

## Neuroscientist Cori Bargmann to join Rockefeller

UCSF researcher discovered 'matchmaker' molecule responsible for linking nerve cells

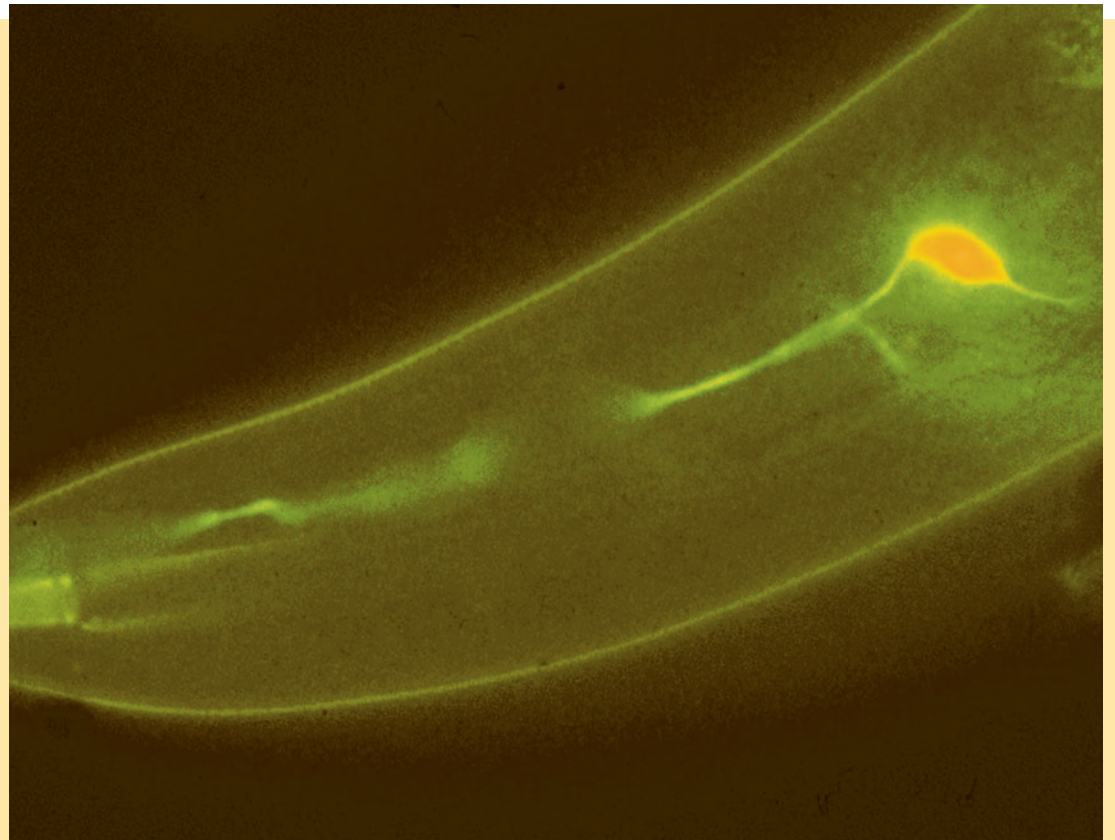
BY ZACH VEILLEUX and CATHY YARBROUGH

Like any matchmaking service, your body's nervous system is only as good as the connections it makes.

Consider the dating scene. Every weekend, people congregate in bars with hopes that the right mate will simply appear. Others stake their faith on internet ads or reality TV.

None of these is reliable enough for the billions of lonely nerve cells in your body. Every thought and movement you make depends on their successfully finding the right mate. When that doesn't happen — when the wrong connections are made — it's the biological equivalent of "Fatal Attraction."

"When two nerve cells don't make the right connection, they make a bunch of wrong connections. They hook up with all kinds of wrong partners," says **Cornelia Bargmann**, one of the country's most respected neuroscientists. It's one of the things that goes wrong in brain diseases such as epilepsy.



'Thoughts' of a worm. A *C. elegans* nerve cell (upper right), in action.

Bargmann, who discovered a "matchmaker" molecule responsible for making the correct connections between nervous system cells called neurons, soon will make a new match of her own. This fall, the University of California, San Francisco scientist will

join Rockefeller University to head one of its over 70 independent laboratories, where she will continue her landmark research to identify and study the genes that shape the assembly and function of the nervous system.

*continued on page 2*

## Of mollusks, mice and stem cells

BY LYNN LOVE and ZACH VEILLEUX

Human stem cells, it turns out, aren't *entirely* human. At least not in the U.S., where human stem cell lines have been grown using "feeder" cells, derived from mice, to preserve their potency.

While suitable for basic research, ultimately these cells are too risky for medical use because they may be contaminated by mouse-associated viruses.

But there may be a work-around.

Rockefeller's **Ali Brivanlou**, working in collaboration with French and Greek scientists, has devised an alternate system for maintaining existing or new human stem cell lines that's based on a Mediterranean shellfish.

It works like this: a new compound, derived from the purple dye of a marine red mollusk — called 6-bromoindirubin-3'-oxime or by its working acronym, "BIO" — has been shown to activate a crucial gene expression mechanism, called the Wnt signaling pathway, in embryonic cells.

