OAK RIDGE NATIONAL LABORATORY

REVIEW

• MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY •

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SYSTEMS BIOLOGY

Advancing at a Breathtaking Pace

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Features

SYSTEMS BIOLOGY: ADVANCING AT A BREATHTAKING PACE

1 Editorial: Unraveling Life's Molecular Mysteries
Our researchers are thinking big while working at the molecular level.

2 New Tools for Analysis
Systems biology enables a leap forward in understanding life.

4 First, the Questions
Tough questions drive systems biology research.

4 Sequencing the First Tree Genome
"What makes a tree a tree?" might be easier to answer now.

6 Pathways Underlying Disorders
Mouse study may shed light on face and skull abnormalities.

8 Piloting the Pipeline
ORNL's toolkit is identifying and characterizing microbial proteins.

9 Neutron-Rich Mecca for Biologists
ORNL's state-of-the-art neutron sources should advance protein research.

10 A Clean Mouse Research Lab
ORNL's new Mouse House is a tribute to Bill and Lee Russell.

12 A Return on Investment
Biology research may bring improved health for ecosystems and people.

14 Providing Access to World-Class Tools
The Joint Institute for Biological Sciences should attract world-class talent.

A RICH PAST

20 Pioneer of Biological Research
Bill Russell's work led to human radiation protection standards.

22 ORNL's Unsung Discovery
Messenger RNA was first discovered in Oak Ridge.

PROFILE

24 Tuan Vo-Dinh: Inventor and Mentor
One of ORNL's most prolific scientists collaborates with younger researchers.

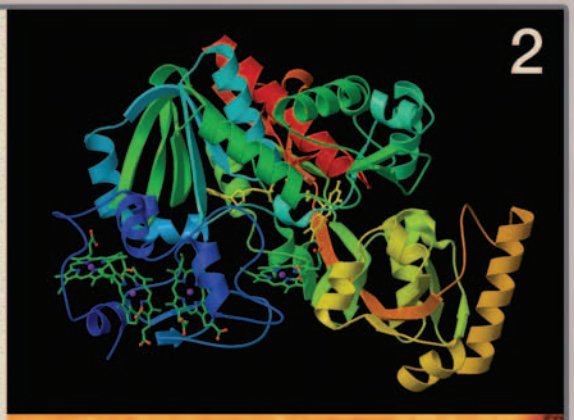
RESEARCH HORIZONS

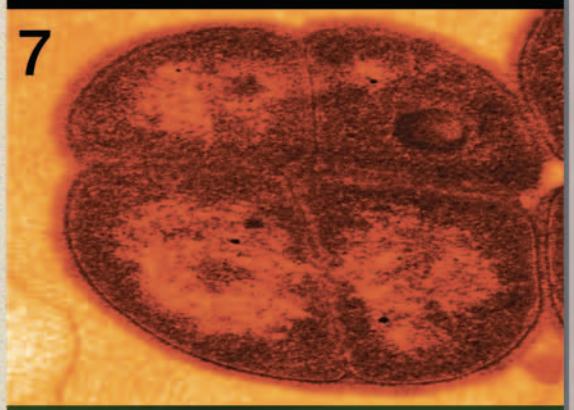
26 Guiding Light
The Photo-Molecular Comb may be used for drug development.

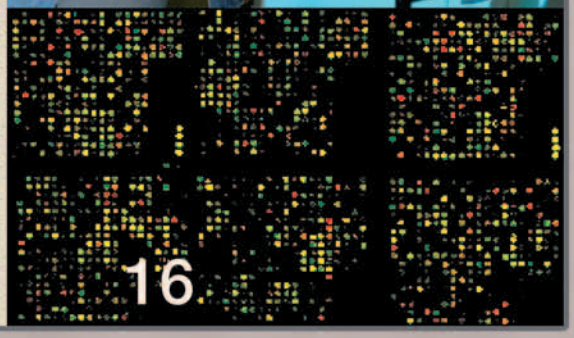
27 Another World Record
ORNL has the world's most powerful microscope, aiding ceramics research.

AWARDS

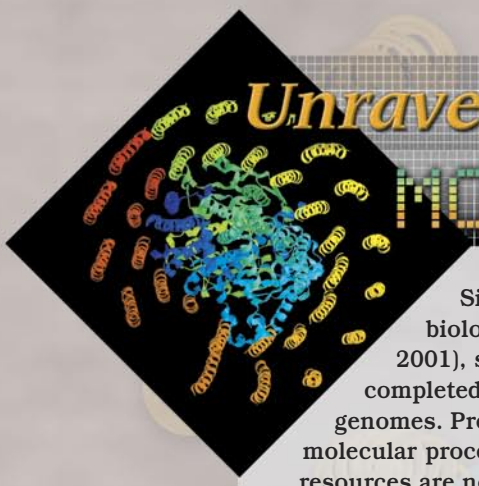
28 And the Winners Are. . .







COVER: Tuan Vo-Dinh, recipient of seven R&D 100 awards (see p. 24). Photo by Curtis Boles and bacterial protein visualization by Pratul Agarwal.



Unraveling Life's MOLECULAR MYSTERIES

Since the ORNL *Review* last presented a snapshot of Oak Ridge National Laboratory's biological research program ("New Biology: Covering All the Bases," Vol. 34, No. 1, 2001), scientific advances have been occurring at a breathtaking pace. Researchers have completed sequences of the human, mouse, and multiple other animal, plant, and microbial genomes. Protein interaction maps have been published for the fruit fly, worm, and yeast, and molecular processes have been observed in cells using quantum dots. Computational tools and resources are now an integral part of biological research. Advancements in our ability to observe biological processes at the molecular level and to derive organizational and operational principles from the data through integrated informatics, modeling, and simulation are revolutionizing our understanding of biology and the environment in which we live.

Because of ORNL's core expertise and worldwide reputation as a center of excellence in environmental and biological research, because of our unique facilities in the physical sciences and in the computational sciences, and because of our long tradition of bringing productive interdisciplinary research teams together, ORNL is helping to drive this revolution. Over the next decade, our research efforts will lead to economical and efficient energy production, sustainable environmental stewardship, and better human health.

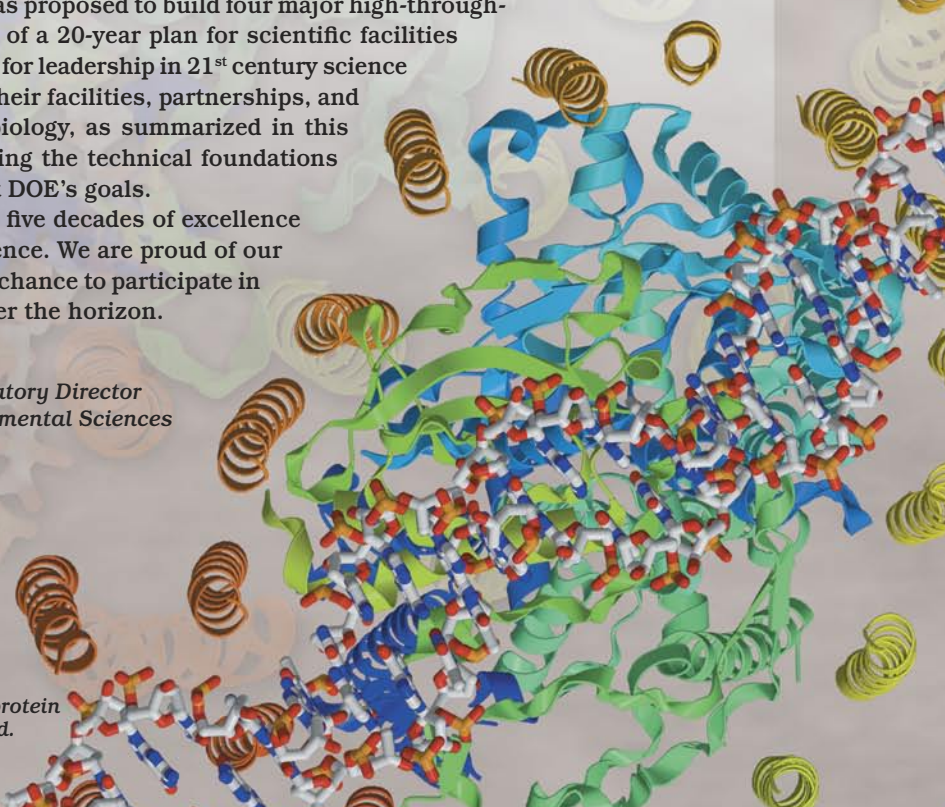
When the Department of Energy initiated the Genomics: GTL program, ORNL offered important capabilities to support the department's mission. Several of ORNL's mass spectrometers are now the workhorses for researchers tasked to establish a high-throughput pipeline for characterization of molecular machines. Cutting-edge research in molecular and cellular imaging is under way using ORNL's world-class electron and other microscopy capabilities. Our researchers are applying their pattern recognition methods and gene-finding skills, along with our high-performance computing resources, to annotations of hundreds of genomes in collaboration with other experts in the scientific community, thus enabling comparative genome analyses and new biological insights.

Working at the molecular level, we are nonetheless "thinking big." ORNL is pioneering the integration of modern biology and ecosystems research, based on the foundation of understanding molecular machines and molecular interactions. We are assembling a world-class team of environmental scientists, biologists, physicists, chemists, mathematicians, computational scientists, and engineers, who can apply the principles of systems science and engineering and knowledge of their respective fields to grasp biological complexity and to apply ultimately the principles and mechanisms of biology in engineered systems.

The DOE Office of Science has proposed to build four major high-throughput biology user facilities as part of a 20-year plan for scientific facilities that will position the United States for leadership in 21st century science and technology. Our scientists—their facilities, partnerships, and research directions in systems biology, as summarized in this issue of the *Review*—are developing the technical foundations for these new facilities to support DOE's goals.

At ORNL we are building on five decades of excellence in biology and environmental science. We are proud of our past, and we are excited about the chance to participate in discoveries that surely lie just over the horizon.

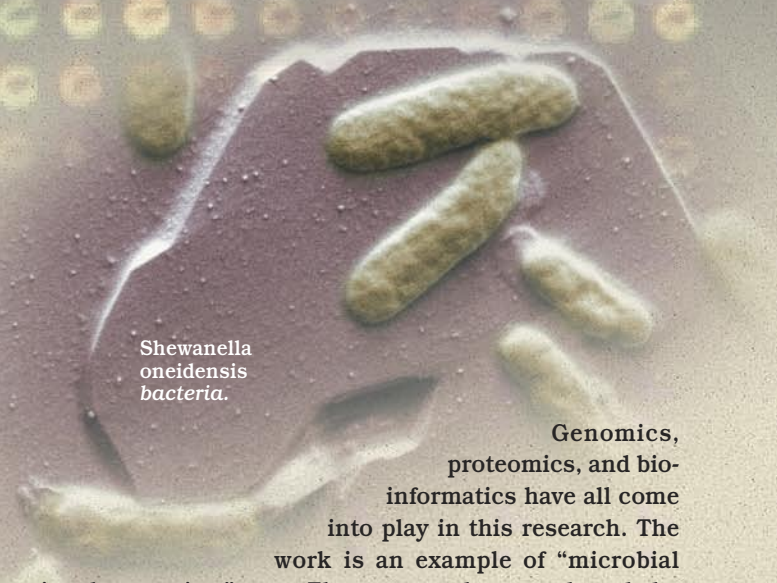
Reinhold Mann
ORNL Associate Laboratory Director
Biological and Environmental Sciences



Visualization of a microbial protein intertwined with a DNA strand.
Visualization by Pratul Agarwal.

New Tools of Analysis

Systems biology enables a leap forward in understanding life.



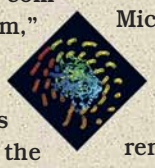
Shewanella oneidensis *bacteria.*

In the mid-1990s at Ohio State University, Dorothea Thompson studied a single gene and a single promoter regulating that gene as part of her doctoral thesis research. Today Thompson, a molecular microbiologist in ORNL's Environmental Sciences Division (ESD), acknowledges that today's Ph.D. candidates in genomics no longer focus on, say, determining the DNA base sequence of a single gene or predicting the structure of one protein formed according to one gene's instructions. Instead, like Thompson, these graduate students are taking a systems-level approach to describing biological organisms. They are engaged in systems biology, invented by physical scientists who apply systems analysis tools to biological problems.

Consider Nathan VerBerkmoes, a third-year Ph.D. candidate in the University of Tennessee-ORNL Graduate School of Genome Science and Technology (GST), who is working with Bob Hettich, a mass-spectrometry expert in the Organic and Biological Mass Spectrometry Group in ORNL's Chemical Sciences Division (CSD). By developing and demonstrating a mass-spectrometry-based technology platform for systems biology studies, VerBerkmoes has been able to contribute as a first author or coauthor on at least 10 scientific journal papers that have been published, are in press, or are under review.

"Many researchers have spent their entire careers investigating a particular protein—its interactions, its regulation, and its pathways," Hettich says. "Systems biology is the opposite of this conventional biological approach because it takes a global view down rather than a reductionist view up. You don't target any particular gene or protein but rather take a snapshot of the whole organism and all of its parts working together."

In the case of a bacterial cell, systems biology attempts to integrate all the DNA information (the genome), the RNA information (the transcriptome), the protein information (the proteome), and the metabolite information (metabolome). "The integration of this global information should provide a composite description of the whole function of the organism," Hettich says.



In a couple of systems biology projects, VerBerkmoes and Hettich collaborate with Thompson, who has obtained microarray data on specific activated genes of the *Shewanella oneidensis* bacterium in the presence of radionuclides and toxic metals, such as strontium and chromium. Using mass spectrometers to analyze *S. oneidensis* as it makes a metal less soluble and more likely to stay put in sediments or soil, VerBerkmoes cranks out lists of proteins and their relative concentrations.

Thompson's microarray data show that the expression level of some genes has risen, and VerBerkmoes' mass spec data indicate an increase in the abundance of corresponding proteins encoded by those genes. They send their data to computational biologists for additional interpretation and analysis. From this kind of collaboration emerges a scientific paper.

Genomics, proteomics, and bioinformatics have all come into play in this research. The work is an example of "microbial functional genomics," says Thompson, who coauthored the first textbook on the subject with three other researchers, including her colleague Jizhong Zhou, a pioneer in the environmental applications of DNA microarray technology. But, broadly speaking, understanding the roles of genes, proteins, protein complexes, regulatory sequence elements, and complex regulatory networks within cells and how they operate together to enable cells to function and survive is systems biology at work. Changes in system components and their interactions can best be studied when the cells are either grown in different ways or stressed by exposing them to toxic or radioactive metals.

From Genomics to Proteomics

Hettich explains that genomics embraces not only the order of the DNA bases but also the location of all the genes in a particular genome. The genome is translated into the transcriptome—the RNA level indicating which genes are turned on and which ones have a high expression level. The next level is the proteome—the proteins produced by the cell in response to instructions from the expressed genes.

The proteins interact with each other and form protein complexes, which carry out much of the work of the cell. Identifying and analyzing protein complexes in two bacterial species—*Shewanella oneidensis* and *Rhodopseudomonas palustris*—is the goal of the Department of Energy's Genomics: GTL Center for Molecular and Cellular Systems, of which Michelle Buchanan is scientific director.

Buchanan, CSD director, calls *R. palustris* "a good bug for DOE's missions because it might be useful for hydrogen production, carbon sequestration, and waste remediation. That's partly why we chose it. Depending on how *R. palustris* is grown, it will turn on different parts of its apparatus to take different pathways to ensure survival. That's also why we want to understand from a systems approach how we can control those metabolic pathways to get the microbe to do all the things we want it to do simultaneously."

Frank Larimer, leader of the Genome Analysis and Systems Modeling Group in ORNL's Computer Science and Mathematics Division, says that computational biologists collaborate with experimenters in an iterative process. "Experimenters help us fine-tune a computer model of a bacterium, such as *R. palustris*," he says, "to get a comprehensive view of this biological system and to predict how best to re-engineer

it to maximize its ability to achieve a desired function, such as producing hydrogen."

Ecosystem Genomics

"Systems biology looks at a microbe as a cell—as a microbial system with all the genes, RNA, ribosomes, proteins, and regulatory sequences that are active all the time as a total system," says Brian Davison, director of ORNL's Life Sciences Division. "Then it examines groups of microorganisms, communities, and ecosystems of microorganisms all working together."

In the past, ecologists have tried to understand the functioning of ecosystems—from forests to wetlands to deserts—in terms of different species, including bacteria, plants, and animals. The approach has not worked. Some ecologists now argue that it may be possible to understand ecosystems by starting with DNA molecules. The concept often elicits laughter, but according to ESD's Steve DiFazio, there are reasons to believe this approach could work.

"Multicellular organisms share the same genetic code and are related evolutionarily. For example, most organisms respire in about the same way. We can exploit this shared ancestry and conservation of function to design genomic tools that can be used to elucidate the underlying rules that govern the organization and functioning of ecosystems.

