explain why our most powerful cancer drugs can't always halt tumors

BY RENEE TWOMBLY

Chemotherapy has a lousy reputation. Even the sickest cancer patients are often wary of its many side effects and failure rates.

But new research from Rockefeller University, published last month in the *Proceedings of the National Academy of Sciences*, may explain why some people don't respond to the powerful drugs.

The study, led by Archontoula Stoffel, research assistant professor in Hermann Steller's Laboratory of Apoptosis and Cancer Biology, examined human cells taken from the tumors of a type of non-Hodgkin's lymphoma. By looking at how specific molecules interact with two different cell growth pathways, Stoffel and her colleagues found a mechanism that may cause chemotherapy drugs to "jam" before they can take effect.

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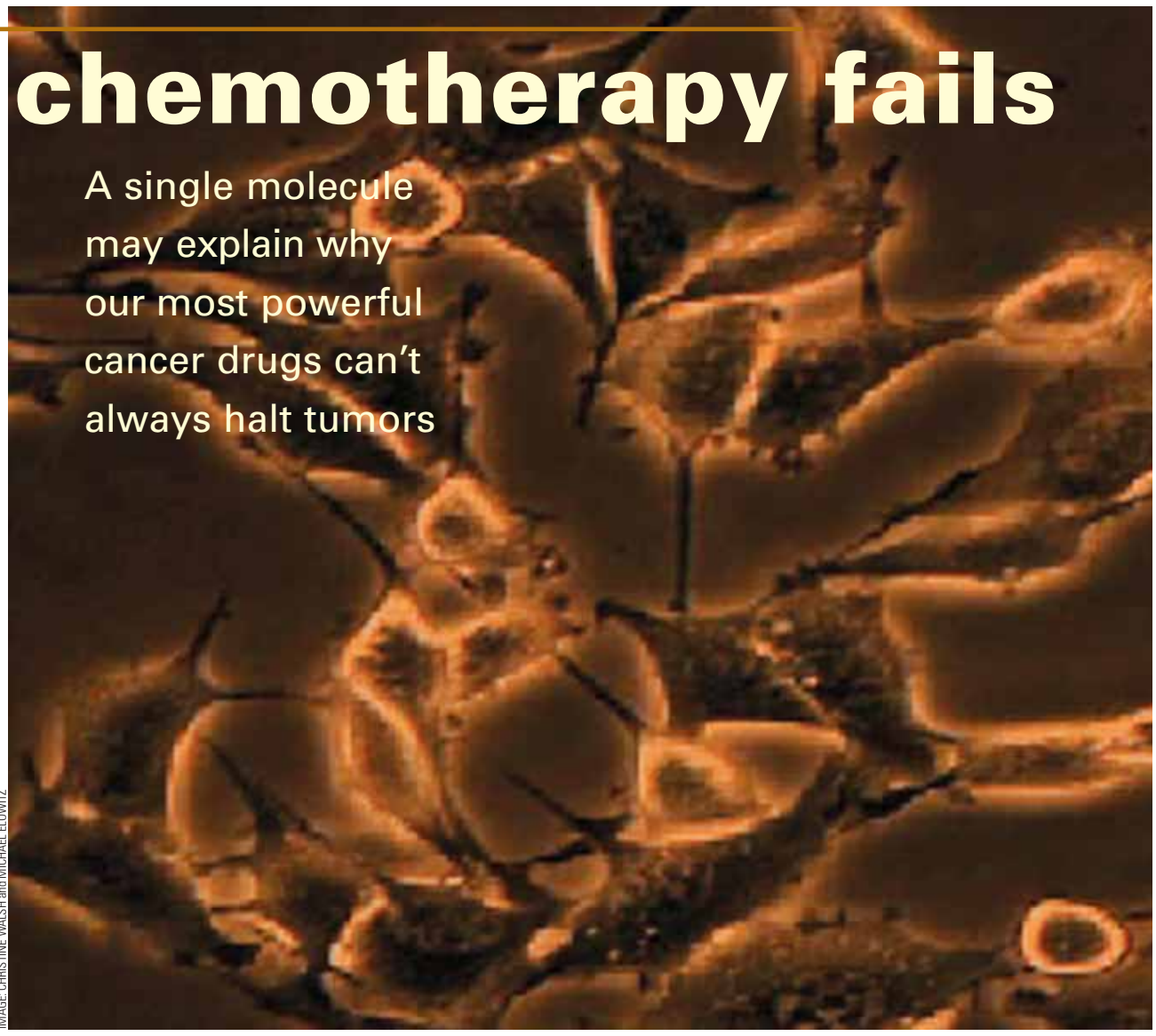

IMAGE: CHRISTINE WALSH and MICHAEL ELOWITZ

A cell before dying. A frame from a movie shows what happens when the p53 tumor suppressor pathway *(highlighted)* functions to kill cells that have been damaged by ultraviolet radiation. Rockefeller scientists led by Archontoula Stoffel have shown that this process is inhibited when a second pathway called NF-κB is activated. To see the entire movie, go to www.rockefeller.edu/scientist.

Carbohydrates on trial

A study just launched at The Rockefeller University Hospital will determine whether high fat diets truly work better than high carb diets

BY JOSEPH BONNER

When the Atkins diet first became a national obsession about two years ago, millions of Americans became carb counters. One American, Rockefeller's **Jan L. Breslow**, had a different reaction.

After reading a July 2002 *New York Times Magazine* cover story on the diet, Breslow began digging through medical literature, looking for evidence that the latest diet craze would work any better than any other low-calorie diet. "I found that there's hardly any good, carefully controlled data out there," says Breslow, Frederick Henry Leonhardt Professor and head of the Laboratory of Biochemical Genetics and Metabolism at Rockefeller.

That won't be the case for long. Breslow will soon enroll his first volunteer in a rigorous study designed to determine which of two low-calorie diets — one high in fat, the other high in carbohydrates — best reduces abdominal fat in overweight and obese people. Subjects who volunteer for the study will lose, on average, 20 pounds, and scientists will gain a better understanding of the effects of these diets on body composition, risk factors for heart disease and stroke, and overall health.

The Atkins craze has led huge numbers of Americans — *Dr. Atkins' New Diet Revolution* has sold over 15 million copies — to abandon low-fat, high-carb diets, which are the basis of the food pyramid recommended by the U.S. Department of Agriculture, the American Heart Association, the American Cancer Society and many other health organizations, in favor of high fat diets. The author of the *New York Times Magazine* article, science writer Gary Taubes, agreed with cardiologist Robert C. Atkins, the late inventor of the diet, that carbohy-

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New treatments for autoimmune disease

The Rockefeller University
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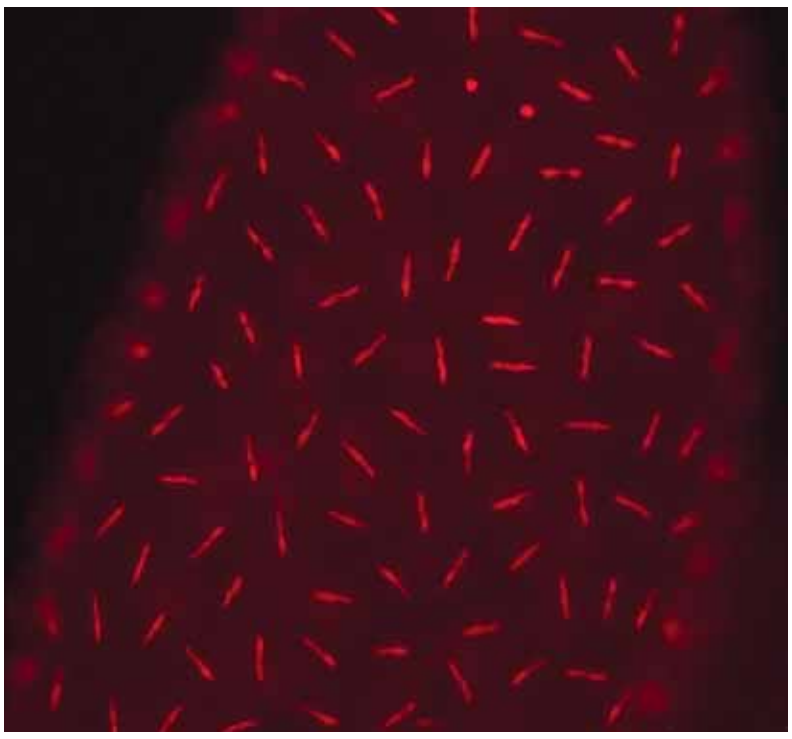

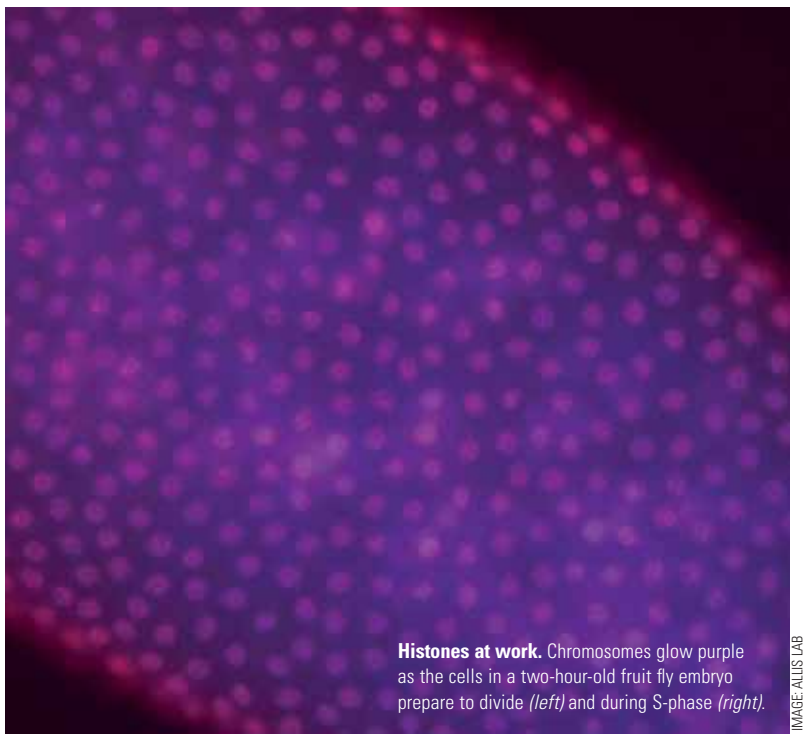

Histones at work. Chromosomes glow purple as the cells in a two-hour-old fruit fly embryo prepare to divide *(left)* and during S-phase *(right).*

