news & notes

FRIDAY, SEPTEMBER 13, 2002 | THE NEWSLETTER FROM THE ROCKEFELLER UNIVERSITY'S OFFICE OF COMMUNICATIONS AND PUBLIC AFFAIRS

TODAY'S EVENTS

Alan Aderem on the immune response

Alan Aderem, an internationally recognized immunologist and cell biologist, gives the Friday lecture today (September 13). His talk, "The Role of Toll Receptors in the Innate Immune Response," begins at 3:45 p.m. in Caspary Auditorium.

Aderem became a research associate at the Laboratory of Cellular Physiology and Immunology at Rockefeller in 1982 and from 1991 to 1996, headed the Laboratory of Signal Transduction. In 1996, he moved to the Department of Immunology at the University of Washington. Aderem is now professor and associate director of the Institute for Systems Biology in Seattle, which he co-founded with Lee Hood and Reudi Aebersold.

At Rockefeller, Aderem began a classical series of studies investigating the function of macrophages, white blood cells implicated in atherosclerosis, cancer and autoimmune disease.

A remarkable feature of the immune system is its capacity to recognize a broad spectrum of pathogens using only genomically encoded receptors, which recognize microbial targets ranging from sugar and protein to toxins and microbial nucleic acids. Although a variety of different types of receptors detect microbes, a new development in understanding pathogen recognition has come from the discovery of a family of receptors, the Toll-like receptors (TLRs) that permit many organisms (humans, mice and even flies) to specifically detect a host of microbes.

TLRs and other microbe-recognition receptors initiate signaling in phagocytes that lead to inflammatory responses specific to the type of pathogen encountered. Aderem's lab was the first to define the differential recognition of microbial patterns by TLRs and to show that this repertoire of recognition is refined by different physical combinations of TLRs.

Dinosaur ancestor's vision possibly nocturnal

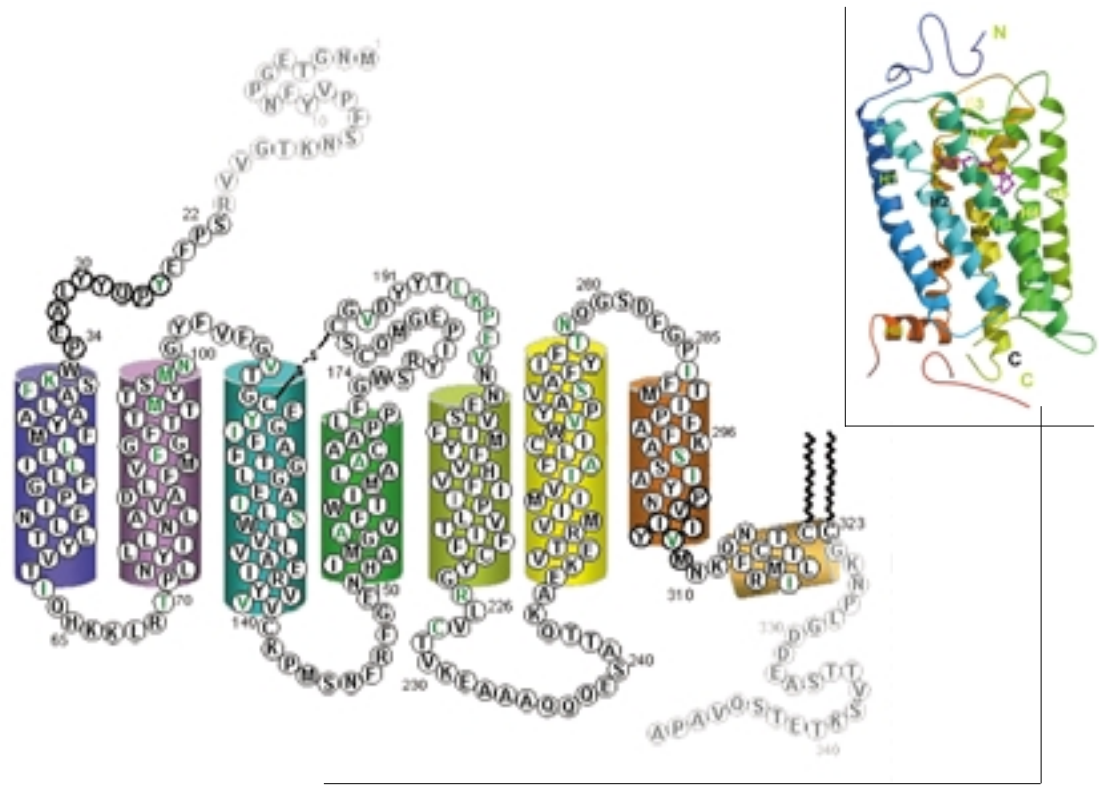
240-million-year-old protein created in test tube