The Wnt pathway, in turn, keeps stem cells in an active, undifferentiated state. As long as Wnt is active, stem cells remain stem cells. Turn it off, and they are free to specialize — to become any of the dozens of types of tissues that make up the human body. In order to be truly useful, cultured stem cells must be capable of self-renewal in an undifferentiated state.

"We know precisely how this compound works — that is, on which enzymes and pathways — and that it is very controllable," says Brivanlou, who is head of the Laboratory of Molecular Vertebrate Embryology. "This knowledge makes the compound useful not only in stem cell research but also, as we are already seeing in the lab, in numerous other research areas."

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## Training 'natural' killers

New RU lab shows immune system cells don't have the killer instinct many thought

BY LYNN LOVE

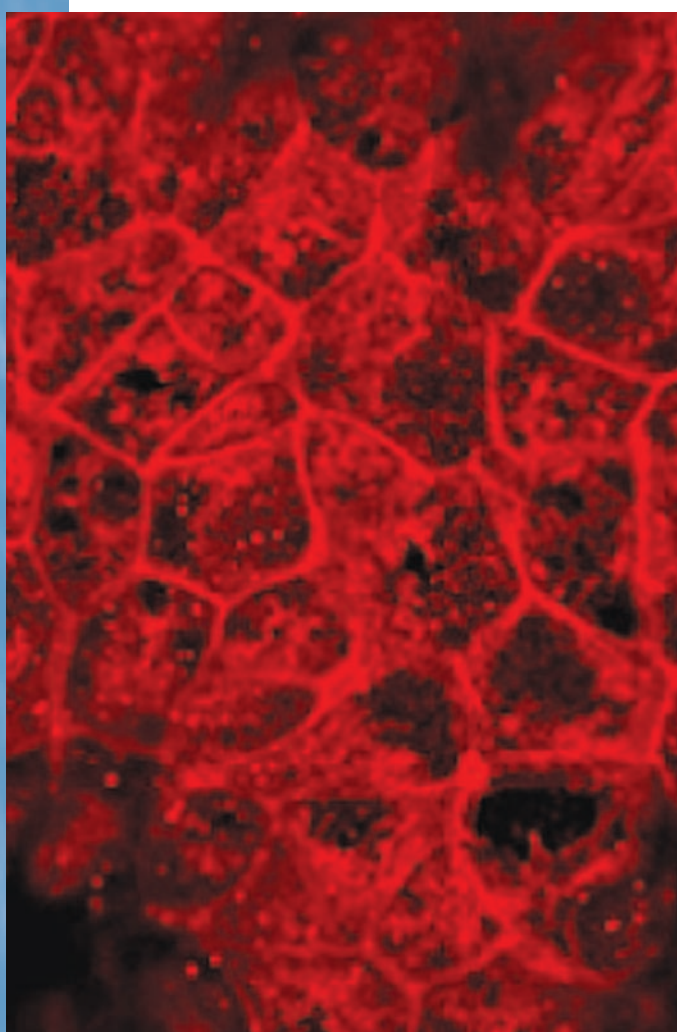
Call it the immune system's version of nature versus nurture.

For years, scientists regarded natural killer cells as the blunt instrument of the immune system, born to kill. They were thought to travel straight from the bone marrow, where they are manufactured, to the sites of early tumors or infectious agents.

Now scientists led by newly promoted head-of-laboratory **Christian Münz** have learned otherwise. Natural killers, they say, have to be raised. In fact, after emerging from the bone marrow, they accumulate in the tonsils, lymph nodes and spleen. There, the natural killer cells await activation before reacting in one of two distinct modes. In one mode, they secrete cytokines, chemical messenger proteins, which modulate emerging responses from other immune system cells. In the other, they are the well-trained killers of tumors and virus-infected cells. While natural killer cells do provide a crucial first defense against many infectious agents and tumor cells, they do so with more discrimination than raw determination.

"Natural killer cells burst forth from the secondary lymphoid tissues, the tonsils, lymph nodes and spleen, and destroy infected and cancerous cells while the T and B cells are still mobilizing," says Münz. "Without

*continued on page 3*



Untainted cells. Human embryonic stem cells glow red after they are stained to show the activity of a new compound called BIO that preserves their usefulness without the need for mice "feeder" cells.

## SCIENCE BRIEFS

BY ZACH VEILLEUX

**Transforming proteins.** A new technique that can alter the structure and function of proteins after they have been manufactured, developed last year by **Tom Muir's** Selma and Lawrence Ruben Laboratory of Synthetic Protein Chemistry, has now been tested in living cells. The technique, called conditional protein splicing (CPS), provides a means to trigger the post-translational synthesis of a target protein from two fragments, thereby controlling the function of that protein. It works by genetically fusing the two protein fragments of interest to each half of an engineered split intein, a polypeptide involved in protein splicing. The engineered split intein is inactive until it binds to a small diffusible molecule, whereupon splicing of the two proteins is triggered. The *in vivo* technique is clean and fast (spliced proteins are observed in minutes), and the amount of product generated can also be controlled. CPS is expected to be applicable to many proteins. Muir and his colleagues say CPS could be especially useful for studying kinetic aspects of protein function *in vivo*.

