Biological Sciences

Learning the genetic causes of human diseases and finding better ways to prevent and cure diseases are long-term goals of ORNL's studies in the biological sciences. Our talented staff and excellent facilities for genetics research, information and computational biology, protein engineering, and structural biology not only help us attain these goals but also support extensive industrial and educational outreach programs.

At our Mouse Genetics Research Facility, where functional genomics research is performed, researchers have determined that the mutant mouse agouti gene has a primary, rather than an indirect, role in promoting skin cancer. Modern technologies are helping ORNL biologists assess hidden genetic defects (from depression to digestive disorders) in the descendants of irradiated mice. ORNL nuclear medicine experts have developed a technique that is expected to benefit heart patients. Clinical tests are being conducted on patients to determine if radiation from the rhenium-188 isotope produced by ORNL's generator prevents the reclogging of coronary arteries after the patients have balloon angioplasty operations. These achievements promise to advance our understanding of the origins of cancer, behavioral disorders, and heart disease and speed the development of ways to treat these health problems.

A normal mouse and an obese mouse that has the mutant agouti gene.

Agouti Gene Linked to Mouse Skin Cancer

ORNL researchers have determined that the mutant mouse agouti gene has a primary role in promoting skin cancer.

The normal, or wild-type, mouse (top) has agouti colored hairs, which are black with a central band of yellow. The mouse at left has completely black hair because it lacks the agouti gene. The mouse at the bottom is mostly black with some yellow hairs because it has a partially inactive agouti gene. The yellow mouse has an overactive agouti gene that is turned on all the time throughout the body. The overactive agouti gene also causes mice to become obese and diabetic and to develop cancer. ***Electronic file of photograph by Tom Cerniglio enhanced by Allison Baldwin.***

Mice, squirrels, and other mammals often have brown fur streaked with gray. The alternating light and dark bands of fur that make mice look grizzled result from the action of the agouti gene (named for the South American rodent). This mouse gene, whose normal and mutant forms were identified and cloned by Ed Michaud and others at ORNL in 1992, is of interest to researchers. The reasons: its mutant form is linked to altered fur color, obesity, diabetes, and cancer in mice, and it has a human counterpart.

When the mouse agouti gene is functioning normally, it turns on briefly in the skin where hair follicles are rooted. There, ORNL researchers found, the gene causes the secretion of the agouti protein, which signals the pigment-producing melanocyte cells to make yellow instead of black pigment for awhile. Then when the agouti gene turns off, the melanocyte cells return to producing black pigment again. Thus, a normal mouse hair is black at both ends, with a band of yellow in between.

But what happens if this coat color gene is mutated or deleted? Michaud and other ORNL researchers found that the mutant agouti gene expresses itself all the time, causing the melanocyte cells to produce yellow pigment endlessly.Thus, mutant agouti mice are yellow. And if a mouse is born without an agouti gene, its coat color will be solid black.

Being yellow is the least of the mutant agouti mouse's problems. It also is obese, diabetic, and more susceptible to getting skin, lung, liver, and mammary gland cancer. An explanation for these widespread disease effects may lie in the ORNL discovery that the mutant agouti gene's single defect is that it codes for the production of normal agouti protein not only all the time but also in every mouse cell. The causes for this ubiquitous, unending expression appear to be additions of DNA not usually found within the gene, such as retroviruses and a promoter usually linked to the nearby Raly gene, which is mostly deleted as part of the agouti gene mutation.

ORNL researchers and collaborators have found that the melanocyte receptor to which a ligand binds to activate the cell's black pigment production is blocked by signals from the agouti protein, resulting in yellow pigment production. It has also been discovered that four receptors in other parts of the body have similar structure and function, suggesting that the receptors' normal activity in regulating body processes may be susceptible to disruption by the agouti protein.

In a long-term quest to identify the mechanisms underlying obesity, diabetes, and cancer in yellow mice, ORNL researchers set out first to prove that overproduction of the normal agouti protein by an overactive gene causes these diseases. To address this point, they made transgenic mice by inserting into fertilized mouse eggs the normal agouti gene linked to a special beta-actin promoter, a DNA fragment that causes the gene to be turned on all the time everywhere in the body. The transgenic mice were born with yellow hair and became obese and diabetic, demonstrating that overproduction of the normal agouti protein causes these diseases.