IMAGE: ALLIS LAB

Nature's backup plan. Scientists have long observed that certain histone-protein molecules — around which chromosomal DNA is looped — undergo a chemical reaction called phosphorylation when their cells divide. Though the purpose of this process is not fully understood, it occurs most dramatically on just two of a cell's five histones, H1 and H3. Now, using an antibody developed for this purpose, researchers in **David Allis's** Laboratory of Chromatin Biology along with researchers at three other institutions have found that phosphorylation also occurs on histones H4 and H2A. The researchers speculate that the process may serve as a redundancy in the system to help ensure successful cell division. The researchers also found that H4 and H2A are phosphorylated to a lesser extent during the S-phase of the cell cycle, when the cell is synthesizing its DNA. This finding would suggest an additional — as yet unknown — role for those histones. Allis is Rockefeller University's Joy and Jack Fishman Professor.

Chromosoma, May 2004

Enzyme guidance system. An enzyme called protein phosphatase 1 (PP1), which regulates the flow of signals between nerve cells and helps determine the shapes of their receiving branches, depends on a host of other proteins to do its work. Scientists in **Paul Greengard's** Laboratory of Molecular and Cellular Neuroscience, using a technique called interaction cloning, have now identified four members of a new class of proteins that bind to PP1 and maneuver it to specific locations within the cell where it is needed. Because the newly discovered proteins work by also binding to structural filaments inside the cell called actin, the researchers named them phosphatase and actin regulators (phactrs) 1 through 4. In studies on rats, Greengard's team detected high levels of phactr-1 in specific sections of the brain: the cortex, hippocampus and striatum — and there was an especially high concentration of the protein at the synapses of nerve cells, which send and receive messages. The study was carried out with colleagues at Yale University School of Medicine. Greengard is the university's Vincent Astor Professor.

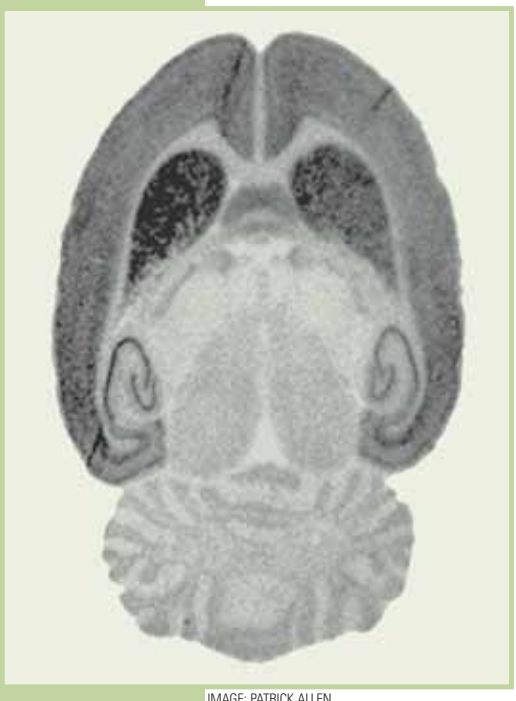

IMAGE: PATRICK ALLEN

Synapse support. Dark areas show where phactr-1 appears in specific sections of a rat brain.

Proceedings of the National Academy of Sciences, May 2004

Syndrome X scan. **Jeffrey Friedman**, **Jan Breslow** and **Markus Stoffel** have been studying obesity related diseases on the Micronesian island of Kosrae for the past decade. Now they're using the island population to launch one of the first large scale genome-wide association studies ever undertaken. Using newly developed "gene chip" technology, the scientists will scan the genomes of more than 3,200 individuals — nearly the entire adult population of the island — in hopes of discovering genetic variations associated with obesity, high blood pressure and diabetes. "We've been wanting to do this experiment for a long time, but simply didn't have tools with the needed power and resolution to get detailed genetic answers to define the associations between specific genes and obesity," says Friedman, the university's Marilyn M. Simpson Professor and head of the Laboratory of Molecular Genetics. Kosrae is an ideal setting for genetic studies because it has a unique mix of Caucasian and Polynesian ancestry and a clear distribution of obesity.

Scent of a gene. The family of genes responsible for producing odorant receptors in mice is among the largest of any mammal — about 1,000 of an estimated 30,000 genes are devoted to smell. These 1,000 genes encode receptors that are expressed in neurons spread throughout the olfactory epithelium, the lining of the nose that detects odors. Junji Hirota and **Peter Mombaerts** have now identified a transcription factor — the third to be discovered in mice — called Lhx2, which has a positive regulatory role in olfactory sensory neuron development. Hirota and Mombaerts speculate that Lhx2 may control both odorant receptor gene choice and olfactory sensory neuron development through distinct mechanisms. Mombaerts is head of the Laboratory of Developmental Biology and Neurogenetics.

Proceedings of the National Academy of Sciences, June 2004

Sex and schizophrenia. A team of researchers led by **Maria Karayiorgou** at Rockefeller and Joseph A. Gogos at Columbia University College of Physicians and Surgeons report on a new schizophrenia susceptibility gene on human chromosome 22. The gene, called ZDHHC8, encodes an enzyme that modifies proteins important for cell-to-cell communication in the brain. Some people with schizophrenia inherit a version of the gene that encodes a defective enzyme. Oddly, female patients with schizophrenia were more likely to inherit the defective gene than males, possibly explaining some of the sex differences observed in the disease. In experiments with mice lacking ZDHHC8, the scientists found that females with one or zero copies of the gene were abnormal with respect to indices of fearfulness and their ability to process sensory stimuli. The researchers conclude that even modest decreases in the levels of proteins expressed by ZDHHC8 may have substantial effects on behavior and the likelihood of schizophrenia. Karayiorgou is head of the Laboratory of Human Neurogenetics.

Nature Genetics, June 2004

Mutation suppression. In the process of translating genetic instructions into proteins, DNA's code is fed through two molecular machines — the spliceosome, which edits pre-mRNA to create mRNA, and the ribosome, which assembles proteins from mRNA's instructions. Research from **Magda Konarska's** Laboratory of Molecular Biology and Biochemistry now suggests that evolution has provided these two machines with similar strategies for coping with imperfect instructions, or mutations in the genetic code. The scientists identified two new forms of a gene, prp8, that suppress intron mutations during the two-step process by which the spliceosome deletes specific segments of pre-mRNA. Following several lines of biochemical and genetic analysis, they propose that intron mutations are suppressed by altering the equilibrium between the first and second steps of the process, when the spliceosome changes shape.

Molecular Cell, May 7, 2004

Senseless failure. In the 1960s, David Hubel and Torsten Wiesel showed that if one eye is deprived of visual stimulation, competition arises between the neurons processing signals from the eyes, and the brain's wiring develops abnormally. Now, Rockefeller's **Peter Mombaerts**, working with colleagues at Columbia University, shows that a similar phenomenon occurs in the olfactory system. The scientists used gene-targeted mice to study the maturation of glomeruli — structures in the brain's olfactory bulb where olfactory nerve cells terminate. The researchers found that when the animals are deprived of scents, the glomeruli do not fully mature. Furthermore, there is a sensitive period during the mice's development during which sensory activity influences the organization of the glomeruli. The specific timing of this sensitive period varies from one odorant receptor to the next.

Science, June 2004

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Carbohydrates on trial *continued*

drates — rice, grains, pasta and sugars — and not fat are responsible for obesity.

To find out if that's true, Breslow and Research Nurse Practitioner Jill Culiner of The Rockefeller University Hospital will enroll 50 obese or overweight subjects over the next five years, with the expectation that 40 of them will complete the protocol. Subjects will live overnight at the hospital for 16 weeks and will eat precise meals prepared by the hospital's bionutritionists Janet Maturi and Diane Meehan.

For the first three weeks of the study, they will eat an average American diet — containing approximately 34 percent fat — to determine the average number of calories they need to maintain their then-current weight. They will spend the next 10 weeks on either a high fat or a high carbohydrate diet. Both diets will represent a 40 percent calorie reduction in order to induce weight loss. In the final three weeks, the subjects will continue on the diets, but calories will be added as needed to maintain their new weights.

The study will be the first to comprehensively record a person's changes in body composition and cardiovascular risk factors over the entire course of a diet, from weight stabilization through loss and maintenance of new weight. Each subject's weight and blood pressure will be checked daily. Periodic measurements will be made of several blood components, including insulin, glucose, lipoproteins, which transport cholesterol in the blood, and leptin, the "obesity hormone" discovered 10 years ago by Rockefeller's Jeffrey Friedman. Body composition before, during and after weight loss will be measured by magnetic resonance imaging at the Obesity Center at St. Luke's Hospital by Steve Heymsfield and Stanley Heschka.

