Human Genome news

Linking Interdisciplinary Contributors and Users of Genome Data and Resources

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Countering Bioterrorism

DOE-Funded DNA-Based Technologies Track Identity, Origin of Biological Agents

In cases of bioterrorist attack such as the recent anthrax outbreaks, decision makers and law enforcement officials need to understand the situation quickly. Early detection and identification of the biological organism and its source are crucial for minimizing the potentially catastrophic human and economic costs of such an attack.

Clues may lie hidden in the weapon itself. Does the bacterium or virus harbor information in its DNA that could lead to its source? Is it resistant or vulnerable to vaccines and antibiotics?

To find answers to these questions, revolutionary new approaches, including many at the national laboratories, are being developed with DOE funding. These strategies combine the knowledge and tools generated in genome programs with advanced computation and microfabrication.

Many projects are funded through DOE's Chemical and Biological National Security Program of the National Nuclear Security Administration (NNSA). NNSA's mission is to develop, demonstrate, and deliver technologies and systems to prevent the spread of chemical and biological weapons. This article describes some examples of the new techniques.

Rapid Detection

Intensive research at DOE laboratories concerning *Bacillus anthracis*, *Yersinia pestis* (causes of anthrax and plague, respectively), and many other causative agents has led to a wealth of genetic information and unique technologies

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Genomes to Life Program Funded for FY 2002

As researchers press toward completing the Human Genome Project by 2003, the DOE Office of Science has taken the next leap forward by launching a program to explore how the static information in DNA "comes to life" to create dynamic living systems. Goals of the new Genomes to Life program, funded at $19.5 million in FY 2002, are to identify and characterize the protein complexes that perform most of the cells' work, the gene regulatory networks that control those processes, and the functional repertoire of natural microbial communities at the molecular level; and to develop computational capabilities for integrated and predictive understanding of biological systems. This new and comprehensive level of understanding will allow scientists to design ways in which the biological capabilities of various organisms can serve DOE missions in energy security, environmental cleanup, and health protection. Specific payoffs include U.S. independence from foreign oil, enhanced protection against biothreat agents, stabilization of atmospheric carbon dioxide to counter global warming, and a savings of billions of dollars in toxic waste cleanup.

(See GTL Funding, p. 3)

for detecting and identifying the genetic strains of these organisms down to their precise DNA fingerprints. The laboratories have focused on tracking selected, highly variable, or very specific DNA "signatures" in the microbial genomes. In some cases, these analyses offer clues to the agent's genetic identity, geographic origin, and genetic modification to enhance its resistance to antibiotics and vaccines.

Researchers at Lawrence Livermore National Laboratory (LLNL) have developed unique reagents for rapid identification of target genome fragments using polymerase chain reaction. The test also may enable the tracking of bacterial infections. In October 2001, researchers Peter Agron and Gary Andersen reported success with a technique to identify a strain known as *Salmonella enteriditis*, which is commonly associated with food poisoning. Their paper appeared in the November 1, 2001, issue of *Applied & Environmental Microbiology*. The successful *Salmonella* testing follows an achievement in May 2001, when unique *Y. pestis* DNA signatures identified by LLNL researchers were used to confirm quickly a naturally occurring outbreak of plague. Made available to federal agencies, this technique may soon lead to identification of some bacteria in hours rather than days or sometimes weeks. These groups are working with the Centers for Disease Control and Prevention to validate assays for distribution to the U.S. public health network.

Researchers at Pacific Northwest National Laboratory and Washington State University are improving microarray sensors that speed the detection of such pathogens as anthrax and smallpox. The new sensors allow direct detection of RNA or DNA from multiple pathogens at improved sensitivity and are expected to make the technology less expensive and more readily available for routine use.

Los Alamos National Laboratory (LANL) researchers developed an amplified fragment length polymorphism tool to analyze *B. anthracis* from naturally occurring anthrax outbreaks around the world. This method uses small DNA fragments to establish a fingerprint that is added to a database where it can be read and interpreted by comparison to others. In recent work on the anthrax threat, researchers analyzed *B. anthracis* strains found in the attack and compared them with a library of more than 1200 different strains. The group continues to work on the rapid identification of drug-resistant strains.

Security at the 2002 Winter Olympics was enhanced by the Biological Aerosol Sentry and Information System (BASIS), which greatly speeds the detection and analysis of airborne biological releases. Especially useful for events involving large numbers of people, BASIS was developed by scientists at LANL and LLNL and sponsored by DOE's National Nuclear Security Administration. BASIS builds on many years of biological science research and development at the DOE national laboratories.