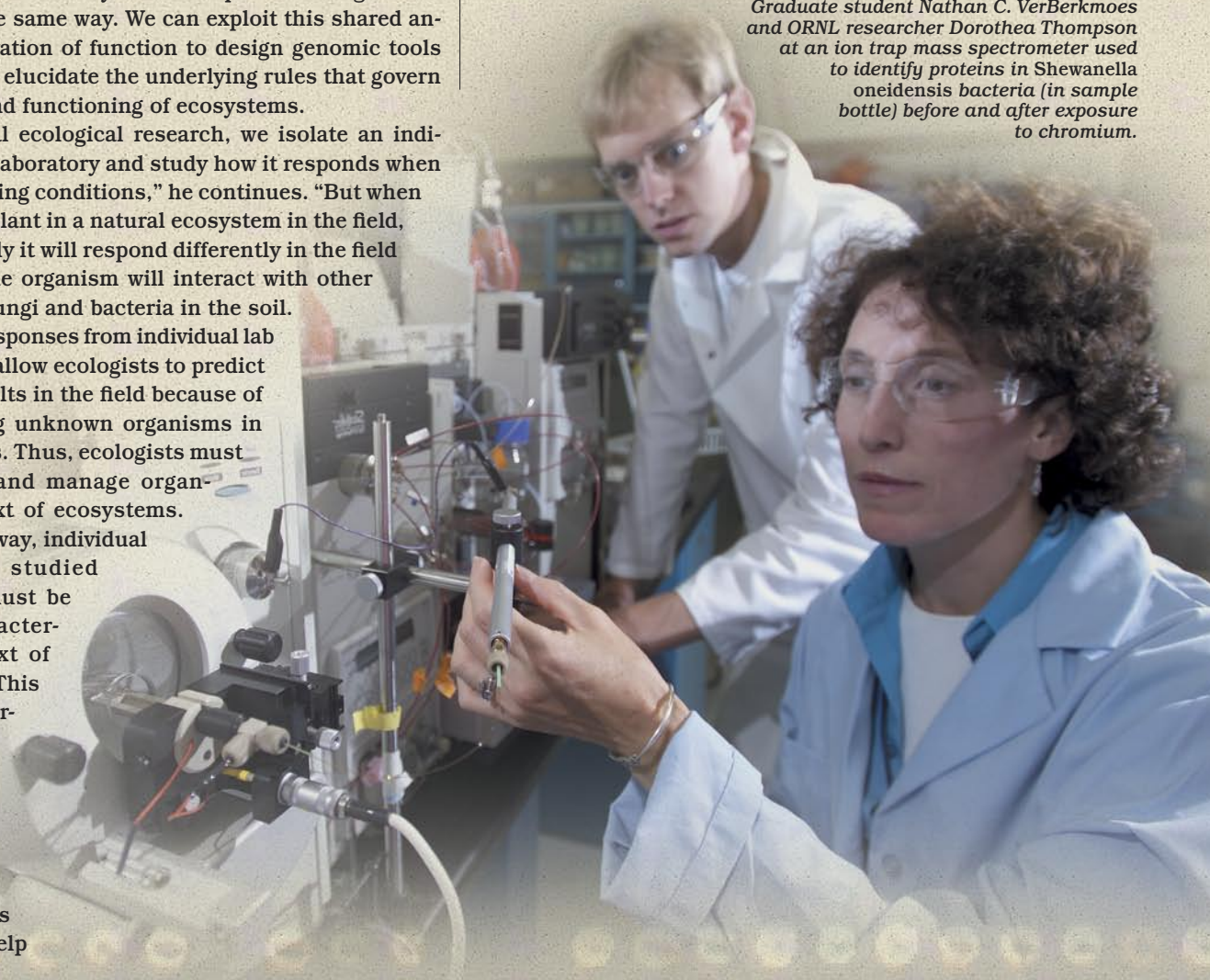
"In traditional ecological research, we isolate an individual plant in the laboratory and study how it responds when grown under changing conditions," he continues. "But when we grow the same plant in a natural ecosystem in the field, we find that typically it will respond differently in the field than in the lab. The organism will interact with other plants, as well as fungi and bacteria in the soil.

"Often, the responses from individual lab experiments don't allow ecologists to predict accurately the results in the field because of interactions among unknown organisms in natural ecosystems. Thus, ecologists must ultimately study and manage organisms in the context of ecosystems. In much the same way, individual genes cannot be studied in isolation but must be functionally characterized in the context of living organisms. This is the signature purpose of systems biology. Just as parts of cells exchange protein subunits, organisms in an ecosystem can exchange metabolites and signals that help each other survive," DiFazio says, explaining that metabolites are compounds containing carbon, nitrogen, and other elements that provide cells with energy and building blocks of proteins.

"For example, certain fungi (mycorrhizae) can survive only by growing on tree roots. Without the fungi, the tree will not grow as well or survive severe drought. Genomics provides tools and information that will allow us to understand the mechanistic basis of these complex interrelationships."

"We are sequencing the fungi that affect the poplar tree's mineral uptake and drought tolerance," says ESD's Jerry Tuskan. "We are beginning to build bioinformatics resources that will allow us to look at how enzymatic systems within organisms interact, how organisms interact among themselves, and how those interactions are shaped by other organisms and physical components in the environment."

To understand ecosystems, researchers may need to get down to the molecular level, to the level of genes and proteins, to understand why components that are similar yet different—like ORNL staff members and UT graduate students—are so dependent on each other. ®



Graduate student Nathan C. VerBerkmoes and ORNL researcher Dorothea Thompson at an ion trap mass spectrometer used to identify proteins in Shewanella oneidensis *bacteria (in sample bottle) before and after exposure to chromium.*

First, the Questions

Tough scientific questions drive systems biology research at ORNL.

Scientists believe they are on the brink of solving some mysteries underlying the miracle of life. The confluence of increasingly sophisticated analytical techniques, more powerful computing capabilities, and multidisciplinary partnerships linking some of the world's best researchers have set the stage for a revolution in biology. This revolution, spawned by systems biology research on the heels of the Human Genome Project, could produce answers to some very profound questions. In addition, it could suggest new questions to ask, based on the flood of data resulting from experimental analysis and computer modeling.

What Makes Species Different

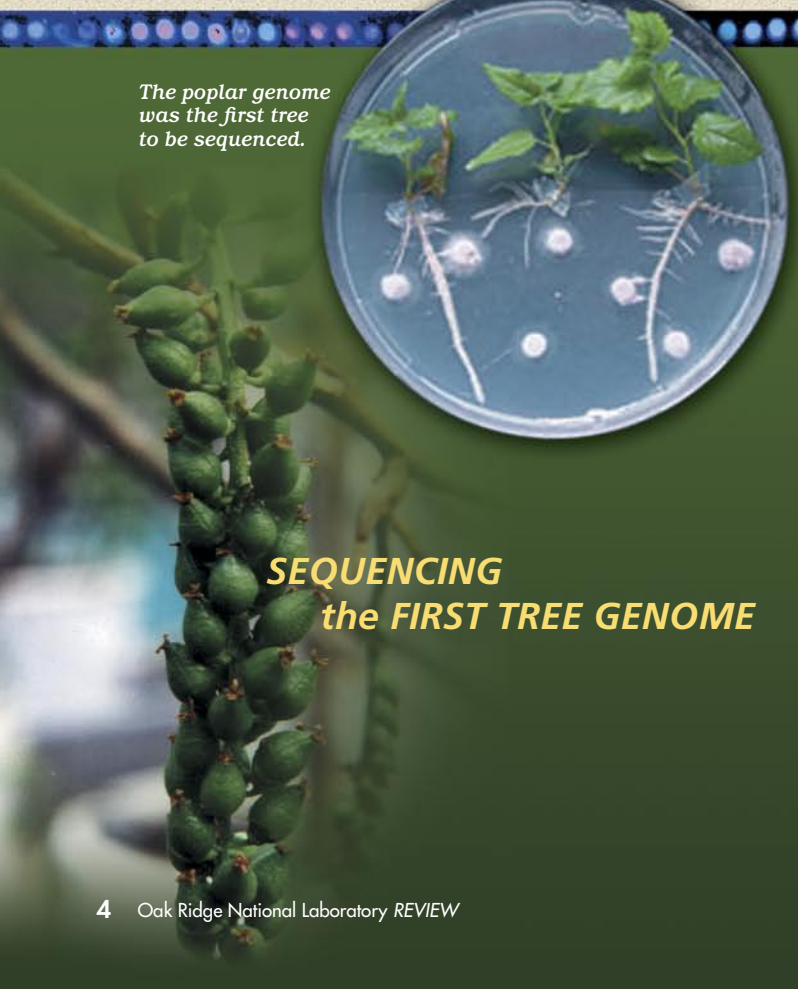
The quest begins with some fundamental questions, such as: Biologically, what makes humans different from mice or flies? Researchers need to use systems biology to resolve these classical questions in biology: How is an organism's complexity created from a single-celled embryo? Why is one human individual more likely than another to develop a certain disease?

The recent sequencing of human, mouse, and other genomes has not yet provided full answers to these questions. What we have learned, however, is that humans and other large mammals share many genes and proteins found in multicellular animals such as worms and mice. So, what makes species different?

"A half a billion years ago, around the Cambrian era, the evidence suggests a huge explosion in the number of different body plans for multicellular animals," says Jay Snoddy, a bioinformatics researcher at the University of Tennessee–ORNL Graduate School of Genome Science and Technology. "The protein-coding part of the genome for genes involved in laying down the body plan of different animals, like humans and insects, did not seem to diverge that much."

Subtle changes, however, do occur in the genome, including the part that helps determine when the RNA and protein for a gene are made. These subtle changes may affect whether a gene in a cell will be silent or active. These changes outside of the protein-coding part of a gene can determine when and where that gene makes a protein in a subset of cells during the development of an organism from an egg. In some sense, the evolution of body plans is often the evolution of changes in development, and changes in development are often initiated by subtle changes in the networks that regulate the expression of genes in cells.

"According to some researchers, what makes humans different from mice does not lie in the protein coding part of the genome," Snoddy says. "The difference often lies in the genome parts targeted by regulatory transcription factors that decide when and where a protein should be made."



The poplar genome was the first tree to be sequenced.

SEQUENCING the FIRST TREE GENOME

In 2004 researchers from around the world finished sequencing the complete genome of *Populus*, the first tree and the third plant to have its molecular "parts list" revealed. Jerry Tuskan of ORNL's Environmental Sciences Division, who led a group that played important roles in the international effort, says the sequenced genome will bolster researchers' chances of answering several important questions. For example, "What makes a tree a tree?"

Researchers have sequenced the complete genomes of two other plants, which are neither trees nor perennial species. One plant is rice and the other, *Arabidopsis*, is an herbaceous weed. Comparison of the genomes of the three plants is expected to provide some answers.

Studying the *Populus* genome under which hybrid poplars, cottonwoods, and aspens fall, could enable scientists to address some questions of interest to the Department of Energy's Office of Biological and Environmental Research. The office funded ORNL's research effort in support of the International Populus Genome Consortium (IPGC).

These questions might be: How do individual genes influence the growth of trees, their adaptation to the natural environment, the functioning of the forest ecosystem, and its response to climate change? Can poplar trees be designed to promote storage of carbon

Snoddy compares genes and proteins to conserved computer hardware and chips, designed millions of years ago. "What has evolved over the centuries has been subtle changes in networked wiring and software—subtle changes in the regulation of genes and in the timing and location of the regulation," he says. "Small changes in gene regulatory networks, cell-to-cell communication, and protein interaction networks are among the forces that have contributed to the huge amount of complexity, diversity, and variability of species on the earth. Understanding these relationships should be a long-term goal for systems biology."

Snoddy and his UT colleagues Bing Zhang, Stefan Kirov, Rob Williams, and Michael Langston are using computer analysis of gene expression data sets to study regulatory networks in the brain. These networks "read out" the genome information and integrate it with other information signals that a cell receives from the extracellular environment during physiology and development. These regulatory networks are key to understanding many fundamental parts of biology. This knowledge is also useful in practical matters, such as biomedical applications; parts of these regulatory networks seem to be affected both by disease and drugs used to treat it.

The rhinoceros, zebra, elephant, and peacock all illustrate the phenotypic diversity that can come from similar genomes.

in the soil for longer times by fixing it into a chemical form that resists microbial degradation, thus enhancing carbon sequestration and slowing the buildup of atmospheric carbon dioxide? Can poplar trees be designed to grow faster and produce higher-quality wood for building products, as well as more biomass that can be converted to liquid biofuels with higher energy content?

"*Populus* was selected as the first tree genome to sequence for several reasons," Tuskan says. "The genome is small, it is easy to clone, a lot of genetic information is available on this species, and a lot of scientists have studied it. The genome is a model perennial woody plant, is fast growing, and has several uses of interest to DOE and the forest industry."

A group of researchers in ORNL's Environmental Sciences Division worked on the *Populus* genome for almost two years. Tuskan served as the point of contact for the IPGC and the three groups annotating the sequence, including the group led by Frank Larimer in ORNL's Life Sciences Division.

"We developed a genetic map of the *Populus* genome and identified 1300 simple sequence repeats, which are important DNA markers, in the map," Tuskan says. "Of 365 million DNA bases in the genome, we linked 265 megabases to the genetic map. Our second contribution was to help IPGC computational biologists 'train' gene-calling algorithms so they can identify genes in the *Populus* genome. We sequenced about 500 full-length cDNAs—expressed DNA sequences—and sent them to the three annotation labs."

These labs—Larimer's group in Oak Ridge, DOE's Joint Genome Institute in California, and the University of Ghent in Belgium—are developing algorithms and training them based on poplar-unique or poplar-specific genomic characteristics. About 95 to 98% of the expressed part of the genome—the part that contains genes—has been sequenced.

"If all three models from these labs predict that a particular sequence is a gene, we will have pretty high confidence that it is, in fact, a gene," Tuskan says. "If only one group predicts that a sequence has function, we may be more skeptical about whether it's a gene.

"We think the number of genes in the *Populus* genome will probably range from 30,000 to 35,000 genes. The process will take a decade or more of research to understand what each gene does."

What makes a tree a tree? Tuskan says researchers are already finding hints as they compare plant genomes. One answer may lie in the regulatory elements that control the expression of the structural genes that code for certain enzymes.

PATHWAYS UNDERLYING DISORDERS

Abnormalities of the face and skull rank among the most common birth defects in humans. Understanding such complex human disorders requires a systems biology approach, according to Cymbeline Culiat, a molecular geneticist in ORNL's Life Sciences Division. She is taking this approach as she investigates a series of eight mutant mouse strains that could serve as animal models for deciphering the complex molecular interactions underlying skull development.

"We found that these eight mutations occurred in the same gene and that this gene codes for a novel cell-signaling protein critical to the development of bones in both the skull and spine," Culiat says. "This collection of mutant mice carrying different changes in the same protein gives us an excellent opportunity to understand that particular protein's various functions."

In the mutations being studied by her group, when one part of the protein is affected by a mutation, a mouse may be born with a deformed skull and face but a normal spine. If another portion of the protein is affected, severe defects in both the skull and spine occur. In this gene's most severe mutation (designated 102DSJ), the amount of protein being made is greatly reduced. The mice with this mutation exhibit extreme alterations in spine curvature and skull anomalies.

"Our mice are potential models for children suffering from craniosynostosis (CS), a condition wherein skull bones grow very fast and fuse prematurely, preventing further brain growth," she says. "Children with CS undergo major skull reconstruction at an early age and can suffer from mental retardation, visual and hearing impairment, and skeletal defects of the limbs and spine.

"Some children with CS manifest the same type of spinal defect observed in our mutant mice and some do not," Culiat continues. "If we can figure out why and how the mutant protein in our mouse models affects both the developing skull and spine, we will better understand this complex human disorder."

The availability of mouse and human genome sequences, rapid advances in technologies for detecting and measuring changes in gene expression, and computational tools for analyzing vast amounts of data are allowing Culiat and her associates to seek answers to systems biology types of questions: Which groups of genes interact and how do proteins interact to ultimately control the development of specific biological structures or perform certain functions? When a mutation occurs in a key gene in a pathway, how does the resulting perturbation in the pathway's other genes ultimately lead to a disease or abnormality?

Culiat established a collaboration with Mark Shannon of Applied Biosystems-Celera, which has developed sophisticated gene expression technology. Shannon wanted to test his company's technology on a large scale, to determine how useful it is for studying biological pathways in a whole organism—such as a mutant mouse.

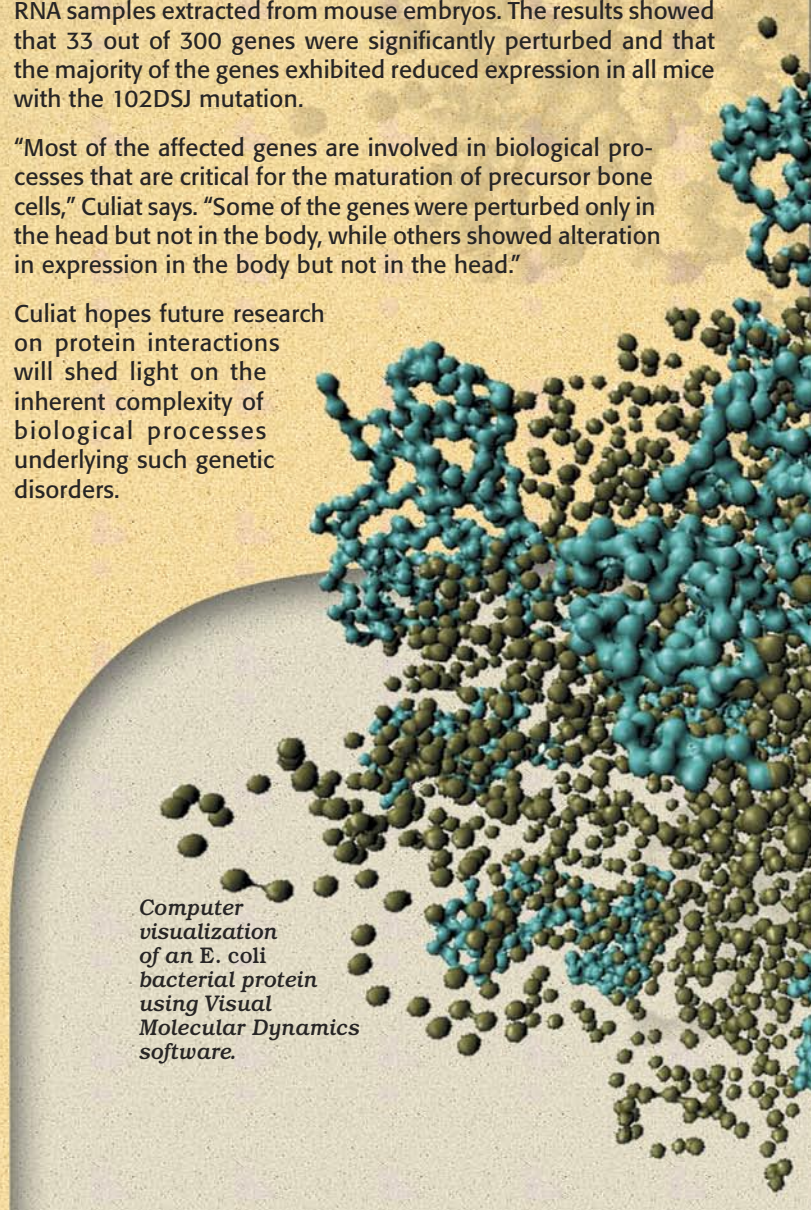
Using bioinformatics data, the collaborators initially studied 300 genes in normal mice, as well as in mutant mice carrying the most severe mutation (102DSJ). The genes are involved in bone, cartilage, and brain development and in cell proliferation and differentiation. The researchers also assayed the expression of genes coding for proteins that could potentially interact with the mutant protein, based on knowledge of the predicted functional domains.