*Journal of the American Chemical Society*, September 2003

**Correcting psoriasis.** When Edmund Lee, **Madhav Dhodaphkar**, head of the Laboratory Tumor Immunology and Immunotherapy, and **James Krueger**, head of the Laboratory of Investigative Dermatology, compared the cells of skin lesions from psoriasis patients with skin cells from people without the disorder, they found that two subunits of an immune system regulator called IL-23 were 12 to 22 times more plentiful in the psoriasis skin samples. IL-23 shares one of these subunits with a related chemical, IL-12, and until recently scientists believed IL-12 played the primary role in the autoimmune skin disease. But after looking at mRNA profiles of diseased skin, the scientists are reconsidering. "We'd now like to suggest that IL-23, not IL-12, is a key cytokine in psoriatic skin lesions," the authors write.

*Journal of Experimental Medicine*, January 2004

**A drug for newborn jaundice.** If not treated quickly, jaundice in newborns can lead to irreversible brain damage when levels of the chemical bilirubin build up beyond normal. The condition, which affects several hundred thousand births each year in the U.S., is especially difficult to treat because it develops quickly and the susceptibility of individual infants to bilirubin is largely undefined. In addition, newborns leave the hospital several days before bilirubin production peaks. **Attallah Kappas**, Sherman Fairchild Professor and Physician-in-Chief, emeritus, with collaborating pediatricians at other institutions, has developed an effective method to inhibit the production of bilirubin before it reaches critical levels. "The method involves using an inhibitor that we have developed which is targeted at the enzyme, heme oxygenase, controlling the production of bilirubin and permitting physicians to rapidly and predictably interdict the progression of jaundice," says Kappas. The inhibitor, called Sn-mesoporphyrin or SnMP, blocks the catalytic site of heme oxygenase where bilirubin production is initiated and entirely replaces the need for cumbersome light-box exposure as the primary intervention in infant jaundice. More than 1,000 newborns have been treated with SnMP to date.