At Sandia National Laboratories, a project is under way to help doctors quickly identify and contain disease outbreaks, especially infectious diseases. The Rapid Syndromic Validation Project (RSVP) is a full-time medical database used to report and give early warning of disease outbreaks. RSVP tracks syndromes (signs and symptoms) rather than positive diagnoses of specific diseases. Real-time syndromic and epidemiological surveillance could significantly speed the process of determining whether a novel disease was introduced naturally or intentionally, where the disease first appeared, how it spread, and the pathogen's origin. A version of RSVP also is being developed for livestock surveillance (*www.cmc.sandia.gov/bio/rsvp/*).

Other genomic technologies developed with DOE funding include multiple-locus variable number of tandem repeat analysis (MLVA) and single nucleotide polymorphism (SNP) analysis. Through the recognition of repeated DNA sequences in the genome, MLVA provides a very high resolution DNA fingerprint of a suspected agent. SNP analysis uses a flexible microsphere array that can target potential antibiotic-resistant genes, toxin genes, and genome sites that may have undergone deliberate genetic modification. Also being used for strain identification, SNP detection eventually may supplant MLVA when enough sequences are available.

Toward a Greater Understanding

A vital next step toward realizing the full potential of genomic advances to neutralize biothreats is to translate their genetic codes into a deeper understanding of how these organisms infect and survive in the host and how they cause toxic effects. This information is needed to understand fully what makes a biological agent a threat and how it can best be found and counteracted.

Exploring the critical life functions of microbes is a goal of the new DOE Genomes to Life (GTL) program (*http://DOEGenomesToLife.org*), whose findings will have applications in biothreat research. A brochure by Jill Trewhella (LANL) and Bert Weinstein (LLNL) highlighting GTL contributions in this area can be accessed via the Web site. ♦

TIGR Sequencing Anthrax Used in Florida Attack

The National Science Foundation is funding The Institute for Genomic Research (TIGR) to sequence to 8× coverage the genome of the *Bacillus anthracis* strain used in last fall's attack on a Florida publishing company. TIGR previously sequenced the genome of a more common strain of *B. anthracis*. Sequence comparisons may help pinpoint the Florida strain's source and determine whether its genome had been manipulated to increase its virulence.

In a commentary that appeared in *Nature* on October 22, 2001, TIGR President Claire Fraser acknowledged the rising concern over the potential misuse of genomic advances for attacking humans and their staple crops or livestock. She urged biologists to discuss the implications of their work in this context and to play a role in generating effective deterrence strategies.

Data and technologies generated in the biology revolution have the potential to be misused by terrorists, but the same breakthroughs may be used to design countermeasures, she pointed out. Genomic progress can counter biological agents in ways that include rapid detection, identification of new vaccine targets, and the design of novel antimicrobial compounds. ♦

In the News

Third Human Chromosome Finished

Chromosome 20 Genes Implicated in Diabetes, Obesity

Chromosome 20 is the first to be completely sequenced since publication of the working draft in February 2001. An effort of the Wellcome Trust Sanger Center (U.K.), this is the third and largest human chromosome finished to the high quality specified by the Human Genome Project. A paper reporting the work appeared in the December 20, 2001, issue of *Nature*. Some genes linked to chromosome 20 are implicated in Creutzfeldt-Jakob disease, severe combined immunodeficiency, Type 2 diabetes, obesity, cataract, and eczema. Other chromosomes completed thus far are 22 and 21, which were published in December 1999 and May 2000, respectively.

At almost 60 million bases, chromosome 20 comprises around 2% of the human genome. About 99.5% of its genetically active ("euchromatic") regions were sequenced. Using analysis methods similar to those for the other chromosomes, researchers added such approaches as comparisons with newly released genomic sequences, including mouse and the pufferfish *Tetraodon nigroviridis*. These analyses can help reveal genes and regulatory elements having essential functions that have remained relatively unchanged by evolution. Authors report that some 727 genes were identified in the sequence.

Finishing the sequence of chromosome 20 (covering over 95% of the euchromatic portion with less then 1 error in 10 kb) was a difficult task requiring a tedious "clone-by-clone" approach to find errors as small as single bases. Researchers suggest that some major discrepancies between the public human genome draft and finished data may be due to large duplicated regions of the genome. Completion of the remaining 21 human chromosomes is expected in 2003. ♦

GTL Funding *(from p. 1)*

For more details, see the April 2001 Genomes to Life plan describing the new program's background, rationale, and goals (*DOEGenomesToLife.org*). Other related documents and graphics on the Web site include Q&As; slide set; backgrounder on GTL payoffs; brochure on "Neutralizing the Biological Threat"; workshop report by cochairs Ken Nealson and J. Craig Venter on The Role of Biotechnology in Mitigating Greenhouse Gas Concentrations, held in June 2001; fact sheets on global warming; related statements by President George Bush; link to a *Washington Post* special report; and report of the August 2001 Workshop on Computational Biology.

