

Inside inflammation

How an enzyme called Csk controls the body's immune response — and may answer questions about inflammation's role in cancer, asthma and heart disease

BY LYNN LOVE

Inflammation, it seems, is a hot topic these days. Even on the cover *Time* magazine. Its February 23 story implicates the biological process of inflammation — the body's own defense against microbe invaders — in diseases from diabetes and cancer to asthma and heart disease. "Hardly a week goes by without the publication of yet another study uncovering a new way that chronic inflammation does harm to the body," the *Time* journalists wrote.

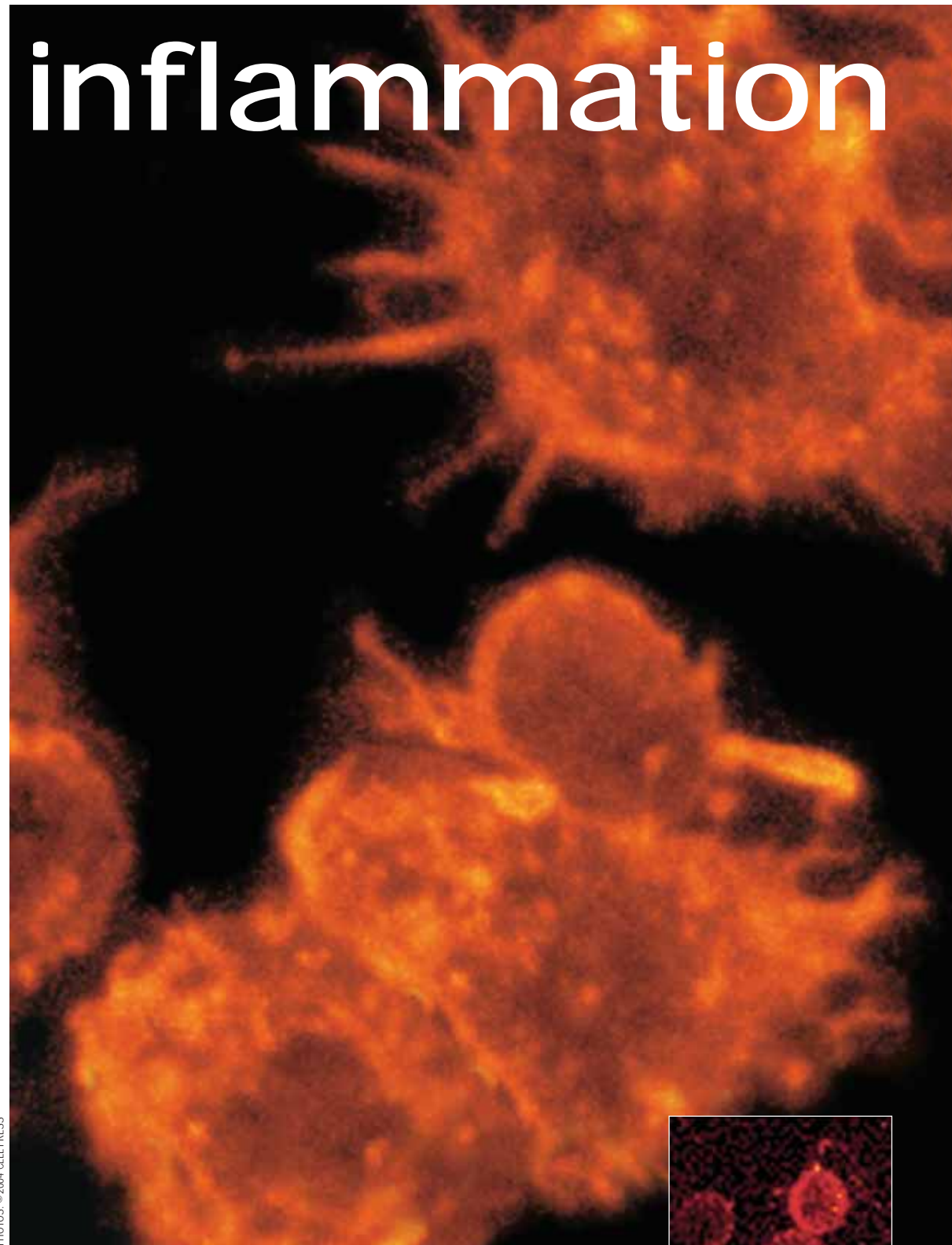
While physicians are struggling to understand how inflammation is linked to disease, Rockefeller scientists, in their typical fashion, are focusing on what goes on at the most basic molecular level.