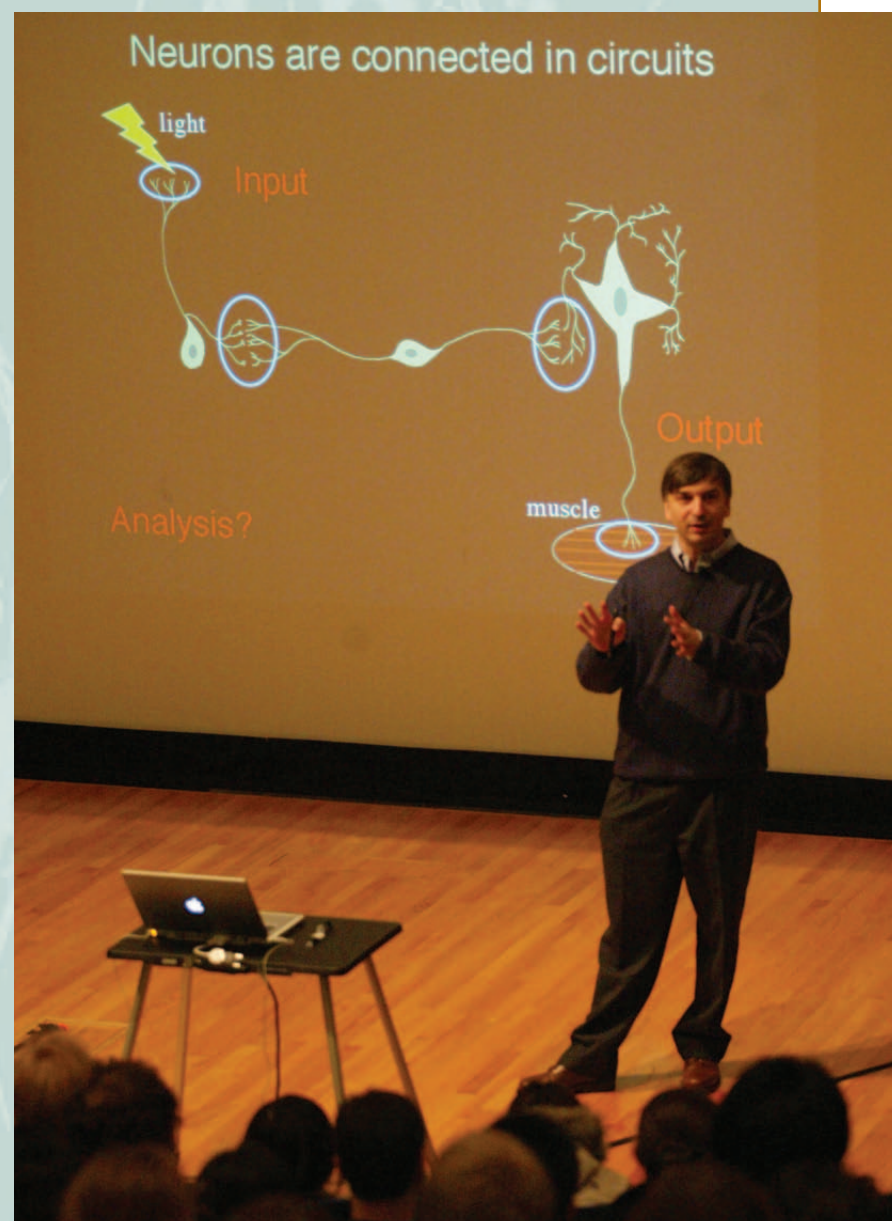
*Pediatrics*, January 2004

# Teenagers, worms gather for science lecture

If extraterrestrials wanted to conquer earth and enslave its inhabitants, what two species of animals should they study before invading our planet?

Rockefeller professor **Shai Shaham's** answer to that question — *Homo sapiens* and *C. elegans*, humans and roundworms — was the introduction to a day-long lecture on science to more than 500 high school students assembled in Caspary Auditorium in December.

Simple worms may teach us a lot about human beings, said Shaham, head of the Laboratory of Developmental Genetics, who studies glial cells in the roundworm's nervous system. His presentation, to the largest group ever to attend in the 44-year history of the holiday lecture series, explained how glia cells in the brains of worms and humans play key roles in many aspects of nervous system function. —Cathy Yarbrough



## Bargmann *continued*

tem. About 14 members of her lab are also making the transcontinental move.

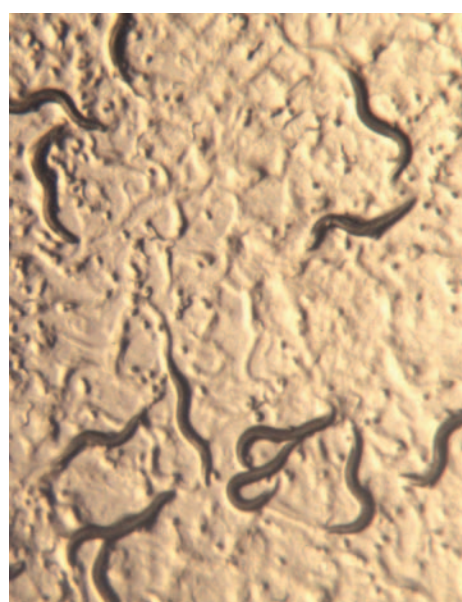
Born in Virginia and raised in Athens, Georgia, Bargmann received her undergraduate degree in biochemistry in 1981 from the University of Georgia. She was awarded a Ph.D. from the Massachusetts Institute of Technology in 1987, studying in the laboratory of Robert Weinberg. Then, after completing postdoctoral training in the MIT lab of Robert Horvitz (winner of the 2002 Nobel Prize for Medicine or Physiology), she joined the faculty of the University of California, San Francisco, in 1991. She was promoted to professor in 1998 and became a Howard Hughes Medical Institute investigator in 1995.

Identifying the specific neurons and mechanisms responsible for relatively complex behaviors is the primary aim of Bargmann's research. "The brain is what makes us human — it's the source of our thoughts, perceptions, memories and desires," says Bargmann. "What could be more interesting than learning how the brain works?"

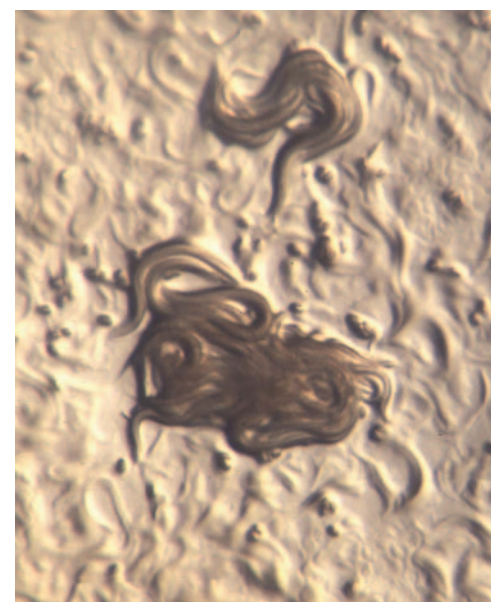
Bargmann conducts her research on the transparent, one-millimeter-long worm *C. elegans*. "I study the brain of a worm instead of the brain of a human for the same reason that you would study a Volkswagen Beetle before you investigate a Boeing 777," says Bargmann. There are only 302 neurons in the worm's entire nervous system; mammals have billions.

And, strange as it sounds, humans and worms engage in many of the same basic survival behaviors. Like us, they use sensory input to perceive and remember their environment as they forage for food and avoid predators. "Insights that scientists learn from the worm allow us to target our questions to understanding the human brain in a much more precise and intelligent way," Bargmann explains.

Because worms are both deaf and blind, Bargmann focuses on their keen sense of smell. Worms can differentiate between thousands of different compounds based on their odor and can even distinguish between two different scents to which they are exposed simultaneously. "We're trying to understand the logic that allows the worm to ignore a pervasive odor and pay attention to one that is coming from a specific source, which might be informative or might predict food," Bargmann says.