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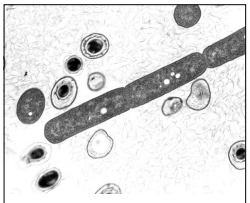
Microarrays Aid Understanding of Anthrax



Bacillus anthracus

DNA microarrays allow the massively parallel, semiquantitative analysis of gene expression at the whole-genome level. Fabricated by robots that deposit near-microscopic spots of DNA onto solid surfaces, a single microarray can carry tens of thousands of unique DNA fragments. By exploiting DNA's ability to form highly specific base pairs, such microarrays allow samples to be checked for the presence and relative abundance of each DNA fragment represented on the array surface.

In an elegant method first reported by Patrick Brown (Stanford University), two samples can be characterized simultaneously with respect to their

(See Microarrays, p. 4)

JGI Completes Draft Pufferfish Genome, Begins Poplar Sequencing

Great Potential Benefits for Health, Environment, Energy Security

Having completed the draft sequence of the pufferfish *Fugu rubripes* genome in collaboration with an international consortium, the Production Genomics Facility* of DOE's Joint Genome Institute (JGI) is sequencing and assembling the genome of the poplar (*Populus*) tree (*www.jgi.doe.gov*). *Fugu* already is providing clues for finding similar genes and control elements in humans; a detailed knowledge of the poplar genome also will lead to significant environmental and energy security benefits. Brief descriptions of both projects follow.

Fugu

Fugu rubripes is one of at least 100 species of pufferfish. It has about the same number of genes and regulatory regions as the human, but these elements are embedded in only 365 million bases as compared with the 3 billion that make up human DNA. With far less noncoding DNA to sort through, finding *Fugu* genes and their controlling sequences should be a much easier task. Although separated by evolution nearly half a billion years ago, both genomes retain the record of distant common ancestors.

Comparing the two vertebrate genome sequences will allow the discovery of new human genes and important elements that control or regulate their activity. *Fugu* is the first animal genome to be assembled by whole-genome shotgun sequencing and made available to the public. The sequencing consortium plans to publish an analysis and is making all sequence information freely available via the JGI Web site.

Populus

With its small genome (550 Mb) and ease of propagation, this hybrid poplar tree is a valuable model organism for molecular studies of tree development,

*Formerly Production Sequencing Facility.

resulting in a trove of resources for conducting further genetic studies. The goal of the poplar genome sequencing project is to obtain 6× coverage by the end of FY 2003, using a whole-genome shotgun approach coupled with cosmid and BAC end sequencing.

The project is sponsored by the Carbon Management and Sequestration Program of DOE's Biological and Environmental Research program. The agency's main interest in this work is to understand and provide the scientific foundation for eventually enhancing the molecular mechanisms involved in capturing and sequestering (storing) carbon.

Counter Global Warming. Carbon capture is important for countering the global climate change caused by rapidly rising carbon dioxide levels from the burning of such fossil fuels as coal, oil, and natural gas for electricity and heat. Dramatically increased amounts of CO_2 released in the last six decades will remain in the atmosphere for hundreds of years unless recaptured. Measured in billions of tons of carbon every year, this global challenge is immense.

Because carbon is stored in plant tissues, large biological systems such as trees are the principal way to sequester CO_2 already in the atmosphere. Fast-growing poplars and other similar plants that produce the most biomass per acre per year are the best choices for carbon sequestration. Furthermore, once genomic data are obtained and the mechanisms involved are understood better, these plants can be modified genetically to take up and retain more carbon in an inaccessible form when they decompose.

Enhance U.S. Energy Security. Carbon stored in poplars has potential energy available for human use. Plants provide raw material either for direct combustion to generate electricity and heat or for conversion to liquid fuel (e.g., ethanol) to power vehicles. Genetic manipulations can alter plants so they will generate greater quantities of biomass and convert easily to biofuels, thus potentially reducing the nation's dependence on foreign oil and enabling energy security. ♦

Microarrays (from p. 3)

relative amounts of mRNA using a two-color fluorescence microarray assay. This approach allows researchers to determine how specific environmental conditions affect gene expression at the level of transcription.

