

## GENETIC DISEASES

# Mutant Mice Mimic Human Sickle Cell Anemia

In this age of genome research, a new disease gene seems to be discovered almost every week—each one carrying its own promise of a cure. But even as researchers bag more and more of the genes at fault in hereditary diseases, sickle cell anemia stands as a sobering reminder that just identifying a mutant gene doesn't necessarily mean the problem is solved. The mutation that causes this painful, debilitating, and eventually fatal disease has been known for 50 years, yet there is no cure and few effective treatments for the hundreds of thousands of people around the world born with the condition each year. But new results described in this issue may help change that.

On pages 873 and 876, two teams, one headed by Tim Townes of the University of Alabama, Birmingham, and the other by Eddy Rubin at Lawrence Berkeley National Laboratory in Berkeley, California, report that they have created mouse models of sickle cell anemia that closely mimic the human disease. As adults, these mice make only the disease-causing, mutant form of hemoglobin, which clumps together when it is

Einstein College of Medicine in New York City, the new mice will be "indispensable" for testing drug and gene-therapy strategies for controlling the disease. Franklin Bunn of Harvard Medical School in Boston, an authority on hemoglobin diseases, agrees, calling the models "a critically important advance."

Until recently, researchers studying sickle-cell disease and looking for new therapies had to conduct their experiments on red blood cells from sickle-cell patients. But all the complex tissue interactions that figure in the disease couldn't be studied in the test tube, nor could the full range of possible treatments be tested. "We obviously needed an animal model," says Townes.

To try to create such models, several research teams in the late 1980s and early 1990s introduced the mutant gene that causes sickle-cell disease into mice, although their efforts weren't completely successful. The mutation underlying the condition is a change of one base in the gene that codes for  $\beta$ -globin, a protein that combines with its partner,  $\alpha$ -globin, to make up the hemoglo-

blunts the sickling effect of the mutant human gene.

Some groups partially overcame that problem by using a "supersickling" version of the human  $\beta$ -globin gene with several mutations. It produces a hemoglobin that induces sickling even in the presence of mouse

**"Having more than one model is a tremendous advantage."**

**—Marie Trudel**

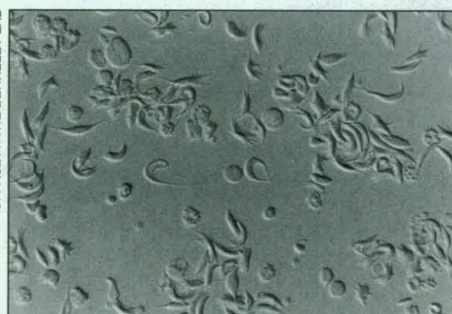
globins. While those mice were useful for studying some aspects of the disease, some researchers became convinced that "we just can't have any mouse globins at all, to make the right model for sickle-cell disease," says Mohan Narla, a member of the LBNL team.

For their models, then, both the Townes and Rubin teams decided to eliminate completely the mouse  $\alpha$ - and  $\beta$ -globin genes, which make hemoglobin in adult mice, in addition to introducing a complement of human genes including the sickling mutant form of human  $\beta$ -globin. Chris Pászty, then a postdoc with Rubin and Narla, and Tom Ryan, then a postdoc with Townes, both set out to do this, unbeknownst to each other.

By early 1995, Ryan had produced "knock-out" mice that were missing the  $\beta$ -globin genes, while Pászty had succeeded in eliminating the  $\alpha$ -globin genes. When the two groups learned of each other's work, Pászty recalls, it seemed "pointless to continue" to make mice the other team had already produced. They shared their knockout mice, and each team bred the two knockouts with each other, then bred the products of those crosses with transgenic mice each team had created that carry both the normal human  $\alpha$ -globin and the mutant human  $\beta$ -globin genes.