**Dinner date.** One of Bargmann's studies identified the gene that underlies worms' tendencies to eat in large groups (*right*) rather than alone (*left*), in order to be less vulnerable to predators.



By studying mutant worms that can detect odors but can't tell them apart, Bargmann was able to pinpoint a gene responsible for odor discrimination — and went on to discover that some odors are sensed by two different neurons that have very subtle differences. "Being a little different, these neurons can compare themselves to each other and sense overlapping, but not identical, odors," Bargmann says.

In other studies, Bargmann has identified a protein that helps control water balance and underlies the sensation of touch (research that was conducted in collaboration with Rockefeller's Jeffrey Friedman), pinpointed a gene called *npr-1* that underlies worms' tendency to feed in social groups rather than alone, and located guidepost molecules that direct neurons to form connections with each other during early development.

Bargmann is currently professor in the departments of anatomy and of biochemistry and biophysics at the University of California, San Francisco. She is also a member of the American Academy of Arts and Sciences and the National Academy of Sciences.

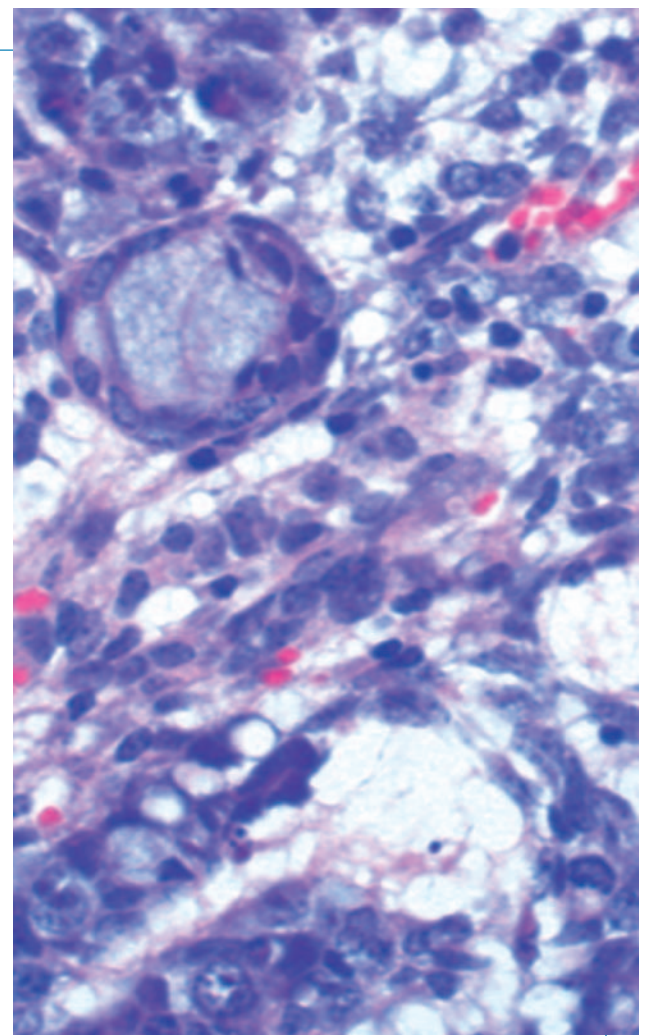
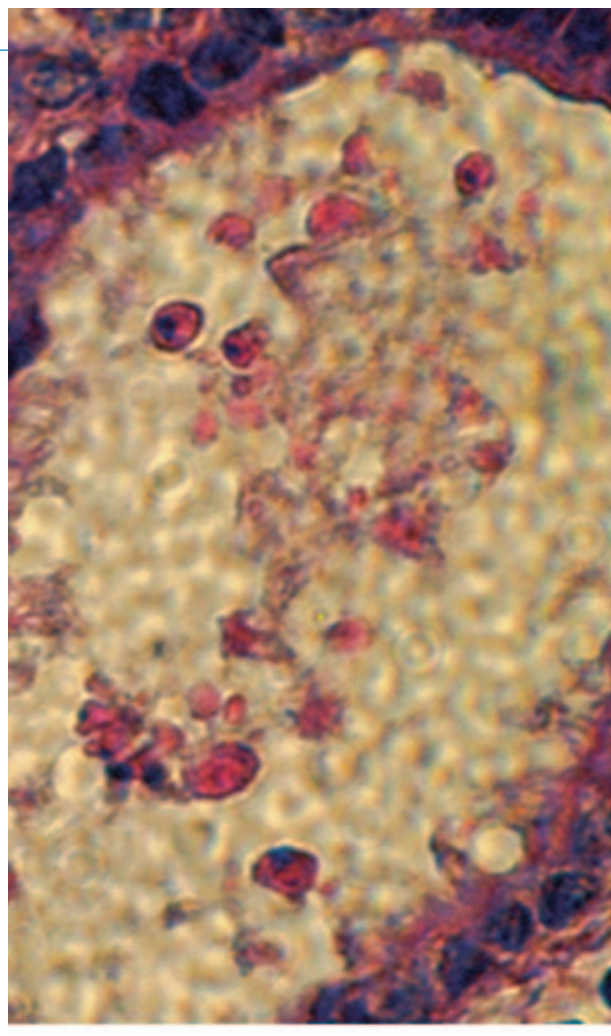
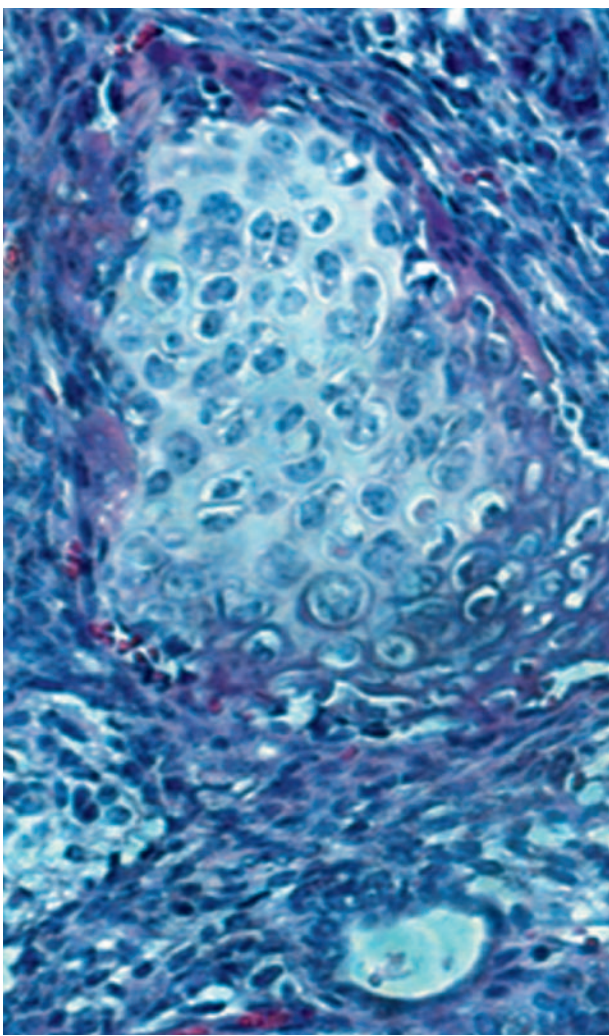
"Cori Bargmann typifies the Rockefeller scientist," says Rockefeller University President Paul Nurse. "She is bold and highly original in her thinking and her approach to studying the brain and other components of the nervous system."