ORNL mice and rabbits (studied elsewhere and shown above) are potential models for children suffering from a condition wherein skull bones grow very fast and fuse prematurely, causing facial abnormalities and preventing further brain growth.

Shannon's lab performed thousands of very sensitive assays of RNA samples extracted from mouse embryos. The results showed that 33 out of 300 genes were significantly perturbed and that the majority of the genes exhibited reduced expression in all mice with the 102DSJ mutation.

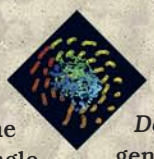
"Most of the affected genes are involved in biological processes that are critical for the maturation of precursor bone cells," Culiat says. "Some of the genes were perturbed only in the head but not in the body, while others showed alteration in expression in the body but not in the head."

Culiat hopes future research on protein interactions will shed light on the inherent complexity of biological processes underlying such genetic disorders.



Computer visualization of an E. coli *bacterial protein using Visual Molecular Dynamics software.*

Microbes on a Mission



The Department of Energy seeks to understand the diverse range of biochemical pathways that enable single-celled organisms to survive under extreme conditions—high temperature, high radiation, and high concentrations of toxic chemicals. DOE is interested in harnessing the genes of these microbes, whose capabilities could help DOE meet its missions in environmental bioremediation, carbon sequestration to slow climate change, and energy production. ORNL researchers and their collaborators are studying these microbes as part of DOE's Microbial Genome Program and Genomics: GTL Program.

Multitalented bacteria of interest to DOE include *Deinococcus radiodurans*, which can withstand high doses of radiation because these cells efficiently repair radiation damage. Like *Shewanella oneidensi*, which is also studied at ORNL, these bacteria reduce certain metals—that is, they donate electrons to toxic metals, like chromium and uranium, so they can extract energy from carbon. When these metals accept the electrons, they often are converted from a soluble to an insoluble state, possibly enabling bioremediation.

Questions that drive some ORNL research include the following: How will these bacteria respond to the stress of a soil or groundwater environment loaded with toxic and radioactive metals? Will some bacterial cells convert radioactive uranium in storage ponds from a soluble to an insoluble form so that this toxic metal sinks into the sediments or stays put in soil instead of dissolving in water that may flow off-site? Can a microbe like *Deinococcus radiodurans* be "designed" so that more of its genes focus on remediating sites with mixed wastes—combinations of radioactive materials and toxic metals? Can a uranium-contaminated site be populated with *Shewanella oneidensi* or some other bacteria "trained" to remove uranium from groundwater and moist soil, saving DOE billions of dollars in toxic waste cleanup activities?

Certain bacteria in the ocean and on land take up carbon dioxide from the atmosphere and perform photosynthesis. Can genes from these bacteria be harnessed to help DOE halt the buildup of atmospheric carbon dioxide from energy production? Can the poplar tree be designed to grow faster and take up more atmospheric carbon dioxide that will be stored in its branches and roots? Can systems biology find ways to ensure that more carbon from decaying roots stays locked up in soil rather than being released back into the atmosphere?

DOE is also interested in microbes that produce clean fuels, such as methane, methanol, and hydrogen. ORNL researchers and their collaborators are focusing on *Rhodopseudomonas palustris* as a potential energy source. Can it be grown in a certain way or can its genes be harnessed to produce hydrogen efficiently? Can enough hydrogen be produced biologically for use in power-producing fuel cells for cars and buildings in the envisioned hydrogen economy?

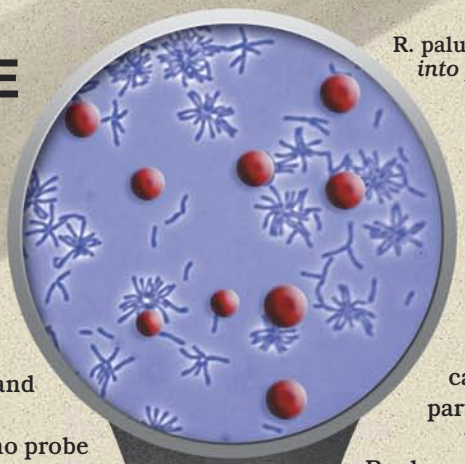
Researchers in ORNL's Environmental Sciences Division are addressing these and other key scientific questions about the microbial community: What is the genetic diversity of microbial communities? How do environmental disturbances, such as contaminants, affect the structure, functional stability, and adaptive capacities of microbial communities? Can the diversity and metabolic capabilities of a microbial community be manipulated to achieve desired functions, such as remediation of mixed-waste contaminants?

To understand how a cell works, researchers must understand how protein complexes do the work of the cell. Questions that ORNL biologists are asking and hope to address using systems biology include the following: Why does a certain protein complex behave the way it does? How far can a protein complex be twisted so that it does something a little different better, cheaper, and faster than it did before? Will the protein complex meet our needs yet still survive? If a certain genetic part of a mutant *E. coli* bacterium is knocked out, will the bacterium suddenly produce more succinic acid for making useful products? If bacteria capable of anaerobic digestion are grown on a particular feedstock, will large amounts of methane be produced all the time, even when the feedstock is changed? To save time and money, could a biology experiment be simulated on a computer and confirmed in the laboratory?

These and other questions are driving biological research at ORNL and fueling the revolution in post-genome biology. ®

PILOTING THE PIPELINE

ORNL is assembling a state-of-the-art toolkit for systems biology research such as characterizing and imaging microbial cells for DOE's genomics research programs.



R. palustris *bacteria might be coaxed into producing hydrogen efficiently.*

R pal. The automated pipeline. Mass spec and proteomics.

These phrases are used by ORNL researchers who probe microbes to determine what these "bugs" are made of and what drives them. The Institute for Genomic Research and the Department of Energy's Joint Genomics Institute (JGI) sequenced these microbes. They are among the 100 microbes annotated by an ORNL group of computational biologists led by Frank Larimer of ORNL's Life Sciences Division (LSD). This group identified and characterized most of these microorganisms' genes.

DOE is seeking more detailed information about the proteins encoded by these genes. Using a systems biology approach, ORNL researchers are trying to determine which microbial proteins, or groups of proteins called protein complexes, carry out a function of interest to DOE.

One microbe of great interest to DOE and ORNL is "*R pal*," short for *Rhodopseudomonas palustris*. This bacterium, which can be grown in many different ways, could possibly be manipulated to produce hydrogen efficiently while fixing nitrogen or to take up carbon dioxide from the air, slowing the buildup of a greenhouse gas. Identifying the protein complexes in *R pal* is an initial goal of DOE's Genomics:GTL Center for Molecular and Cellular Systems, of which Michelle Buchanan is scientific director. Buchanan, director of ORNL's Chemical Sciences Division (CSD), says ORNL has the task of identifying and testing tools that will be used to rapidly identify and characterize protein complexes in many different microbes. The idea is to determine which proteins do the work of a bacterial cell and keep it alive under different growth states and environmental conditions. She talks about analyzing a microbe a day by running it through an automated "pipeline" that incorporates an "arsenal of methodologies."

The workhorse instrument in the pipeline is the mass spectrometer. "Mass spec" is considered the world's leading tool for "proteomics," which entails rapidly identifying and characterizing proteins and the changes they undergo—called post-translational modifications (PTMs)—when a microbe is grown differently or is exposed to a toxic material that could reduce its ability to render a desired service.

"This year we are focusing on high-throughput, automated analysis of protein complexes in a large format process so we can do many things at one time in a massively parallel way using mass spectrometers and microscopes," says Buchanan. "The concept is not to follow a biological pathway from beginning to end. Rather, we are 'jumping' on a microbe and trying to identify as many of its protein complexes as we can as fast as possible. Once we obtain the parts list, biologists can use it to figure out how the parts interact."

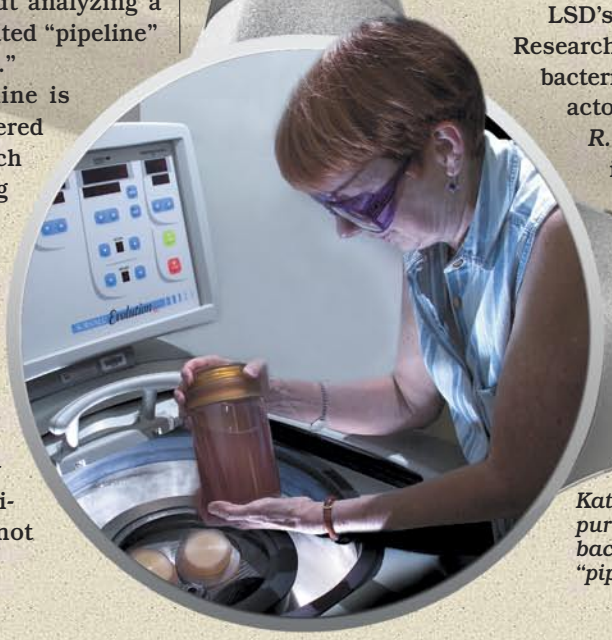
The ORNL pilot project led by Buchanan involves growing microbes in different ways with special tags; extracting their protein complexes; identifying and characterizing the protein complexes using mass spectrometers and imaging tools, such as fluorescent microscopes; and sending mass spectra and other data to bioinformaticians and computational biologists for interpretation. These specialists write algorithms, improve supercomputer codes, and annotate genome sequences. One goal might be to identify the *R. palustris* protein most involved in hydrogen production.

ORNL researchers hope the project will strengthen the Laboratory's effort to compete for one of the DOE Office of Science's proposed new genomics user facilities—the Molecular Machines Characterization and Imaging Facility. CSD's Greg Hurst and Bob Hettich anticipate that the facility will have at least 60 mass spectrometers to meet DOE's goals.

"We will need various methodologies to characterize the interactions of protein complexes with each other and with other components of bacterial cells," Hettich says. "A high-throughput pipeline will be anchored around mass spectrometry, but there will also be lower-throughput parallel lines, such as imaging and neutron scattering. These technologies will be very important for targeting specific pieces of information for these biological systems."

Growing R Pal

LSD's Biochemical Engineering Research Group grows masses of bacteria in various ways in bioreactors for use in research. "If *R. palustris* is grown so that it receives energy from light and carbon from organic molecules, it will produce hydrogen," says LSD director Brian Davison. "If, however, R. palustris is grown so it gets en-



Kathy McKeown grows and purifies cultures of R. palustris *bacteria as a part of the "pipeline."*

NEUTRON-RICH MECCA FOR BIOLOGISTS

Biologists can image proteins using electron and atomic force microscopes. They can visualize the three-dimensional structure of proteins—amino-acid sequences folded in complicated ways—by using X rays at ORNL and other DOE labs. They also can identify proteins using mass spectrometers and predict their structure using supercomputers.

What they cannot "see" with these tools are a protein's most active and abundant components, their interactions, and their locations in protein complexes. These components are hydrogen atoms, and they can be seen only with neutrons. In two years Oak Ridge will offer biologists two powerful sources of neutrons.

The sources are the High Flux Isotope Reactor, which is already the best steady source of slow neutrons, and the accelerator-based Spallation Neutron Source, which will be the world's best source of pulsed fast neutrons, starting in 2006.

"HFIR and SNS will both have small-angle neutron scattering instruments, which will enable biologists to understand the structures and activities of proteins, lipids, and sugars," says Dean Myles, director of DOE's Center for Structural Molecular Biology (CSMB) at ORNL. "The SNS will also likely have a protein crystallography instrument and a reflectometer for better understanding cell-to-cell communication."

Because CSMB is offering all these tools to scientific users, Oak Ridge may someday become a neutron-rich mecca for biologists.

Sam McKenzie checks the SNS accumulator ring.

Photo by Lynn Freeny, DOE

ergy from light and carbon from carbon dioxide, *R pal* could be used to slow the buildup of atmospheric CO_2."

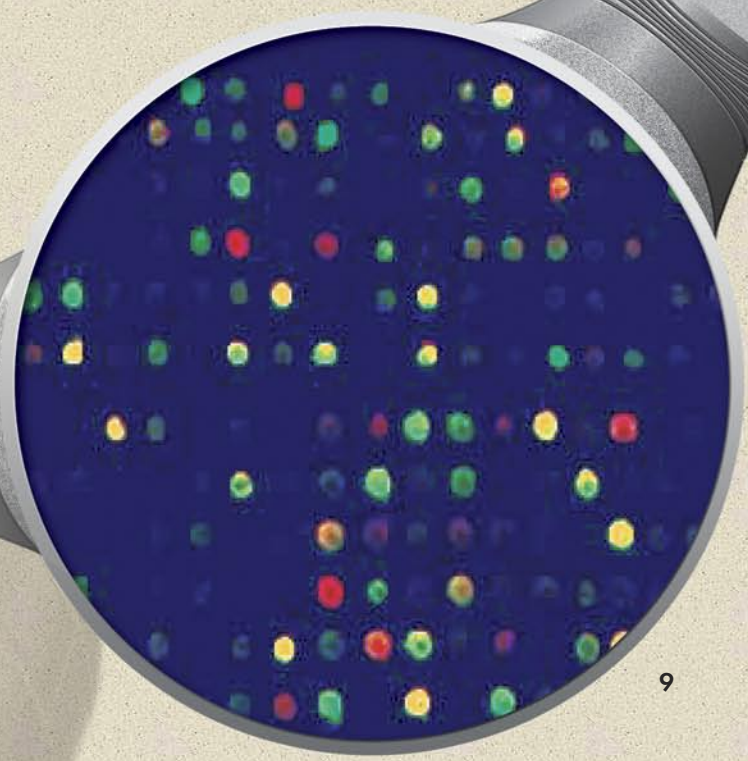
Extracting Protein Complexes

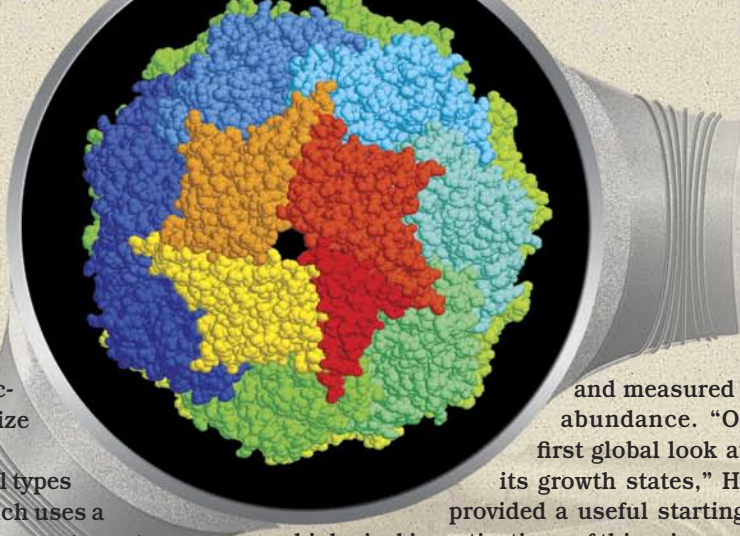
LSD's Dale Pelletier and his colleagues perform molecular biology to induce bacteria to express proteins that are tagged, so that protein complexes can be fished out or imaged inside live cells. One trick Pelletier's group uses to fish protein complexes out of bacterial cells is what Buchanan calls "selective Velcro." Multiple copies of special genetic sequences are added to *R pal* cells reproduced at ORNL. Within each cell, a protein called a 6-histidine tag grows as an attachment to a protein complex. The 6-histidine protein has an affinity for nickel.

Upon disruption of the cells' membranes, affinity reagents made of beads coated with nickel are mixed with the cell contents. "The 6-histidine binds to the nickel," Pelletier says. "We fish out the beads and out come protein complexes."

The goal is to create a library of antibodies that individually pair with specific microbial proteins. The antibodies can be used to extract target proteins and their partners.

Microarray data on bacteria can indicate increases in protein production.





Bacterial proteins are identified using mass spectrometers and visualized using computers.

A Leading Analytical Tool

Pelletier's group purifies the protein complexes extracted from the *R pal* cells and hands them over to CSD's GTL mass spectrometry effort, led by Hurst. This group uses liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify and characterize microbial proteins and protein complexes.

The GTL-MS effort focuses on two general types of measurements. For the first approach, Hettich uses a Fourier transform ion cyclotron resonance mass spectrometer to do "top-down" identification of intact proteins in microbes. For the second approach, Nathan VerBerkmoes, a doctoral candidate at the University of Tennessee–ORNL Graduate School of Genome Science and Technology, has been a driving force in using CSD's three ion trap mass spectrometers for "bottom-up" identification of the components of proteins. ORNL researchers are integrating the top-down and bottom-up approaches to get the most comprehensive proteome information.

"Using LC-MS technology, we can identify a substantial portion of the *R. palustris* proteome," Hettich says. "In the more common bottom-up MS approach, the complex protein sample from *R. palustris* is digested with the protease trypsin, which selectively cuts all the proteins into smaller pieces called peptides. We identify the individual peptides by investigating their fragmentation using tandem MS, and then assemble the information to identify the original proteins present in the sample."

"First, we identify and catalog proteins in the *R pal* bacterium," Hettich continues. "Then we try to determine how much of each protein is present when *R pal* is grown under different conditions. Mass spec is the best tool for not only identifying proteins but also for characterizing their PTMs."

Recently, Hettich, Hurst, VerBerkmoes, and postdoctoral associate Michael Brad Strader identified and characterized the 54 proteins that make up the *R pal* ribosome, the cell's protein "factory." VerBerkmoes and his collaborators also catalogued all the proteins produced in *R pal* by its various growth states and measured changes in their abundance. "Our study is the first global look at *R pal* under all its growth states," Hettich says. "We provided a useful starting point for many biological investigations of this microorganism. We identified proteins that were either unknown previously or were not expected to be so important under different growth states."

