

## Good riddance for bad cholesterol

Scientists ID gene that regulates 'bad' cholesterol in mice

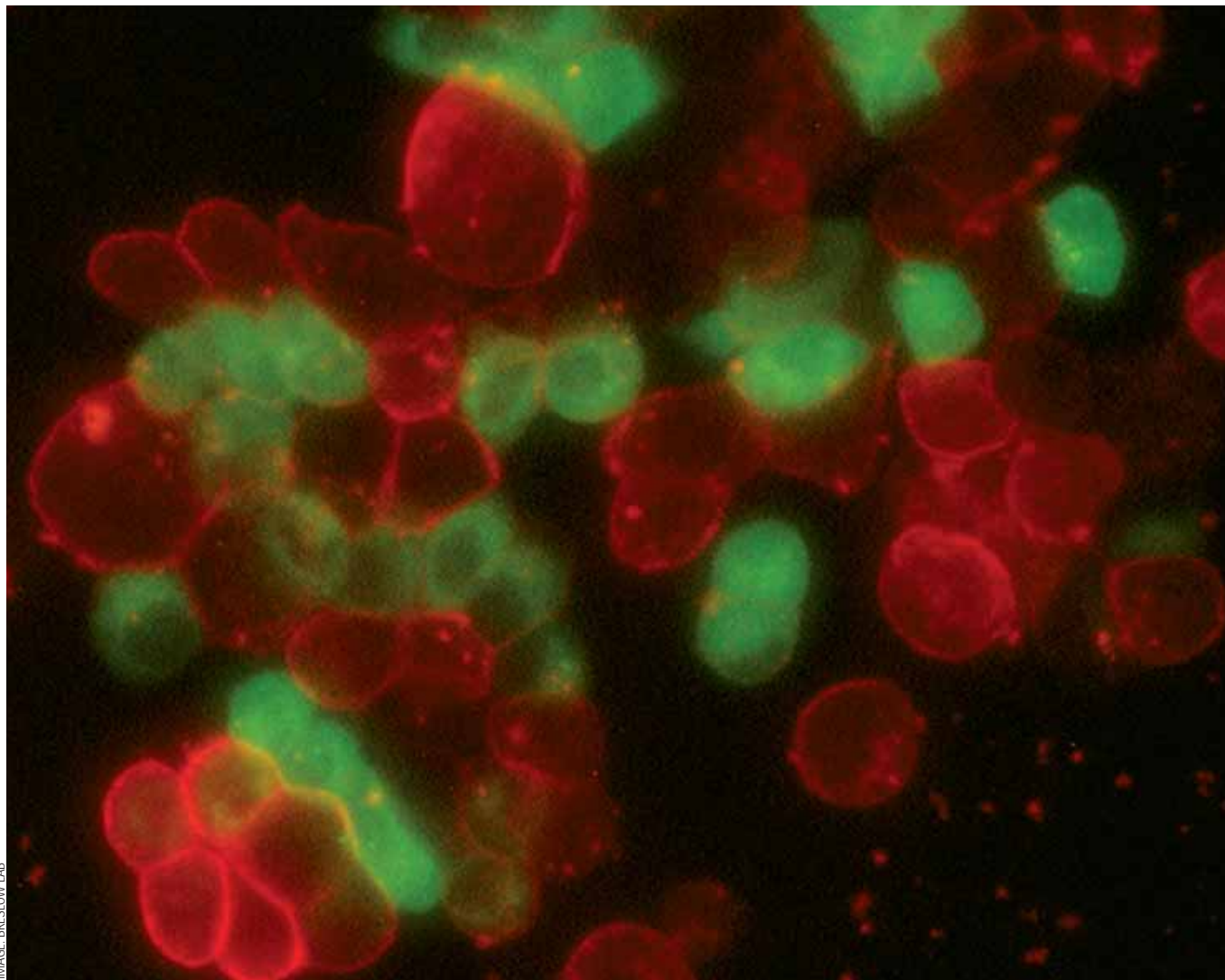
BY TIEN-SHUN LEE

Even cholesterol is bound by the basic laws of economics. When the inventory of fatty, artery-clogging molecules in your bloodstream is too high — that's the case for 105 million Americans — the best options are to either reduce supply or increase demand.

Dietary changes, exercise and statin drugs are ways to lower the bloodstream's supply of cholesterol. Now, Rockefeller researchers are pursuing another approach — getting rid of cholesterol in the bloodstream by increasing demand for it in the liver.