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**Forming tissues.** To test whether stem cells maintained using a new compound called BIO could successfully form other body tissues after BIO is removed, researchers injected them into mice. The cells performed

as expected: mouse embryonic stem cells have formed the precursors to cartilage (*above left*), and two different kinds of epithelium, the cells that cover internal and external surfaces of the body (*center and right*).

## Stem cells *continued*

As is often the case with discoveries, this one was something of an accident. Red mollusks were not on Brivanlou's scientific radar screen until early in 2003, when Laurent Meijer, a biochemist from the Roscoff Marine Biology Institute in France, came to Rockefeller for a sabbatical in Rockefeller Nobel laureate Paul Greengard's Laboratory of Molecular and Cellular Neuroscience. Meijer asked Brivanlou to test a new compound that he and a Greek collaborator, Leandros Skaltsounis, had recently isolated from red mollusks. The

two scientists wanted to know whether the compound inhibited GSK-3, an enzyme associated with both the Wnt pathway and several neurodegenerative disorders.

"Protein kinases like GSK-3 are very promising targets for the discovery of new therapeutic agents," says Meijer. "In particular, pharmacological inhibitors of GSK-3 have great potential for application to treat Alzheimer's disease as well as sleep disorders and depression."

Brivanlou and Alin Vonica, a postdoc in his lab, confirmed that the synthetic compound mimicked Wnt acti-

vation. And in the process, they discovered that the compound arrested differentiation of stem cells in frog embryos.

Thus far, Brivanlou has applied the BIO compound to frog, mouse and NIH Registry human stem cells, with favorable results. So far, it has demonstrated superior stability over other methods designed to circumvent the need for mouse feeder cells. And the stem cells appear to progress and differentiate normally after the compound is removed.

## 'Natural' killers *continued*

them, threatening conditions can get a strong foothold before any adaptive response kicks in."

This new insight, published in two separate papers in the February issue of *The Journal of Immunology*, may lead to new ways for scientists to harness natural killers for therapeutic purposes. Leading oncologists treating human leukemias already track natural killer cell activities after bone marrow transplants. James Young of Rockefeller's neighboring Memorial Sloan-Kettering Allogeneic Bone Marrow and Stem Cell Transplant Service, is one of them. "The emerging data on natural killer cells are helping to move their potential role in transplantation and cancer from conjecture to sound hypotheses," he says.

Münz's newly established Laboratory of Viral Immunobiology not only explains why the natural killer burst is important — it likely results from mobilization of the cells from lymphoid tissues, and these activated immune cells are discriminating enough to recognize virus-infected and tumor cells — it also affirms a potential strategic change in bone marrow donor matching.