"Most of the other inpatient diet studies in the literature are shorter, and only a few measurements are taken, usually when people are starving," Breslow says.

Recently, two outpatient studies published in the journal *Annals of Internal Medicine* provided evidence that the low carbohydrate Atkins diet is more effective than a low fat diet during a six-month period, although the weight-loss results even out for both diets over the course of a year.

"The focus of the two new studies and many others in the literature is outpatient adherence to the two diets," says Breslow. "This involves many complex behavioral issues and is epitomized by the question 'What do you do at 11 p.m. when standing in front of the refrigerator?'"

Although behavioral aspects of dieting are important, Breslow explains, behavioral studies depend on many factors, such as education, physician follow up, availability of certain foods, family situation and economic status, among others. "To adequately address these issues would require a totally different study design than we have pursued and is really not our expertise," says Breslow. "The Rockefeller Hospital excels in carefully monitored metabolic studies." (See "Precision nutrition," *right*.)

Breslow is primarily interested in evaluating how much visceral fat each patient loses during the course of the study. Visceral fat, which accumulates around the organs in the abdomen, is much more strongly associated with insulin resistance, diabetes, heart disease and stroke, hypertension and gout than total body fat or subcutaneous fat.

The study also should help answer some myths regarding high fat diets. For example, it's commonly accepted, without any scientific evidence, that the low blood levels of insulin that accompany a high fat, low carbohydrate diet have special metabolic effects that promote weight loss. "But the truth is, we really don't know," says Breslow. "It's never been studied."

Because each study participant can expect to lose about two pounds a week on either diet, for a total loss of 20 pounds by the end of the inpatient period, Breslow calls it a therapeutic study. "The subjects can view their participation as a good start toward maintaining a healthy weight," says Culiner. "They will learn what good portion sizes are and how to eat healthfully. And because the meals are based on natural foods, they will learn the best kinds of food to eat."

Precision nutrition

Why The Rockefeller University Hospital is uniquely qualified to conduct dietary studies

BY BETSY HANSON

Cereal science. Trays of carefully portioned meals await delivery in the hospital's bionutrition department.

Jan L. Breslow isn't the first Rockefeller scientist to take on obesity. The Rockefeller University Hospital has been famous since the 1950s for research that requires special diets to study metabolism or nutrients.

They've gotten quite good at it.

"This type of protocol — studying a small number of people with tightly controlled variables — is the hospital's forte," says Breslow.

While other research hospitals are weighed down with the beaurocracies associated with routine patient care, Rockefeller is free to focus on research studies initiated by scientists.

The bionutrition department, for example, will be critical to Breslow's research. Instead of preparing thousands of daily meals for patients with diverse needs, as might be the case elsewhere, Rockefeller's bionutrition team will devote its resources to making precise, personalized meals for just a few patients at a time. A staff of 11 will produce 24 different meals for three diets. Each portion of every food — bacon for a high fat, high protein diet, spaghetti for a high-carb diet, etc. — will be weighed to one-tenth of a gram before it is packaged. The patients will receive customized, color-coded servings based on their specific calorie requirements.

Though this is work the hospital staff have been doing for half a century, this study's five-year timeframe presented a particular challenge. Because the nutrient content of fruits and vegetables can vary widely depending on where they were grown and when they were harvested, the staff will purchase large quantities of food, then measure and freeze individual portions. In this way, the scientists can be sure that the first volunteers to enroll in the study eat exactly the same foods as the last ones.

The country's only free-standing research hospital outside of the National Institutes of Health, The Rockefeller University Hospital this year completed its first major modernization in decades. Upgraded laboratory and patient care facilities, strengthened support services and new initiatives to ensure high quality care and high quality science have made the hospital one of the nation's premier facilities for conducting clinical research.

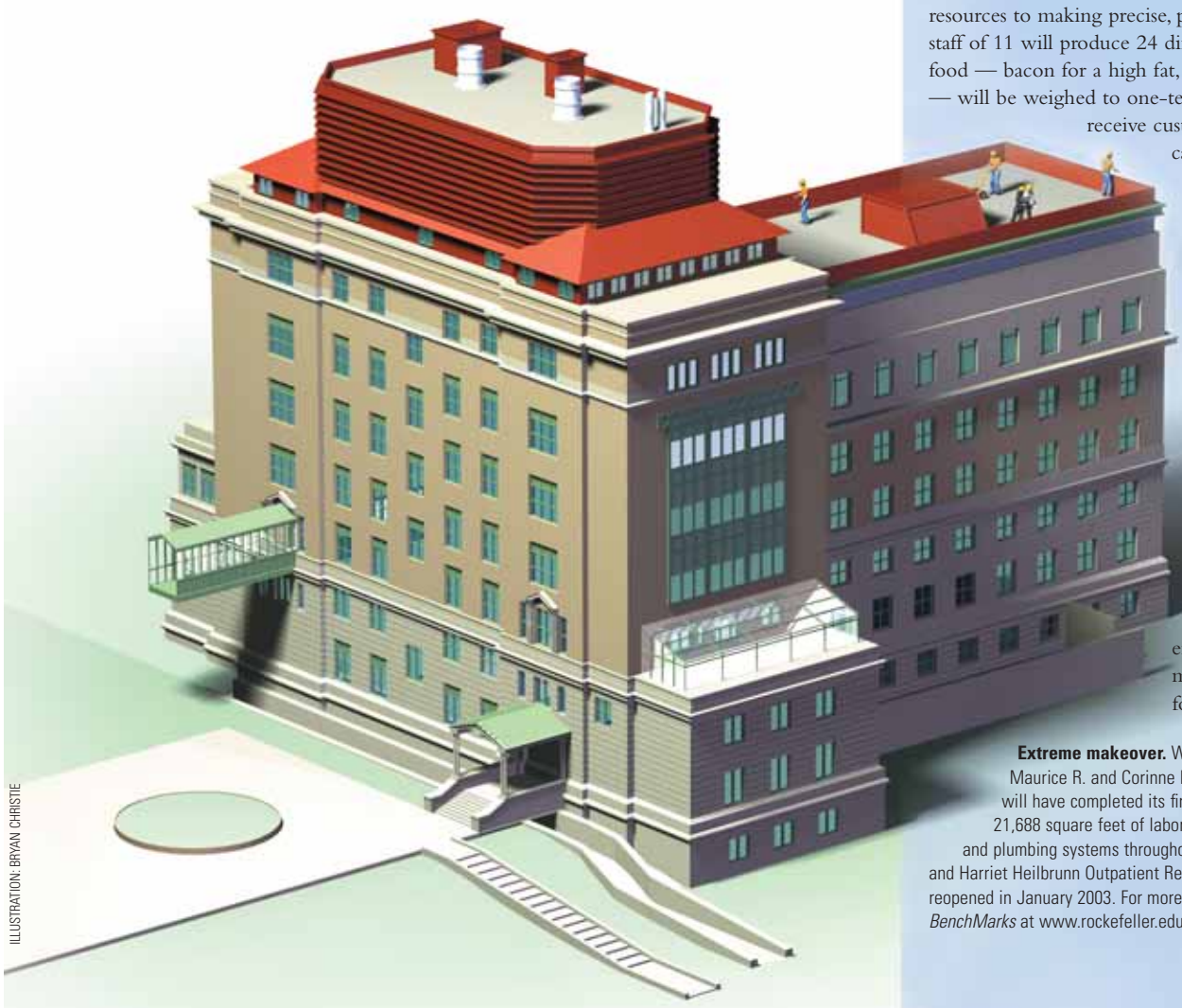

ILLUSTRATION: BRYAN CHRISTIE

Extreme makeover. When the scaffolding comes down in August 2005, Rockefeller's Maurice R. and Corinne P. Greenberg Hospital, where Breslow's study is being conducted, will have completed its first major modernization in decades. The work has renovated 21,688 square feet of laboratory space and overhauled the heating, ventilation, electrical and plumbing systems throughout the building. Patients have visited the newly renovated Robert and Harriet Heilbrunn Outpatient Research Center on the A level nearly 10,000 times since it reopened in January 2003. For more on the hospital's revitalization, see the June 7 issue of *BenchMarks* at www.rockefeller.edu/benchmarks.

Working the (immune) system

How four Rockefeller labs are joining forces to cultivate treatments for autoimmune disease

BY LYNN LOVE

As far as **Ralph Steinman** is concerned, we are alive right now because — among other reasons — our immune systems are allowing it.

As you read this newsletter, your T cells, B cells, natural killer cells — each of the immune system's warriors that circulate in your bloodstream — are allowing your body's heart, brain, pancreas, joints and other tissues to exist and function without harassment or interference. This remarkable process is called tolerance.

But in autoimmune disease, that's not the case. The legendary German scientist Paul Ehrlich described the body's capacity to turn on itself as "horror autotoxicus." Ever since Ehrlich's observations at the turn of the 20th century, scientists have tried to figure out what causes the biological system designed to protect your body from disease to revolt against it or break immune tolerance.

Now, for the first time, several Rockefeller University scientists are devising novel and specific ways of treating lupus, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, psoriasis and other autoimmune diseases by unraveling and exploiting the biological mechanisms of tolerance and autoimmunity.

The progress is not only an example of how science, creatively practiced, can shed light on the body's most complex and mysterious workings — it's also a model of how collaboration among Rockefeller labs can solve problems that might be too big for a single lab to tackle.

Four of the university's seven immunology labs, headed by Steinman, **Jeffrey Ravetch**, **Michel Nussenzweig** and **Alexander Tarakhovsky**, all part of the university's Christopher H. Browne Center for Immunology and Immune Diseases, are tackling different but related pieces of the autoimmunity puzzle. The interactions are a testament to how Rockefeller's small size and flat administrative structure can make the best use of limited resources to answer big questions. The labs have formed a de facto working group: what's revealed in one lab is often incubated in the next.