To keep the resulting transgenic mice from dying of sickle-cell disease before they were born, the researchers also introduced an extra set of human genes. Like humans, mice have a set of embryonic globin genes that are shut off partway through fetal development, but they lack an equivalent of the fetal hemoglobin that tides humans over until their adult genes switch on after birth. Mice normally just turn on their adult hemoglobin after the embryonic genes shut off. Consequently, both teams worried that if the altered mice switched to making the sickling form of adult human hemoglobin midway through gestation, they wouldn't survive to be born. "We wanted them to switch like humans," says Townes. So the teams introduced normal human fetal globin

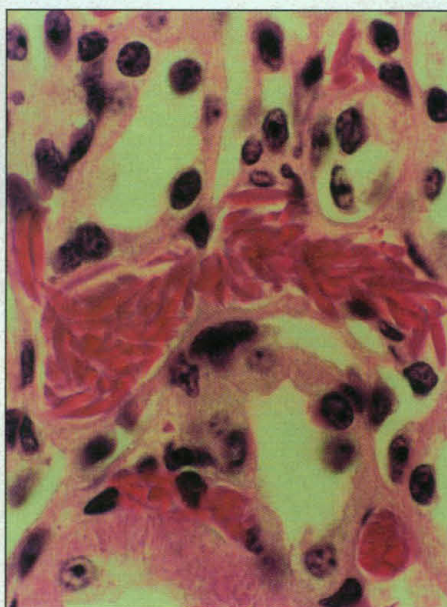
C. PÁSZTY/THE BERKELEY LAB



**Logjam.** The micrograph above shows the sickled red blood cells from a genetically altered mouse, while the one at right shows the consequences of the sickling: The deformed red blood cells clog blood vessels and cause tissue damage in the kidneys of the mutant mice.

not bound to oxygen, distorting red blood cells into a sickle shape. The animals also seem to have the same dismal array of symptoms as human sufferers. They develop severe anemia as a result of the premature death of the abnormal blood cells. Their blood vessels become clogged with sticky, sickle-shaped red cells. And their organs show damage from the resulting oxygen and nutrient starvation.

As a result, says Mary Fabry, who works on other sickle cell mouse models at Albert



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bin molecule that carries oxygen in our blood. If just one of an individual's two  $\beta$ -globin genes carries this mutation, the person doesn't get sick because the good hemoglobin keeps the red cells from sickling. Similarly, when researchers put various mutant forms of the human  $\beta$ -globin gene into mice, they found that the normal mouse globin



genes into their mice as insurance to get the mice through fetal development.

That move was a bit more successful in Ryan's mice than in Pászty's: Ryan's mice make human fetal hemoglobin until after birth, whereas Pászty's mice turn off the human fetal gene before birth, causing many of them to suffer severe sickling just hours after they are born, and die. But the surviving mice from both labs show a fuller complement of sickle-cell symptoms than earlier mice. They have much more severe anemia and also much more infarction, tissue death resulting from clogged blood vessels. "In Dr. Pászty's model, we are actually seeing infarcts much more frequently than in other models," says Elizabeth Mancini, a pathologist at the University of South Alabama Medical Center in Mobile who has studied human sickle-cell disease and the mouse models for many years.

Researchers can use the mice to study the changes in the red-cell membranes that make them stick to blood vessel walls, says Bunn, and perhaps devise new strategies for blocking the process. What's more, unlike earlier mouse models that used supersickling  $\beta$ -globin, the new mice have exactly the same mutant  $\beta$ -globin (known as  $\beta^s$ ) as the vast majority of humans with the disease, notes University of California, San Francisco, biologist Y. W. Kan, a pioneer of research on hemoglobin diseases. That, he says, makes them ideal subjects in the search for drugs that may inhibit the hemoglobin clumping that causes red blood cells to sickle.

Bunn notes that one of the most promising ways to block sickling is to keep the normal fetal globin genes switched on after birth, to provide a source of normal, antisickling hemoglobin. One drug, hydroxyurea, has already proven effective at keeping the genes active, but researchers hope to find better ones. "The fact that this current mouse model expresses fetal hemoglobin during fetal development, with a switch to adult  $\beta^s$ -globin," Bunn says, "opens up the opportunity to expose these animals to various agents, to ask which ones may [prolong fetal] globin expression."