A gene recently identified in mice by Jan L. Breslow's Laboratory of Biochemical Genetics and Metabolism

*continued on page 3*



**Watching one's cholesterol.** A cholesterol regulating gene called Pcsk9 (*green*) prevents LDL cholesterol (*red*) from binding to the surfaces of liver cells. If drugs were developed that blocked the activity of Pcsk9, they could cause "bad" cholesterol to be absorbed by the liver and removed from the body.

## A window on the brain

*New technique affords a view inside the brains of mice as they sense odors*

BY JOSEPH BONNER

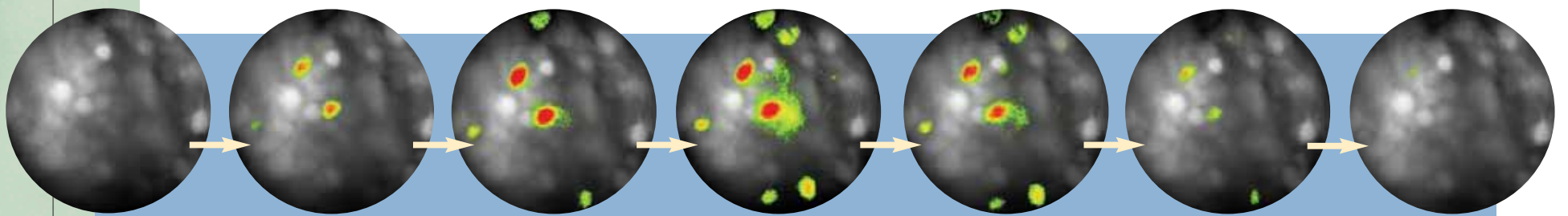
"The anatomical definition is superb," says Rockefeller University's Peter Mombaerts, watching a computer monitor as tiny blobs of green and red appear, then intensify over a black-and-white image of a mouse brain. "We can identify each of the structures beautifully."

Mombaerts and research associate Thomas Bozza are reviewing a movie of the brain of a living mouse as it is exposed to odors. Each time the mouse gets a whiff of a chemical called hexanal, which smells something like freshly cut grass, specific sections of the mouse's brain light up. Mombaerts and Bozza are watching as mice, quite literally, process scent.

Making this possible is a technique, developed by Mombaerts, Bozza, and colleagues at Boston University, to monitor the flow of information from one brain cell to the next. The scientists can essentially see the tiny bursts of light as messages pass from one neuron to the next. The technique allows researchers to see 10 to 20 percent of the 2,000 structures in the brain's olfactory bulb, which receives smell information from the nose (the other structures are too deep within the mouse brain to be seen).

The system promises to advance research on how animals, as well as humans, sense odors, and it may also help scientists develop new drugs that target a family of

*continued on page 4*



**Scent of a neuron.** A series of images shows what happens in a mouse's brain when the animal processes odors. As information is transmitted to the olfactory bulb, the section of the brain responsible for receiving smell information from the nose, clusters of neurons become active (*red and green areas, above*).

IMAGES: MOMBAERTS LAB/BOSTON UNIVERSITY



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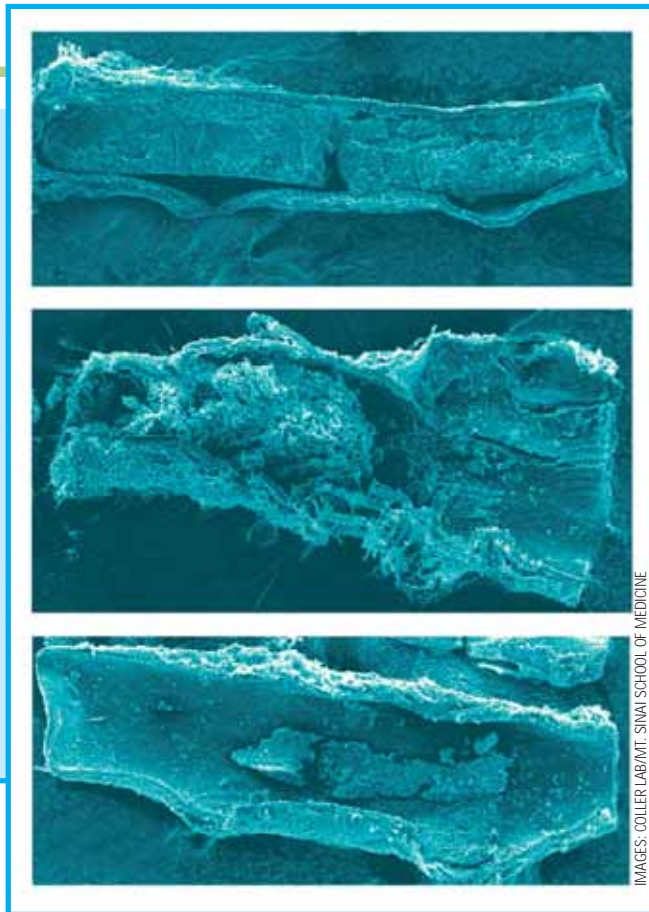
**Fine-tuning fibrinogen.** The apothecary for treating heart disease contains several separate classes of drugs to deter the potentially fatal clots that trigger heart attacks and strokes: anti-coagulants that prevent clots from forming, anti-platelet medication that prevent platelets from clumping, and dissolving agents that aid in breaking up existing clots. Now, Rockefeller University scientists have produced an antibody that acts on all three pathways.