Call it "Triassic Park"— with statistics, instead of amber-preserved DNA, researchers at the Howard Hughes Medical Institute at The Rockefeller University and Yale University recreated in the test tube a functional pigment that would have characterized the eyes of archosaurs ("ruling reptiles") and allowed these direct ancestors of dinosaurs to see in dim light.

The pigment, rhodopsin, was recreated based on the scientists "inferring" its protein sequence.

Their findings, reported in the September issue of *Molecular Biology and Evolution*, offer the first look at a protein that has not been seen in 240 million years, and pave the way for scientists to study how the structure and function of vision pigments — and ultimately other biologically important molecules — have changed over the course of evolutionary time.

"Visual pigments trigger the critical first step in the biochemical cascade of vision in humans and other animals and obviously were present in now extinct species," says senior author Thomas P. Sakmar, head of the Laboratory of Molecular Biology and Biochemistry.



Protein sequence of the reconstituted ancestral archosaur rhodopsin drawn as a schematic on the basis of the crystal structure for bovine rhodopsin (inset).

"Recreating the inferred visual pigments of the archosaur ancestors in the laboratory should be a first step toward a better understanding of what they could see — and not see," adds Sakmar, professor and HHMI associate investigator.

In their paper, Sakmar and his colleagues report that archosaurs may have had a class of visual pigments that would support dim-light vision. "This is consistent with the intriguing, though controversial, possibility that nocturnal, not diurnal, life histories may have been the ancestral state in amniotes, which are birds, reptiles and mammals whose embryos are protected with a fluid-filled sac," says Belinda S.W. Chang, first author and research assistant professor at Rockefeller. "We are doing further biochemical studies on this recreated pigment to clarify this issue."

Chang turned to existing databases and employed sophisticated statistical methods to infer

continued on page 2

Microbiology makes page 1

When *Nature* published a research paper by Professor Vincent Fischetti and his colleagues, postdoctoral researchers Raymond Schuch and Daniel Nelson, that identified a phage enzyme that kills the deadly anthrax bacterium, the journal showcased the article on its August 22 cover. The research also attracted international press attention, with reports on the findings appearing on the front pages of *The New York Times* and *USA Today*.

"I can't remember the last time a microbiology research paper made the cover of *Nature*, let alone the front pages of two major dailies," Fischetti says. "Microbiology is now looked upon as playing a particularly important role, both in fighting bioterrorism, as well as emerging and re-emerging infectious bugs."

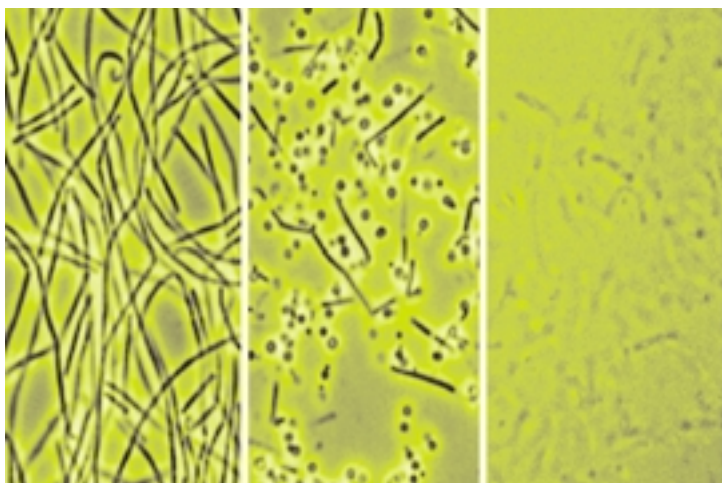
Scientific and media interest in phage enzymes is not new — it attracted attention in the early 20th century (a 1925 *New York Times* story is headlined, "The Virus that Eats Bacteria"), and phage therapy was moderately successful in the Soviet Union in the 1930s. However, bacteria develop resistance to phage as they do to antibiotics, eventually rendering the treatment ineffective.