"ORNL has identified more than a dozen protein complexes so far," Buchanan says. "Our target for 2005 is to identify and characterize 500 protein complexes through work with our collaborators, especially DOE's Pacific Northwest National Laboratory."

RNA and Microarrays

Another bacterium that could be useful to DOE is *Shewanella oneidensis* because of its potential for converting radioactive uranium compounds into a less soluble state so that they sink into the sediments or stay put in soil. A DOE objective is to prevent uranium contaminants from dissolving in groundwater and flowing off site, where the uranium could endanger public health. DOE is interested in knowing how well *Shewanella* responds to stress from exposure to toxic metals. The concern is that the presence of toxic metals might make Shewanella less effective in immobilizing uranium.

To help answer questions about *Shewanella*, DOE has sought help from the group led by Jizhong Zhou, a pioneer in the environmental applications of microarrays and a group leader in ORNL's Environmental Sciences Division. A microarray is the only available tool for capturing genome-wide, or global, information about the intricate timing and coordination

A CLEAN MOUSE RESEARCH LAB

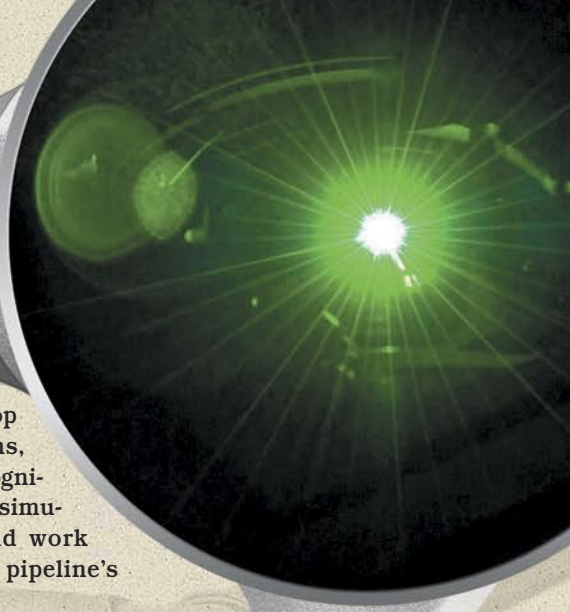
The new "Mouse House" is a tribute to Bill and Lee Russell, the husband-and-wife team who conducted genetic research at ORNL for nearly 50 years.

Made with the mouse in mind. That is one way to describe the William L. and Liane B. Russell Laboratory for Comparative and Functional Genomics. The $14 million Russell Lab is a new 36,000 ft_2 building on ORNL's life and environmental sciences campus, where researchers will determine the biological functions of a subset of some 30,000 mouse genes, 85% of which are identical to human genes.

"This Department of Energy user facility was designed from the mouse's point of view," says Dabney Johnson, leader of the Mammalian Genetics Group in ORNL's Life Sciences Division. "With its air locks and high-tech air filtration, the facility is pathogen-free, unlike our old Mouse House. The Russell Lab is perceived as a source of clean mice because it poses no risk of contamination, enabling our biologists to collaborate more with outside scientists."

The vivarium, which can house up to 60,000 mice, operates more efficiently and has lower utility and maintenance costs than the old Mouse House. The savings allows ORNL to stretch its research dollars farther, compete more effectively for funds from the National Institutes of Health, and attract new research talent.

Only designated researchers and animal care contractors can enter the Russell Lab. They must take "air



Certain bacterial proteins may be located using ORNL's confocal laser scanning microscope.

of gene regulation at the level of RNA in bacterial cells. With a grid of red and green dots of different brightnesses, a microarray indicates which genes encode a high level of protein production and which ones instruct the host cell to produce little or no protein. The process allows scientists to compare gene activity in different microbes and their mutants when exposed to toxic metals such as uranium, strontium, and chromium.

"We have created 40 different mutants of *Shewanella* bacteria," Zhou says. "Mutant bacteria are important to the understanding of the functions of genes. We are using microarrays to determine which bacterial genes encode proteins under different conditions. That way we will find out which genes enable a bacterium to effectively reduce a target contaminant despite the presence of other toxic materials."

Imaging Live Cells in Action

A novel way to observe which proteins are together in a complex is live-cell imaging. Mitch Doktycz and his colleagues in LSD are developing ORNL's imaging capability. Recently, Doktycz's group used an atomic force microscope to take images of *R pal* grown both in air and in an oxygen-free liquid. They observed different shapes and surface characteristics of the bacteria, depending on how they were grown.

showers" to remove debris from their clothing, put on special shoes, and "suit up" before they handle mice. Mouse food, bedding, cages, glassware, surgical equipment, and anything else brought into the facility must be spray-disinfected, fumigated, or sterilized in steam in an autoclave there.

Starting in the 1980s at the old Mouse House, ORNL biologists collected, froze, and catalogued embryos, sperm, and ovaries from more than 1400 mutant mouse strains, each of which has a unique set of genetic mutations. Many of these frozen embryos will be brought back to life in the Russell Lab.

Purchased, certified clean female mice introduced to the facility are being mated with vasectomized male mice. Trained staff members surgically implant thawed embryos in the oviducts of these surrogate mothers. By October 2004, some 75 strains of healthy mice should be thriving in DOE's major animal research center.

Doktycz's group has an epifluorescent microscope and a recently acquired confocal laser scanning microscope, now the standard tool for live-cell imaging. The instrument enables researchers to see which proteins are interacting with each other and with other molecules inside a live cell in real time.

The Computer Connection

Researchers in LSD's Genome Analysis and Systems Modeling Group, led by Frank Larimer and Ed Uberbacher, are a key part of the pipeline. These researchers are also part of the Computational Biology Institute in DOE's Center for Computational Sciences at ORNL, which houses several supercomputers. They develop and apply algorithms, models, pattern recognition programs, and simulation methods and work on automating the pipeline's computational part.

The major emphasis of the group has been to identify genes in sequenced DNA. The researchers found genes in 100 microbes and in human chromosomes 5, 16, and 19 after they were sequenced by JGI. In addition, the group has developed the PROSPECT algorithm to predict the three-dimensional structures of proteins—important clues to their functions. To support the Genomics: GTL project, this group of computational biologists is improving the flexibility, efficiency, and accuracy of peptide identification algorithms.

Existing software is designed to handle relatively small data collections. The proposed GTL facility, which will house 60 mass spectrometers, could produce approximately two million spectra a day. Uberbacher is leading the development of a new suite of workstation tools to process that volume of data.

To better understand what protein complexes do in bacterial cells, Larimer and his colleagues characterize and describe the components of a cell and its environment. They "guess" which proteins are processing specific metabolites, which include sources of energy, carbon, and nitrogen needed by cells. Then they build a model to describe how the organism works, while characterizing the functions of its components. "We may build a systems model of a bacterial community," Larimer says, "and predict what the community will do if a toxic metal is added."

Systems biology remains a tough challenge. ORNL is counting on new state-of-the-art tools and facilities, combined with an excellent staff and collaborators, to demonstrate the feasibility of assembling and operating an automated pipeline. ORNL researchers are increasingly confident that these assets will help lead them to significant scientific discoveries about biological systems. ®

UT graduate student Claretta Sullivan images bacteria using an atomic force microscope.

A Return on Investment

Understanding responses to environmental changes and improving the health and lifespan of ecosystems and people are among the potential benefits of systems biology.

As ORNL researchers seek answers to life sciences' persistent questions, some are struck by how systems biology applies to life on a variety of scales. Rich Norby, a physiological ecologist in ORNL's Environmental Sciences Division (ESD), is one of those scientists. At the annual American Association for the Advancement of Science meeting in 2004 in Seattle, Norby heard LeRoy Hood, pioneer of the DNA sequencer, "talk about systems biology in a way that made me think, 'That's what I do.'" Hood studies living cells and immunology while Norby focuses on forests and global change.

"Dr. Hood was talking about DNA, proteins, and underlying networks in a live cell and I talked as a panelist at the AAAS conference about trees, roots, microbes, air temperature, and carbon dioxide in a small forest ecosystem. There's a huge gulf in scale there, but I think the basic principles are still the same. In both cases the objective is to identify components of the system and analyze their interactions to reveal emergent properties of the system as a whole."

Systems biology research at ORNL has applications at different scales, as shown in a few examples in this article. The research described relates to ecosystems and global change, energy production, bioremediation, and human health.

Ecosystems and Global Change

Norby is one of many ESD researchers who studied individual seedlings in chambers to determine how their physiology was affected by exposure to one pollutant such as ozone. The scientists studied a simple, controlled system that was a long way from today's endpoint of a global forest.

FACE experiment. After a number of years experimenting with small tree seedlings in growth chambers and saplings in field chambers, Norby became involved in the much larger, more complex but controlled Free-Air Carbon Dioxide (CO_2) Enrichment (FACE) experiment, a Department of Energy user facility at ORNL. The purpose of this experiment, now in its seventh year, has been to help DOE understand more completely the consequences of elevated atmospheric CO_2. Whereas in the past, the focus of experiments was on individual components of the system, the current focus is on integration of component organisms and processes into a system-level response.

In the FACE experiment, tons of CO_2 are pumped into plots of sweetgum trees, so that the concentration of CO_2 in the tree stand is almost 50% higher than the ambient level. Norby and his colleagues then compare the responses of the forest ecosystems exposed to elevated levels of CO_2 with the responses of the forest in ambient air.

Each year, ORNL staff measure net primary productivity (NPP), or the total amount of carbon fixed into organic matter in the ecosystem, above and below ground. They have found that the NPP of the plots exposed to elevated CO_2 was enhanced by about 23% annually over six years. But how that carbon was allocated changed over time.

"In the first year," Norby says, "the trees exposed to elevated CO_2 had a 35% increase in wood production—aboveground trunks and branches. In the second year it was 15% and then in the past four years we measured only a 5 to 7% annual increase in wood production. Instead, the NPP shows up in fine root production. Our technician Joanne Ledford has measured and documented increases in production of fine roots over six years, which is a significant and unprecedented response."

Fine roots are an important component of a forest system because they regulate the

The effects of elevated levels of carbon dioxide on a forest are being determined at this ORNL sweetgum stand.

A new experiment at ORNL is measuring the growth of roots of seven species of grasses, herbs, and weeds.

cycling of carbon, water, and essential nutrients. Unfortunately, for DOE's interest in removing CO_2 from the air, fine roots do not store carbon for much more than a year, unlike the wood in tree trunks, which can store carbon for decades.

"Fine roots have a short life, and when they die, microbes digest them to get energy" Norby says. "Much of the carbon in fine roots is returned to the atmosphere as CO_2. That's not a good story if your interest is net removal of CO_2 from the atmosphere."

However, because considerable carbon is moving through the soil system, an opportunity exists for some of it to be trapped in longer-lived soil organic matter pools. One important research challenge at the FACE facility is to quantify the amount of carbon that remains in or gains access to these pools. Researchers are seeing indications that, compared with the ambient plots, the FACE plots show an increase in 'protected' carbon—soil carbon that will not decompose right away.

"Through understanding the interrelationships between components within the system and how they work together to get an integrated response, we will have a stronger basis from which to project the responses of forests to global change," Norby says. "Systems biology applied at a large scale can help DOE better understand biological impacts of atmospheric and climatic change."

Underground activities. The FACE experiment demonstrated the importance of whole-system analysis, including responses below the ground. The experimental system, however, is very simple—one dominant species (sweetgum) and one environmental change (CO_2).

A new study at ORNL is investigating the responses of a more complex community to multiple environmental change factors. Near the FACE facility is the Old-Field Community, Climate, and Atmospheric Manipulation (OCCAM) experiment, a new joint project between ORNL and the University of Tennessee. ESD's Steve DiFazio is the principal investigator of an internally funded Laboratory Directed Research and Development Program project on ecosystem genomics that makes use of the OCCAM experiment.

DiFazio is testing genomics in a systems-level approach on abandoned land to determine the amount of growth of roots of seven different species of grasses, herbs, and weeds subjected to three different treatments—ambient and elevated atmospheric CO_2, ambient and increased temperature (higher by 3°C), and ambient and decreased soil moisture. The ORNL researchers are interested in how the different combinations of treatments change the composition of the plant community, and how this transformation alters ecosystem responses.

The different species are not difficult to distinguish above ground, but observing community composition changes below ground presents a challenge. "In the OCCAM plots, you cannot tell which species the intertwined roots belong to because there is no easy way to distinguish, based on a root's appearance, which of the aboveground species it came from," DiFazio says.

To solve the problem, he and his colleagues are taking a novel approach based on the new field of ecosystem genomics. The approach views ecosystems not as a web of habitats for a variety of species but rather as a stage on which genes, proteins, and living cells interact.

"We are investigating a method in which characteristic DNA from the roots of the individual plants is used to identify the species," DiFazio says. "We will take a plug of soil, grind it up and, using our DNA-based technique, determine the relative abundance of each species present. By comparing the different treatments, we will know more about how different species respond to changes in carbon dioxide levels, temperature, and soil moisture."

Another aspect of the project is an assessment of the indirect effects of the plant responses to ecosystem perturbations. For example, researchers are uncertain how microbial populations will respond to increased productivity and competitiveness of individual plant species. Also, if the plants have a higher rate of photosynthesis under changing conditions, will microbial populations that fix nitrogen for plants adapt fast enough to meet plants' nutritional needs?

To address these questions, ESD's Jizhong Zhou is using microarrays to assess the responses of microbial populations in this experiment. His team will determine how the microbial populations change in response to the treatments, and whether changes in plant populations are reflected in the composition and functioning of the microbial communities. Detailed, integrated studies such as these are required to achieve a systems-level understanding of the effects of climatic change.

Improving the efficiency of biological hydrogen production is an ORNL research goal.

Energy Production

A systems biology approach to understanding a protein complex could unlock a source of energy, according to Brian Davison, director of ORNL's Life Sciences Division (LSD). One complex is hydrogenase, an enzyme that can take electrons and protons from other enzymes and compounds and use them to release hydrogen for use in power-producing fuel cells. "This is a way to produce energy in the form of hydrogen using a biological system," Davison adds.

Systems biology may enable researchers to find smarter ways to harness the ability of microbes to produce hydrogen under certain limited conditions. "Everything in the life of some microorganisms has tended to limit their ability to produce hydrogen," Davison says. "Some microorganisms, we think, use hydrogenases to deal with excess energy and prevent the buildup of protons and other free radicals floating around inside their cells. We want the process to pro-

PROVIDING ACCESS TO THE BEST BIOLOGICAL TOOLS

A unique investment by the state of Tennessee will help ORNL and the University of Tennessee attract some of the world's best biological researchers.

ORNL's modern biological and environmental sciences campus will soon have a new addition: the Joint Institute for Biological Sciences. This joint institute of ORNL and the University of Tennessee, which is scheduled for construction in the spring of 2005, will have a single mission: to enable joint faculty appointees, senior staff scientists, graduate students, postdoctoral researchers, and UT research associates to perform world-class research in systems biology and biotechnology, taking advantage of ORNL's user facilities and other world-class tools. Both parties expect that some of this research will lead to the founding of new companies, spurring economic development in the region.

Funded in whole by the state of Tennessee, the joint institute will be located close to ORNL's Laboratory for Comparative and Functional Genomics, other biomolecular sciences research laboratories, and the Environmental Sciences Division buildings. The three-story building will provide offices, conference rooms, classrooms, interaction space, and molecular biology and biochemistry labs. The facility will be the home for the ORNL-UT Graduate School of Genome Science and Technology.

Researchers and students at the institute will have access to DOE's existing world-class user facilities at ORNL, including the Russell vivarium, the Free Air Carbon Dioxide Enrichment facility, Walker Branch Watershed, the Natural and Accelerated Bioremediation Research Facility, high-performance computing resources at the Center for Computational Sciences, and neutron sources at the High Flux Isotope Reactor and the Spallation Neutron Source (which comes on-line in 2006). For characterizing proteins and protein complexes in cells, researchers will also apply ORNL's extensive mass spectrometry instrumentation, neutron scattering and diffraction at HFIR and SNS, X-ray diffraction equipment, and advanced microscopes and other imaging tools.

The institute was conceived as a unique program to encourage multidisciplinary, collaborative research in the biological and environmental sciences. UT and ORNL researchers will specialize in microbial functional genomics, comparative genomics, plant genomics and physiology, biophysical chemistry, nanobiotechnology, bioengineering, structural biology, bioinformatics and computational biology, and ecosystem genomics for environmental change sensing and forecasting.

"This state-of-the-art institute will catalyze world-class interdisciplinary research in modern biology and attract top scientists and engineers to UT and ORNL," says Reinhold Mann, associate laboratory director for biological and environmental sciences. "We will leverage this state investment and partnership to advance biology with applications in clean energy, environmental stewardship, and human health."

duce hydrogen all the time, so we are studying the enzyme's active site and its partners to see how hydrogen is generated."

Scientists have found evidence that when hydrogenase is used to produce hydrogen from water, the oxygen that is formed can hurt the hydrogenase. Research at ORNL and elsewhere seeks to solve this problem to improve the efficiency of biological hydrogen production.

"When we fully understand natural systems," Davison says, "I believe we can coax them to do our bidding in a smarter, better way than in past biological approaches where success came from doing experiments and getting desired results by accident."