In the past, bone marrow donors were selected based on their blood profiles: the closer the match to the patient, the better. But that's less important when doctors can harvest, multiply and reinject the patient's own natural killer cells to fight both residual cancer and immune system cells of the patient. Certain mismatches between donor and recipient can actually encourage the donor's natural killer cells to deliver an extra punch to the cancer and to graft-versus-host disease, the updated logic goes.

Münz and his colleagues did not develop this strategy, but part of their aim in understanding where and how natural killers hang



**Killer research.** New Rockefeller head-of-lab Christian Münz.

out was to determine more effective ways to combat emerging diseases in the body. The Rockefeller scientists are in close contact with clinicians interested in tailoring immune cells including natural killer and dendritic cells, to treat human leukemias.

The research may also help Münz and colleagues battle a menace known as the Epstein-Barr virus, a member of the herpes family of viruses. Though most infections are latent, active Epstein-Barr is the source of infectious mononucleosis in many teenagers.

Epstein-Barr was also identified as the first human cancer-causing virus. Epstein-Barr transforms the immune system's B cells and is associated with B cell tumors such as Hodgkin's disease and Burkitt's lymphoma. Münz and his colleagues know that the natural killer response is important in establishing immune control against Epstein-Barr.

"We have seen that Epstein-Barr transformation of B cells can be delayed by a strong natural killer burst," says Münz. "Now we are studying how this herpes virus may be targeted by natural killer response." By learning both what molecular signals activate natural killers in their dialogue with dendritic cells and how viruses can be targeted by natural killers, Münz and his colleagues may be able to artificially stimulate natural killers to heighten their effect and ward off emerging Epstein-Barr associated malignancies.

"We're trying to get a sum of all signals that activate natural killer cells against viruses and tumors and do not cause harm to human tissues," says Münz. "In the past five years, we've learned enough about these cells to extend hopes of their eventual usefulness in medical treatments."

Before this, natural killer cells were simply the immune system's natural born killer.

## Academic VP named

**Michael Young**, who studies the biological clocks of fruit flies, may need to adjust his own biological clock this spring. In March, Young will make room in his schedule to become vice president for academic affairs.

"Rockefeller University has never been stronger," says Young, head of the Laboratory of Genetics, explaining his decision to accept President Paul Nurse's request and to take on the new responsibilities. "It will be exciting to work closely with Paul and my faculty colleagues to plan the university's next 10 years."

Young will set aside his activities in various committees and task forces, but isn't scaling back his research. "Science is still the primary reason I'm at Rockefeller," he says.

Since becoming president last fall, Nurse has met with the heads of nearly every major laboratory at Rockefeller. "When I asked which faculty member would best serve as VP for Academic Affairs, I heard Mike's name over and over. He has the respect of everyone as a scientist, leader, mentor and 'citizen' of the university," Nurse says.

Among Young's new responsibilities: guiding and mentoring tenure track faculty; strengthening research infrastructure; assisting the president with scientific planning and operations; and assuming overall responsibility for colloquia, lectures and seminars that serve the community.

PHOTO: ZACH VELLEUX

# A love/hate relationship with telomeres

Enzyme both protects and destroys the tips of chromosomes, research shows

BY RENEE TWOMBLY

The study of telomeres is not often compared to the study of Roman mythology. Yet Titia de Lange's latest discovery, the identification of an enzyme that both protects and destroys the ends of chromosomes, revealed a cellular component that exhibits many of the same traits as Janus, the two-faced Roman god of beginnings.

De Lange, who is head of the Laboratory of Cell Biology and Genetics, is now pushing to understand how this duplicitous enzyme, known as ERCC1/XPF, is itself managed. The answer, she says, might unlock the mystery of how a cell controls its chromosomes — a process crucial to every organism's development and day-to-day function.

While the chromosomes of bacteria are circular in shape, the chromosomes of humans and

other more complex organisms are linear and are sealed at their ends by specialized protein-DNA complexes known as telomeres. In addition to protecting the ends of chromosomes — think of the plastic at the end of a shoelace that keeps it from fraying — telomeres act as a sort of molecular clock, ticking down the number of times a cell can replicate. With every cell division, telomeres shorten in length, and de Lange has shown that a cell ceases to reproduce itself when its telomeres become too short to protect the ends of chromosomes.

Four years ago, de Lange and her group, in collaboration with researchers at the University of North Carolina at Chapel Hill, found that telomeres form closed loops, called t-loops, at their ends. The loops act as protective caps on the end of telomeres, which in turn protect the end of chromosomes. A protein complex called TRF2 protects the telomeres, probably by stimulating formation of these protective loops. Without the loops, cells mistake the exposed chromosome ends for sites of DNA damage; when they attempt to repair them, the cells die. (A related protein complex, TRF1, regulates telomere length.)