The molecule, a monoclonal antibody called 7E9, binds to a special region of a blood protein known as fibrinogen, which is responsible for forming the platelet clumps and fibrin clots that choke off the blood vessel. "In normal health, platelets and fibrinogen in your blood do not interact. But in every instance of abnormal clot formation, the interaction of platelets and fibrinogen plays a role. If you give 7E9, you globally

decrease the interacting capacity of fibrinogen," says first author Marketa Jirouskova, a research associate in Barry Collier's Laboratory of Blood and Vascular Biology.

In a plenary paper published in the journal *Blood*, Jirouskova compared how readily blood clots formed in normal mice and mice treated with the 7E9 antibody. 7E9 dramatically reduced the formation of dangerous clots. "Dr. Jirouskova's data with 7E9 provide important new insights into the mechanism of clot formation and provide exciting new potential therapeutic targets," says Collier, the university's David Rockefeller Professor.

**Beat the clot.** In experiments designed to test clot formation in the arteries of mice, those treated with the new 7E9 antibody (*bottom*) fared better than control mice (*top*) and mice completely lacking in fibrinogen (*middle*), the protein responsible for sticky, fibrous coagulation of the blood.



IMAGES: COLLIER LAB/MT SINAI SCHOOL OF MEDICINE

**Proteins that stitch skin.** The skin epidermis acts as Saran Wrap for the body, sealing microbes out and body fluids in. Now, new research from Elaine Fuchs' Laboratory of Mammalian Cell Biology and Development sheds light on how the individual cells in this flexible wrapper adhere to one another.

To form a sheet of skin, epithelial cells make so-called adherens junctions at the points of stable cell-cell contact. At these junctions, the cells assemble linear cables of actin polymers, which are zipped together with the help of a protein called alpha-catenin. The new research, led by postdoc Agnieszka Kobiela, shows that a

later. Mice treated with a fibrin-depleting drug derived from snake venom also showed

improvement: demyelination was greatly reduced compared to untreated MS mice.

These results suggest that fibrin could potentially be a target for treating MS and are consistent with the Strickland lab's earlier studies showing that fibrin interferes with new myelin formation during regeneration of peripheral nerves.

*Proceedings of the National Academy of Sciences*, April 2004

**Cracking the neuron code.** Like a telegraph that transmits the dots and dashes of Morse code, the body's neurons transmit pulsed electrical signals with distinctive rhythms. Scientists have long suspected that the frequency and rhythm of this neuron firing translates into meaningful messages within the brain, but so far the meaning has remained a mystery. Recently, George Reeke, head of the Laboratory of Biological Modelling, and research associate Allan Coop devised a new method for analyzing patterns of neuron firing.

Unlike previous methods, which divide a neuron's recorded activity into regular, clock-like intervals in which each neuron either did or did not fire, Reeke and Coop's new method is based on the measured times between firings. Information statistics are calculated from a theoretical distribution that is fit to these data. This has several advantages: it eliminates the need to impose a clock on the firing record, it greatly reduces the amount of data otherwise required, and it gives more accurate results for neurons firing in a regular pattern.