The Berkeley Lab and Alabama teams haven't compared their mice side by side yet, but it is already clear that the disease progresses somewhat differently in each one. That, too, could be an asset; each may have particular strengths and weaknesses for testing therapies. "Having more than one model is a tremendous advantage" for double-checking the effectiveness of any experimental treatment, says Marie Trudel of the Institut de Recherches Cliniques in Montreal, who created some of the earlier mouse models. Perhaps this multiplicity of models will usher in a better array of treatments for sickle cell patients.

—Marcia Barinaga

## HUMAN EVOLUTION

# Y Chromosome Shows That Adam Was an African

In the beginning, there was mitochondrial Eve—a woman who lived in Africa between 100,000 and 200,000 years ago and was ancestral to all living humans. Geneticists traced her identity by analyzing DNA passed exclusively from mother to daughter in the mitochondria, energy-producing organelles in the cell. To test this view of human origins, scientists have been searching ever since for Eve's genetic consort: "Adam," the man whose Y chromosome (the male sex chromosome) was passed on to every living man and boy.

Now, after almost a decade of study, two international teams have found the genetic trail leading to Adam, and it points to the same time and place where mitochondrial Eve lived. Described this month at a symposium on human evolution at Cold Spring Harbor Laboratory in New York, the genetic trail is so clear that it allows researchers to compare the migration patterns of men and women tens of thousands of years ago (see sidebar). It even pinpoints the living men whose Y chromosomes most resemble Adam's: a few Ethiopians, Sudanese, and Khoisan people living in southern Africa, including groups once known as Hottentots and Bushmen.

The findings, by teams based at Stanford University and the University of Arizona, are the latest in a series of genetic studies that point to Africa as the recent birthplace of modern humans—people who then spread around the world and replaced other human populations. "It's very comforting to see the Y is giving us the same picture as mitochondrial Eve," says molecular anthropologist Mark Stoneking of Pennsylvania State University in University Park, who was part of the team that identified "Eve" as an African. "There's no doubt that there's some clear event of modern humans coming out of Africa." Now the question is whether the wave of modern humans from out of Africa completely replaced the people on other continents, or whether there was some interbreeding.

In science, unlike the Old Testament, Eve came before Adam—specifically, in 1987, when Stoneking and other researchers in the lab of the late Allan Wilson at the University of

California, Berkeley, announced that they had found our mitochondrial ancestor. They had compared mitochondrial DNA variants found around the world and traced a common ancestor by sorting out the variants on an ancestral phylogenetic tree. But Eve ignited fierce debate, as some scientists challenged the methods and assumptions used to place her in Africa from 100,000 to 200,000 years ago (*Science*, 14 August 1992, p. 873). Even if the Wilson team was right—and intense debate continues—"the mitochondrial DNA is just one gene lineage," says University of Michigan, Ann Arbor, paleoanthropologist Milford Wolpoff. Different genes might trace back to different ancestors on a different continent.

The only way to test the story of mitochondrial Eve was to trace the ancestry of other genetic lineages in the nucleus of the cell to see if separate lines of evidence also led back to African ancestors. And the obvious place to start was the Y chromosome, which is passed from fathers to sons and therefore provides an independent test of the mitochondrial Eve hypothesis. The bulk of the Y chromosome remains unchanged through generations, except for rare mutations hidden in regions

that don't code for proteins. By comparing variation at the same site in different individuals' DNA (known as polymorphisms), geneticists can sort out which populations are most closely related and then build a phylogenetic tree that traces the descent of men, just as the mtDNA traces the descent of women. And by using average mutation rates for nuclear DNA, they can estimate how long ago particular mutations appeared, and thus how long ago each limb of the tree branched off.

Sketching the branches of a Y chromosome tree has been painstakingly slow, however, because few polymorphisms in the Y chromosome had been found (*Science*, 26 May 1995, p. 1183). But new tools to speed the detection of variation in DNA sequences have recently given a boost to research on the Y. One big break has come at Stanford University, where biochemist Peter Oefner and



**Son of Adam.** Y chromosome links some Ethiopians to a genetic "Adam."