In a recent set of experiments, Michaud and his colleagues next asked if the mouse agouti gene has a primary role in promoting skin cancer or if the cancer is a secondary effect of obesity and diabetes (e.g., from excessive levels of insulin produced to clear glucose from the blood). To tease out this information, they used different transgenic mice that had an active agouti gene only in the skin. In this case, the normal agouti gene was linked to a keratin promoter that causes genes to be turned on only in skin. These transgenic mice have yellow hair but are not obese or diabetic. In experiments involving large numbers of transgenic and control mice (black mice with no active agouti gene), ORNL researchers found that only 3% of the control mice developed skin cancer but 20% of the transgenic mice got the disease. Thus, ORNL demonstrated that overproduction of the agouti protein by the overactive agouti gene has a direct role in causing skin cancer.

Funding for the research came from DOE's Office of Biological and Environmental Research.

Observing Subtle Changes in Mutant Mice

Modern technologies help ORNL biologists assess subtle genetic defects in the descendents of mice exposed to radiation and chemicals.

Dabney Johnson watches a mutant mouse afflicted with "behavioral despair" (left) make no effort to escape the water in a tank while the normal mouse swims rapidly in an effort to get out of the water during the Porsolt swim test. *Photograph by Tom Cerniglio.*

Shortly after World War II, geneticists Bill and Lee Russell established a mammalian genetics program at ORNL to assess the genetic effects of ionizing radiation and a wide variety of chemicals in mammals. Many mutations that cause visible changes in the color or form of the mouse were generated, as were genetic changes that cause embryos to fail to develop or mice to die after birth. In some cases, the DNA damage that caused these visible or lethal mutations also caused more subtle genetic changes that would not traditionally have been recognized. To evaluate mutants for behavioral and biochemical changes, ORNL researchers are developing new technologies and increasing the sophistication, efficiency, and throughput for these tests.

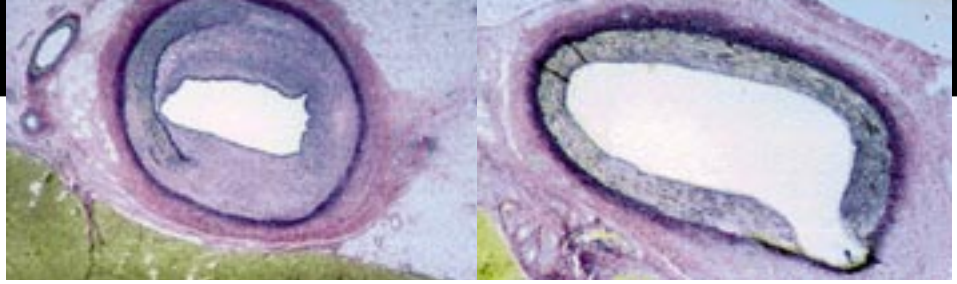
Using recombinant DNA techniques to identify and characterize genes from two small regions of chromosome 7 near the pink-eyed dilution (*p*) gene, ORNL researchers have identified less obvious defects in mice caused by the absence of certain genes in the *p* neighborhood. These defects include memory loss and depression.

Noticing that some of the genes in the *p* neighborhood are involved in regulating the activity of cells in the central nervous system, ORNL researchers led by Dabney Johnson recently gave strains of mice missing these genes two behavioral tests. The first examines the mouse's ability to process and store new information—that is, to remember an unpleasant experience. This test consists of a gentle foot shock accompanied by a sound cue; the mouse is then retested without the shock to see if it is afraid of both the sound that accompanied the shock and the chamber in which the shock took place. Normal mice fear both the sound and the chamber, while mice with the mutation near *p* fail to remember that the sound and chamber are associated with an unpleasant experience. ORNL researchers are focusing on this small region of the mouse genome to identify this gene, and eventually its entire associated biochemical pathway, which affects learning and memory.

The second test is the Porsolt swim test for behavioral despair, the equivalent in mice of human depression. Mice are put into a water tank from which they cannot escape. During the 7-minute test, a normal mouse will try constantly to climb out, but mice with the mutation near *p* exhibit despair and make little effort to escape.

Biochemical tests have shown that a particular region of the brain in these mutant mice has low levels of serotonin, a neutrotransmitter that promotes communication among brain cells called neurons. Low levels of serotonin have been associated with depression in people. Because the antidepressant drug Prozac raises the level of serotonin in humans, the ORNL biologists will give Prozac to the defective mice to see if it improves their performance in the Porsolt swim test. The research could lead to a more-targeted, lower-dose treatment that raises the serotonin level only in a specific part of the brain, rather than in the whole body, to dispel behavioral despair.