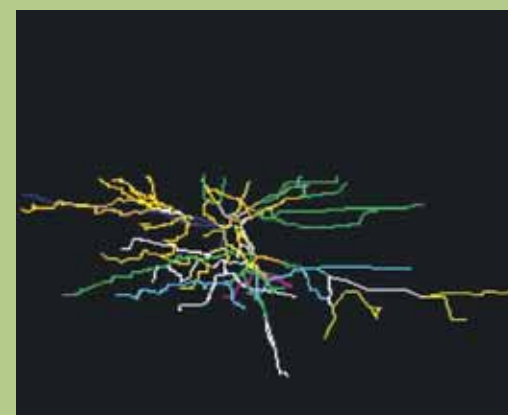
*Neural Computation*, May 2004

**How stress rewires the brain.** Studies in rats, carried out in Bruce McEwen's Laboratory of Neuroendocrinology, have found that high levels of stress may cause atrophy to certain neurons in an area of the brain called the medial prefrontal cortex, a section that in humans is often implicated in post-traumatic stress disorder. The cells, known as apical dendrites, became 20 percent shorter and had 17 percent fewer branches after the rats were subjected to stressful conditions for a 21-day period (*see images, below*).

The scientists suggest that such cellular changes may impair the ability of the medial prefrontal cortex to respond to hormones released in stressful situations. Further studies with this experimental model may yield clues to the structural basis for post-traumatic stress disorder in humans.

The research was done in collaboration with colleagues at Mount Sinai School of Medicine and the National Institutes of Mental Health Center for Fear and Anxiety. McEwen is the university's Alfred E. Mirsky Professor.

*Neuroscience*, March 2004



IMAGES: MCEWEN LAB

**A brain lapse.** Computer-generated images of brain cells, derived from Bruce McEwen's data, show the difference between normal rat neurons (*left*) and the neurons of rats exposed to stressful conditions (*right*). The stressed-out rats had shorter branches and fewer of them, which the scientists suggest may impair their ability to respond to stress hormones.

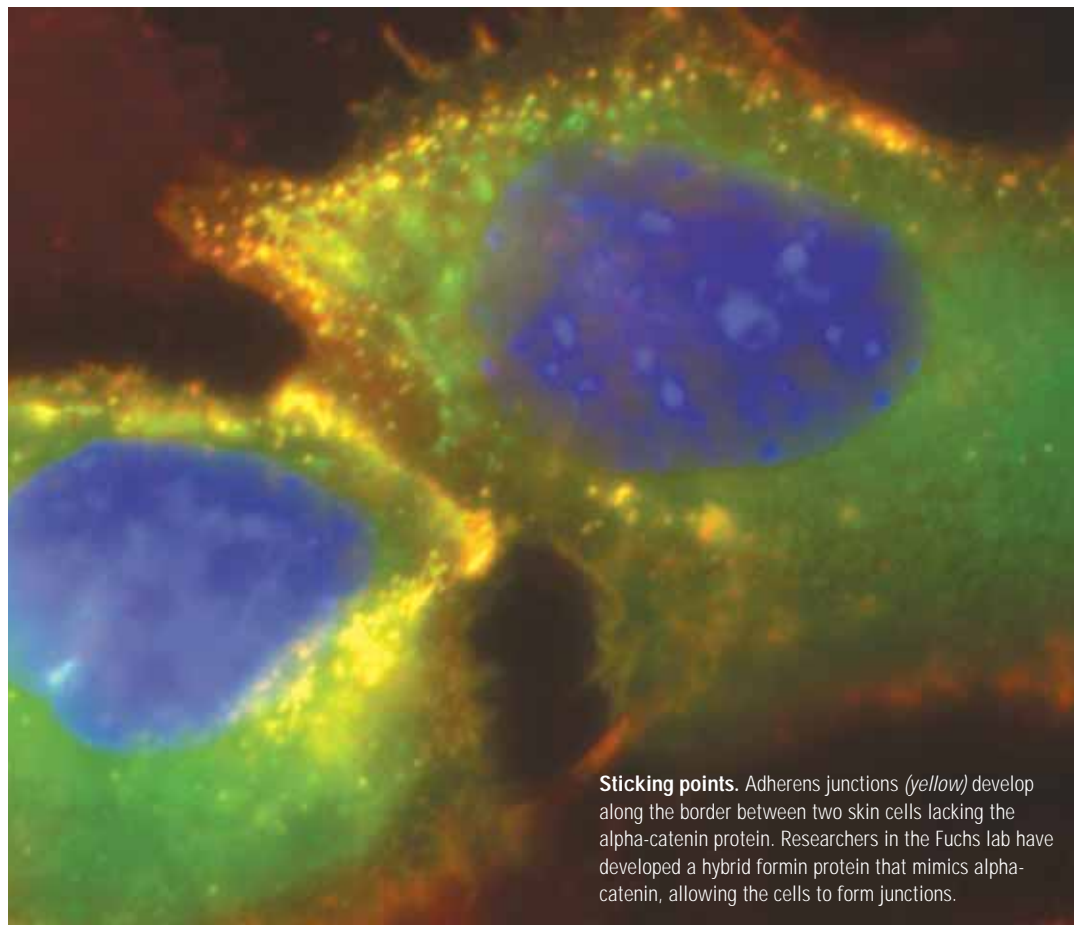


IMAGE: FUCHS LAB

**Sticking points.** Adherens junctions (*yellow*) develop along the border between two skin cells lacking the alpha-catenin protein. Researchers in the Fuchs lab have developed a hybrid formin protein that mimics alpha-catenin, allowing the cells to form junctions.