Bioremediation

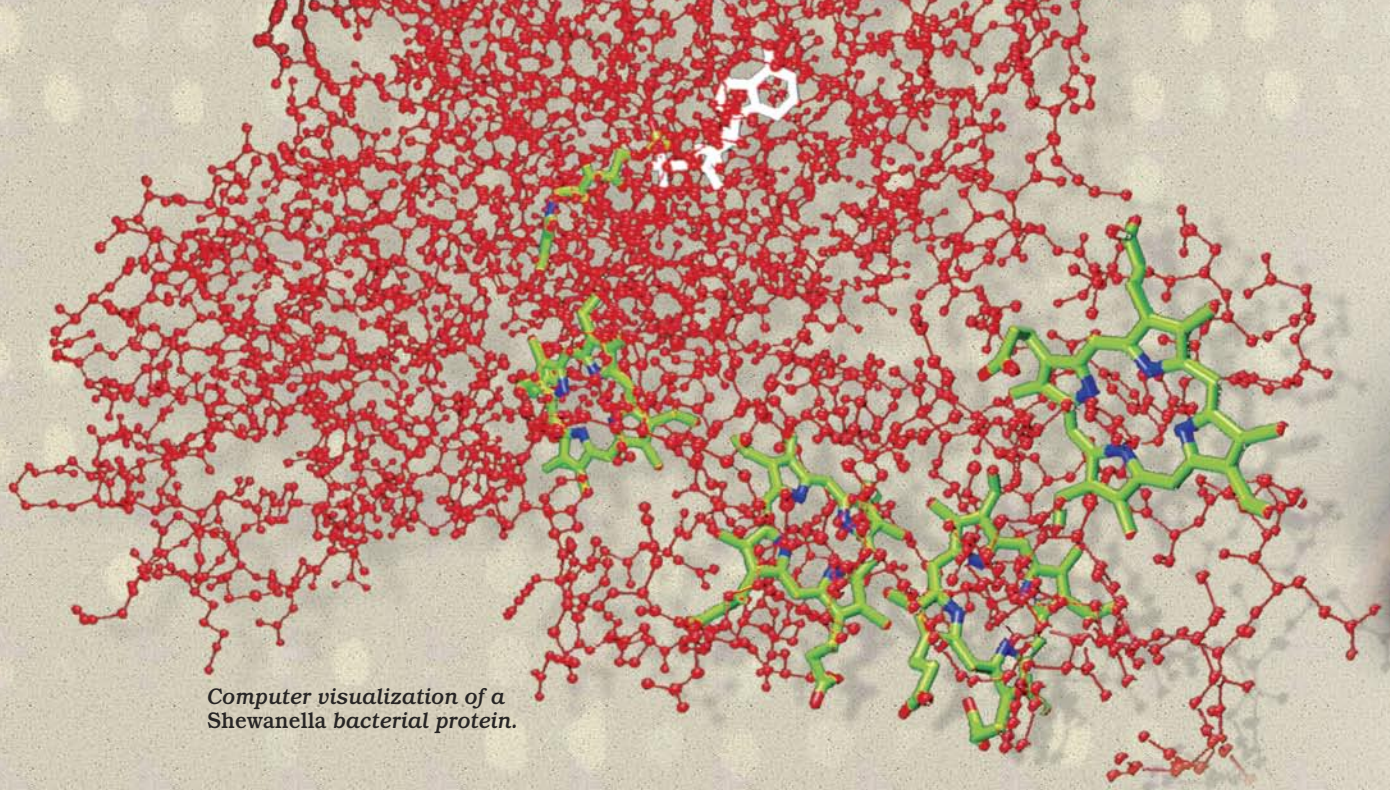
Bioremediation is the use of microorganisms to eliminate, contain, or reduce the concentration of contaminants in soil and water. One of DOE's missions is to use bioremediation to clean up waste sites or immobilize wastes so they do not migrate. Using a systems biology approach, ORNL researchers have been helping DOE identify bacteria that can immobilize and make less bioavailable any compounds containing radionuclides, such as uranium and strontium, and toxic metals, such as chromium, technetium, and mercury.

ORNL researchers are helping DOE search for bacteria that show great promise for changing uranium compounds from a soluble to an insoluble chemical state. Such transformed uranium compounds are more likely to stay put in soil or sediments rather than dissolve in groundwater and flow off-site. If scientists can find a bacterium that is especially effective at reducing uranium compounds, the discovery could well meet DOE's environmental goals and save millions of dollars in potential cleanup costs.

Researchers could characterize the capabilities of this new bacterium and try to identify the genes that enable the reduction of each uranium atom by donating two electrons. Such an interaction with metal enables the bacterium to extract energy from carbon.

Jizhong Zhou, Dorothea Thompson, and others on Zhou's team have been studying the bacterium *Shewanella oneidensis* strain MR-1, whose genome was completely sequenced by The Institute for Genomic Research (TIGR) and annotated by TIGR as well as LSD's Frank Larimer and others. *Shewanella* is able

Joint Institute for Biological Sciences



Computer visualization of a Shewanella *bacterial protein.*

to make uranium less soluble in the laboratory, but further research is needed to determine how well the bacterium responds at a toxic waste site.

"Because of DOE's mission, we are trying to understand how *Shewanella* responds to environmental stresses such as high and low pH, high temperature, high salt, and metal toxicity," Thompson says "DOE wishes to know how *Shewanella* transforms, detoxifies, and reduces metals in the environment. DOE also seeks to understand the relationship to environmental stresses and which stresses make the process less effective in bioremediation."

DNA microarray technology allows Zhou's team to place an array of at least 20,000 DNA probes, each corresponding to a single microbial gene, on a glass microscope slide. They can look at the global expression—all the responses of genes at the messenger RNA level—in a bacterial cell exposed to a toxic metal such as strontium or chromium. Then, by interacting with the mass spectrometry group at ORNL, they can determine if the switched-on, or up-regulated, genes produce corresponding increases in the encoded protein products.

Working with Steve Brown, an ESD postdoctoral researcher, Thompson found in some cases that a limited set of genes in *Shewanella* revealed greater than a hundredfold increases in expression in response to exposure to strontium. Some of these differentially expressed genes encode enzymes that synthesize siderophores, low-molecular-weight compounds that show a high affinity for binding iron.

"By disrupting a gene in *Shewanella*, we have produced a mutant that is unable to produce the siderophore," Thompson says. "We found this mutant displays an increased sensitivity or lower tolerance to strontium than the normal bacterium, suggesting that siderophores may be involved in the resistance mechanism."

In Zhou's laboratory, researchers have built microarrays for a mixture of microbes in contaminated samples to determine which genes in bacteria have been turned up or down by exposure to the contaminated site. They have found that many of the different bacteria have adapted to the contaminated site by changing the amounts of specific proteins produced.

The researchers have determined how the microbe population changes in soil when nitrogen is added or when a contaminated site is remediated. No other group has been able to characterize in detail how a community of different species of microbes changes in a contaminated soil or groundwater sample and how that community differs from a community of the same species in a clean reference sample.

Zhou and his colleagues have been analyzing microbes present in groundwater at an Oak Ridge site that has legacy wastes with a high concentration of toxic metals and radionuclides. The site is a Field Research Center (FRC) of DOE's Natural and Accelerated Bioremediation Research (NABIR) Program, located at the Y-12 National Security Complex on DOE's Oak Ridge Reservation. Zhou believes that microarray analysis of many samples taken from the NABIR site will produce the microbe that is the most effective at reducing uranium.

"It is very expensive to pump and treat groundwater, so DOE would like to speed the growth of bacteria that can convert uranium and technetium compounds to materials that are less mobile and less toxic," says ESD's David Watson, manager of the FRC in Oak Ridge. "We hope that this bioremediation strategy for wastes containing metals and radionuclides will economically reduce risks to human health and the environment."

Human Health Benefits

Live-cell nanobiosensor. The first observation of programmed cell death in a single live cell, or apoptosis, was made recently at ORNL by Corporate Fellow Tuan Vo-Dinh and two colleagues. Vo-Dinh has led the development of "nanobiosensor" technology for investigating vital biomolecular processes, including interactions between proteins in living cells.

Vo-Dinh, leader of LSD's Advanced Biomedical Science and Technology Group; Paul Kasili, a Ph.D. degree candidate at the UT-ORNL Graduate School of Genome Science and Tech-

ORNL's nanobiosensor penetrates a cell without destroying it and targets a specific protein.

nology, and postdoctoral researcher Joon Myong Song recently published papers on the optical nanobiosensor for measuring apoptosis in a single living cell in the *Journal of the American Chemical Society* and in *Nature*.

"This minimally invasive nanotechnology allows scientists to go inside a live cell and follow its molecular processes in real time," Vo-Dinh says.

The nanobiosensor is a tiny fiber-optic probe drawn to a tip of only 40 nanometers (nm) across—a thousand times smaller than a human hair. Experiments have demonstrated that such a probe is small enough to be inserted into a cell and withdrawn without destroying it. Light from a laser can be directed through the fiber-optic probe.

Because the 40-nm width of the probe tip is much more narrow than the 400-nm wavelength of the light, only molecules near the tip are excited by the laser signal. In this way, scientists can target specific molecules inside the cell, such as proteins, enzymes, or DNA strands.

Vo-Dinh and his colleagues have demonstrated that a fiber-optic probe with a bioreceptor molecule at its tip can be manipulated inside a cell to find a target protein. When the protein binds to the bioreceptor, a laser signal excites the target molecule, causing it to fluoresce. The resulting glow is detected.

The team recently detected the signaling process involved in apoptosis—a key process in an organism's ability to prevent disease. The programmed cell-death mechanism causes the cell to self-destruct before it can introduce disease to the organism.

"When a cell in our body receives insults such as toxins or inflammation and is damaged, it kills itself so that it does not propagate," says Vo-Dinh. "The loss of cells' ability to undergo apoptosis is one cause of uncontrollable cell growth leading to cancer. For the first time we have seen apoptosis occur within a single live cell."

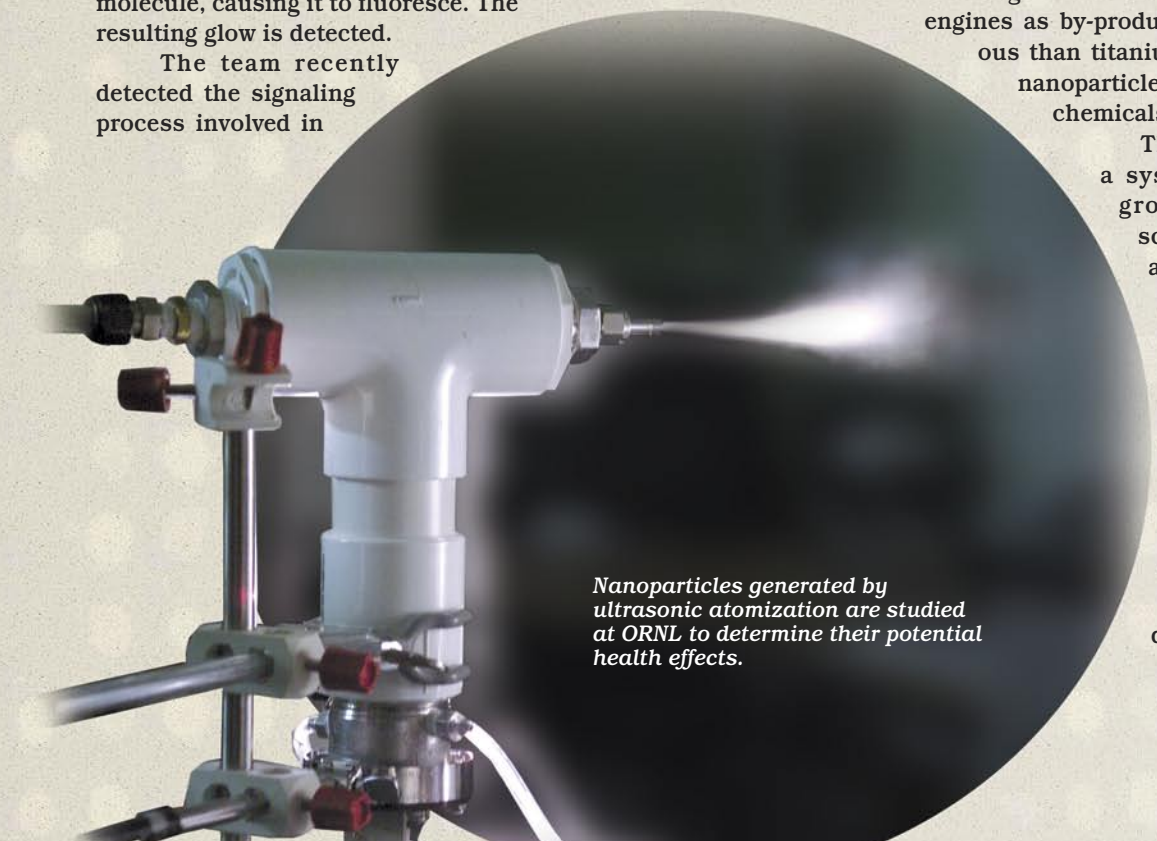
Environmental and engineered nanoparticles. Some particles in the air we breathe are smaller than one 100 billionths of a meter in diameter. To improve our understanding of these "nanoparticles" and their impacts on human health and the environment, ORNL researchers led by ESD's Mengdawn Cheng have developed special technologies. Their inventions produce well-defined nanoparticles of a known size and composition and measure the responses of lung cells to particles of different sizes. The research is of particular importance to DOE because emissions from internal combustion engines in automobiles, trucks, off-road vehicles, and aircraft are known to contain nanoparticles.

"Our research suggests that the environmental and health effects of nanoparticles are different from the impacts of particles in the micrometer size range, even when particles of different sizes have an identical chemical composition," Cheng says.

"Using a direct air-cell exposure approach, we found that human lung cells exposed to 10-micron titanium dioxide particles showed little damage. But other lung cells died when exposed briefly to 20-nanometer titanium dioxide particles. It appears that the size of the particles, their surface properties, and area of exposure affect cellular response and increase the nanoparticles' toxicological potency toward biological tissues."

Cheng believes that nanoparticles emitted from engines as by-products are potentially more dangerous than titanium particles. The reason: engine nanoparticles are complex mixtures of organic chemicals and toxic metals.

The work of Cheng's group has a systems biology flavor when the group applies precision aerosol science and technology to detect and characterize the biomarkers generated by human lung cells exposed to nanoparticles. The group collaborates with university researchers as well as scientists at DOE and Department of Defense laboratories. Cheng expects results from the research will have significant impacts on future emissions controls, environmental and occupational health regulation, and defense work.



Nanoparticles generated by ultrasonic atomization are studied at ORNL to determine their potential health effects.

Combating blindness. Plant proteins might someday provide higher-resolution vision for the legally blind than current and near-term artificial retina implants such as electrode arrays. Recent research at ORNL showing that plant molecules can be fused with mammalian cells suggests this exciting possibility could be realized soon. The research was led by Eli Greenbaum, a corporate fellow in ORNL's Chemical Sciences Division (CSD) and former leader of DOE's Artificial Retina Program, which involves research by several national laboratories.

"Mammalian and plant systems have been separated by two billion years on the evolutionary time scale, but we showed it is possible to combine them," Greenbaum says. "This work fits well into systems biology because large multicomponent systems are involved."

Greenbaum and CSD's Tanya Kuritz showed that a spinach protein—a light-absorbing pigment, or "photosynthetic reaction center," called Photosystem I (PSI)—could be incorporated in a liposome, an artificial membrane made of lipids. In collaboration with Professor Ida Lee of the University of Tennessee, the team demonstrated that a voltage high enough to make a nerve cell fire is generated by PSI inside a liposome when exposed to light. They then inserted the PSI-containing liposomes into membranes of retinablastoma cells, which are cancerous versions of cells in the eye's retina. The process demonstrated that the presence of PSI molecules is essential to making eye cancer cells respond to light.

"What we do not know is whether these spinach proteins are stable enough to last a long time and whether they would undergo immune rejection by the eye," Greenbaum says.

Courtesy of Lawrence Livermore National Laboratory.

Longevity and genes. "Aging is a perfect example of systems biology," says Dabney Johnson, leader of LSD's Mammalian Genetics Group. "Like mice, people are predetermined to live a long life or a short life, depending on whether they have a network of longevity or 'shortevity' genes."

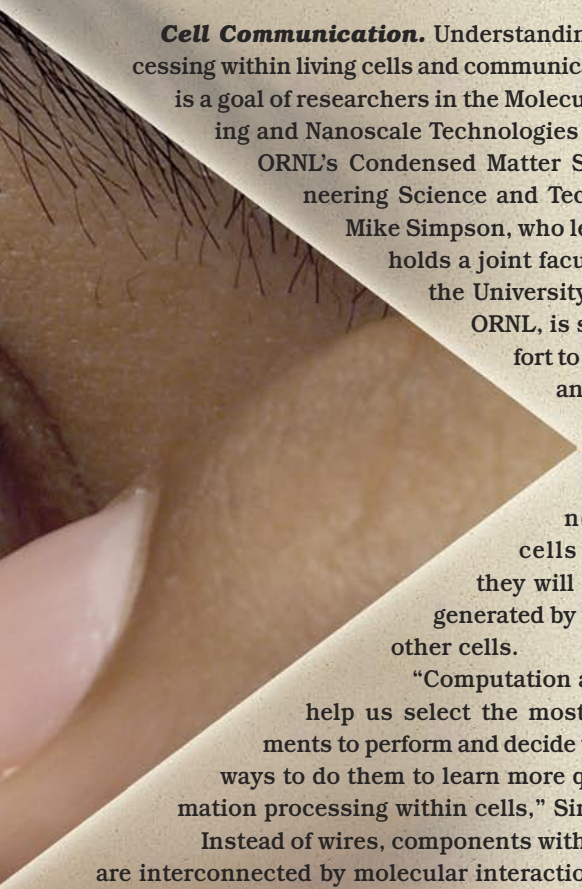
Two years ago, members of the Tennessee Mouse Genome Consortium (TMGC) were surprised to learn that the National Institute on Aging was less interested in knowing which diseases shorten a lifespan or impair health and wellness and more interested in finding out which genes increase a healthy lifespan. The NIA was responding to findings that 200 genes in a recently sequenced worm are related to lifespan and that making a mutation in any one of these genes will alter the worm's longevity. The NIA funded a TMGC study to identify mouse genes that affect lifespan. TMGC researchers targeted different longevity genes for mutation in individual mice by using ENU, the chemical mutagen discovered 25 years ago at ORNL.

The offspring affected by the ENU treatment are being aged to their full lifespan at a UT mouse facility. Researchers compared the blood chemistry and other characteristics of the mice to try to predict which mice might live longer. Johnson says that two factors related to a network of genes are known to affect the lifespans of mice, as well as worms, fruit flies, and—probably—people.

"Individuals who are smaller and thinner live longer," she notes. "Individuals that are big, heavy, bulky, and tall for their species tend to die young.