Fischetti, however, is using phage in a new way.

Phage (for bacteriophage — "bacteria-eating" virus) has been battling anthrax and other species of bacteria for billions of years. By isolating one of their primary weapons, an enzyme called a lysin, the scientists developed a powerful new agent that can specifically target and wipe out millions of anthrax bacteria in seconds.

"We're looking at a new platform: nature vs. nature," he says. "Rather than engineering an antibiotic, we're using nature to control the environment."

Working with Rockefeller's BioImaging Resource Center, headed by Alison North, the



This series of electron micrographs shows the new phage enzyme completely wiping out a colony of *Bacillus cereus* (left to right), the cousin strain of anthrax used in this study.

Fischetti lab produced a stunning video of the phage enzyme destroying a noninfectious cousin of the anthrax bacterium.

Several TV reporters used the clip to illustrate their stories. Fischetti also e-mailed the clip to Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, who presented it to Health and Human Services Secretary Tommy Thompson and his Advisory Council on Biodefense on August 27.

To read the news release issued by the Office of Communications and Public Affairs and view the video clip, please visit: *www.rockefeller.edu/pubinfo/082102.php.*

—*Joseph Bonner*

What inspires yeast cells to divide? New findings by Rockefeller scientists shift focus to new model

Often in science a novel set of experiments comes along that forces researchers to abandon old models for new ones that better fit their observations. This is the case in a recent *Nature* report by Rockefeller University researchers, which finds that past models of cellular division in the simple yeast organism were focused on the wrong protein.

Until now, scientists thought that yeast cells began dividing into two separate cells upon the destruction of a "cyclin" protein called Clb5. But the new research shows that a related protein called Clb2 is in fact the real trigger.

"To our surprise, the current model of cyclins and cellular division in yeast does not appear to hold true," says Ralph Wäsch, a postdoctoral researcher at Rockefeller and first author of the paper. "We found that replicating cells do divide in the presence of Clb5, which means that its destruction cannot be the signal for division. What's more, we show that replicating cells cannot divide in the presence of Clb2."

In addition to providing fundamental insight into the "cell cycle," the process by which all cells from yeast to human create exact duplicates of themselves, the findings have implications for treating cancer — which is characterized by a cell cycle gone awry.

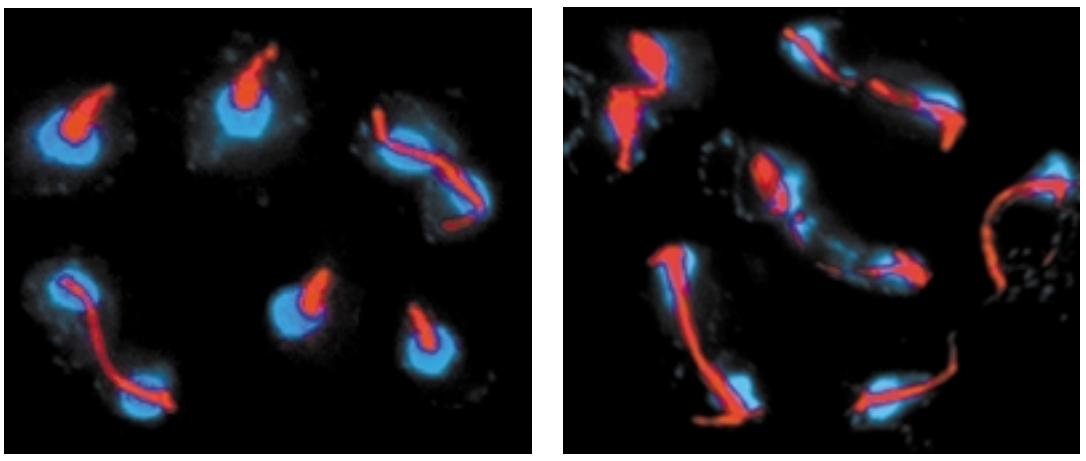
"Yeast and human cells share many of the same cell cycle mechanisms," says Frederick R. Cross, head of the Laboratory of Yeast Molecular Genetics and principal author of the paper. "Because of this and because they are easier to work with, yeast organisms are ideal models for studying how the cell cycle may normally work in humans, as well as how it might malfunction in cancer."