protein called formin-1 binds to alpha-catenin to initiate the assembly of actin cables. In cells that lack alpha-catenin, the researchers found that a hybrid protein — composed mostly of formin-1 with only a small amount of alpha-catenin — rescues their ability to make contacts (*see image, above*).

Fuchs's findings, which show that formin-1 can regulate actin polymerization, raise the interesting possibility that actin polymerization may also be required for limb development, a process in which formin-1 is also known to play a role. Fuchs is the university's Rebecca C. Lancefield Professor and investigator at the Howard Hughes Medical Institute.

*Nature Cell Biology*, January 2004

**New ideas about MS.** People with multiple sclerosis have severe problems with nerve signaling because they are missing myelin, an insulating material that normally forms a sheath around nerves. Mice manipulated to have an MS-like disease typically die a few weeks after birth because myelin breaks down within their brains and spinal cords, causing paralysis.

In a recent study, Sidney Strickland, head of the Laboratory of Neurobiology and Genetics, and colleagues found that MS mice with lower levels of fibrin, a blood clotting factor, fare much better. When MS mice were mated with mice lacking a gene for making fibrin, the offspring showed less myelin degradation and less inflammation around their nerves. They also lived longer and developed symptoms of MS



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# The rise of telomeres

## Rockefeller scientist proposes a theory of how telomeres evolved from tiny loops at the ends of chromosomes

BY RENEE TWOMBLY

“As scientists get older, they often start thinking about evolution or decide they have to figure out the brain,” says **Titia de Lange**, head of Rockefeller’s Laboratory of Cell Biology and Genetics.

de Lange is starting to think about evolution.

Her recent opinion piece, published by *Nature Reviews*, constructs a framework for the evolution of one of the cell’s most mysterious and complex systems, telomeres, which de Lange has spent her career studying.

Telomeres are specialized protein–DNA complexes that cap the ends of chromosomes. Like the plastic sleeves that stop shoelaces from unraveling, they protect the sequences that are needed for DNA to replicate when cells divide. Telomeres are enormously complicated machines made up of specialized DNA, an enzyme called telomerase, and protein complexes that interact with DNA. They function to regulate the lifespan of a cell by shortening with each cell division until they become too small to serve their function and cause the cell to cease dividing.

“But things started out very simply,” de Lange says. She suggests that tiny structures called telomeric-loops (“t-loops”), which she and her collaborators discovered four years ago, are actually remnants of the original telomere system that served to protect the ends of the first linear chromosomes found in early microorganisms. The investigators have shown that without these little loops, cells mistake the exposed chromosome ends for sites of DNA damage and when they attempt to repair them, the cells die.