Sasha Tarakhovsky, head of the Laboratory of Lymphocyte Signaling, working with a team at the Windeyer Institute at University College London, has now discovered an enzyme that halts inflammation in mice.

Their findings, which were featured as the lead article in the February issue of *Immunity*, may prove to be an important milestone in the understanding of how inflammation may contribute to disease.

Inflammation isn't always bad. When the body responds to the presence of a pathogen, early responder immune system cells called granulocytes migrate amoeba-style into the infected tissue. Once in place, these granulocytes engulf and destroy invaders.

This process, the basic cycle of inflammation, fights microbes of all types before the body's adaptive immune response, the second level, kicks in. But if unchecked, the inflammation response has devastating potential. If allowed to continue indefinitely, it can



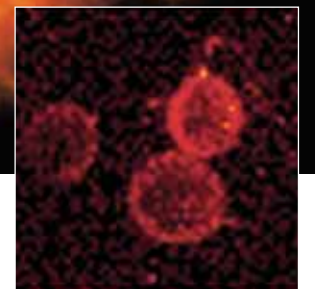
cause serious consequences such as sepsis and death.

Tarakhovsky's studies now show that it's up to an enzyme called Csk to serve as the crucial check on the system. Enzymes play important facilitating roles in the body: they drive metabolism, replicate DNA, read genes, convey signals from the outside of the cell to

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Uncurbed enthusiasm.

White blood cells from mice lacking the Csk enzyme show what happens when inflammation runs out of control. Unlike cells from normal mice (*inset*), the Csk-deficient cells aggressively adhere to uncoated glass and have distinct thread-like projections, called filopodia, that under normal conditions help them attack invading microbes.



Putting a smell theory to the sniff test

BY RENEE TWOMBLY

Of the five senses, only one remains a mystery to scientists: scent. Despite decades of study, the theories of how we detect odors remain, at least on a molecular level, theories.



PHOTO: ZACH VEILLEUX

Now, in an attempt to cast new light on how the nose works, Rockefeller's Leslie Vosshall has, for the first time, put a controversial scientific theory of smell to the test. Her results should lay to rest a debate over the "vibration theory" of scent that has until now largely been based on speculation and hearsay.

There are two theories of smell. Most researchers believe that the shape of a chemical determines how it smells. That is, the sense of smell works like a lock and key: the shape of a chemical (the key) fits into odorant receptor proteins on the outside of cells (the lock) that are dedicated to the sense of smell.

Then there's the vibration theory. It states that molecules in every substance generate a specific vibration frequency that the nose interprets as a distinct smell.

Though both theories are unproven, vibration theory has recently received some press attention, first by a

BBC-TV documentary, then by a book titled *The Emperor of Scent*. The book's author, Chandler Burr, argues that the biophysicist who came up with vibration theory, Luca Turin, is a pioneering researcher who is being ignored by the smell research community because of his unconventional ideas. Many reviews of his book parroted that theme.

Turin, a physiologist by training and a recognized expert on perfumes, expanded upon a theory first offered in the 1930s that suggested smell was dependent on intramolecular vibrations, or the stretching of chemical bonds, within an odor molecule. He hypothesizes that the receptors lining the nose function as a biological "spectroscope" to measure the vibrational energy of odorant molecules.