"The other factor associated with longevity is resistance to stress. Individuals who are usually on an even keel live longer. The theory is that individuals subjected to prolonged stress make oxygen radicals inside their cells. The radicals damage proteins, DNA, and lipids inside cells. Damage accumulated over a lifetime diminishes the functioning of cells."

In about two years, the researchers will know which of the mice with "mutated longevity genes" lived a significantly long life. They then can determine which gene or genes are involved in longevity.



DOE's Artificial Retina Program, previously managed by ORNL, focuses on construction of microelectrode arrays that would directly stimulate surviving retinal tissue in people who become blind as a result of retinal degenerative diseases.

Cell Communication. Understanding information processing within living cells and communication between them is a goal of researchers in the Molecular-Scale Engineering and Nanoscale Technologies Research Group of ORNL's Condensed Matter Sciences and Engineering Science and Technology divisions. Mike Simpson, who leads this group and holds a joint faculty appointment at the University of Tennessee and ORNL, is spearheading an effort to use computational, analytical, and experimental tools to simulate genetic circuits and genetic networks in cells and predict how they will respond to signals generated by the environment or other cells.

"Computation and simulation will help us select the most important experiments to perform and decide the most intelligent ways to do them to learn more quickly about information processing within cells," Simpson says.

Instead of wires, components within a genetic circuit are interconnected by molecular interactions such as regulatory protein–DNA interactions to control gene expression; RNA polymerase–DNA interactions that produce messenger RNA (mRNA) during gene expression; and ribosome-mRNA interactions that produce proteins that carry out cellular functions. A small subset of interconnected reactions that carry out a single function is considered a genetic circuit; a genetic network, which hooks together multiple genetic circuits, is responsible for complex cellular functions.

Simpson's group has written mathematical expressions to represent the components of genetic circuits and genetic networks to understand better the biological functions of bacterial cells. The approach is one of many used in systems biology, an emerging discipline that endeavors to apply analytical tools and approaches more familiar to the physical sciences to biological problems.

For a U.S. Defense Advanced Research Projects Agency project funded jointly with the National Science Foundation, UT graduate students in Simpson's group have developed software tools that simulate and analyze "stochastic fluctuations"—random noise in cellular biomolecular populations that may be important to genetic circuit fluctuations. For example, stochastic fluctuations are vital to the decision-making process in the phage virus's infection of *E. coli* bacteria. To examine such processes and gain new insight into biological function, the UT-ORNL collaboration developed and published papers on the Exact Stochastic Simulator that simulates noise and its effect on biological systems.

The group is refining an experiment in which genetic components of a cell-cell communication system found in the marine bacterium *Vibrio fischeri* are inserted into *E. coli*. *V. fischeri* cells do not give off light unless a large enough population of cells is present, such as a squid's "light" organ that offers them a nutrient-rich environment. When populated with *V. fischeri*, this otherwise dark organ suddenly becomes luminescent. Thus, the squid's predators lurking below cannot distinguish the "camouflaged" squid from starlight above.

Simpson's group is interested in the genetic circuits that process cell-cell communication like that found in *V. fischeri*. "Cell-cell communication is the mechanism that allows groups of cells to coordinate their activities and produce the complex group behaviors that lead to infection, biofilm formation, and functioning tissues, organs, and organisms," Simpson says. "By looking at the more primitive communication systems in bacteria, we hope to develop an understanding of information processing in cellular communication systems of more complex organisms, especially those that impact human health." ®

Dabney Johnson and her colleagues are studying old mice to determine which genes increase a healthy lifespan.

P I O N E E R of Biological Research

Editor's note: Bill Russell (1910-2003) was an internationally renowned ORNL biologist and member of the National Academy of Sciences, whose research led to human radiation protection standards. At the October 4, 2003, memorial service for Bill Russell, Dabney Johnson, leader of the Mammalian Genetics Group in ORNL's Life Sciences Division, delivered this tribute to her mentor.

I didn't even realize for years after I met Bill at a gathering of the Tennessee Citizens for Wilderness Planning he helped found that this light-hearted and gentle man was a famous scientist who turned his agile and funny brain to serious subjects—like estimating the genetic risk that people face following exposure to radiation and chemicals. My discovery of the "other Bill" came one day at his Watts Bar Lake cabin, a decade after Bill had retired and after I had joined the Russell group as a "young" graduate student.

Bill passed the rainy afternoon explaining to me the "specific locus test," which measures the frequency of transmitted gene mutations induced in mouse cells that are ancestors of sperm. I was awed as I began to understand the impact of the test and the creative thinking that Bill put into it. I saw Bill in a different light after that day. He always remained a friend and colleague who could be comfortable on my level, but I began to understand that he also occupied an intellectual realm that I (and few others of us) could ever access.

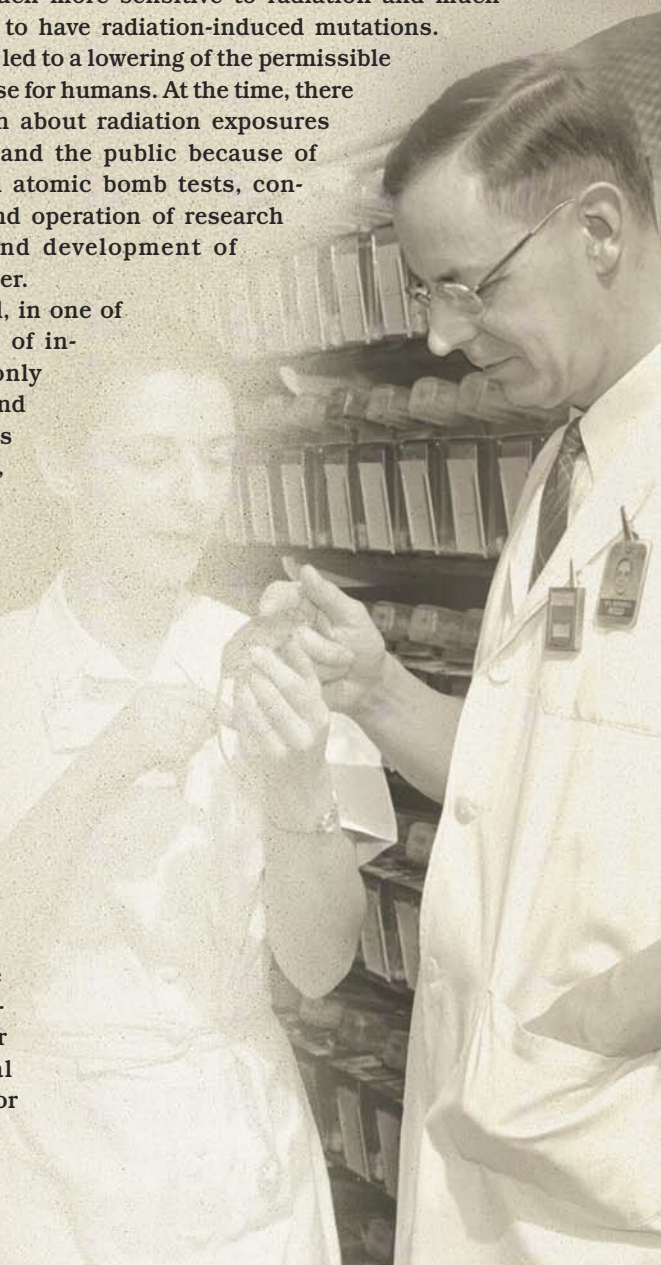
I never lost the "first Bill," of course. How many Ph.D. celebration parties have included a Gilbert and Sullivan–style serenade written and sung by a member of the National Academy of Sciences?

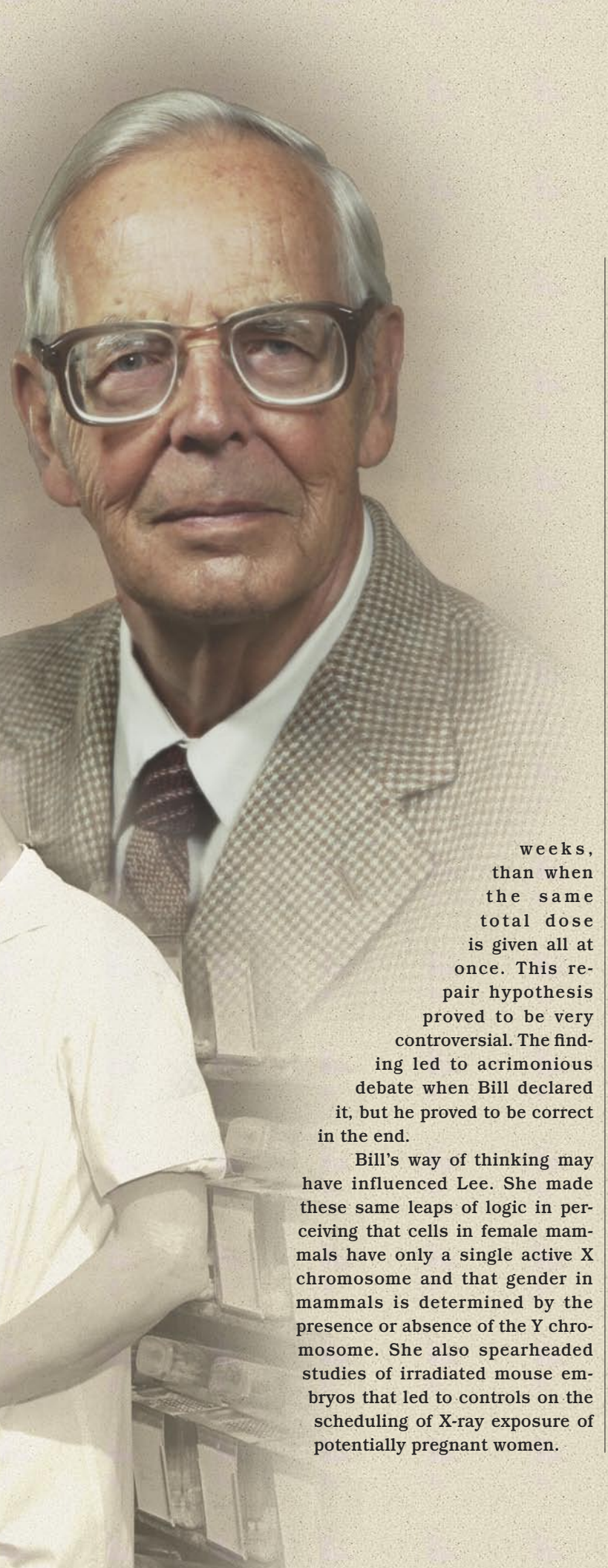
In 1936 Bill earned his Ph.D. degree at the University of Chicago, under the famous population geneticist Sewall Wright, who helped Bill set the stringent scientific attitude and standards that were hallmarks of both Wright and Russell. From Chicago, Bill moved to the Jackson Laboratory in Bar Harbor, Maine, switched from guinea pigs to mice, and began his illustrious career. I never knew Tibby Russell, Bill's first wife, but have heard from others who did know her that she was as special as Lee is. Bill once told me, "I am unique; all my wives are members of the National Academy of Sciences."

Beginning in 1947, with Lee at this side, Bill organized and presided over the Mammalian Genetics Section of the Biology Division at ORNL. The section's initial goal was to explore the genetic hazards of radiation to humans. Earlier work in using fruit flies as the model organism had established a set of radiation genetics principles that were thought to be well understood and assumed to be universally applicable. Bill, however, conceived and developed the specific-locus test to measure both the biological and physical factors that influence mutation frequency in mammals. Experiments designed and overseen by Bill led to the first determination of how frequently X-rays induce mutations in mammals. These experiments led to a number of important discoveries. I've selected three discoveries that I consider most important, although several others could easily make the list.

First, he discovered that, compared to fruit flies, mammals are much more sensitive to radiation and much more likely to have radiation-induced mutations. This finding led to a lowering of the permissible radiation dose for humans. At the time, there was concern about radiation exposures of workers and the public because of fallout from atomic bomb tests, construction and operation of research reactors, and development of nuclear power.

Second, in one of those leaps of insight that only very open and agile minds can make, Bill realized that some reproductive cells in mammals must be able to repair genetic damage. His experiments showed that the mutation rate is lower when the dose is protracted over an interval of days or





weeks, than when the same total dose is given all at once. This repair hypothesis proved to be very controversial. The finding led to acrimonious debate when Bill declared it, but he proved to be correct in the end.

Bill's way of thinking may have influenced Lee. She made these same leaps of logic in perceiving that cells in female mammals have only a single active X chromosome and that gender in mammals is determined by the presence or absence of the Y chromosome. She also spearheaded studies of irradiated mouse embryos that led to controls on the scheduling of X-ray exposure of potentially pregnant women.

The third of Bill's findings—that different mutagens cause different kinds of mutations—has proved important for research that continues to this day. Bill's discovery that the chemical ENU is the best mutagen for producing point mutations in mice has formed the basis for huge research programs currently supported by many sponsors interested in mouse models for human genetic diseases. ORNL conducts biological research using ENU. Millions of dollars a year are spent worldwide on genetics research using ENU.

For this body of work, which led to the realization that mutation frequency can be mitigated by repair of DNA damage caused by radiation, Bill was twice nominated for a Nobel Prize.

I could not give a précis of Bill's career at ORNL without including the famous trip that he and Gene Oakberg made in the 1950s to the site of an aboveground atomic bomb test. They stacked cages of mice in an old Ford and drove from ORNL to Nevada. Because they needed females in various stages of gestation, they had to check vaginal plugs (a sign of mating) along the way, sneak the mice into motels, and fill water bottles for the mice in bathroom sinks wherever they stopped. Once at the Nevada test site, they spent days practicing quick recovery of the cages from exposure chambers built into the desert floor. Obviously, the environment for both mice and scientists would be highly radioactive after the bomb test, so only a few minutes of whole-body exposure in getting the mice out of their cages could be tolerated. Bill and the mice returned to Oak Ridge on a DC3 airplane, arriving just in time to be accused of contaminating practically the whole city until it was realized that the fallout cloud had accompanied them home. The offspring of those mice carrying radiation-induced mutations from that Nevada experience are still in use in our research program.

Bill was also very interested in why genetically identical mice often show variable traits. He did some fascinating work transplanting ovaries, even from female fetuses, to see if the maternal environment might be responsible for some of the observed variations. His famous paper "Offspring from unborn mothers" was a report of this type of work. Lee has said that his extreme near-sightedness made the handling of the tiny embryonic ovaries easier for him than for most people.

During his career, Bill served on numerous national and international committees, and he was invited to give presentations all over the world. He won many awards, most notably the Roentgen Medal (jointly with Lee) and the Fermi Award, DOE's highest honor, which Lee also won years later. Such solemn occasions gave his light-hearted side an outlet. At a Fermi Award congratulatory dinner given by Union Carbide, ORNL's managing contractor then, he professed to being glad about the "economic savings for the company, because my wife is also my supervisor, and I presume she's invited in both capacities but can eat only one dinner."—*Dabney Johnson* ®

ORNL's Unsung Discovery

Two ORNL researchers "discovered" messenger RNA in 1956, but the Nobel Prize went to other researchers who rediscovered it later.

The original discovery of messenger RNA (mRNA) by two Oak Ridge National Laboratory scientists "has never received the acclaim it deserves," says Alvin M. Weinberg, former ORNL director and a distinguished fellow of Oak Ridge Associated Universities. Weinberg is referring to Elliot "Ken" Volkin and Lazarus Astrachan's 1956 discovery of what they called "DNA-like-RNA," which François Jacob and Jacques Monod later identified as "messenger RNA."

The discovery, for which Jacob and Monod received a Nobel Prize, was "next to the original discovery of the molecular structure of DNA, probably the most important event in the history of molecular biology," Weinberg says. Paul Berg, winner of the 1980 Nobel Prize in Chemistry, calls the ORNL research an "unsung but momentous discovery of a fundamental mechanism in genetic chemistry" and a "seminal discovery [that] has never received its proper due."

Messenger RNA is the life-sustaining ribonucleic acid (RNA) that serves as the living cell's template for protein synthesis. Volkin and Astrachan first discovered the acid three years after James Watson and Francis Crick determined the structure of DNA, which makes up genes. For this 1953 discovery Watson, Crick, and Maurice Wilkins received the Nobel Prize for Medicine or Physiology in 1962.

In the mid-1950s scientists knew that genes contained the coding that dictates the molecular structure of proteins, the tens of thousands of fundamental molecules of living cells necessary for the proper functioning of an organism. They also knew that proteins were synthesized on miniature factories called ribosomes, which are found outside the cell's nucleus in the region called the cytoplasm.

Not understood was how the information from inside the nucleus is conveyed to the protein factories in the cytoplasm. One theory at the time was that each ribosome is made in the nucleus, endowed with the DNA code required to direct the assembly of a specific protein, and then exported to the cell's outer region. Some scientists speculated that RNA, a sister molecule of DNA, was involved in protein assembly because RNA is the chief ingredient of ribosomes. However, little experimental information supported the notion that RNA could carry information from the cell's nucleus to its periphery, until Volkin and Astrachan made their discovery.

Elucidation of RNA Structure at ORNL

Using radioisotopes and the ion-exchange chromatography technique developed for the separation of fission products at ORNL's Graphite Reactor, Waldo Cohn was able to isolate uniformly each of the four chemical bases of DNA and RNA molecules. He found that each base bound to the ion-exchange column in a different position, according to its unique ionic strength, depending on the pH of the solvent used to extract one material from another. Because DNA and RNA are organic molecules with pentose-phosphate backbones, Cohn and Volkin incorporated radioactive carbon and phosphorus into these molecules to help determine their structure.

By detecting and measuring the beta radiation of the chemical degradation products, scientists could obtain considerable knowledge about the structure of RNA. However, Volkin convinced Cohn that more insight could be gained using enzymatic hydrolysis.

"Ion-exchange analysis of the digestion products of the enzyme, pancreatic ribonuclease, made it possible to more clearly define the composition of RNA and, in fact, even allowed a partial sequencing of the RNA," Volkin says. Cohn and Volkin then used other enzymes to show that the principal products were mononucleotides with phosphate groups attached to the fifth carbon atom of ribose. These experiments are considered to have been essential to establishing the structure of the ribose-phosphate chain of RNA.