When eukaryotic cells — those with

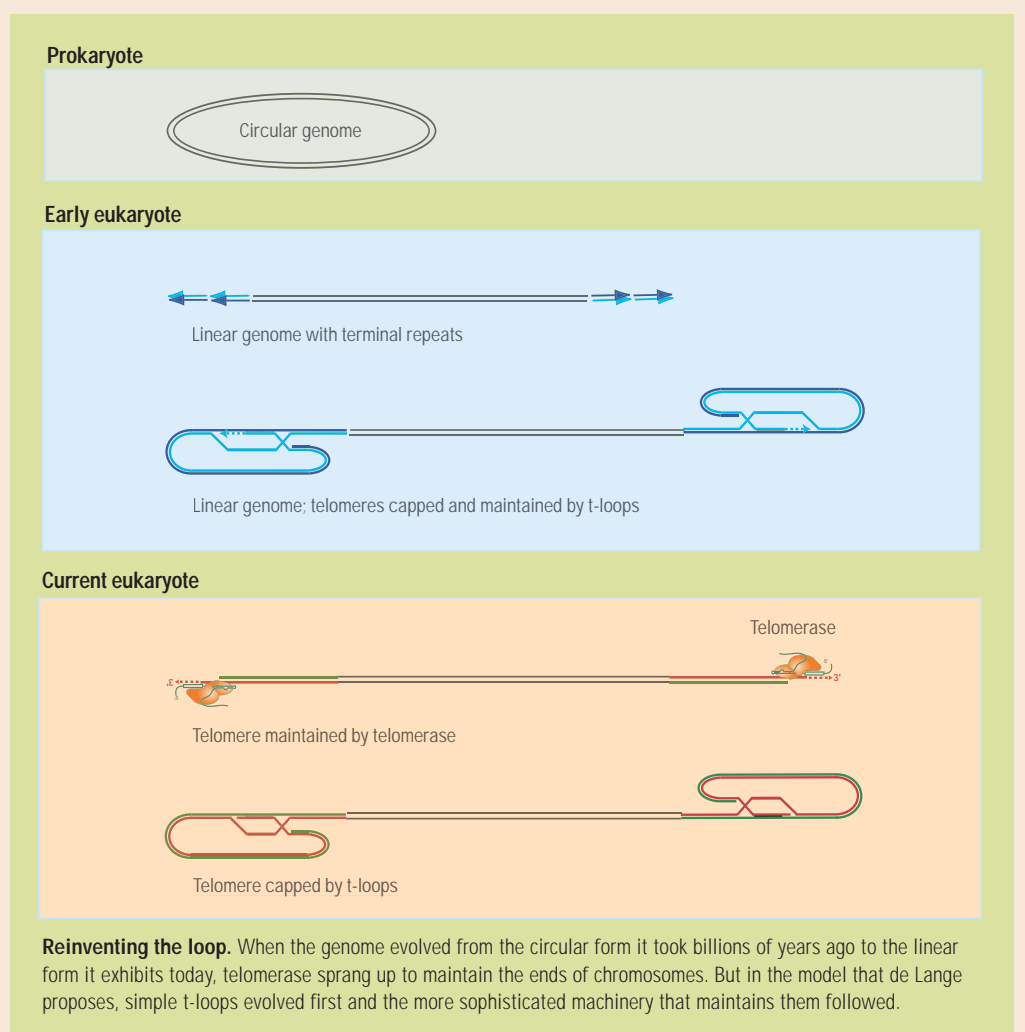
distinct nuclei — first developed about 1.8 billion years ago, their chromosomes evolved to become linear in shape, rather than circular, as they were in prokaryotic cells, which lack nuclei. Many biologists have theorized that telomerase was born at the same time in order to protect the newly exposed ends of linear chromosomes.

But de Lange says t-loops may have existed even before telomerase evolved. “The eukaryotic t-loop looks a lot like a structure that could have been formed in prokaryotes before eukaryotes evolved,” she says.

If the chromosomes of a microorganism, such as the bacterium that gave rise to eukaryotes, were linear, a t-loop could easily be formed from just a few repeats at the end of the chromosome, de Lange says. All the enzymes to make t-loops were already available; they were used for regular DNA replication in bacteria. “When *E. coli* is replicating its DNA, occasionally the newly synthesized fork collapses, leaving the end of the new DNA hanging out of a half-replicated *E. coli* genome.”

*E. coli* has a mechanism to deal with this. Enzymes take the extruded DNA and reinsert it back into the genome. This reaction is similar to the formation of a t-loop. “Through regular DNA synthesis, you have the chance to extend the end of the inserted DNA in the t-loop, just as the telomerase enzyme does,” de Lange says. “All you need to get this primitive telomere system to work are a few repeats at the end of the linear DNA.”

As proof of this notion, de Lange points to several proteins that currently act at telomeres that have evolved from the



enzymes in *E. coli* that are involved in replication restart events. There are also prokaryotic relics of this system — de Lange calls them living fossils — in which linear chromosomes have repeats of varying sequences and sizes at their ends. These living fossils survive with linear chromosomes because they have miniature t-loops, de Lange says.

“What I’m suggesting is that the first eukaryotes had t-loops made by these replication enzymes, and that’s all they needed,” says de Lange, the university’s Leon Hess Professor. “Later, telomerase arose from evolutionary pressure to select cells that came up with the more elegant solution we have today.”

Not only can telomerase make new

telomeres where none are present, but it can ensure that all chromosome ends have the same sequence. “In this early t-loop evolution model, every end could have had a different sequence, a form of telomere anarchy. When you have the same sequence at all ends — in most eukaryotes it’s TTAGGG — you can evolve proteins that specifically bind to that sequence,” says de Lange.

“Now you can start regulating the whole thing, and begin fussing over events, building a bureaucratic committee of proteins that bind to the TTAGGG sequence and help regulate how telomeres behave. This is what eukaryotes like to do: control things through the use of large committees.”