Yet Turin never undertook a series of experiments

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SCIENCE BRIEFS

BY ZACH VEILLEUX

Afrikaner clues to schizophrenia. Founder populations, which originate from relatively few individuals and tend to remain isolated over many generations, offer scientists unique opportunities to study the genetics of disease. Two recent studies led by **Maria Karayiorgou** report on the Afrikaner population, descendants of South African settlers originally from The Netherlands and other parts of northern Europe. Karayiorgou's first study, on 260 Afrikaners with schizophrenia, reports on the genealogy of this population and evaluates the potential it has to yield clues about genes that are linked to the disease. When Karayiorgou compared the clinical data on the Afrikaner patients with data from a sample of American patients with schizophrenia, she found many similarities. "The comparison suggests that our genetic findings in the Afrikaner study will be applicable to other populations and schizophrenia in general," she says. In the second report, Karayiorgou and her colleagues performed a genome-wide scan on multiple members of 143 of the families, and found specific locations on chromosomes 1, 9 and 13 that may have links to schizophrenia — including a locus on chromosome 1 that had not previously been identified. Furthermore, the researchers identified one patient carrying a uniparental disomy of his entire chromosome 1, a finding that may prove extremely valuable in their efforts to isolate the culprit gene from chromosome 1.

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), January 2004, and *American Journal of Human Genetics*, March 2004.

A new tool for membrane channel studies. A toxin derived from the polyps of soft-bodied marine animals called zoanthids could help scientists understand how sodium and potassium ions are pumped — that is, moved against their natural tendencies — across the membranes of all animal cells. But before this toxin, called palytoxin, can be scientifically exploited, researchers need to know more about how it works. **David Gadsby**, head of the Laboratory of Cardiac and Membrane Physiology, and postdoc Pablo Artigas, have now conducted extensive studies of how palytoxin interacts with sodium/potassium pumps in human kidney cells and guinea pig muscle cells. Their results indicate that the pump's affinity for palytoxin varied over several orders of magnitude depending on the ratio of the potassium and sodium ions on either side of the membrane and on the metabolic state of the cell. The researchers also showed that the narrowest part of the palytoxin-induced ion pathway through the pump is about 0.75 nanometers wide and that prior or simultaneous exposure to a cardiotonic steroid weakens palytoxin's ability to bind to it. Gadsby and Artigas say their research will help them, and other scientists who want to use palytoxin, to examine the structure of gates in the pump that regulate the uphill passage of sodium and potassium ions through practically all cell membranes.

Journal of General Physiology, March 2004.

Characterizing kinases. In the neuroscience community, there's a tremendous amount of interest in kinase inhibitors, which block the activity of specific proteins (called kinases) believed to contribute to Alzheimer's disease, Parkinson's Disease and other neurodegenerative disorders. Now Laurent Meijer, a visiting professor in **Paul Greengard's** Laboratory of Molecular and Cellular Neuroscience, has characterized how a class of kinase inhibitors known as indirubins — chemicals derived from indigo-producing plants, certain species of mollusks and various bacteria — bind to kinases. Meijer's studies, which were conducted in collaboration with colleagues in France, Italy, Greece and the United Kingdom, provide scientists with additional tools to investigate the functions of two specific kinases — glycogen synthase kinase-3 (GSK-3) and cyclin-dependent kinases (CDKs). The scientists also hope their research will eventually lead to new therapeutic compounds useful in treating Alzheimer's, cancer and other diseases in which CDKs and GSK-3 are implicated.

Journal of Medicinal Chemistry, February 2004.

Smell theory *continued*

that he said, in a theoretical paper, would prove his theory. "Since Turin's theory was based solely on his unverified reports about the smell of certain odors, the scientific community rejected it as a universal theory of smell based on one man's olfactory impressions," says postdoc **Andreas Keller**, first author of a report on the research published in the April issue of *Nature Neuroscience*.

But problems exist with the shape theory as well: humans can detect many more smells than there are odorant receptors, and even if the locks are a little "loose," the shape theory can't explain how two chemicals, each with a unique shape, can smell essentially the same.

"There are cases that are not intuitive for the shape theory, and that is why scientists have been looking for alternative theories for a very long time," says **Vosshall**, head of the Laboratory of Neurogenetics and Behavior.

A few months ago, Keller and Vosshall — who normally study olfaction in fruit flies — decided to conduct the human studies that Turin never did.