How cells reproduce

All eukaryotic cells (cells that contain a nucleus) must undergo some form of a cell cycle to grow and reproduce. During this process, two crucial events must occur within a cell's nucleus: replication of the DNA, called S-phase, and separation of the resulting chromosomes into two groups, called mitosis or M-phase. Completing the cell cycle are two periods of rest, which take place just before both S- and M-phase, and are called G1 and G2, respectively.

Only when the cell senses that these events have transpired without error will it exit mitosis and divide into two daughter cells. At this point, the process either begins anew, or a cell enters a state of dormancy, called G0.

How a cell moves from one phase to the next depends on periodic waves of cyclins: low levels prepare DNA for replication, higher levels trigger S-phase and mitosis, and a drastic drop in cyclin number signals the cell to begin dividing. Equally important to this process are the proteins that cyclins bind to and activate, called cyclin-dependent kinases (CDKs). Once activated, CDKs carry out the specific cellular tasks required for growth and division.



Rockefeller researchers discovered that the destruction of a protein called Clb2 triggers replicating yeast cells to divide. Normal cells can be seen in all stages of cellular division (left), whereas cells containing a mutant version of the Clb2 protein — one that cannot be destroyed — fail to divide (right).

Cancer arises when the body fails to properly regulate this process. For example, healthy cells respond to DNA-damaging agents, such as sunlight or cigarette smoke, by halting their cell cycle while the damage is repaired, or by committing a type of cell suicide called apoptosis. But cancerous cells have lost this system of checks and balances, resulting in uncontrolled cell growth, DNA damage and eventually tumors. This breakdown in the cell cycle is caused by genetic mutations that lead to abnormal quantities of cell cycle proteins, such as the cyclins.

Cellular oscillators

The latest findings also suggest a new way of thinking about a yeast cell's "oscillators." Oscillators are protein complexes that control the ebb and flow of cyclins within a cell's nucleus, thereby ensuring an orderly progression through the cell cycle. During mitosis, they signal the cell to destroy certain cyclins, which then forces it to exit mitosis and begin division. In both human and yeast cells, there are two oscillators: the Cdc20 oscillator and the Cdh1 oscillator.

Previously, scientists thought that the Cdc20 oscillator controlled chromosome separation as well as mitotic exit via elimination of Clb5, while the Cdh1 oscillator was thought to complete exit from mitosis by destroying Clb2.

But the new *Nature* report tells a different story. It shows that the Cdc20 oscillator dictates exit from mitosis via elimination of Clb2, not Clb5.

"Previous experiments showing the destruction of Clb5 to be the primary trigger for cell division were not flawed," says Wäsch. "Rather, the conclusions drawn from them were incorrect. We can now go back and reinterpret those experiments as meaning only that the elimination of Clb5 can act as a trigger for mitotic exit under experimental conditions. But we now know that the essential trigger is the direct destruction of Clb2 by Cdc20."

The researchers say that the destruction of Clb5 may instead be required for proper chromosome maintenance.

Interestingly, the results also suggest how the two oscillators may have evolved. According to the researchers, the first oscillator appears to control both chromosome separation and mitotic exit, while the second mainly oversees the break between cycles of growth and division, G1. Because G1 provides higher organisms with the ability to create different types of cells, the researchers speculate that this second oscillator may represent a necessary step in the evolution of both yeast and humans.