## Good riddance for bad cholesterol *continued*

suggests that increasing the number of LDL cholesterol receptors in the liver can help eliminate more LDL, the “bad” form of cholesterol that is a risk factor for heart attacks and strokes. LDL receptors snatch up LDL into liver cells, depleting the bloodstream’s inventory of it.

It was Kara Maxwell, a graduate student in Breslow’s lab, who first identified the LDL-regulating gene. Her initial experiment, which compared gene activity in mice that were fed normal diets to mice that were fed high-cholesterol diets, found that the previously unknown gene, now named Pcsk9, was expressed at a much lower level in the mice eating high-cholesterol food.

To find out what Pcsk9 does, Maxwell inserted the gene that codes for this protein into a virus that targets the liver and injected the virus into normal mice, where it made Pcsk9 protein inside liver cells. The blood vessels of the mice were quickly flooded with cholesterol; four days after injection, the animals’ LDL levels were five times the normal level, and their levels of total cholesterol in the bloodstream had doubled.

Maxwell suspected that the Pcsk9 protein was raising LDL levels by decreasing the number of LDL-snatching LDL receptors on the surface of liver cells. To test this hypothesis, she injected the Pcsk9-carrying virus into a strain of mice that do not have LDL receptors because of a genetic defect. Their cholesterol levels did not change — with no LDL receptors to eliminate, there was no room for further increase in LDL cholesterol.

Another experiment, in which Maxwell injected Pcsk9 into normal mice, confirmed that the protein reduced the number of LDL receptors in their livers.

“LDL receptors are the main way of getting LDL out of the blood,” says Maxwell. “Knowing that Pcsk9 modulates LDL receptors is critical to our understanding of how LDL levels are regulated.”

The results of the mice studies, published last month in *Proceedings of the National Academy of Sciences*, are

highly relevant to humans because mutant forms of the Pcsk9 gene have been linked to people with one form of autosomal dominant hypercholesterolemia, a group of genetic disorders characterized by excessive levels of cholesterol in the bloodstream. (This finding, discovered last year by a group in France, did not identify the function of Pcsk9.)

“If the same mechanism Maxwell found in mice pertains to humans, and we could inhibit Pcsk9 with drugs, this should increase the number of LDL receptors on cells and in this way lower LDL levels in the blood. This might offer another therapeutic approach to the high blood cholesterol levels that are a major risk factor for heart disease,” says Breslow, the university’s Frederick Henry Leonhardt Professor and a former national president of the American Heart Association.

High levels of LDL cholesterol in the blood lead to heart attacks because the waxy LDL molecules build up inside the walls of arteries, causing damage to blood vessels and leading to clots that block the flow of blood to

the heart muscle.

“The question is, is your cholesterol at the right level?” says Breslow.

“There are a huge number of people that fall into the category in which their LDL should be lowered. A lot can be done with diet and exercise but many people also need drugs.”



Pursuing cholesterol. Maxwell (left) and Breslow.

PHOTO: ZACH VEILLEUX

# How leptin rewires the brain

## Study sheds new light on how the fat hormone works to control appetite

BY REBECCA PERL

It's been nearly 10 years since Rockefeller's Jeffrey Friedman discovered leptin, a hormone found in fat tissue that's critical to regulating weight. Yet despite a wealth of clinical data from patients who have undergone treatment with leptin in The Rockefeller University Hospital, scientists understand very little about how the hormone actually works.

Now, in research published in the April 2 issue of *Science*, Shirley Pinto, a postdoc in Friedman's Laboratory of Molecular Genetics, has discovered that leptin affects both the architecture and function of neural circuits in the brains of mice, changing the brain's wiring by controlling the inputs and outputs of brain cells that regulate feeding behavior.

Scientists know that brain maps of cells can be quite plastic. Pinto, whose scientific training is in the area of learning and memory, wanted to know if the same was true when it came to feeding behavior. "Feeding turns out to be a really good model to test plasticity because it's much simpler to study than the system that controls learning or memory," she says.

For her research, Pinto used a strain of mice that lack leptin; they grow to twice the size of normal mice and contain five times the fat. Six hours after administering leptin to these obese mice, changes could be seen in the synaptic connections between brain cells. Forty-eight hours later, they were eating less and in 12 days they were beginning to lose weight.