"This is a theory that has been universally rejected by every scientist, so you might ask why we bothered," Vosshall says. "We felt that his theory has been given, by virtue of press coverage, some degree of credibility although it was never been put up to scientific scrutiny."

Keller designed a series of three controlled, double-blind human tests with several dozen human volunteers. In the Rockefeller University Hospital Heilbrunn Outpatient Clinic, he asked volunteers to smell different odors presented in coded vials and answer a series of questions.

In the first experiment, Keller tested

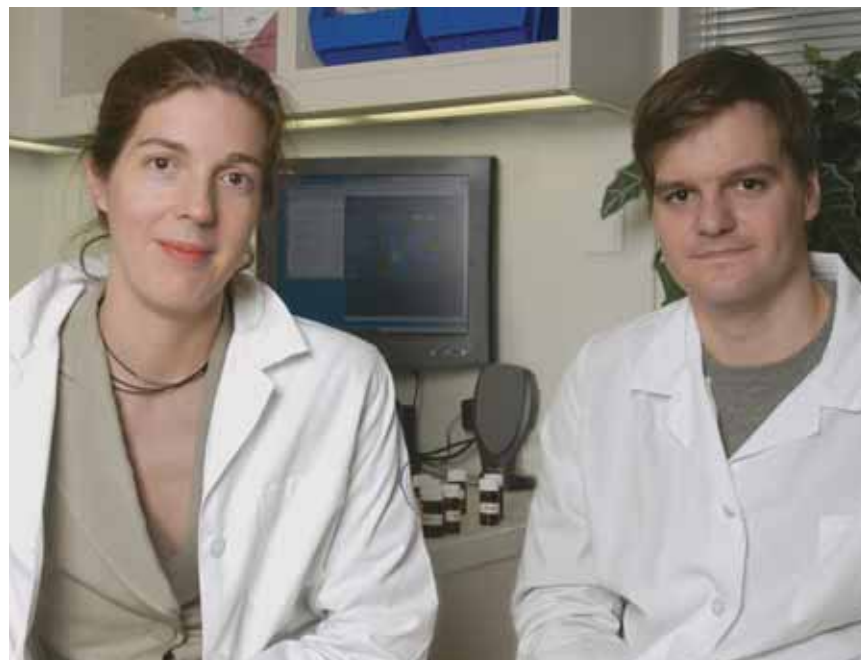


PHOTO: ZACH VEILLEUX

Scent science. Vosshall (left) and Keller in the Rockefeller University Hospital Heilbrunn Outpatient Clinic, where their odor study was conducted.

Turin's prediction that if two different chemicals, one that smells like smoke and one that smells like bitter almond, were mixed together, they would smell like vanilla, because their combined molecular vibrations would match those of vanilla. None of the volunteers agreed.

A second experiment tested whether aldehydes (the major components of Chanel No. 5 perfume), composed of an even number of carbon atoms, smell different from those with an odd number. Turin hypothesized that they would because vibrational frequencies between the two groups would be different. But the participants did not detect such a trend.