A key element of the understanding they are developing is one of active maintenance of tolerance on a daily basis, in the "steady state." As long as the immune system's killer cells tolerate the body's tissues, health is preserved.

"This tolerance is literally what keeps us alive," says Steinman, head of the Laboratory of Cellular Physiology and Immunology. "Studies in immunology have most often emphasized the immune system's role in defense against infections and tumors, but tolerance is just as critical, even in the steady state. If one could learn how to induce authentic immune silencing, or tolerance, to the causes of autoimmune disease and allergy, it would constitute a revolution."

The spiny dendritic cell, discovered by Steinman and

Eluding lupus

In the case of lupus, the immune system attacks not a single organ but the skin, joints, blood and kidneys. As many as 1.5 million Americans have been diagnosed with lupus.

The problem is antibodies. Antibodies, Y-shaped proteins that lead the immune system to specific invaders, start forming in humans from infancy and hang out inside the body. When appropriate, they bind to microbes and other foreign material, called antigens. This connection between an existing antibody and an antigen starts a process called clonal expansion, in which the body's B cells produce great numbers of antibodies. Some of these new antibodies will have a tighter fit with the existing antigens than the original antibody did.

With bacteria or other invaders, a tight fit with an antibody makes the pathogen easier for other immune cells to identify and destroy. But during clonal expansion, antibodies are also created that bind to the body's own cells (see illustration, *right*). These antibodies are known as autoreactive antibodies. Ideally, the body's immune system tolerates these autoreactive antibodies. But when it doesn't, the results are disastrous.

"Autoreactivity is not the exception, it's the rule," Ravetch says. Yet the body maintains tolerance and autoimmunity is held off. Why?

One of the immune system's antibodies, known as immunoglobulin G or IgG, actually controls the activities of other antibodies that are prone to autoreactivity. In most cases, IgG inhibits the potential onset of the autoimmune cascade we know as lupus and it performs this role via the Fc receptor that forms its, and every other antibody's, Y shape.

But not always: In 2000, Ravetch, who is an expert on the Fc receptor, and postdoc Silvia Bolland discovered that a defect in the IgG Fc receptor will cause spontaneous autoimmunity in mice that are genetically predisposed to lupus.

"Many people who could develop lupus never do," says Ravetch. "But the animals we studied broke tolerance and developed full-blown lupus. So we immediately wanted to know how this inhibitory receptor contributes to the maintenance of tolerance."

Once Ravetch and his colleagues further characterize IgG's inhibiting role in lupus, they may be able to develop a means of repairing faulty Fc receptors that lead to the onset of the disease.

Tolerance in the face of d

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know that the checkpoint system has faile
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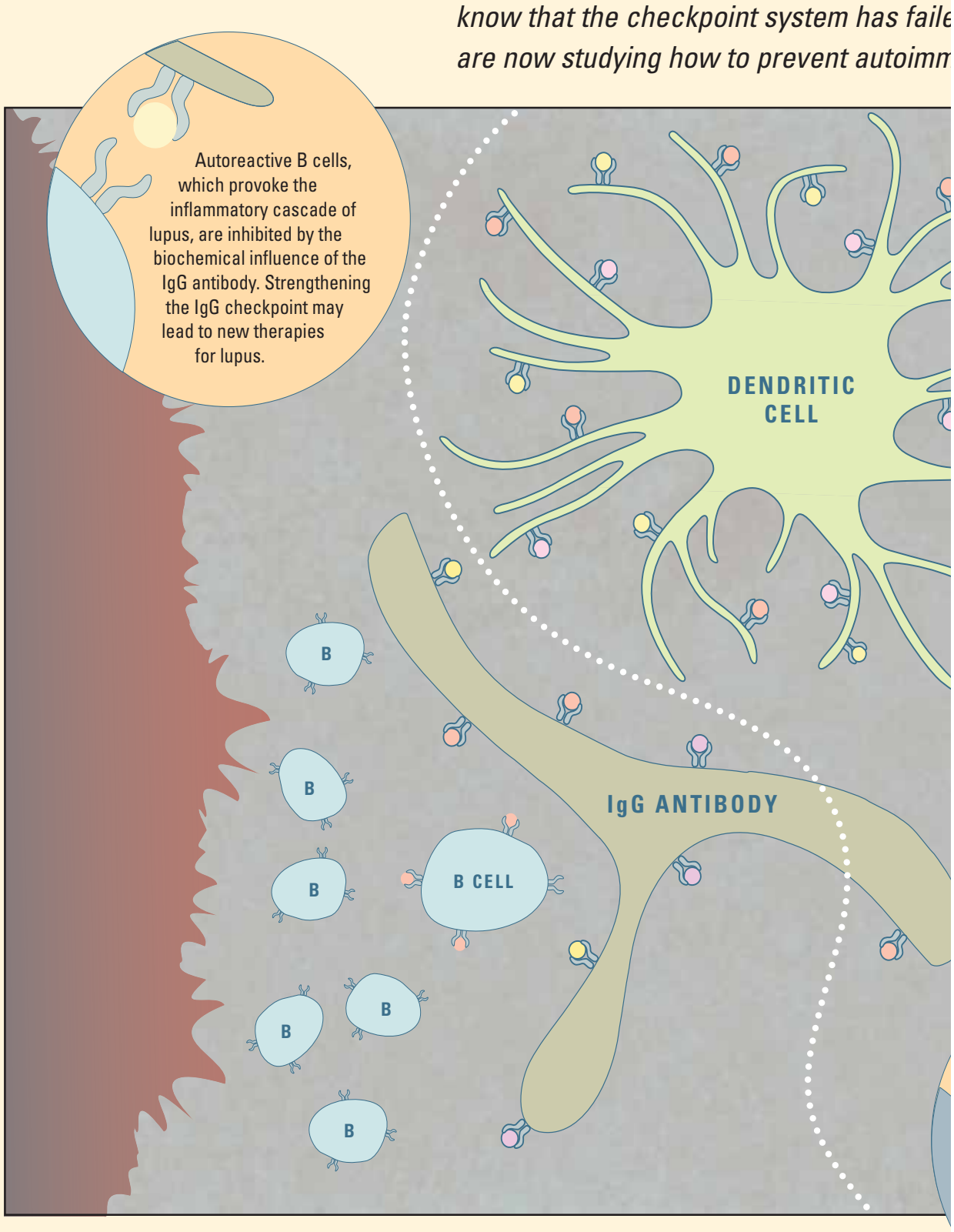

colleagues in 1973, is a nexus for clues about how the immune system functions: the specialized cell carries out both the protective work of the immune system and its preventative work of maintaining tolerance. This is because the dendritic cell is what's known as an antigen-presenting cell. It picks up foreign molecules — bits of the invading bacteria, for example — then evaluates them and relays messages about how best to respond to other immune system cells such as T and B cells that then carry out the dirty work. Through this biologically didactic process, dendritic cells can recognize an enormous variety of antigens and either encourage or subdue an immune system attack.

The scientific community was not prepared to consider a new cell type in the immune system when Steinman made his famous discovery 30 years ago. "We discovered the dendritic cell because we had new microscopes and other cell biological methods to pursue things we saw that did not fit into accepted categories," says Steinman, who is the university's Henry G. Kunkel Professor. "When we discovered it, we could not envisage how important the dendritic cell would be in understanding both the immunity and tolerance aspects of the immune system." It wasn't until the 1990s that the dual role of the dendritic cell was fully appreciated.

Soon after the dendritic cell discovery, Michel Nussenzweig, now Rockefeller's Sherman Fairchild Professor and head of the Laboratory of Molecular Immunology, came as a graduate student to the laboratory then headed by Steinman and the late Zanvil Cohn. "It was a perfect time for me, a curious young medical student, to enter the field. The dendritic cell seemed like the most exciting and natural project to devote myself to," says Nussenzweig.

Nussenzweig has since emerged as a leader in his own right in the field of autoimmunity. In 2001, for example, he and graduate student Daniel Hawiger proved that the dendritic cell is as responsible for establishing tolerance to the body as it is to getting the body to eradicate pathogens — a finding that cleared the way for dendritic cells to be exploited in the treatment of autoimmune disease.

However, the dendritic cell alone does not tell the whole story. In fact, it's just one of several checkpoints the immune system has developed to ensure that its force isn't unleashed on the wrong target.

Think of it as an electric circuit: In order for a circuit to be completed, a series of switches need to be closed. "At a basic level, the immune system is a checkpoint system," says Jeffrey Ravetch, Theresa and Eugene Lang Professor and head of Rockefeller's Laboratory of Molecular Genetics and Immunology. "From the earliest days of B cells to the way that T cells are informed by dendritic cells in the periphery, health is maintained in a series of tolerance checkpoints along the way."

An additional checkpoint was discovered by Nussenzweig and postdoc Hedda Wardeman in 2003. Their research found that a majority of early immune system B cells — which are important to antibody production during infection — are self-reactive. If allowed to mature, these misguided B cells would predispose the body to severe autoimmune disease, most likely, lupus. Yet because of an immune system checkpoint, these self-reactive B cells are killed off before they fully mature in the bone marrow.

Another series of checkpoints, identified by Rockefeller's Alexander Tarakhovsky, head of the Laboratory of Lymphocyte Signaling, are signaling proteins, such as one discovered by Tarakhovsky and postdoc Ingrid Mecklenbrauker called protein kinase C delta, which can establish or foil the steady state of tolerance.