— *Whitney Clavin*

Dinosaur's ancestor *continued*

the most likely DNA sequences that the ancestral archosaur would have had for its rhodopsin.

"From the databases, we pulled rhodopsin gene sequences for such animals as dogs, rats, cows, birds, teleost fish, eels and amphibians. Then we aligned them," says Chang. "Using our knowledge of how these vertebrates are related to each other, the sequence alignment and a model of how often certain types of genetic changes occur over time, we calculated the most likely gene sequence."

In her calculations, she used maximum likelihood phylogenetic statistical methods.

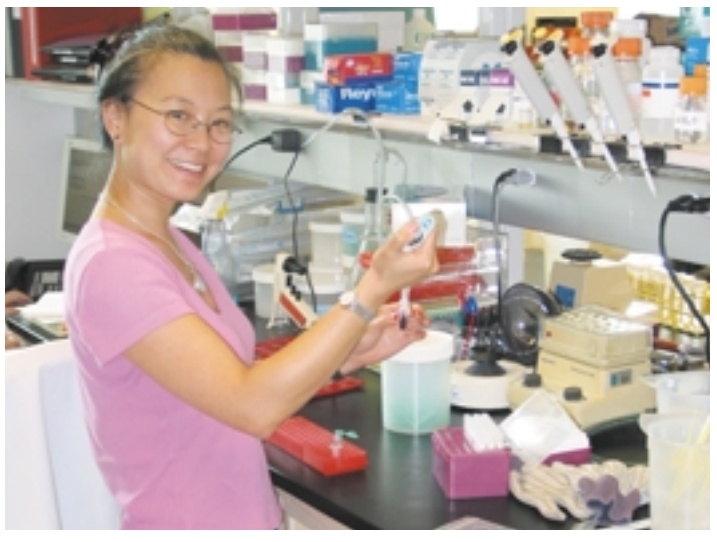
Chang and her colleagues next took the inferred DNA sequence for archosaur rhodopsin and reconstructed a gene, which they then inserted into mammalian tissue cell cultures — a standard method for producing rhodopsin in the lab. As expected, the gene instructed the cells to generate rhodopsin in the mammalian tissue.

But, did the protein structure and biological function of the artificially produced archosaur rhodopsin resemble "natural" rhodopsin? To answer this question, the researchers showed that it binds to a molecule called 11-*cis*-retinal, gives a characteristic absorption spectrum in the visible range and activates in response to light.

To see color, humans have three types of visual receptors, sensitive to red, green and blue light, but the primary molecule involved in all of these is a form of vitamin A called 11-*cis*-retinal.

"We found it does bind 11-*cis*-retinal and produces a very beautiful absorption spectrum with a maximal sensitivity at slightly red-shifted wavelengths when compared with our control in the laboratory, which is bovine rhodopsin," says Chang. "Although we don't know why the archosaur rhodopsin is shifted toward the red end of the spectrum, it is closest to the spectrum measured for bird rhodopsins."

The final piece of evidence that the researchers had produced a functional rhodopsin was that the activated form of rhodopsin triggered the rest of the signal transduction cascade in the photoreceptor cell; in other words, it interacted with the "second messenger," the G protein transducin.



Belinda S. W. Chang

"Characteristics of rhodopsin determine characteristics of vision directly, so from this we can infer things about how archosaurs actually saw at night and under dim-light conditions," says Chang. "We can infer that their night vision was, at least on the level of their rhodopsin and its activation of G protein, basically as good as mammalian rhodopsin, which is surprising, since mammals went through a nocturnal phase."

— *Joseph Bonner*

A year later, communications head looks back ... and to the future

This week Cathy Yarbrough completes her first year as Vice President, Communications and Public Affairs. When she joined the university on September 10, she had already worked for several national and international organizations such as Novartis Pharmaceuticals, the Human Genome Project, American Heart Association and Yerkes Primate Research Center.