The funding for this research came from DOE's Office of Biological and Environmental Research.



Inhibition of restenosis (artery reclogging) using rhenium-188 was demonstrated in a swine coronary balloon overstretch injury model. Left: Control artery 30 days after balloon overstretch injury. Right: injured artery 30 days following rhenium-188 irradiation. *Images courtesy of Judah Weinberger.*

ORNL Isotope Offers Hope for Heart Patients

Radiation from the rhenium-188 isotope produced by ORNL's generator may prevent the buildup of smooth muscle cells (restenosis) in coronary arteries after balloon angioplasty.

Harry and Matilda are among the unlucky 30%. Both had mild heart attacks because a coronary artery had been narrowed by the accumulation of fatty deposits. Both had a procedure called coronary angioplasty to restore full blood flow to the heart muscle. In this procedure, a catheter equipped with a tiny uninflated balloon at the tip is inserted into a femoral artery in the leg and then threaded through the clogged coronary artery. Then the balloon is inflated to clear the blockage and widen the artery. But six months later, as happens with at least 30% of the 450,000 Americans who have the angioplasty procedure each year, Harry's and Matilda's treated arteries developed a different type of blockage. They were reclogged by the buildup of smooth muscle cells in response to balloon-induced vessel damage, a condition known as restenosis.

Most people in the unlucky 30% undergo additional angioplasty or heart bypass surgery to unclog their reclogged arteries. Nationally, these necessary second operations add $1 billion to the $4 billion cost per year of the initial angioplasty procedures. Fortunately, a promising cure for restenosis is radiation.

Although many radioisotopes and delivery systems are being evaluated for this purpose, a tungsten-188/rhenium-188 generator system developed at ORNL for cancer therapy has distinct advantages. This cylindrical system uses the radioisotope tungsten-188 from ORNL's High Flux Isotope Reactor. At the top of the cylinder, tungsten-188 binds tightly to aluminum oxide powder saturated with acidic saline. As it decays, the tungsten turns to rhenium-188, which lets go of the powder. A solution of rhenium-188 is obtained by washing the radioisotope down from the top.

Using a tiny iridium-192 wire source threaded through the coronary artery, cardiology researchers at Columbia University had demonstrated in pigs that high radiation doses inhibit muscle cell proliferation in newly unclogged arteries. Because of difficulty in centering the wire, the radiation dose was not being uniformly delivered to the damaged arterial wall.

Arnold Beets and Russ Knapp show a mockup of a tungsten-188/rhenium-188 generator system for use in preventing a heart patient's coronary arteries from reclogging after balloon angioplasty. *Photograph by Jim Richmond.*

To deliver a more uniform dose, Judah Weinberger, a Columbia University cardiologist, conceived of using a radioisotope solution to inflate the balloon at low pressure following high-pressure balloon inflation with saline to unclog the artery. Searching for the radioisotope with the best properties for this application, he called ORNL's Russ Knapp, who proposed rhenium-188 because ORNL's generator system can produce hundreds of doses of rhenium-188 in solution over several months in a hospital. Rhenium-188 also has nearly perfect beta particle energy for optimal vessel irradiation. Also, because rhenium would be rapidly excreted from the bladder in the unlikely event of balloon rupture, it is safe for patients and should receive regulatory approval.

ORNL is providing Columbia University with the radioisotope generator. ORNL researchers also developed a simple, efficient method for concentrating the rhenium-188 solution obtained from the generator because of the small volume of the balloons and the high radiation levels of rhenium-188 required for the short irradiation times. The patented ORNL method provides a high enough concentration of rhenium-188 to deliver the proper dose. Exclusive rights to this method were licensed in July 1997 to Mallinckrodt Medical, Inc., an international radiopharmaceutical manufacturer headquartered in St. Louis.

Use of rhenium-188 for human patients in clinical trials at Columbia University Medical Center in New York City was approved by the Food and Drug Administration. By March 1998, several patients had been treated with the rhenium-188 angioplasty procedure. Similar clinical studies are being carried out at the Royal Perth Hospital in Perth, Australia, and in the departments of nuclear medicine at the universities of Dresden and Ulm in Germany.

This partnership involving private business and university and government researchers is expected to benefit many thousands of people and improve their quality of life. If Harry's and Matilda's need for angioplasty had arisen a few years later, they might have been among the luckier patients.

The project was funded by DOE's Office of Biological and Environmental Research.