"We can almost think of the immune system as a symphony, or a cascade, of signaling, which brings about certain changes to individual cells and their ability to recognize antigens," says Tarakhovsky. "When that signaling fails to maintain tolerance, the symphony takes on a dissonance that it normally avoids."

Understanding the checkpoints — and being able to hear the symphony — will eventually lead to new ways of corralling the immune system when it misfires and may eventually help combat some of our most perplexing diseases.

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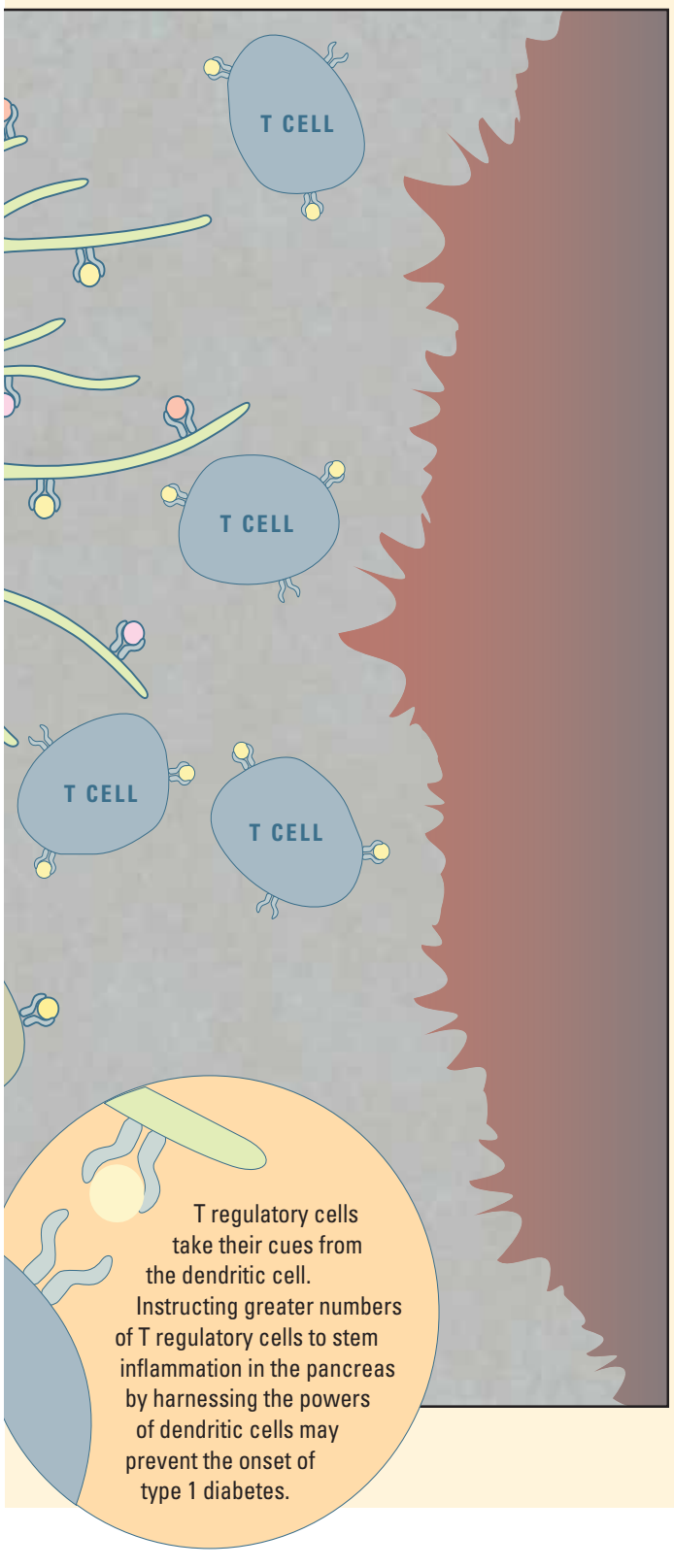
ype 1 diabetes and lupus, scientists ed. Rockefeller University researchers unity from occurring in the first place.



Halting diabetes

When an out-of-balance immune system attacks the pancreas, the result is type 1 diabetes. Over 5 million people worldwide rely on insulin injections to metabolize the sugars that their own bodies can no longer process.

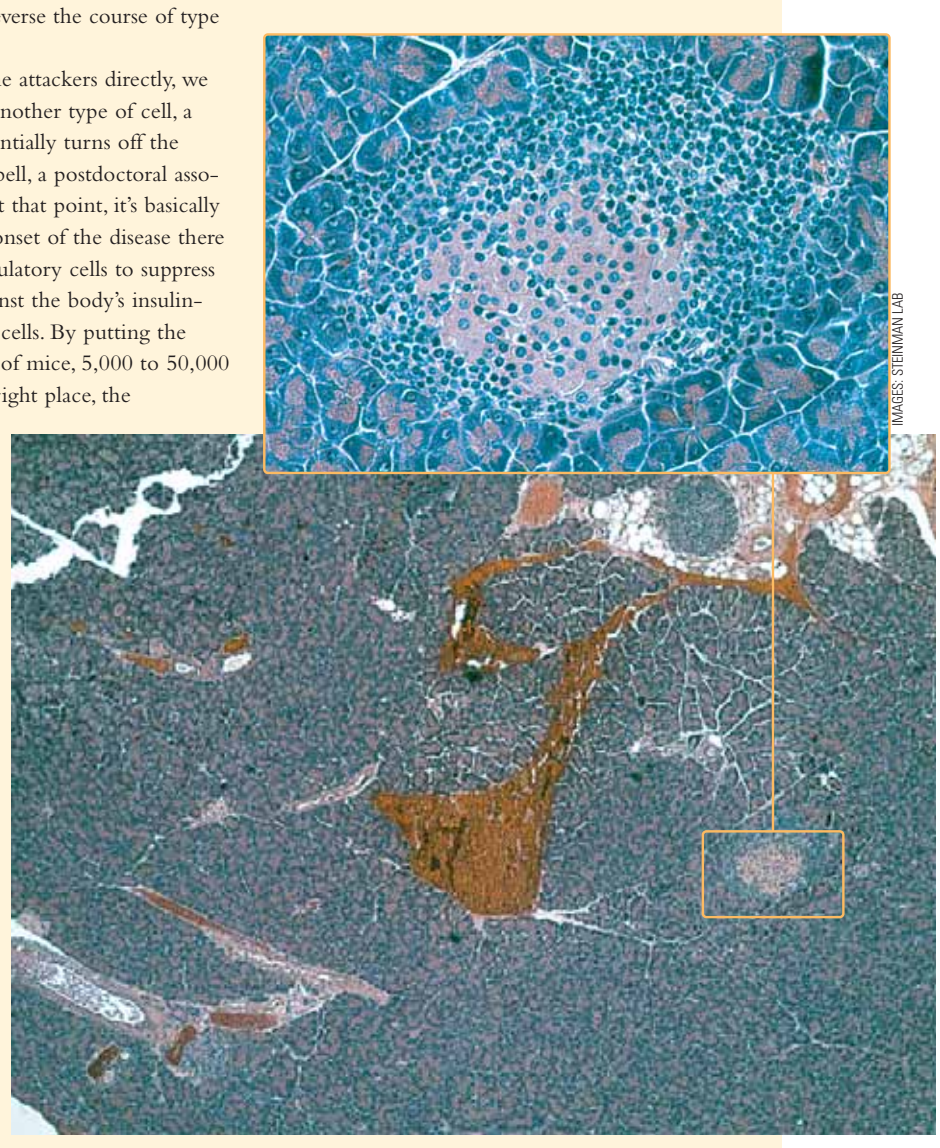
But remarkable new research from Steinman's lab now shows that it may be an imbalance of immune system T regulatory cells that triggers the onslaught. These cells work as suppressors: they turn off the body's immune response. Steinman's study in mice, reported in the June 7 issue of the *Journal of Experimental Medicine*, shows that dendritic cells can be used to expand functional T regulatory cells, which can actually reverse the course of type 1 diabetes in mice.

"Instead of silencing the attackers directly, we learned how to generate another type of cell, a suppressor cell, which essentially turns off the attackers," says Kristin Tarbell, a postdoctoral associate in Steinman's lab. "At that point, it's basically a numbers game." At the onset of the disease there are not enough of the regulatory cells to suppress the immune response against the body's insulin-producing pancreatic islet cells. By putting the right number (in the case of mice, 5,000 to 50,000 regulatory T cells) in the right place, the researchers arrested the process (see illustration, *right*).

Steinman and Tarbell's study used mice that were genetically predisposed so that their suppressor T cells, once activated, would home in directly on the pancreatic islet cells. Now, the scientists need to run the same experiment in mice with normal T cells. This critical next step will determine whether the research can be moved to clinical studies in humans.

Even now, the findings prove an important biological principle that could lead to prevention of type 1 diabetes in humans: autoimmunity can be reversed if the immune system's mechanisms for tolerance — recognition and acceptance of the body's own cells — can be repaired, and dendritic cells mediate this repair.

Take it a step further, and the research could have implications beyond prevention of the disease. Islet cell transplantation, for example, a still experimental technology, is far from foolproof. "At the moment, the problem with islet cell transplants is that the same process that destroyed the first set will destroy the second," Tarbell explains. But with the ability to restore balance in the immune system, islet cell transplant could succeed.



IMAGES: STEINMAN LAB

Restoring the pancreas. An infusion of T suppressor immune system cells mixes with existing, faulty T cells *(blue)* and halts the inflammation in a mouse pancreatic islet *(pink)* that otherwise leads to type 1 diabetes in the animal.