Discovery of Messenger RNA at ORNL

Volkin then became interested in working with bacteriophage, a virus that infects only bacteria. Other researchers had determined that no net synthesis of RNA takes place in these microorganisms. "It occurred to me that no other biological system has both active DNA and protein synthesis but not active RNA synthesis," he says.

Volkin infected bacterial cells of *Escherichia coli* with the bacteriophage virus, added phosphorus-32, isolated nucleic acid from the preparation, and hydrolyzed it with sodium hydroxide to make alkaline products that were separated using ion-exchange chromatography. The results of experiments with phosphorus-32 were confirmed using a carbon-14 precursor that was specifically incorporated into the nucleic acid bases. Larry Astrachan joined Volkin in performing these experiments, which led to the discovery of messenger RNA, but they called it "DNA-like RNA."

According to Berg, the ORNL researchers "discovered that the virus 'turns off' the [bacterial] cell's machinery for making its own proteins and 'instructs' the cell's machinery to make proteins characteristic of the virus. That instruction entails making a new kind of RNA, a copy of the virus's DNA. This discovery revealed a fundamental mechanism for gene action: the coding sequences of genes are copied into short-lived RNAs that are transported out of the nucleus into the cytoplasm, where they are translated into proteins. Because such RNAs transport

information from genes in the nucleus to the cytoplasm, they are designated as messenger RNAs."

Disputed Recognition

Salvatore Luria, who became a Nobel laureate, convinced Volkin and Astrachan to publish their first paper on RNA research in the *Journal of Virology* in 1956. The paper announcing the discovery of a new kind of RNA is titled "Phosphorus Incorporation in *E. Coli* Ribonucleic Acid After Infection."

In an interview at his Oak Ridge home in late 2003, Volkin recalled his conversation with Sydney Brenner at Cold Spring Harbor Laboratory in New York, where Volkin conducted research on the hot topic of bacterial viruses during the summers in the late 1950s. "I can well remember sitting on the lawn at Cold Spring Harbor and telling Sydney Brenner about our experiments," Volkin says. "I gave a presentation on our RNA research to the group there." In a 1977 issue of *Nature*, renowned biophysicist T. H. Jukes wrote that in 1956, "I had squeezed my way into a doorway of a packed room to hear a paper by Volkin and Astrachan on DNA-like RNA."

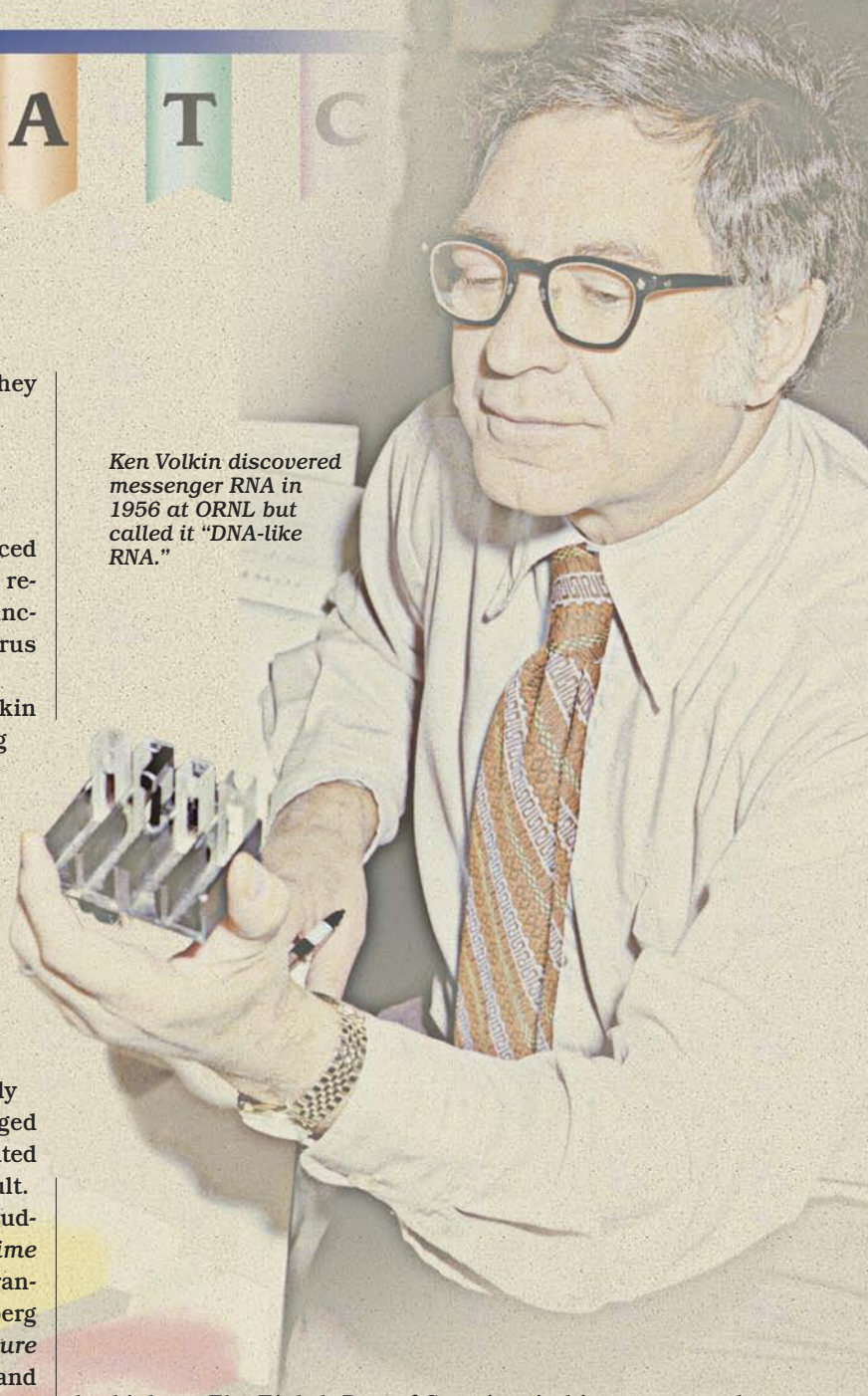
According to Volkin, the ORNL findings were not widely accepted by the biology community because they challenged prevailing theory. Nevertheless, the ORNL researchers repeated their experiment several times and achieved the same result.

In a book review in a 2001 issue of *Nature*, Horace Judson, a renowned historian of science who contributed to *Time* magazine, attributed the discovery of messenger RNA to François Jacob, Sydney Brenner, and Matthew Meselson. Weinberg published a letter in the November 29, 2001, issue of *Nature* disputing this claim. "In fact," he writes, "Jacob, Brenner, and Francis Crick, at an informal meeting on Good Friday 1960, suddenly 'discovered' the unique RNA found first in 1956 by Elliot Volkin and Lazarus Astrachan. Good accounts of this event can be found in *The Statue Within* by Jacob and *What Mad Pursuit* by Crick.

"In several publications from 1956 through 1958, Volkin and Astrachan thoroughly described the unusual properties of this RNA, which they termed DNA-like RNA. These were precisely the properties that Jacob and Jacques Monod sought to assign to the unstable intermediate (which they called X), necessary for the synthesis of galactosidase.

"Out of that Good Friday discussion on the lactose operon came the realization that Volkin and Astrachan's DNA-like RNA was indeed the genetic messenger, hence the messenger RNA (mRNA)."

In his August 2, 2003, obituary for Astrachan in the *New York Times*, Nicholas Wade cited Judson's history of molecular biology, *The Eighth Day of Creation*, in his statement that Brenner, in that 1960 meeting in Cambridge, England, with Jacob and Crick, "realized there must be a missing ingredient that carried information from the DNA in the cell's nucleus to the ribosomes in its periphery. This ingredient, he conjectured, must be the same as the transitory form of RNA seen in the Volkin-Astrachan experiment."



Ken Volkin discovered messenger RNA in 1956 at ORNL but called it "DNA-like RNA."

In 1965 French scientists Monod, Jacob, and Andre Lwoff received the Nobel Prize in Physiology or Medicine for elucidating the nature of mRNA from their observation of protein synthesis by genes of mutated bacteria in the presence of lactose. Brenner (a 2002 Nobel Prize winner), Crick, and Jacob were internationally acclaimed for the discovery of mRNA. Although these giants of molecular biology are properly credited for their accomplishments, Berg and Weinberg believe that Volkin and Astrachan have never been appropriately recognized for their original discovery. ®

PROFILE Tuan Vo-Dinh: *Inventor and Mentor*

Tuan Vo-Dinh, leader of the Advanced Biomedical Science and Technology Group in ORNL's Life Sciences Division, is one of ORNL's most prolific researchers. Born in Vietnam and schooled in Europe, he conducted research that has brought him considerable recognition through seven R&D 100 awards, six licensed technologies, more than 300 scientific journal articles, and six books. Recently, he was elected a fellow of the American Institute for Medical and Biological Engineering. A UT-Battelle corporate fellow, he is frequently invited to speak at scientific conferences. He relishes the role of mentoring young scientists. A humble person, he readily shares the credit for his impressive catalog of achievements.

I still believe that science, from time to time, needs to question its purpose and "reinvent itself...."

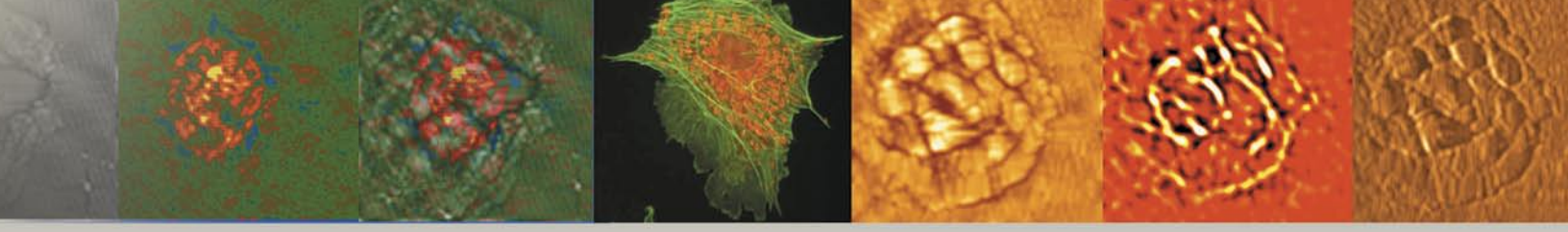
Q. When and why did you decide to become a scientist?

My parents had instilled in me the value of education and my interest in science, even when I attended high school in Vietnam. My father used to tell me that, "unlike material wealth, which can be lost any time, an education will remain with you for the rest of your life." In graduate school I really started to seriously consider a career in research. Following undergraduate studies in physics, I did my Ph.D. thesis work in biophysical chemistry in Zurich at the Swiss Federal Institute of Technology, known as ETH (Eidgenosische Technische Hochschule), where I received my real first introduction to research. ETH is where Einstein completed his formal scientific education and where Wolfgang Pauli did his work. The school was also one of Europe's epicenters for quantum physics that changed our worldview, and the department there where I did my graduate work had several Nobel Prize winners. This was the early 1970s, just after the May 1968 student revolution, which began in France and later spread throughout Europe. We, as students, were interested in so many life topics, and we often questioned the meaning and purpose of existence. In classes we read physics and chemistry books, but out of class we were immersed in books by Albert Camus, Jean-Paul Sartre, Carl Jung, and Jiddu Krishnamurti. During that period almost every student thought or dreamed, often in a naïve and innocent way, that he or she was going to "reinvent the world." In some respect, this "existentialist period" of my student life has continued to influence my thoughts about scientific research. I still believe that science, from time to time, needs to question its purpose and "reinvent itself" in order to refresh itself from outdated beliefs and old paradigms.

A decade ago, Tuan Vo-Dinh was "going nano when nano wasn't cool."

Q. What has been the most notable turn, or change, in your research over the past decade?

While the overall goal of our group has always been directed at the development of advanced technologies for the protection of the environment and improvement of human heath, there has been some gradual evolution in



our research activities from an environmental to a biological focus over the past two decades. Since the mid-1990s systems biology, an approach promoted earlier by a few forward-looking scientists, is now emerging as an important way to study and control complex systems. We know now that all biological components in the human body, from individual genes to entire organs, function and interact together in a well-orchestrated network of biological processes involving a series of intricate and interconnected pathways, to promote normal development and sustain health. In this area of research, my group is now investigating advanced tools such as nanobiosensors, optical tweezers, near-field nanoprobes, and nanoimaging systems, which have the potential to provide powerful ways to diagnose diseases noninvasively, interrogate the cell at the gene level, and fight diseases at the molecular level. I believe that systems biology is an idea whose time has come.

Q. Did your previous research prepare you for ORNL's nanotechnology thrust, what's been termed "nano-bio-info"?

At ORNL our research group was already "going nano when nano wasn't cool." About a decade ago, one of my previous co-workers, Jean-PierreAlarie, and I developed the first nanobiosensor with an antibody probe for the detection of a cancer-causing agent, benzo[a]pyrene. Recently, my graduate student (and now postdoctoral fellow), Paul Kasili, and I completed the development of a nanobiosensor capable of detecting in real time a molecular signaling process in a single human cell following treatment with an anticancer drug.

Q. Of what research are you most proud?

Many people, including coworkers, postdoctoral fellows and graduate students in my research group, have contributed to my research, and they share with me all the credit that we, as a team, have received over the years. Our group has developed several novel technologies—the dosimeter for toxic gas, the PCB spot test, the SERODS optical data storage device, the biochip to detect genetic diseases, the laser-based optical biopsy technique to instantaneously diagnose cancer without surgery, the SERS gene probe for medical diagnostics, the nanobiosensor for single-cell analysis. I really have no favorite because each of these technologies is the product of a lot of effort, intellectual perseverance, and passionate pursuit. All these technologies have a special place in my heart.

Q. Your research involves people at the beginning of their careers—graduate students and postdoctoral researchers. Do you seek them out or do they find you?

Usually, we receive inquiries and applications. I am very proud that our research group has provided an opportunity to many postdocs and students who have not only contributed to our research but also acquired some experience here that is, hopefully, useful to their careers. It is quite satisfying to see, for example, one of my former graduate students become a successful researcher in industry and one of my postdocs become a well-known professor in academia. I'm pleased that some of my students are now becoming established scientists, continuing the scientific legacy. This is quite a powerful and morally satisfying thought.

Q. What do you hope these students take away from the experience of working with you?

I used to tell to my students and postdocs: "A scientific career requires imagination, dedication, and passion. You have to love what you do. If you love your job, then the long hours, the frustration when experiments do not work (which happens quite often), and the tedious effort to apply for research funding are just a small price to pay for an intellectually fulfilling career.

Q. You have also done research projects with distinguished scientists, such as the late Carl Sagan.

Yes, I collaborated some with the late Carl Sagan on a project aimed at searching for extraterrestrial life in the universe. Our group used fluorescence techniques to analyze samples Sagan produced in his labs by simulating the atmospheres of Saturn's moon Titan and of Jupiter (the pre-biotic soup conditions of the early universe). We did detect in those samples polyaromatic hydrocarbons, compounds believed to be the precursors of biological species and indicators of early life in the universe. This work, which for the first time hinted at the possibility of biological life outside our planet, was published well before NASA's announcement of the possibility of life on Mars. That was very interesting, thought-provoking, soul-stimulating research, and it was also quite fun.

Q. What advice would you give researchers who want to commercialize their technology?

Have patience, be persistent, and think long term. ®

Guiding Light

ORNL's Photo-Molecular Comb technology may be used to develop drugs that combat disease more effectively.

In the first-generation lab-on-a-chip device, invented at ORNL 10 years ago, researchers separated chemicals inside channels etched into glass while under the influence of an electric field. In ORNL's latest lab-on-a-chip device, separation occurs on the surface of a silicon chip under the influence of laser light.

The invention, the "Photo-Molecular Comb," has been licensed exclusively to Protein Discovery, Inc., and should be commercially available in 2005 for select researchers working in drug discovery. So says Chuck Witkowski, chief executive officer and president of the Knoxville-based startup company. While earning his M.B.A. degree at the University of Tennessee, Witkowski founded Protein Discovery in 2001, with the assistance of Lee Martin and Dan Kuban of the Tennessee Technopreneurial Leadership Center.

The inventor of the Photo-Molecular Comb is Thomas Thundat, leader of the Nanoscale Science and Devices Group in ORNL's Life Sciences Division. For his invention Thundat was selected as ORNL's Inventor of the Year in 2003 and honored by the Battelle Memorial Institute in 2004. He was recognized "for the development of a new paradigm for achieving biomolecular transport and separation using optical manipulation of surface charge."

"The Photo-Molecular Comb can be used to rapidly concentrate, separate, and analyze molecules," Thundat says. "The device has the advantages of high resolution, low cost, small size, and low power requirements." A 9-volt battery can power the device, which includes a laser diode.

When light from the laser diode shines on one type of semiconductor coated with a gel and put under a positive electric potential, negatively charged electrons from the chip rush toward the spot on the gel surface where the light falls. With a different coating on the semiconductor and negative electric potential, positively charged holes go to the spot of illumination. Negatively charged molecules, such as DNA, placed in the gel are attracted to the holes, while positively charged proteins are attracted to the electrons.

The Photo-Molecular Comb consists of a gel sandwiched between a silicon semiconductor chip and a piece of conducting glass. In one application, proteins scattered throughout the gel are attracted to the concentrated electrons so they accumulate where the light is parked. The "photo-accumulated" proteins can be visualized by scanning the laser light in a parallel-line, or raster, pattern to create a photocurrent map of the surface.

"We are reducing the diameter of the light spot from 30 microns to 3 microns," Thundat says. "Then we can further concentrate molecules of one type for analysis. Also, we can see how much two different molecules, such as a disease protein and potential drug, interact at the illuminated spot by measuring changes in the photocurrent level."