Traditionally, News&Notes *profiles new VPs soon after their arrival. "However, I wanted to focus first on revamping* News&Notes *before being in it!" she says, jokingly. This internal newsletter now contains more articles about research — "the university's strategic driving force," says Yarbrough. An improved design, with full color photographs and scientific images, draws attention to those articles.*

In summarizing her first year, she recalls, "September 10 was a fairly routine 'first day at work'; I hit the ground running on September 11 and spent the rest of the year in what often seemed like a marathon."

Here, she recalls that second day and shares her thoughts about communications.

What was it like for you on September 11?

Unlike so many people, I was not directly affected by the tragedy. No one I personally know died or was injured. Still, I was stunned — and scared. But I had to mentally store away those fears to work, with my colleagues in Communications and Public Affairs [C&PA], on generating news updates to faculty, staff and students about the terrorist incidents, blood donations and housing arrangements for those who were stranded here due to the transportation shutdown.

What did you learn about the university on Sept. 11?

During crisis situations, public relations professionals like myself typically see the 'heart' of an organization — by how decisions are made. You get 'close to the flame and see how it burns.' The Rockefeller flame is very bright and steady.

That's because so many people here are dedicated to this university. The scientists certainly are. They're the reason Rockefeller is a stellar institution. On September 11, I was particularly impressed by Security, Plant Operations including the boiler room, the hospital and of course C&PA. Most C&PA staff stayed until late to help inform their colleagues on campus about what to do. I left for my home, then in New Jersey, at about 7 p.m., impressed with the university's spirit, but depressed of course about the loss of life that day.

Since your university education is in journalism, how did you become so interested in science?

I avoided the sciences in college, mainly because my high school science classes were dreadful. While a reporter in the early 1970s at *The Atlanta Constitution* I covered women's health. I soon realized that great science is behind great medicine and that biomedical research has advanced civilization.

Why should any institution communicate?

People identify closely with their places of work. Thus, informing them about what is accomplished where they work is good business. A robust internal communications program contributes to employee morale, productivity and retention. And, open and speedy communications helps reduce rumors. In the absence of information, people tend to speculate. That's why Tom Sakmar's interview on the trustees' decision about construction (see *News&Notes*, May 24) was so laudatory.

Communications to the external world — the 'public' — is essential too. It helps buy 'good will.' People tend to support organizations about which they know. George Goodwin, a public relations pro and former Pulitzer Prize-winning reporter, told me in 1983, 'people are *down* on what they are not *up* on.' His advice helped me cope with the first animal rights campaign targeted at the Yerkes Primate Center where I then worked. The public, including the news media, were not *up* on or informed about the rationale for animal research and thus were vulnerable to animal rightists' misinformation campaigns. That's why it was and is still important to engage in public outreach via the news media, tours, lectures and community relations.

At one time or another, bad luck afflicts most institutions, even great ones, in the form of events such as a major protest, an explosion that injures or kills employees or visitors, or accusations of misconduct. An organization needs 'good will' even if it hasn't done anything wrong. If good will exists, people will give the institution the benefit of the doubt — allow it time to gather and communicate the facts about the situation.

Cathy Yarbrough says robust communications generates public "good will."

In addition to 'buying' good will, effective external communications contributes to recruitment of patients for clinical trials, recruitment of students, faculty and staff and fundraising.

continued on page 4

Rockefeller researchers provide first functional evidence for mammalian pheromone receptors

Pheromones — chemical signals that influence social and reproductive behaviors — have been studied since the 1950s, but the molecules in the mammalian nervous system that actually detect pheromones have remained elusive.

Now, a research team led by Associate Professor Peter Mombaerts and graduate student Karina Del Punta provides the first functional evidence for molecular receptors for pheromones in mammals. Their findings contribute to understanding the brain's function in orchestrating social and reproductive behavior as well as helping explain why sexual reproduction typically occurs only within a species. Ultimately, the findings may explain how species form.