Observing Radiation Effects

Microarrays containing over 12,000 human cDNAs fabricated in the state-of-the-art facility at Los Alamos National Laboratory (LANL) are providing a fruitful platform for understanding the cell's response to low doses of direct and indirect ionizing radiation. Using primary human cell culture and acute radiation doses, Robert Cary's laboratory at LANL is applying bioinformatics approaches to discover new regulatory pathways responsible for transcriptional response. In collaboration with Bruce Lehnert (LANL), investigators are exploring the response to reactive oxygen species generated as a by-product of ionizing radiation exposure. This work will point the way to identifying key gene products, biochemical pathways, and regulatory networks responsible for DNA damage recognition and repair, cell-cycle checkpoints, and other metabolic responses that determine individual susceptibility or resistance to ionizing radiation.

Understanding Anthrax Infection

LANL scientists also are using DNA microarrays to better understand microbial virulence, as in *Bacillus anthracis*, the cause of anthrax and thus a key threat as a biological weapon. *B. anthracis* is poorly understood with respect to the molecular mechanisms of pathogenesis, which is known to require the presence of the two large virulence plasmids pX01 and pX02. Using LANL-fabricated microarrays and an attenuated *B. anthracis* strain incapable of causing the disease, investigators have characterized the expression of 143 predicted open reading frames (ORFs) on the pX01 plasmid. Although this plasmid contains many predicted ORFs, only four previously had responded at the transcriptional level to changes in temperature and carbonate concentration—two manipulable environmental parameters that can mimic conditions encountered by the microbe during infection. Interestingly, these four ORFs play central roles in pathogenesis, which suggests that identifying additional plasmid ORFs with similar expression profiles will provide important new clues to the molecular mechanisms of *B. anthracis* pathogenesis.

Focusing on the expression pattern of 143 pX01 ORFs, a series of studies has been conducted to examine transcription-level changes in response to temperature and carbonate. Experiments conducted at LANL by Cary and James Pannucci, a postdoctoral fellow working with Cheryl Kuske, have revealed marked changes in RNA levels for a large number of ORFs. Strikingly, these experiments have revealed that 35 pX01 ORFs, in addition to the 4 previously characterized plasmid genes, respond to increases in temperature and carbonate concentration by increasing gene transcription. These ORFS provide an exciting starting point for follow-up studies designed to characterize their role in *B. anthracis* virulence. Data from these studies undoubtedly will play a fundamental role in revealing new key factors in *B. anthracis* pathogenesis. *[Robert B. Cary, LANL]* ♦

DOE Genome Abstracts

Proceedings from all DOE Human Genome Program contractor-grantee workshops, including this year's Oakland meeting, are on the Web:

- *www.ornl.gov/hgmis/publicat/publications.html*

The 2002 proceedings also contain abstracts of DOE's Microbial Genome and Low Dose Radiation Research programs. ♦

Meeting Report

Genes, Justice, and Human Rights

International Judiciaries Consider Opportunities and Challenges of New Technologies

In an increasingly globalized world, the impact of scientific discoveries and applications quickly transcends national boundaries through the rapid exchange of products, people, and ideas. Such advances in the genomics arena have spurred impassioned debate on complex and often contentious issues likely to affect every corner of the world. Questions concern privacy and ownership of human genes; access to revolutionary agricultural, reproductive, and medical technologies; the presence of genetically enhanced products in the world's food supplies; the exploitation of natural resources, including human DNA sequences, from indigenous populations; and the release of novel organisms into the environment.

Without doubt, many disputes relating to these issues will end up in the courts before scientific uncertainties are resolved and national legislatures and international bodies develop comprehensive policies. What remains unclear is just how courts will manage these new challenges in an evolving science with few precedents to guide them. In the first of three planned international meetings, conferees began anticipating and grappling with these issues.

From left, Justice Michael Kirby (Supreme Court of Australia), Vijaya Melnick (University of the District of Columbia), and Judge Barbara Rothstein (U.S. District Court for the Western District of Washington).

Some 80 judges and 40 scientists from Europe, Asia, Canada, and the United States gathered in July 2001 in Kona, Hawaii, for the Courts First International Conversation on EnviroGenetics Disputes and Issues. They considered the potential impact of genomics on courts as well as possible mechanisms for facilitating dispute resolution.

The new paradigms faced in global economics and communications offer new opportunities for discovering and processing truth, Artemio Panganiban (Justice, Supreme Court of the Phillipines) said, but are the judiciaries of the world ready to greet this challenge?

"The complexity of the science these days cries out for international cooperation and judicial education," said Claire L'Heureux-Dube (Justice, Supreme Court of Canada). She noted that "the law cannot lag behind science; in the best-case scenario they will complement each other and thus serve the public interest optimally."