Deaf fish yields gene linked to blindness

Zebrafish that can't hear provide clues to deafness and a model for studying a human eye disorder

BY TIEN-SHUN LEE

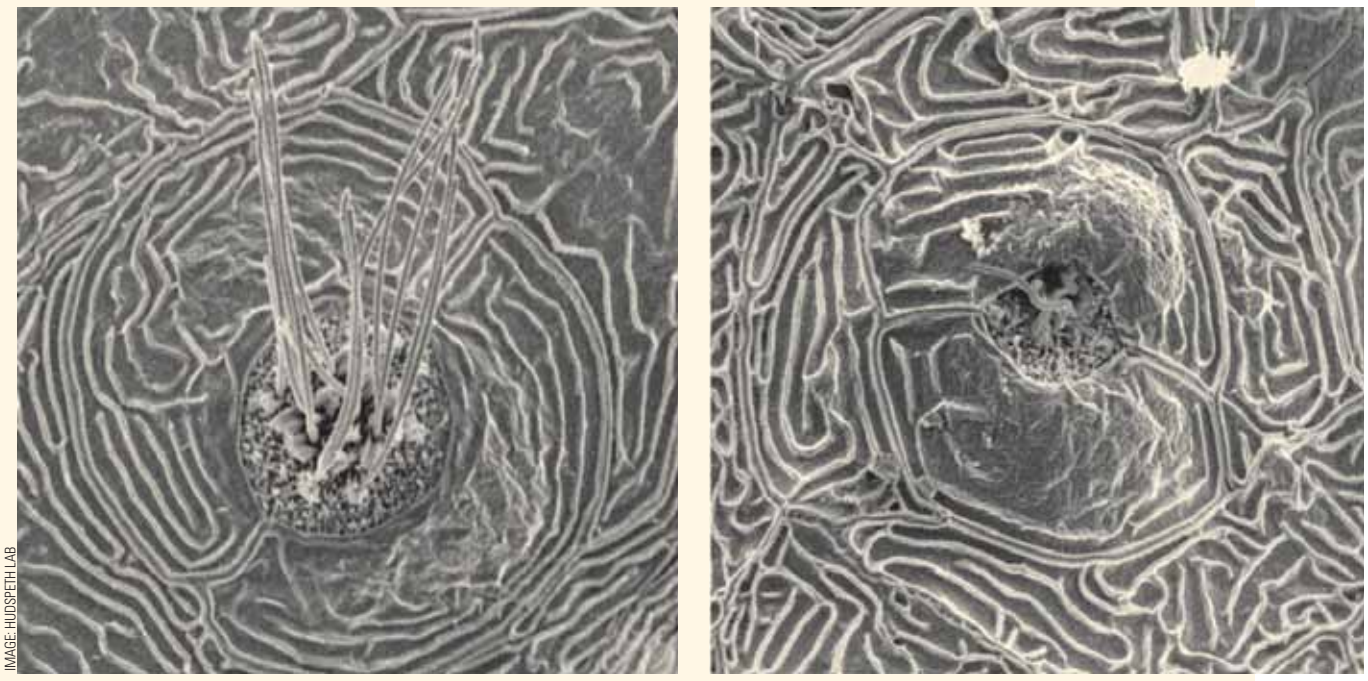


IMAGE: HUDSPETH LAB

Shedding hair. Images from an electron microscope show the center of a normal zebrafish neuromast with healthy hair cells *(left),* and the center of a neuromast from a zebrafish lacking the chm gene *(right).* Though the surrounding cells are normal, the mutant has only two tiny hairs in its hearing organ.

For most men, hair is something you want more of on your head and less of in your ears. Not necessarily so for Rockefeller's **Jim Hudspeth**.

Hudspeth studies the tiny bundles of hair, buried deep inside our ears, that translate sound waves generated by mechanical forces — the drawing of a bow across strings, the crashing of a car through a window — into electrical signals that can be processed by the brain. New research in his lab has now identified a gene critical to the process by which these hairs develop.

To better understand hearing, Hudspeth's Laboratory of Sensory Neuroscience has long studied bullfrogs, whose extra-large ear hairs are well suited for analysis. In recent years, however, Hudspeth's team has also been focusing on zebrafish, which are excellent for genetic studies. The scientists wanted to find a deaf fish that would lead them to key genes and molecules involved in hearing.

Six years ago, Hudspeth, who is the F. M. Kirby Professor and director of the F. M. Kirby Center for Sensory Neuronscience, and Catherine Starr, then a graduate student in Hudspeth's lab, began the painstaking process of screening thousands of genetically mutated zebrafish. To find out whether or not the fish could hear, Starr repeatedly tapped on the sides of their tanks. Normal zebrafish will swim away from the sound. Starr was looking for a fish that did not respond.

When she eventually found one, Starr paired the deaf fish with a normal fish, and then used genetic mapping techniques to identify a mutant gene in deaf offspring. Because the mutant gene is similar to a human gene called *Choroideremia*, Starr named her fish gene choroideremia, or chm.

To confirm she'd found the right gene, Starr injected the RNA product of the chm gene into fertilized fish eggs. She found that 50 percent of genetically mutant fish, which would normally have ended up deaf, were "rescued" by the chm RNA. Like normal fish, they quickly swam away when their container was tapped.

Starr also created chm knockout zebrafish by injecting modified anti-chm RNA snippets called morpholinos into eggs of normal zebrafish. The offspring responded poorly to sound and had fewer hair cells in their hearing organs.

Starr's zebrafish are the first animal models for studying a human disorder called choroideremia, but the immediate implications are less for hearing, and more for sight. "In humans, none of the patients with mutations in this gene had hearing problems," Starr says.

Mutations to the human *Choroideremia* gene lead instead to a disorder characterized by degeneration of the choroid and retina, portions of the eye that provide nourishment and light sensitivity. People with the genetically inherited disorder progressively lose their vision, beginning with a ring of irregular sight that gradually expands both toward the center and toward the periphery of their fields of view.

The human *Choroideremia* gene codes for Rab escort protein 1 (REP1), a well-studied molecule that plays a key role in the transport of other proteins to their intended destinations within cells. "The next step is to find out how REP1 affects hearing and vision," says Starr.

Others in Hudspeth's lab are investigating that, as well as related questions. Former graduate student James Kappler, for instance, has examined the physical differences between Starr's deaf fish and fish with normal hearing. He immersed live zebrafish larvae in a fluorescent dye that is absorbed by hair cells in their hearing organs, called neuromasts. His findings: normal zebrafish larvae show 30 spots representing fluorescent neuromasts on one side of the body, while mutant larvae showed only two.

Meanwhile, Avani Sinha, a high school student, looked at the hearing organs of larvae under a scanning electron microscope and saw that normal larvae had about 15 hair cells in each neuromast while mutant neuromasts had only one or two, if any.

"Some people who are deaf have no hair cells in their ears. Some develop hair cells fine, but the ionic balance in their ear is off, so the cells can't signal," says Starr. "The same is true of fish."

In fact, there is another deaf fish in Hudspeth's lab that can't hear even though it has hairs in its hearing organs. "That mutant is in the works," Starr says.

Why chemotherapy fails *continued*

Most chemotherapy drugs target what was thought to be a discrete pathway responsible for cell destruction, such as the well-known p53 tumor suppressor protein. But Stoffel and her colleagues showed that proteins that control the function of p53 are also involved in the NF-kappa B (NF-κB) signaling pathway that is responsible for pushing cells to grow uncontrollably. If p53 is managed or inhibited by proteins that also promote tumor development, chemotherapy will not be effective. (The same interactions could characterize other cancer types beyond lymphoma, the researchers say.)

But it might be possible to target one or both of the genes that link the two pathways.

"Now that we know the proliferation pathway can jam the p53 suicide pathway, we might be able to block specific sections of those pathways," says Stoffel, whose coauthors include former Rockefeller president and professor Arnold J. Levine, now at the Institute for Advanced Study and the University of Medicine and Dentistry, both in New Jersey.

The trick will be to block only selected portions of the pathways, Stoffel says. Though the NF-κB pathway is linked to a growing list of cancers, it also plays a vital role in the body's normal immune and inflammatory responses. "It is not possible to shut down NF-κB without causing systemic problems, so we need to find out how to disarm its carcinogenic properties down the signaling pathway, while maintaining its useful functions," she says.

The Rockefeller University study also presents the first molecular description of a cancer caused by bacteria, and thus represents a model system of how the environment and genetics can lead to a cancer, Stoffel says.

The cancer the researchers used as their model is known as mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), which consists of tumors that originate from cancerous growth of immune cells. MALT lymphoma most often occurs in the stomach and usually arises when immune system B cells respond to inflammation provoked by the bacterium *Helicobacter pylori*. Infection by this bacterium is one of the main risk factors for developing gastric cancer, the world's second most common cancer.

In some people with chronic *H. pylori* infection, the immune cells that respond to the infection acquire genetic changes, called chromosomal translocations, which produce MALT lymphoma. The most common translocation occurs when a gene known as the apoptosis inhibitor 2 gene, API2, on chromosome 11 breaks in half and moves over to a similarly broken gene, MALT1, on chromosome 18. The MALT1 protein helps activate NF-κB in immune cells.

The so-called fusion protein created by this translocated gene was key to understanding the two different pathways. The researchers discovered that the fusion protein acts like a cancer-causing gene, capable of promoting unrestricted cell growth. When they studied the proliferating cells by microchip gene expression analysis, they found more than 80 percent of the genes were involved in regulating proliferation and were known to be involved in NF-κB immune responses. "That makes sense because lymphoma is a tumor of the immune system and abnormal activation of the NF-κB pathway, which is normally involved in setting up an immune response, leads to abnormal proliferation," Stoffel says.