Two sets of neurons, called NPY and POMC, in particular were affected. Both are found in the hypothalamus, an area of the brain that controls appetite. NPY stimulates food intake and increases body weight while one of the active products of POMC, a peptide called alpha-MSH, has the opposite effect. Pinto and her colleagues discovered that leptin changes the number of connections that either excite or inhibit NPY and POMC neurons in the hypothalamus.

In other words, leptin inhibits NPY neurons that encourage the animal to eat and reserve energy and, at the same time, activates POMC neurons that curtail feeding. And it does this by altering the synaptic inputs of these cells — the points where the cells connect and communicate.

"This is a very dynamic effect that's quite dramatic and somewhat surprising," says Friedman, a Howard Hughes Medical Institute investigator and the university's Marilyn M. Simpson Professor. "In response to leptin, the cells create new connections."

In addition, the researchers showed that leptin

**Appetite adjustment.** Two kinds of neurons, POMC (green) and NPY (red), respond to leptin by changing the strength and number of their synaptic connections. Rockefeller researchers have now shown how these structural changes impact feeding behavior in mice.

alters the electrical activity of the connections between these neurons and the rest of the brain — not only does the structure of the brain change, but so do the patterns by which it operates.

Obesity is now the second leading cause of death in the United States, according to the Centers for Disease Control and Prevention in Atlanta, and it's associated with more than 400,000 fatalities a year — second only to tobacco as a preventable cause of

death.

Treatment with leptin reduces weight in some individuals but not in others. So learning more about the hormone's mechanism of action could be critically important in understanding why some people do not respond to the hormone. "The malleability of these feeding circuits by leptin suggests the possibility that the brain's wiring may be different in lean versus obese individuals," Friedman notes.

## A window on the brain *continued*

molecules in the brain known as G protein-coupled receptors (GPCRs). About a third of drugs on the market today, from Zyprexa (for schizophrenia) to Claritin (for allergy relief), act on GPCRs.

"Look," Bozza says, pointing to his monitor, "it's easy to see which specific parts of the olfactory bulb are being used as the mouse responds to the odors."

Mombaerts, who is head of the Laboratory of Developmental Biology and Neurogenetics, and Bozza collaborated with Boston University researchers John P. McGann and Matt Wachowiak. The mouse strain used is genetically modified to produce a molecule called synapto-pHluorin in its olfactory sensory neurons. The molecule is a fusion of a pH-sensitive green fluorescent protein — the same type used by many scientists to track gene activity in cells — and a nerve cell protein called VAMP2. Bozza developed the mouse strain to study olfactory physiology in a live animal.

The researchers anaesthetized the mice, then surgically thinned their skulls until they were virtually transparent. They then placed the mice under a microscope and shined blue light — the wavelength that causes the green fluorescent protein to glow — onto them. The microscope

snapped a series of digital images through each mouse's skull as a nozzle sprayed short bursts of hexanal nearby.

The initial imaging was done by Wachowiak at Boston University. Bozza is reproducing the system in the Mombaerts lab in the Bronx building.

The technique allows the researchers to return to the same mouse repeatedly to track changes in brain activity. This option is not available with other imaging techniques because the animals must be euthanized before their brains can be analyzed.

(Anticipating a wide demand, the genetically engineered mice have been sent to The Jackson Laboratory in Bar Harbor, Maine, which will distribute them to researchers later this year.)

The olfactory system is very suitable for this type of imaging because of its unusual organization, Mombaerts explains. Each neuron releases information into the synapse through the cell's structure called an axon. "Several thousand axons, all of the same specificity, terminate in the same area of the brain, so the density of synapses is extraordinarily high. That's probably why we can see the signals so clearly," says Bozza.

To understand the sense of smell, scientists relate

molecules, called ligands, to odorant receptors, which are either stimulated or blocked by the ligands. Currently, scientists have identified only a handful of ligands that bind to odorant receptors in mice, rats and people.

"Ideally, we would like to have an enormous data set with 100,000 chemicals and 1,000 odorant receptors — a hundred million combinations — and figure out exactly at a given concentration what receptor is stimulated by what odorant," says Mombaerts. "At that point, we will be able to understand the sense of smell because we will be able to predict the quality of an odor, which, apart from a few exceptions, no chemist can do now."

The next step is to transfer that knowledge to drug developers who need better data about the locations of specific receptors in order to design drugs that target them. Ligand-less receptors are known as orphans in the pharmaceutical industry. The technique of visualizing the brain as it senses odors also may help clarify the roles these orphan receptors play in many body processes.

"As we find more ligands and better understand the structure and function of GPCRs, in the long run that's going to be useful for drug development," says Mombaerts.

IMAGE: FRIEDMAN/LAB