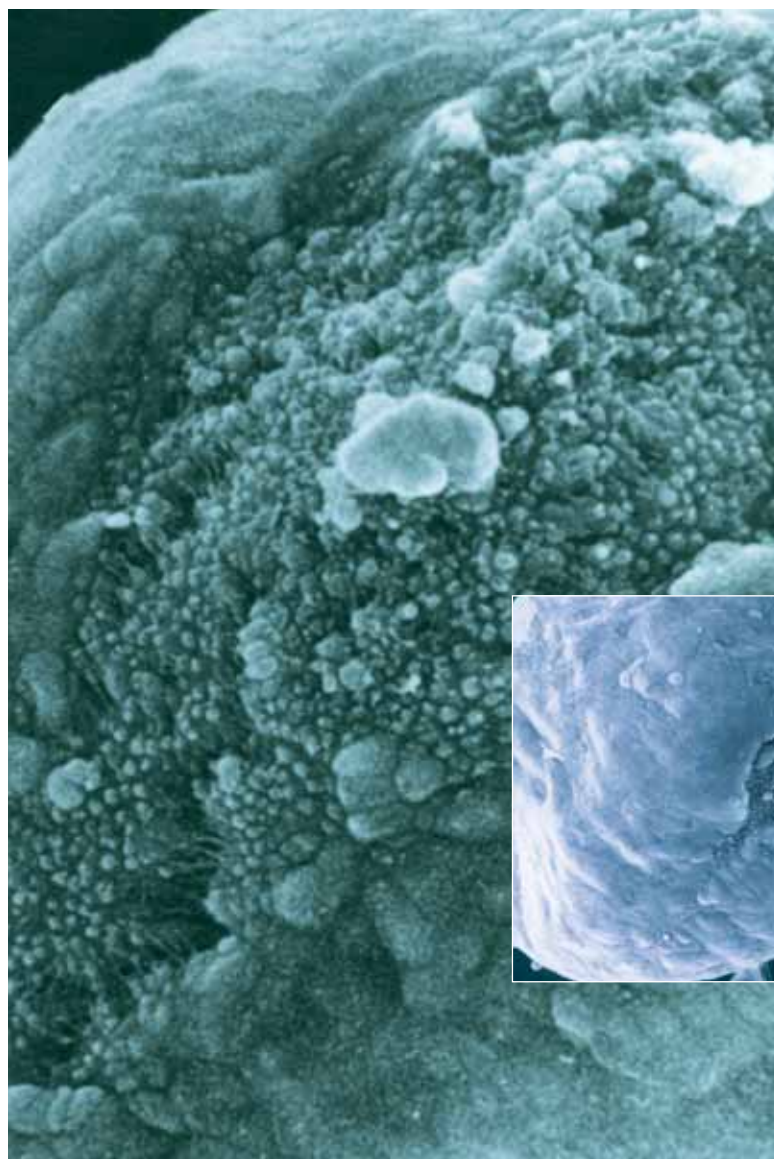
The final experiment was based on Turin's proposal that two chemicals which have almost identical shapes but markedly different molecular vibrations would have distinct smells. In several different tests, none of the subjects could tell the difference between the similarly shaped chemicals.

"In order for science to have integrity, you have to do studies properly. You can't just sniff the substances yourself, decide in advance what the answer is supposed to be, confirm by testing it yourself and then publish a paper," says Vosshall.

"I did the boring work of actually doing Turin's experiments and showing what the real answer is," says Keller, with a laugh.

Because the study was not designed to prove either theory, the results say only how smell *doesn't* work. They don't bring us any closer to knowing how it *does* work.

"This is a paper of solely negative results," Vosshall says. "We didn't disprove the vibration theory, we just didn't find anything to support it. The results show that molecular vibrations alone cannot explain the perceived smell of a chemical. And while all of our data are consistent with the shape theory, they don't prove the shape theory."



PHOTOS: FUCHS LAB

Defective development. Unlike a normal mouse embryo (*inset*), mice lacking the Tcf3 protein often exhibit severe defects, such as the duplicated embryonic node shown in the electron micrograph above. In many cases, the mutant embryos also developed multiple heads and tails.

Turning off embryonic defects

In **Elaine Fuchs's** Laboratory of Mammalian Cell Biology and Development, there's a lot of discussion about two proteins known as Lef and Tcf, which regulate biological processes such as tissue development, stem cell maintenance and tumor formation. Three of the four Lef/Tcf genes of the mouse have been shown to function by switching on the Wnt signaling pathway. To learn more about what the remaining Tcf gene does, Brad Merrill, a postdoc in Fuchs's lab, produced a line of mouse mutants that lack Tcf3. The mutant embryos proceeded normally in their earliest stages, but displayed defects in

the process that shapes the basic architectural plan of vertebrate embryos. This was surprising, because it suggested that the normal function of Tcf3 might be to keep the Wnt pathway turned off rather than to turn it on. When Merrill and Fuchs studied the mutant embryos in more detail, they found that several genes associated with the formation of the basic body plan did not function correctly in the absence of

Tcf3. As a result of these molecular defects, Tcf3 mutant mouse embryos exhibited stunning abnormalities, including multiple heads and tails. The scientists conclude that during early embryogenesis, Tcf3 acts as a repressor rather than an activator of the Wnt signaling pathway and its repressor function restricts the induction of the anterior-posterior axis in the developing embryo.