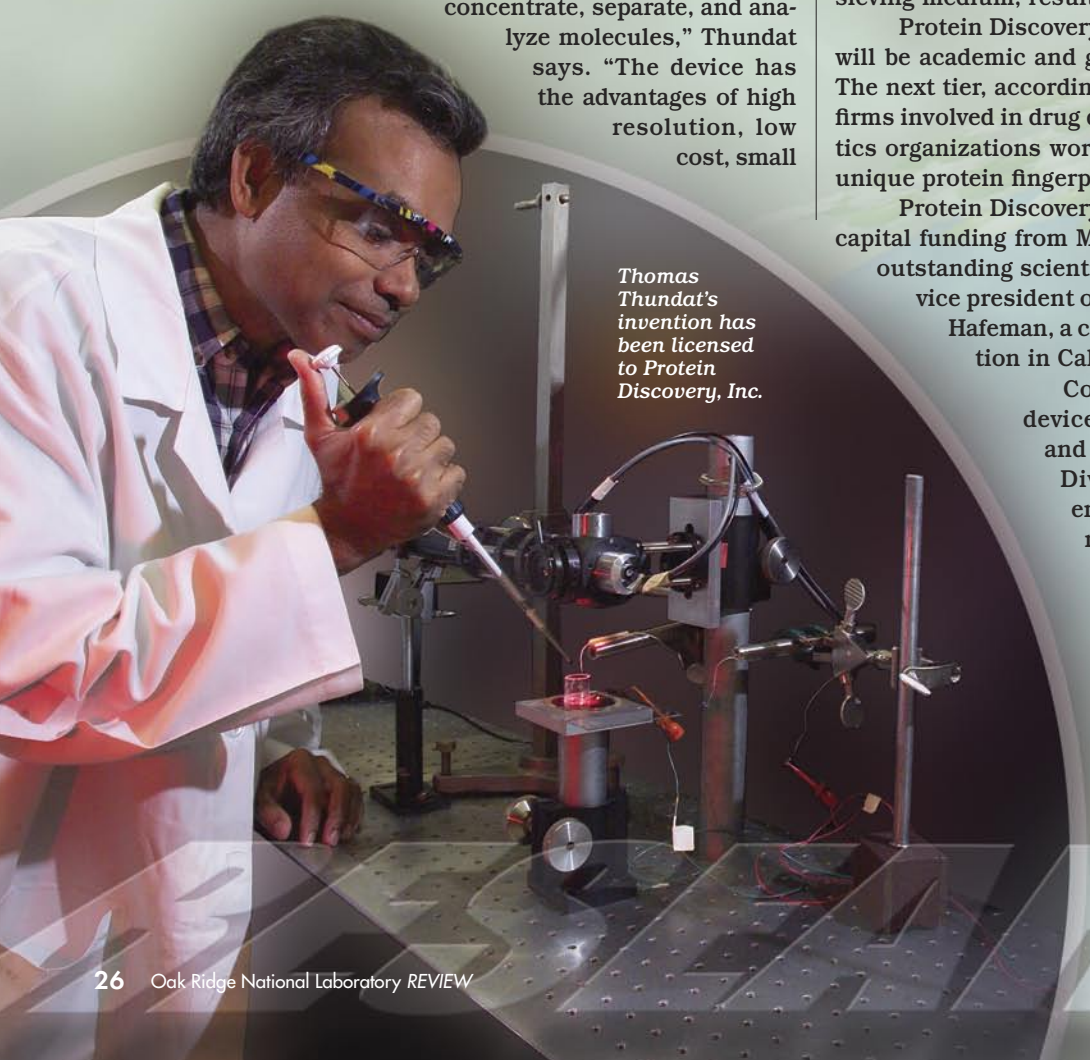
The device can also separate proteins in a gel containing a sieving medium. When the light is scanned, the proteins follow the light, like hair following a comb. The smaller proteins flow farther and faster than the larger, heavier proteins in the sieving medium, resulting in separation.

Protein Discovery expects that its first tier of customers will be academic and government laboratory research teams. The next tier, according to Witkowski, will be pharmaceutical firms involved in drug discovery. The third tier will be diagnostics organizations working in clinical proteomics to discover unique protein fingerprints for disease states.

Protein Discovery has received its first round of venture capital funding from MB Venture Partners, has assembled an outstanding scientific advisory board, and has hired a new vice president of research and development. He is Dean Hafeman, a co-founder of Molecular Devices Corporation in California.

Contributors to the development of the device are Tom Ferrell of Thundat's group and Gil Brown of ORNL's Chemical Sciences Division. Several molecular biologists employed by Protein Discovery collaborate with Thundat under a cooperative research and development agreement, funded by the National Cancer Institute, the National Science Foundation, and the company's equity capital.

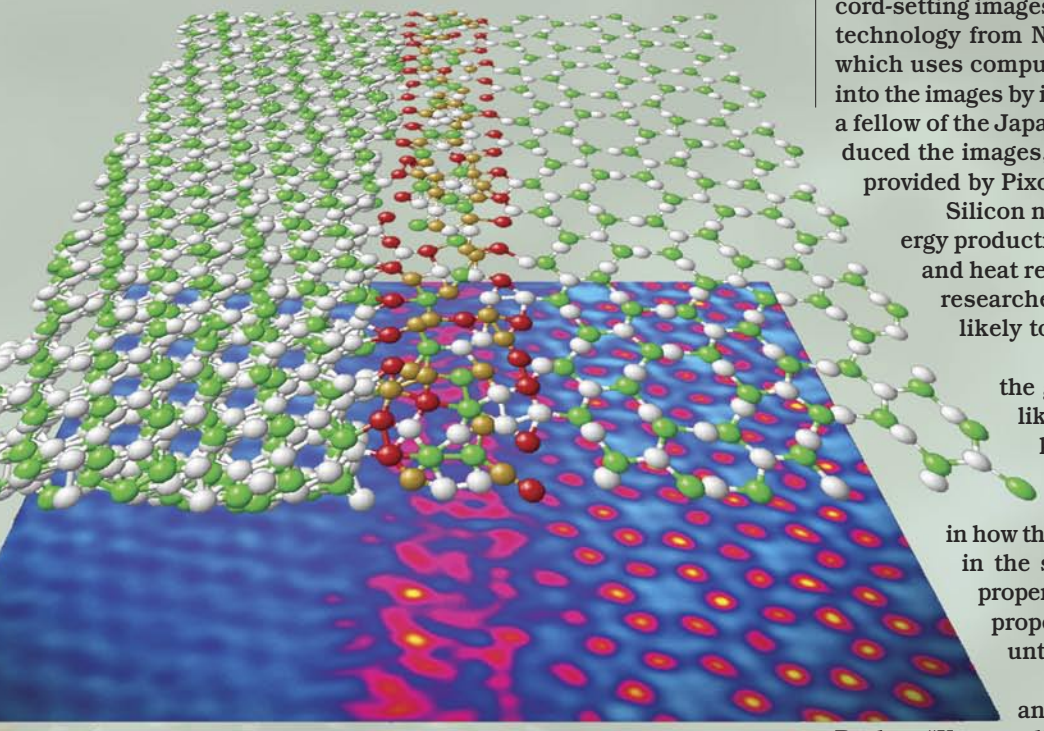
Among biomedical lab-on-a-chip devices, the Photo-Molecular Comb may prove to be a microscopic invention with enormous financial potential. ®



Thomas Thundat's invention has been licensed to Protein Discovery, Inc.

Another World Record

ORNL's high-resolution microscope is making possible tougher ceramics needed for devices that will power future buildings and vehicles.



Sharp microscope image of film between silicon nitride grains shows attached lanthanum atoms.

ORNL has achieved a new world record in electron microscopy, attaining 0.6-angstrom (Å) resolution using a 300-kilovolt Z-contrast scanning transmission electron microscope (STEM) housed in a new $6 million facility. ORNL Corporate Fellow Steve Pennycook, Matt Chisolm, Albina Borisevich, and Andy Lupini, all of ORNL's Condensed Matter Sciences Division (CMSD), eclipsed the Laboratory's previous world record of 0.78 Å established in 1999, thanks to funding from ORNL's Laboratory Directed Research and Development Program.

Incredibly sharp, atom-scale images give researchers a leg up in predicting and modeling the properties and behavior of advanced ceramic materials. A paper in the journal *Nature* by Pennycook; Gayle Painter and ORNL Corporate Fellow Paul Becher, both of ORNL's Metals and Ceramics Division; and visiting researcher Naoya Shibata, illustrates the advantage the Z-contrast STEM gives to researchers seeking to develop strong, heat-resistant materials.

The work reveals the preferred location of "dopant" atoms—atoms added in small amounts to influence the host's properties—within a silicon nitride ceramic. Where specific atoms reside is key to the properties of the materials. The atom-scale images match, almost exactly, the positions predicted by theoretical calculations.

"With this new confidence in our theories, we will soon model materials on a computer screen and predict their properties," Pennycook says. "We will be able to minimize the difficult and expensive task of fabricating and evaluating a large number of samples."

Images of atoms in silicon nitride, along with CMSD's record-setting images, were obtained with the help of an emerging technology from Nion Company called aberration correction, which uses computer technology to correct errors introduced into the images by imperfections in the electron lenses. Shibata, a fellow of the Japan Society for the Promotion of Science, produced the images, which were then refined using technology provided by Pixon LLC of Setauket, New York.

Silicon nitride could be useful for highly efficient energy production devices because it is strong, lightweight, and heat resistant. But it is also intrinsically brittle, so researchers are searching for ways to make it less likely to fracture.

One way to toughen the material is to induce the growth of whisker-like grains that act much like reinforcing rods in concrete. Researchers know how to form whisker-like grains by adding certain rare-earth "doping" agents such as lanthanum oxide. However, slight changes in how the doping agents eventually situate themselves in the silicon nitride ceramic affect the materials' properties. In the past, researchers seeking the best properties have had to try different combinations until they arrived at the best material.

"Rare-earth elements like lanthanum and lutetium have quite different effects," says Becher. "You get different looking microstructures with different properties. Our question was, 'why do these elements cause different changes?'

"Theoretical calculations led by Painter predicted that these elements had different preferences for locating themselves at the silicon nitride grain surfaces. Atoms like lanthanum were seen to want to go to the grain surfaces, causing long, thin grains to form. On the other hand, lutetium was predicted to be less likely to locate next to the grain surface, allowing the grains to grow fatter.

"We know that the particular microstructure we obtain and the nature of the amorphous film strongly affect silicon nitride's properties. So knowing 'the why' is critical to the development of new materials."

Because of the presence of amorphous films around each silicon nitride grain, "it is very difficult to see these dopant atoms in a microscope," Pennycook says, adding that this was a "good problem" for his world-record-holding Z-contrast STEM. Shibata's Pixon-enhanced images corresponded to Painter's theoretical predictions so closely that Pennycook and Becher believe future researchers will be able to confidently design materials by computer, significantly speeding up the development of new advanced ceramic materials.

"Now we know, at the atomic level, why things are happening," Becher says. "The world's most powerful microscope will enable the creation of materials that are tougher and stronger. Those materials will be found in advanced microturbines and auxiliary power systems for aircraft and trucks."—*Bill Cabage* ®

...and the WINNERS are...

Accomplishments of Distinction at Oak Ridge National Laboratory

ORNL in 2004 received ***three R&D 100 Awards*** from *R&D Magazine*, bringing the Laboratory's total to 119 awards and enabling the Laboratory to maintain a lead over all Department of Energy national labs since the competition began in 1963. The awards are given to the 100 most significant innovations of the year. Sharing ORNL's awards were 15 ORNL researchers and one UT technician. The ORNL winners are **Baohua Gu, Gilbert Brown, Bruce Moyer, Peter Bonnesen,** and **Paul Schiff** for a ***highly selective, regenerable perchlorate treatment system*** consisting of a unique, highly specific resin that uses selective ion exchange to trap and break down perchlorate—a chlorine-oxygen compound found in solid rocket propellant that disrupts thyroid gland function—and to regenerate itself without getting contaminated so it can be reused; **Craig Blue, Puja Kadolkar, Greg Engleman, Randy Howell, Jackie Mayotte, Vinod Sikka,** and **Evan Ohriner,** and others for an ***advanced heating system for high-performance aluminum forgings,*** which uses an optimized combination of radiant and convection heating to more quickly process materials—such as heat treating or joining aluminum, steel, titanium, and nickel-based alloy components in automotive and aerospace systems—using less energy than conventional techniques; and **Thomas Thundat, Lal Pinnaduwage, Tony Gehl, Vassil Boiadjiev,** and **Eric Hawk** (with David Hedden of UT and others) for ***SniffEx,*** a compact, low-cost, highly sensitive and specific explosive vapor sensor for detecting and locating plastic-based and other explosives. SniffEx may be used for counterterrorism, law enforcement, airport protection, and humanitarian efforts such as landmine removal.

Major General Dennis K. Jackson, director of logistics transformation in ORNL's National Security Directorate, received the ***National Cargo Security Council's highest award*** for his "skillful management of the largest, most successful, and efficient transfer of materials and equipment in the shortest time span in military history, as the Director of Logistics and Engineering for all of Southwest Asia, with emphasis on Operation Enduring Freedom and Operation Iraqi Freedom in the liberation of Afghanistan and Iraq."

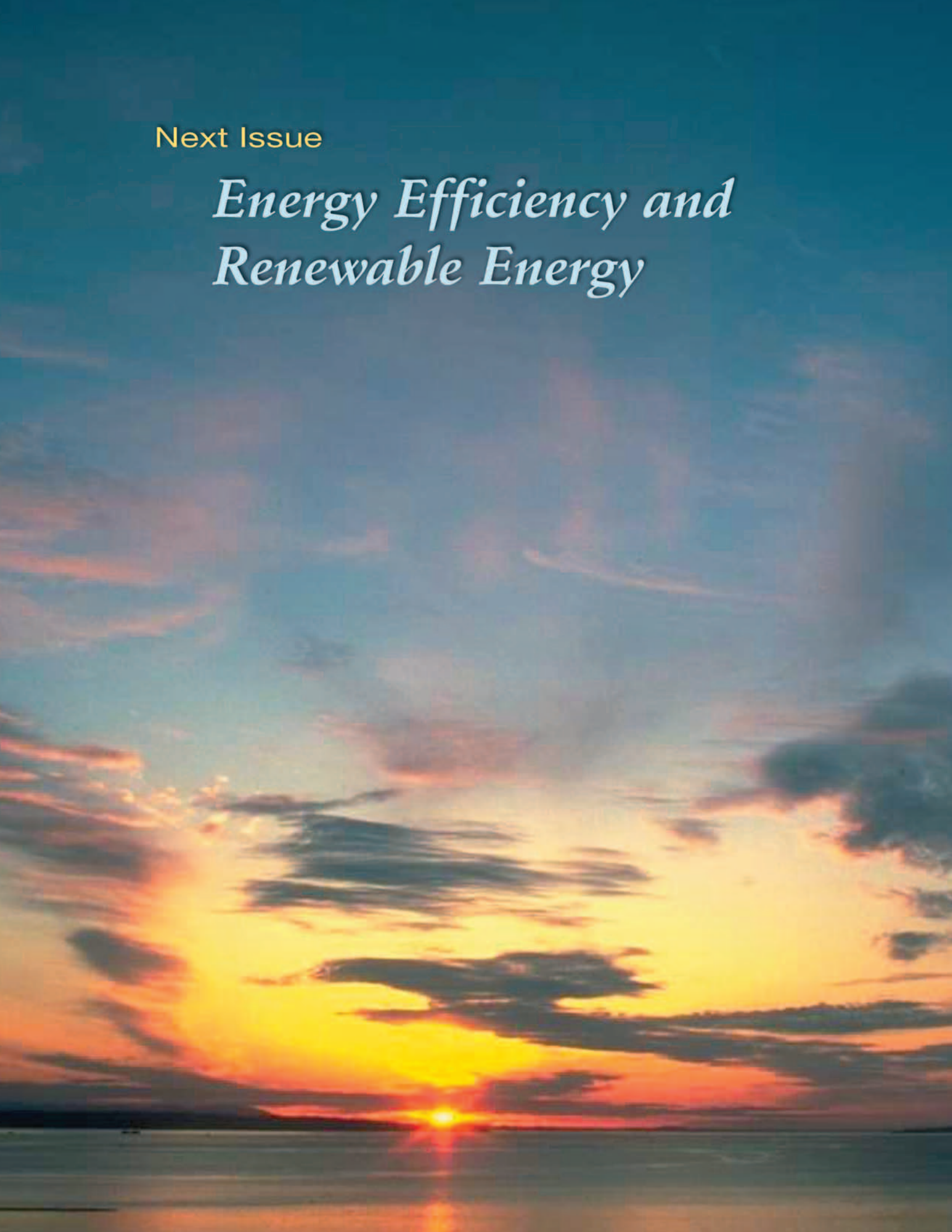
Juske Horita received the ***2004 Geochemical Society of Japan Award,*** in recognition of "his outstanding contributions in the area of experimental studies of stable isotope partitioning at elevated temperatures and pressures."

Stuart Daw, a pioneer in modeling vehicle emission controls, was recognized by the ***Department of Energy's Office of FreedomCAR and Vehicle Technologies*** for his "dedication in creating and coordinating CLEERS (crosscut lean exhaust emission reduction simulation) and leading the Lean NO_x Trap Focus Group."

James R. Beene and **Steven J. Zinkle** have been named ***UT-Battelle corporate fellows*** for 2004. **Beene,** director of ORNL's Holifield Radioactive Ion Beam Facility, was recognized for his leadership in making it "a forefront facility for nuclear science" and for his pioneering work in nuclear structure physics that led to a quantitative understanding of the excitation and decay of radioactive, neutron-rich nuclei. **Zinkle** is considered an international authority in the study of radiation effects on materials. He has written a series of critical review articles summarizing fundamental radiation effects aspects in a broad range of metals and ceramics used in fission and fusion energy systems.

Sergei Kalinin, Thomas Maier, David Silvermyr, Brian D'Urso, and **Vicky D'Urso** (Brian's wife) have been named ***Eugene P. Wigner Fellows.*** ®

R&D 100 Award winners (all left to right): (top) Gilbert Brown, Peter Bonnesen, and Baohua Gu; (middle) Greg Engleman, Jackie Mayotte, Randy Howell, Craig Blue, Vinod Sikka, Evan Ohriner, and Puja Kadolkar; (bottom) Vassil Boiadjiev, Eric Hawk, Lal Pinnaduwage, Thomas Thundat, and Dave Hedden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