More startling was the scientists' finding that five genes were known to be involved in a cell suicide process called apoptosis. When they checked with databases of gene products, the researchers found that three of these five have been shown to block the function of the p53 protein.

"That surprised us," says Stoffel. "We began to think that maybe the fusion proteins work in a bilateral sense, by turning on NF-κB and inhibiting p53. There have been suggestions in previous research that these pathways might be interrelated, but no one has seen that in a model of cancer."

To check whether fusion proteins inhibited p53, Stoffel exposed cells to ultraviolet radiation, which is known to stimulate the activity of p53 and kill cells. "But our cells did not die. That meant something was controlling the action of p53," Stoffel says.

Next, the researchers treated the cells with molecules they knew would block NF-κB and again exposed them to ultraviolet radiation. This time, the cells died.

"The NF-κB pathway, which is the hallmark of all immune responses, tells cells to grow, and abnormal activation of this pathway leads to abnormal proliferation and cancer," Stoffel says. "If this pathway also inhibits p53, which normally helps clear cells that are damaged, then you can't eliminate these cells."

The team concluded that p53 was not inhibited by the NF-κB pathway. The fusion proteins were promoting cancer development by encouraging cell growth through the NF-κB pathway, which, in turn, repressed p53 and inhibited cell death.

The results suggest different ways to inhibit the NF-κB pathway for cancer control. "Many people are now trying to knock out NF-κB, but that produces systemic effects in patients because this pathway plays such an important function in normal cells. It is active in disease but also needs to be intact for normal immune and inflammatory responses," Stoffel says.

The goal now is to identify molecules downstream of the NF-κB pathway that could be targets for new drugs able to activate tumor suppressor genes without upsetting the body's other cells.

Tails of transcription

Activation of tumor suppressor gene p53 is much more complex than previously believed

BY JOSEPH BONNER

It's the biochemist's twist on the old light bulb joke: how many proteins does it take to activate a gene?

In the case of p53, a widely studied gene linked to tumor suppression, the number keeps growing. Rockefeller scientists now know that as many as five are involved in just the first step of the activation process. (Another 70 or so are later involved in transcribing the gene's DNA.)

Reporting in the June 11 issue of the journal *Cell*, **Robert Roeder**, with first author Woojin An and graduate student Jaehoon Kim, provides the first direct evidence that chemical changes to packaging proteins called histones regulate the activation of p53 and other target genes. It's a finding that has major implications for the treatment of human diseases including cancer.

"The regulation of gene expression is one of the most important and actively studied areas in biology today, and an understanding of the central role of histone modifications in gene activation has enormous implications for understanding both normal and abnormal cellular processes," says Roeder, head of the Laboratory of Biochemistry and Molecular Biology and last year's recipient of the Albert Lasker Award for Basic Medical Research. "The p53 protein is an important tumor suppressor, and by looking at key target genes, we have found that there is a surprising complexity to p53-mediated gene activation, which occurs as a direct result of the function of several histone-modifying enzymes."

The p53 gene is mutated in many cancers, and scientists are trying to determine the consequences of this abnormality and also how the p53 gene product, called a tumor suppressor, normally acts in the human body to monitor and to avoid harmful consequences of damaged DNA. When p53 is activated in response to DNA damage, it binds to and activates so-called target genes, which produce proteins that execute one of two possible responses. These proteins either arrest cell growth so that the cell can repair the DNA damage and grow normally or they kill the cell before it can generate a tumor. Scientists still do not properly understand the conditions that influence one DNA-damage response over the other.

In 1997, Roeder and Wei Gu, then a postdoc in Roeder's lab, showed that an enzyme called p300/CBP, known to modify the chemical composition of histone tails, serves as a transcriptional coactivator for p53. Coactivators are regulatory proteins that, together with activator proteins, are required to turn genes on.

But Roeder and Gu also showed that p300 could chemically modify and alter the function of p53 itself, thus showing for the first time that histone-modifying enzymes can also modify regulatory factors. This finding, which is now commonly observed, raised questions regarding the functions of the enzymes that are most important for transcription.

Scientists believe that histone modifications are crucial actors in the activation and repression of gene expression. Histones help to package DNA, the hereditary material of life, into each cell's nucleus. The double-helical strand of DNA wraps around a ball of histones consisting of four distinct proteins: H2A, H2B, H3 and H4. This fundamental unit, called a nucleosome, is repeated at regular intervals throughout the length of DNA and, under a microscope, resembles beads on a string. Strings of nucleosomes coil up further to form a more compact chromatin and even further to become the familiar X-shaped chromosomes of human cells.

Beginning with early observations by Rockefeller University's Vincent Allfrey, research by Rockefeller University scientist C. David Allis and others provided a large body of evidence that correlated chemical modifications at specific locations on the tails of histone proteins with gene activation.

In 2002, An, Roeder and their Rockefeller colleagues provided important new information about the role of histone tails in gene activation. Scientists knew that histone tails repressed gene activation by preventing transcription factors — proteins that help read out the information encoded in DNA — from gaining access to DNA. An and colleagues, using a test tube system of coiled up chromatin created from engineered or recombinant histones and DNA, showed that, just as Allfrey and Allis had predicted, histone tails and associated modifications are required for reversing the repression of transcription and that p300 plays an important role in this process as a histone-modifying enzyme.

Scientists elsewhere had previously shown that two other histone-modifying enzymes, called CARM1 and PRMT1, worked with p300 to mediate the function of a class of transcriptional regulatory proteins called nuclear hormone receptors. And like p300, CARM1 and PRMT1 can modify both histones and regulatory factors.

To determine if CARM1 and PRMT1, alone or in conjunction with p300, also are involved in gene activation by p53, An and his co-workers put all these proteins in a test tube with pure p53 and tested their function on a target gene wrapped up in a chromatin structure with histones.

An found that the three proteins worked synergistically in mediating gene activation by p53. And surprisingly, An also observed that the proteins followed a specific order: activation of the p53 target gene GADD45 — a gene involved in the repair of damaged DNA — was strongest when An added PRMT1 first, p300 second and CARM1 third. Moreover, when analyzed with recombinant chromatin containing mutated histones that could not be modified, he failed to see gene activation by p53 — thus proving that histone modifications are indeed necessary for p53 function.

"There have been many studies correlating histone modifications and transcriptional regulation, but none of the studies directly showed that histone modification itself actually regulates transcription," says An. "Our results show — as was generally assumed — that chemical modifications of histones indeed are required for the activation of gene transcription."

To show that the results he obtained in test tube studies also occur in living cells, An exposed cells to ultraviolet light, which inflicts DNA damage and causes GADD45 to be highly expressed, and watched the recruitment of cofactors. To do this he used an antibody-based assay called ChIP (chromatin immunoprecipitation), which enabled him to look at proteins sitting on the GADD45 gene in a living cell. Consistent with the test tube results, the first complexes to arrive at the scene, within two hours of exposure to UV light, were p300 and PRMT1. CARM1 showed up within four hours of UV irradiation. Somewhat surprisingly, the assay also showed p53-dependent accumulation of at least two other histone-modifying enzymes on the GADD45 gene during the activation process.

"It is surprising that so many proteins are required in the initial events — the histone modifications — leading to activation of a single gene," says Roeder, who is Arnold and Mabel Beckman Professor. "Now the stage is set to determine if all these proteins are used by all p53-responsive genes, or during all of the different stress responses that activate these genes, and how they influence the outcome — for example cell death versus cell growth arrest — of the DNA-damage responses."

Structure of a DNA attack

BY TIEN-SHUN LEE

After two years and hundreds of gallons of buffering solution, scientists in **C. Erec Stebbins**' laboratory have visualized the molecular structure of a genotoxin used by 10 different disease-causing bacteria to attack human DNA.

The image they obtained of the cytolethal distending toxin (CDT) *(right)* will help scientists understand the activity of this potential carcinogen and to design new drugs to fight a range of bacterial diseases that are caused when the toxin creates lesions and breaks that cause cells to stop dividing and die.

"More CDT-containing bacteria are discovered each year," says Stebbins, head of the Laboratory of Structural Microbiology. "Many of these bacteria cause very different kinds of diseases and colonize different tissues. But they all have CDT. To me, that argues that it's playing an important role."

Stebbins' structure of CDT visually confirms that it is made up of three subunits, including one called CdtB that cleaves, or cuts, DNA. According to Stebbins' model, the three-unit toxin contains a long, deep groove, a cluster of ring-shaped molecules, called the aromatic patch, and a dangling protein tail that can block a key portion of the CdtB subunit that is necessary for damage to the host cell genome.

"We're not sure what the role of the cleavage-blocking protein tail is, but the structure helps us to understand how to interact with the active site of CdtB to impair its activity, which could give us some ideas for achieving the same thing with a drug molecule," says Stebbins.

Stebbins has solved the structures of over 10 other proteins, including the cancer-related VHL tumor-suppressor and several other bacterial toxins.