Development, January 2004.



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The 'Sleeper' project

A mouse produced by cloning from a single neuron yields answers about the genetics of olfaction

BY JOSEPH BONNER



PHOTO: PAUL FEINSTEIN

Mighty mouse. Harvey, who in a nod to the Woody Allen movie "Sleeper" was created from the DNA in a single olfactory neuron, is teaching scientists about odorant receptor genes.

Like your nose, your immune system is primarily a detection device. Its chief job is to detect and identify molecules. Just as there is an almost infinite number of smells, there's a nearly infinite number of microbial invaders that must be properly distinguished from one another in order for your body to resist infection.

Yet both the olfactory and immune systems can detect this infinite number of substances by using a finite number of genes.

Scientists know how the immune system accomplishes this task: it combines pieces of genes in a process called DNA rearrangement, which results in irreversible changes to the genetic material of the cell. What they didn't know was whether or not the smell-detecting nerve cells in the nose do the same thing.

To find out, scientists led by Rockefeller's Peter Mombaerts did something amazing: they used cloning technology to produce an entire mouse from the DNA in

just one of the animal's neurons. They then traced the spread of that neuron's nucleus as the mouse embryo developed and eventually as the mouse itself grew.

Harvey, as they called their mouse (that's him above), gave them their answer.

"We and many other scientists have been looking for changes in genetic material of olfactory neurons that, it now turns out, do not occur," says Mombaerts, who is head of the Laboratory of Developmental Biology and Neurogenetics.

To conduct their study, Mombaerts, along with postdocs Jinsong Li and Tomohiro Ishii and research associate Paul Feinstein, chose an odorant receptor gene called M71, one of the 1,000 odorant

Nose knowledge. Two different odorant receptors neurons, M71 (green dots, left) and P2 (yellow dots, right), are expressed in different regions of the olfactory epithelium, the layer of sensory cells in the upper-rear portion of a mouse's nose. Even though the mouse itself was derived solely from the DNA of a M71-expressing neuron, other odorant genes are still expressed in its olfactory system.

receptor genes in the mouse genome. They permanently linked a green fluorescent protein to M71 so that every cell in which M71 is active lights up green under the microscope.

After inserting the nucleus of the M71 nerve cell into a mouse egg, the scientists were able to literally watch the green spread as the DNA housed in the nucleus was replicated with each cell division.

"Our cells were labeled with fluorescent

protein continuously, so even after the nucleus was pulled out and put into an egg, the egg is green, the embryo's stem cells are green, everything else in the mouse embryo is green, because it's driven from a promoter in the DNA that's active in all cells," says Mombaerts. "We have an irreversible marker that shows us that we started with that particular cell."

Then, using a technique called *in situ* hybridization, the scientists showed that in addition to producing M71 neurons, cells expressing the green protein also generate other types of olfactory neurons in different parts of the lining of the nose.

Therefore, Mombaerts says, olfactory sensory neurons that contain the same DNA that once belonged to an M71-expressing cell are able to express other receptor genes — not just the M71 gene from which they are derived.

The findings, therefore, do not support the hypothesis that irreversible changes in DNA occur when an olfactory neuron's DNA is expressed.

Perhaps just as important, the scientists found that a neuron that no longer divided could still be coaxed into cell division.

"This may prove to be important for the nascent field of therapeutic cloning to produce custom-tailored cellular therapies for people with diabetes and other disorders," says Mombaerts.

"It shatters the concern that we cannot use post-mitotic cells for nuclear transfer."

Although therapeutic cloning is not the main line of Mombaerts' research, his lab is at the frontier in their research with mice. With the publication of 16 cell lines in this study, the total of published mouse cell lines produced by cloning technology has reached 65. Of this worldwide production, 41 — two-thirds — were established in Mombaerts' Rockefeller lab.