In the September 5 issue of *Nature*, Mombaerts, Del Punta and colleagues at Rockefeller, University of Maryland School of Medicine and Monell Chemical Senses Center report significantly less aggressive and sexual behavior in laboratory mice engineered to lack a particular cluster of genes that previous research from the Rockefeller lab had linked to pheromone detection. They also show that the nerve cells of mutant mice are unable to detect certain pheromones. These pheromone receptors are found in the lining of the animals' vomeronasal organ (VNO), a part of the olfactory system thought to specialize in the detection of pheromones.

"We know that the VNO is involved with pheromones because if it is surgically removed from the animals, several abnormalities in their mating behavior and aggression patterns arise," says Del Punta, the paper's lead author. "We found that deleting the cluster of genes that produce pheromone receptors replicates some aspects of the surgical removal of the VNO in these animals."

Normally, nursing females are aggressive toward other lab mice that intrude or invade their nest. The nursing mutant females, however, were less aggressive when confronted by an intruder: there were fewer attacks, the first attack was delayed, and the total time spent attacking the invader was much less, when compared to the behavior of the females' normal counterparts in the same test situation.

The researchers studied four behaviors in the male mutant mice, and found two of these affected in the mutant mice. A first assay tested if male mutants emit 70 kilohertz ultrasounds when first exposed to a female. Removing the VNO reduces this behavior, but in the mutant mice it was unaltered. Second, male aggression against other males was also unchanged in the mutants.

A third test focused on male-male sexual behavior. Socially inexperienced mice are often observed to exhibit sexual behaviors toward other males until they become more experienced and learn to distinguish males from females. Socially inexperienced mutant mice, surprisingly, made fewer sexual advances toward males, suggesting that the mutants are better at distinguishing between sexes without prior experience, or that their sex drive is reduced.

The fourth behavioral test analyzed sexual behavior of males toward females, also dependent on a functioning VNO. Compared to their normal counterparts, the mutant males tended to mount females fewer times, and the more they were exposed to females, the less they mounted.

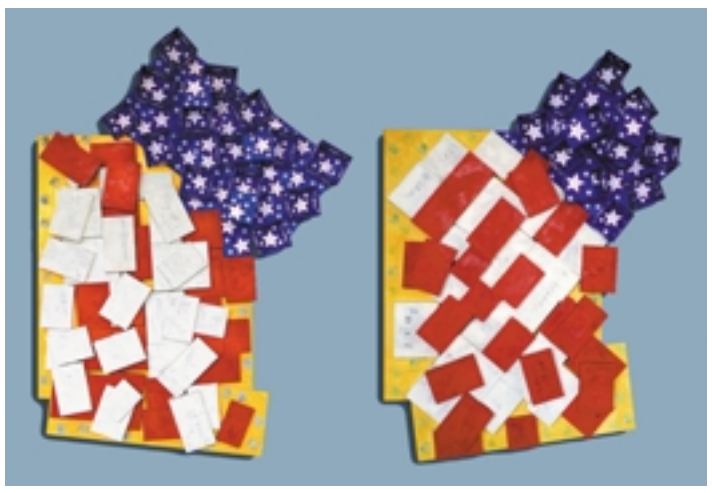
Whether a functional VNO is present in humans is controversial. The role of pheromones in human behavior also is not clear. The Mombaerts team had shown earlier that the human genome harbors five putative pheromone receptor genes that could be functional. The mouse genome, by contrast, has at least 140 receptor genes of this type, and the mutant strain of mice described in the *Nature* paper misses precisely 16 of these.

"For the first time our work has shown that there are pheromone receptors in mice. This, in turn, will stimulate research in functional characterization of the counterparts of these genes in humans," says Mombaerts.

For more information visit http://www.rockefeller.edu./pubinfo/090402.php.

— *Joseph Bonner*

Art reflects on 9/11



"South Tower, North Tower," a commemorative work of art reflecting on the September 11 attack on the World Trade Center, will be on display in the Abby Aldrich Rockefeller Dining Room beginning the week of September 16. Currently on display in the Weiss Research Building lobby, "South Tower, North Tower" was conceived in Paris by New York City-based artist Robert Lambert.