Normally hidden within a t-loop is a single strand of DNA that acts like a latch to secure the loop structure. But de Lange and her team noticed that when telomeres lose TRF2, this latch quickly disappears. "We were keenly interested in trying to find out what is responsible for making this strand disappear," she says, "and thought it might be a nuclease, which can quickly degrade DNA." De Lange has already identified several interacting partner proteins within the TRF1 and TRF2 complexes that play very specific roles in telomere regulation.

The group tested several recognized DNA nuclease enzymes in a series of experiments, led by first author Xu-Dong Zhu, then a postdoctoral researcher in de Lange's lab and now assistant professor at McMaster University. The scientists found, to their surprise, that the enzyme responsible for destroying the overhang strand was ERCC1/XPF, a well-known repair tool that cleaves away portions of DNA strands that have been damaged by ultraviolet light. The rare individual born with defective ERCC1/XPF suffers from a skin disease called xeroderma pigmentosa, often accompanied by persistent skin cancer.

"We never expected ERCC1/XPF to be the culprit nuclease because it had only been studied in the context of UV damage. These proteins had never been implicated at telomeres in human cells or cells from any other organism," says de Lange who is Rockefeller's Leon Hess Professor. "Yet we found that when TRF2 is inhibited and telomeres lose their protection, ERCC1/XPF is one of the factors involved

in repairing what the cell thinks is a site of damage. It removes the overhang and then the ends of the chromosomes are fused together."

De Lange and Zhu turned to researchers in The Netherlands and Denmark for ERCC1/XPF deficient mouse cells with which to run further tests. For de Lange, this alliance was a scientific homecoming of sorts: not only was she born and raised in The Netherlands, but her collaboration was with Jan H.J. Hoeijmakers of Erasmus Medical Center, who identified in 1980 the genes that first drew her to the study of telomeres. "The first experiment I did with those genes showed us they had located at telomeres, and that is how my interest in telomeres began," de Lange recalls.

Using the deficient mouse cells, the researchers found that the overhang remained intact even without TRF2, pinpointing ERCC1/XPF as the protein responsible.

But further analysis by Zhu found ERCC1/XPF was not only associated with telomeres in the cell, but also within the TRF2 complex. "This was a big puzzle. Here is a nuclease that can threaten telomeres when things are off, when TRF2 is inhibited, and yet TRF2 brings that protein to the telomeres," says de Lange. "So this nuclease must also have a protective role to play otherwise it wouldn't be located in the telomere protein complex."

Zhu then noticed that when ERCC1/XPF-deficient cells divided, they produced loose pieces of chromosomal material with telomeric DNA on them. De Lange speculates that these small, so-called double minute chromosomes were created when the repeat sequence of DNA found in telomeres recombined with similar sequences elsewhere on the chromosome. The role of ERCC1/XPF must therefore be to prevent these recombination events by cleaving away these mistakes. That was the protective effect: cells with the ERCC1/XPF did not produce the mutant chromosome bodies.

So, unexpectedly, ERCC1/XPF plays two very different roles at chromosome ends. Under normal circumstances it prevents errant recombinations during chromosome division. But when the telomere cap is no longer in place, ERCC1/XPF likely has a very negative effect, seeming to destroy the telomere by consuming its latch.

"It's a delicate balancing act," says de Lange. "The telomere seems to both protect itself from DNA repair factors but also, at the same time, use those DNA repair factors for its own purposes.

"Now we have to sort out what controls this nuclease, to learn how it knows when to perform which of its functions. The whole key to understanding telomeres is in understanding how this regulation occurs."

**Lonely telomere.** Telomeric DNA (green) is usually attached to chromosomes (red). But when cells without the ERCC1/XPF enzyme divide, they produce loose pieces of telomeric DNA (in box, below). It was this discovery that led de Lange and her colleagues to conclude the enzyme had more than one role.

## Aaron Diamond research team reinterprets immunity data

*Though alpha-defensins aren't produced by immune system T cells, they still have activity against HIV*

After learning that part of their interpretation of data from a September 2002 publication is inaccurate, **David Ho**, Linqi Zhang and their colleagues at The Aaron Diamond AIDS Research Center have published a "Retraction of Interpretation."

"In our follow-up studies on the blood of long-term non-progressors of HIV, we learned that alpha-defensins are not produced by CD8 T cells, as we originally concluded," says Ho. "We feel this retraction of interpretation is the right thing, the honorable thing, to do in this situa-

tion," says Ho. The retraction was published in the January 23, 2004 issue of *Science*.

The specialized CD8 T cells collected over the years from these patients are not the source of anti-viral alpha-defensins 1, 2 and 3, as Ho and colleagues proposed in the original *Science* 2002 publication. Instead, the "feeder" cells used to experimentally stimulate these blood samples are the true source of the alpha-defensins.

"There were many findings in our original publication and two main conclusions," says Ho. "One of those

conclusions has been reinterpreted; the other conclusion, that alpha-defensins are active against HIV, is true."

Ho, Zhang and their colleagues were the first to report alpha-defensins' anti-viral capabilities against HIV. Other scientific teams have subsequently reported the same results about the anti-HIV properties of alpha-defensins.

"We will continue to publish our research on alpha-defensins," says Ho. "Their mechanism of anti-viral activity is worthy of pursuit."

—Lynn Love

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