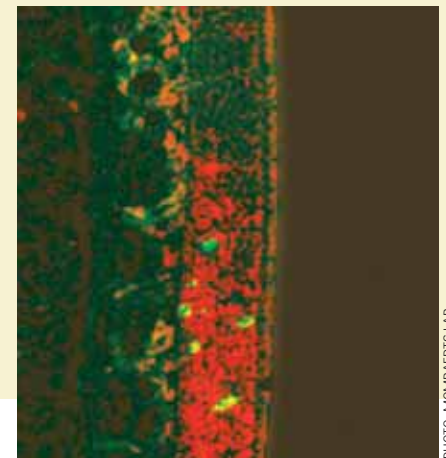
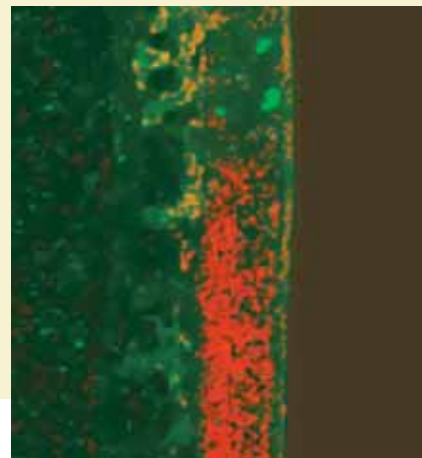


PHOTO: MOMBAERTS LAB

Collins, von Rydingsvard headline Rockefeller public events



Each spring, Rockefeller University hosts a series of public events designed to stimulate discussion about current topics in science. In the past two years these events — including "Stress in the City," "String Fever" and last year's "Compelled to Create?" — attracted huge audiences.

As in past years, these events are free and open to everyone. However, admission this year will require reservations, which will be taken on a first-come first-served basis. Tickets will then be mailed to you. For more information, please call (212) 327-7625. This year's events are:

The Earth: An Intimate History (2003 Rockefeller University Lewis Thomas Prize for Writing about Science) (April 8) Throughout history, scientists and writers have sought to communicate with one another, despite barriers of language and process. This Rockefeller University prize honors the rare individual who bridges both worlds. Richard Fortey, distinguished as both a paleozoologist and geologist, and senior paleontologist at the Natural History Museum in London, will give the prize lecture.

Life in a post-genomic world: Where are science and technology taking us? (April 13)

In the several years since scientists deciphered the human genome, we are faced with many questions. Addressing some of them will be Francis Collins, director of the National Human Genome Research Institute, National Institutes of Health; Troy Duster, professor of sociology, New York University and Chancellor's Professor, Sociology, University of California, Berkeley; Ruth Fischbach, professor of bioethics and director of the Center for Bioethics, Columbia University; and Rockefeller President Paul Nurse. The discussion will be moderated by Michael Waldholz, health and science news editor, *The Wall Street Journal*.

Ereia: A gale force performance of rock, classical and jazz (May 4)

Musical notes, unlike cells, do not typically arrange themselves. But if they did, the result would sound something like Ereia, an avant-garde musical composition combining jazz, classical and rhythm and blues in a score generated in part by algorithms. Ereia's composer, Nick Didkovsky, systems administrator for Rockefeller University's Gensat project, is the composer and conductor. Didkovsky's band, Doctor Nerve, joins the Sirius String Quartet for the performance. Rockefeller scientists and Gensat leaders Nat Heintz and Mary Beth Hatten also will speak.

Compelled to Create? Artists and scientists on the process of discovery (May 18)

Can a sculptor get writer's block? What might a novelist see in a petri dish? The quest for the eureka moment — from the lab bench to the painter's atelier. Panelists are Ursula von Rydingsvard, sculptor; Wendy Wasserstein, playwright; David Small, information designer; Sarah Sze, installation artist; and Rockefeller's Titia de Lange, cell biologist. The moderator will be Ira Flatow, host and executive producer of NPR's "Talk of the Nation: Science Friday."