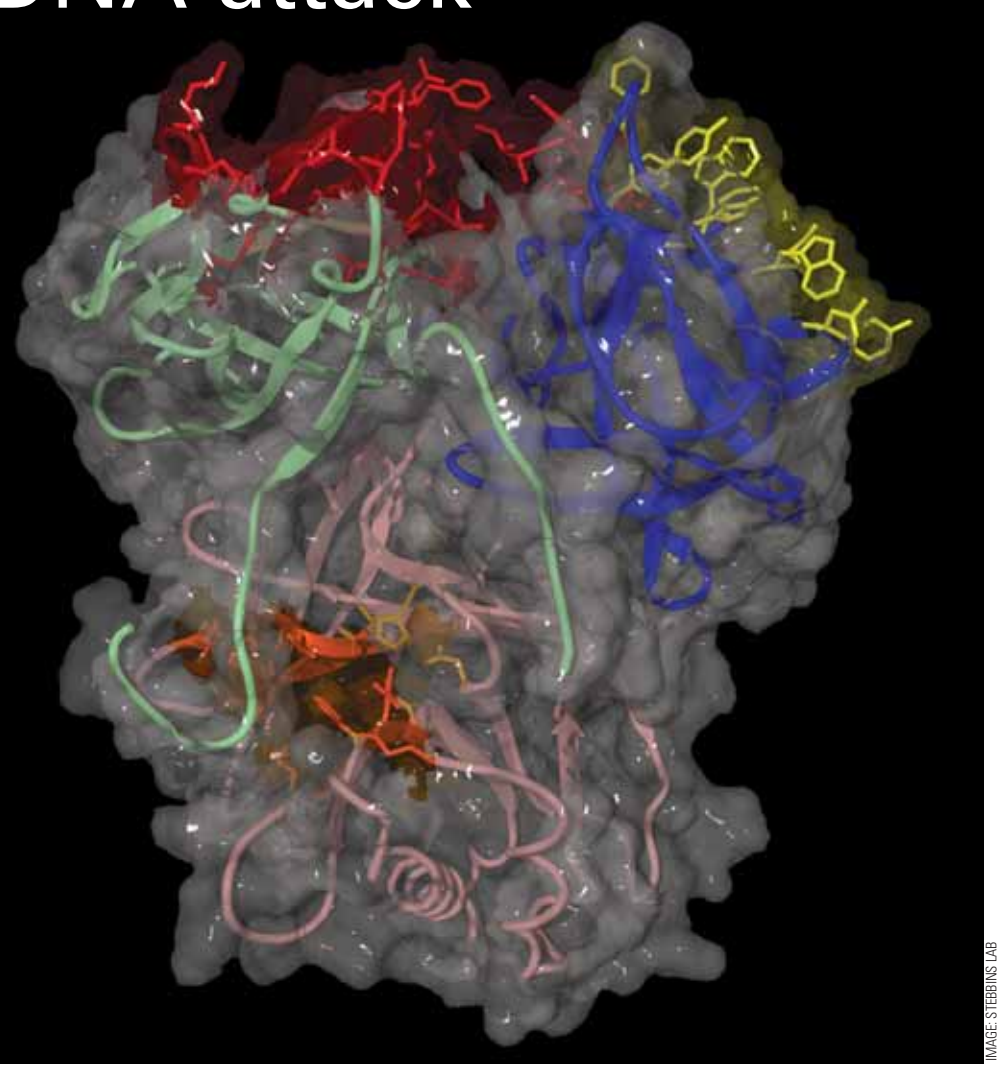


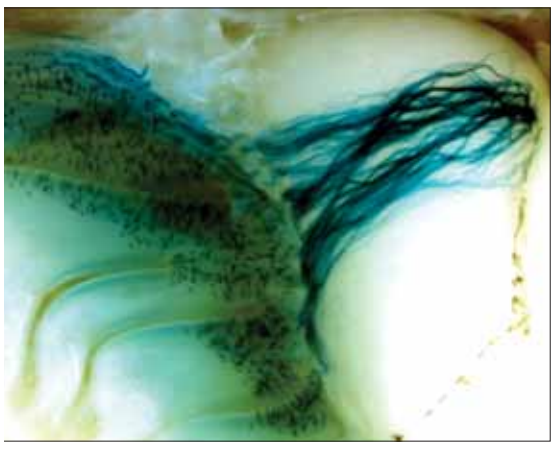
IMAGE: STEBBINS LAB

IMAGES: MOMBAERTS LAB

Tracing axons

How developing nerve cells in the nose find their way to a single tiny spot in the brain

BY RENEE TWOMBLY



The spotless mind. Olfactory neurons — visualized here in blue — normally express an odorant receptor molecule called M71 and have extensions that coalesce into a single spot, a glomerulus, on the surface of the brain's olfactory bulb *(above)*. Rockefeller's Peter Mombaerts genetically forced the neurons to instead express a different receptor, the β2 adrenergic receptor, and found the projections also coalesced into a single glomerulus, though in a different spot *(top)*.

Peter Mombaerts and Paul Feinstein think about the wiring of the olfactory system in terms of drones and airliners.

The accepted wisdom is that, during development of an embryo, the nerve cells located in the back of the nose extend long tentacles that are drawn to relay stations in the brain just as drones land at a specific airport because signals told the drones to do so. The destination is in charge.

But Mombaerts, head of the Laboratory of Developmental Biology and Neurogenetics and part of the F. M. Kirby Center for Sensory Neuroscience, and Feinstein, a research associate in Mombaerts' lab, are turning that notion upside down. They now liken the nerve cells involved in smell to commercial airliners whose pilots determine where the planes fly and land within the confines of air and earth. The neurons are piloted by odorant receptors that control the cells' course and destination.

The difference is fundamental and it suggests a new explanation for how the olfactory system wires itself to perceive smell. The model may also have implications for understanding how other parts of the brain are constructed.

In two papers published back-to-back in the June 11 issue of *Cell*, Mombaerts, who has a commercial pilot's license, and Feinstein propose that odorant receptors tell olfactory nerve axons, the hair-like extensions of nerve cells, what to do, thereby dictating how the olfactory bulb in the brain is structured. The studies, which take up 29 pages in *Cell* and cover more than 40 different gene targeting studies, are the culmination of eight years of research.

"This is a new way of thinking about brain wiring," says Mombaerts. "We propose that the olfactory bulb is almost a blank slate; there are no targets for the axons to navigate to, but the axons themselves are targets for each other and thereby sort themselves out in reproducible patterns."

Even with its mind numbing complexity of millions of neurons, olfaction appears to be one of the simpler brain systems, the Rockefeller researchers say. Odor molecules entering the nasal cavity travel to the olfactory epithelium, a patch of tissue almost level with the eyes. There, thousands of receptors stud the fine hair-like cilia of nerve cells embedded in the tissue. Neurons that serve these receptors each send an axon back to one of two olfactory bulbs in the brain, and "like-minded" axons — those originating from neurons that link to the same odorant receptor — terminate together into brain structures known as glomeruli.

When an odor molecule interacts with a receptor, impulses travel up the neurons to the glomeruli, some 2,000 of which reside within each olfactory bulb. The glomeruli act as relay stations, processing passing nerve signals from the olfactory epithelium to other regions of the brain. About 5,000 axons form a glomerulus and branch into 50,000 axon terminals that synapse with the dendrites of 25 or so second-order neurons, all in the space of a sphere with a diameter of one-tenth of a millimeter.

The question for Feinstein and Mombaerts: How do all these axons find their way to such a tiny and precise spot in the brain?

The prevailing view is that axons navigate to glomeruli via positional cues or signaling information secreted by cells in the olfactory bulb, says Feinstein: "People view glomeruli as existing structures, targets which axons were aiming for." But glomeruli are not seen when the olfactory system begins to develop. How can a developing axon be drawn to a structure which does not yet exist?

In 1996, Mombaerts published findings that odorant receptors were capable of organizing "like" axons into glomeruli. It was a major hint.

"But we had no model for how they could do that," Mombaerts says. "It was a bizarre notion that receptors could somehow be involved in telling axons where to navigate. As far as anyone knew, the only role odorant receptors had was to smell. We had no proof of another function."

The *Cell* studies now detail that proof. The first paper tests whether odorant receptors themselves carry enough information to drive the identity of the axons. Feinstein carried out a series of "gene swaps" between odorant receptors. By implanting these receptors into the genetic material of mice, he was able to trace axons from neurons expressing associated receptors. He found that when the "identity" of the axon, given to it by its receptor, changed, the wiring also was altered.

"The first *Cell* paper sets forth the concept that axonal identity is primarily determined by the odorant receptor sequence," says Feinstein. "However, the axonal identity is revealed by what other axons exist nearby during the process of axonal extension to and within the olfactory bulb. We do not believe that the olfactory bulb contains the information for axonal identity; rather than following positional cues, we suggest that growth cones of axons sort themselves."

The conclusion: "Our experiments reveal that the odorant receptors exist not just at the ends of nerve cells in the olfactory epithelium, but also along their entire length," Mombaerts says. "We hypothesize that axons with odorant receptors appear to interact stronger with axons carrying similar odorant receptor structures and repel those that are different. As a result, the system self-organizes such that axons eventually coalesce into like-minded bundles."

The second *Cell* paper, by Feinstein, Mombaerts and research associates Thomas Bozza, Ivan Rodriguez and Anne Vassalli, picks up where the first paper leaves off to ask how odorant receptor proteins modulate axonal identity. The team genetically substituted the odorant receptor with an unrelated receptor, a G-protein coupled receptor (GPCR) called the β2 adrenergic receptor. "The expressed β2 adrenergic receptor also supported the formation of glomeruli, which indicates that this receptor, which has nothing to do with the smell system, can also work to guide an axon," Mombaerts says. "That further suggests that the olfactory bulb is not drawing axons in."

"This paper confirms several predictions of axonal identity," says Feinstein. "We show that odorant receptors, including the β2 adrenergic receptor we utilized for the gene swap, are expressed in extending axons. We further show that these GPCRs may also promote axon outgrowth, and speculate that these processes of axon identity and axon outgrowth may be a more general phenomena throughout the brain governed by other, similar proteins."

From an evolutionary perspective, the new model makes perfect sense. "The olfactory system has to be very plastic to accommodate new genes encoding odorant receptors and new populations of neurons," Mombaerts says. "If you have a rigid view that axons are attracted by existing cues, it would be much more difficult to explain how these new populations of neurons wire themselves."