A scientist's 'frog art'

Brivanlou images featured at International Center of Photography

"I've always thought of my science as artwork," says Ali Brivanlou, head of Rockefeller's Laboratory of Molecular Vertebrate Embryology. Until May 30, thousands of others also will have the opportunity to do so.

"The Art of Science," now on exhibit at the International Center of Photography in New York, features several of Brivanlou's images, displayed not as figures buried deep within the pages of *Nature* or *Cell* but in the center of a gallery on two huge flat-screen monitors.

Creating images is a crucial part of many scientific investigations. Scientific images, in conjunction with other kinds of measurements, literally can reveal new insights in biology. Brivanlou's lab uses quantum dots — microscopic, light emitting crystals that can be injected into cells — to illuminate embryonic development as it occurs in animals. This technique renders an opaque mass of cells transparent as they grow and divide. The scientists also use more traditional fluorescent labeling techniques to study embryonic development. In the image at left, part of a time-lapse movie, a tadpole has been engineered to express green fluorescent protein, so scientists can study how the organism's nervous system develops, one neuron at a time.

"Science in the 21st century is increasingly driven by astonishing methods of visualization," says Carol Squiers, who curated the exhibit. "Scientists look at images not so much as documentation but as sites of discovery."

"Creating beautiful images was never the goal, it was a byproduct of the way talented people in my lab express their science," Brivanlou says. "It was an honor to be asked to exhibit my work in this way."

The International Center of Photography is located on Sixth Avenue and 43rd Street. Brivanlou's images are also available at xenopus.rockefeller.edu.

Inside inflammation *continued*

their destinations within and perform many other functions. In the case of inflammation, Csk tells early responder immune cells when to stand down.

"The important role of Csk is an unexpected finding," says Tarakhovsky. "The enzyme is part of a family of enzymes we know to be involved with a different kind of immune system function. Its presence in inflammation runs counter to what we would have predicted."

Tarakhovsky should know. He's one of the world's experts on immune system signaling pathways, and in particular, Csk and its family of enzymes, known as the Src family kinases (SFKs). Because Tarakhovsky has been so successful in understanding the role of Csk in T cells, Jürgen Roes and Richard Thomas, both immunologists based in London, sought his expertise to determine whether Csk plays a role in inflammation.

"We know that Csk is a negative regulator of many processes, and that it is a key enzyme in the adaptive immune response," Tarakhovsky explains. "We wanted to find out whether Csk plays an important role in innate immune response." (The innate immune response is an immediate defense against potentially harmful microbes, while the adaptive immune response is a more versatile set of defenses that often prevent reinfection with the same pathogen.)

To find out, the scientists genetically inactivated Csk in granulocytes, the diverse collection of white blood cells that initially migrate to sites of infection or inflammation. Inactivating Csk

entirely from a living organism, such as a mouse, is not possible. Instead, Tarakhovsky and his colleagues' skill at creating conditional knockout mice using a site-specific inactivation method called Cre-loxP recombination aided the research. In mice without Csk in their granulocytes, the inflammatory response was abnormal. These Csk-deficient mice developed spontaneous inflammation; their granulocytes were hypersensitive and overly aggressive.

In other words, without Csk, the biochemistry of inflammation runs amok.

"Csk limits the biochemical signaling that tells cells to change their shape and texture from non-adhesive to adhesive — the enzyme restores non-inflammatory status to an anatomical locale," Tarakhovsky says.

What's more, Csk is found in every organism from hydra to humans, suggesting a crucial role that may be similar across many species.

Now the research team wonders whether some people have a stronger or weaker genetic predisposition to the Csk signal. Targeting drugs to Csk or the enzymes it inhibits may be a possibility in establishing better inflammatory controls in the immune system.

While Tarakhovsky and his team study the signaling of immune system cells, the research group at UCL plans to continue the search for inhibitory mechanisms of inflammation which, in concert with Csk, ensure that the body's powerful initial response to microbes stays focused.

PHOTO: BRIVANLOU LAB

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