The meeting was cochaired by U.S. District Court Judges Andre Davis (District of Maryland) and Gladys Kessler (District of Columbia). Meeting cosponsors were the DOE Human Genome Program (HGP) and the NIH National Institute of Environmental Health Sciences (NIEHS).

The conference was organized by the Einstein Institute for Science, Health, and the Courts (EINSHAC), under the leadership of Franklin Zweig. An educational and research organization serving the judiciary, EINSHAC is a longtime grantee of the Ethical, Legal, and Social Issues program of the DOE HGP.

Surveying a Changing Terrain

The meeting flowed around a series of plenary sessions, science workshops, and breakout discussion groups. In the opening session, NIEHS Director Kenneth Olden observed that the Human Genome Project's output presents a major societal challenge to use the new information and technologies to improve the quality of human existence. The scope of this challenge, he said, is expanded further when the question is asked about who will benefit most from these advances and who will bear the greater share of the risk.

Discussions that followed the plenaries were far ranging, generally going beyond the suggested topics of genetically modified (GM) foods and agriculture, bioscience and criminal jurisprudence, biological property, genetic testing, and human subjects in biomedical research. During these sessions, judges related how their nations' courts have managed science and technology issues and the problems they have encountered. As groups attempted to anticipate issues likely to arise in the next two decades, researchers in their turn offered opinions on the current state of the science as well as some forecasts of advances on the near horizon.

Several participants emphasized the need to recognize the varying political, economic, and social realities faced by different nations, particularly disparities between developed and undeveloped countries.

Highlights of sessions on GM technologies are found in articles on pp. 6 and 7.

Dispensing Justice

A common vision among those gathered at the meeting, observed Shiranee Tilakawardena (Justice, Sri Lanka Court of Appeals), was their concern that justice be "carried out in the language of human rights, with fundamental guarantees." It is a judicial burden, she emphasized, to stand guard for the people.

And, in doing so, judges often must make decisions about competing scientific opinions, said Timothy Evans (Presiding Judge, Circuit Court of Illinois for Cook County). To resolve disputes, they first look to existing laws to decide relevance but must then listen to scientists.

But in the new and rapidly changing world of genomics, legal precedent and accepted scientific consensus may not exist. Future judges may need to search for truth in other ways, Evans said, on a case-by-case basis in partnership with scientists, until the necessary guidelines are passed into law.

(See Justice, p. 6)

Meeting Report

Debate Over GM Products and Technologies

Safety, Food Security, and a Looming "Genomic Divide"

At the Courts First International Conversation on EnviroGenetics Disputes and Issues, held in Hawaii in July 2001, scientists and judges discussed technical and societal issues surrounding the genetic modification (GM) of plants and other organisms (for an overview of the meeting, see p. 5).

GM technologies offer dramatic promise for meeting some areas of greatest challenge in the 21st century (see box, p. 7). As with all new technologies, they also pose some risks, both known and unknown. Although scientific consensus is not always clear, the world's courts increasingly will be called upon to evaluate disputes involving these technologies.

The most controversial issues fueling worldwide debate on GM products focus on human and environmental safety, labeling and consumer choice, intellectual property rights (patenting), ethics, food security, poverty reduction, and environmental conservation.

GM Technology—Challenges and Progress

Humans have directed crop breeding throughout civilization, beginning some 10,000 years ago when grass species were fused to create a useful variety of wheat. Daphne Preuss (University of Chicago) noted that nothing in the produce department of a grocery store today remotely resembles its wild relative.

Many current products were created using "fry-and-try" methods that induce multiple mutations via mass irradiation to produce plants and microbes with special characteristics. Todd Klaenhammer (North Carolina State Unversity) explained that the new genomic technologies are, by contrast, more direct: they often minimize secondary effects and introduce only one or a few gene alterations at a time. Issues that need addressing, he continued, include more precise targeting of genes, cross-pollination by GM organisms in the wild, effects on biodiversity and ecology, and labeling.

Scientists participating in the meeting presented different viewpoints on current methodologies to evaluate the human and environmental risks of GM products and talked about the need for strengthening protective regulations.

Antibiotic resistance. A controversial aspect of GM technology is the use of antibiotic-resistance genes as markers to help locate cells in which gene transfer was successful. The concern is that such genes may be transferred to intestinal bacteria in humans, but there is little evidence of this occurrence. Advances are being made to eliminate the use of these genes. ➠

Justice *(from p. 5)*

"It is essential that we develop dispute-resolution methods that are as intelligent as the discoveries themselves," added Richard Guy (retired Chief Justice, Supreme Court of Washington). Guy observed that mediation is very effective in resolving the vast majority of disputes and reduces costs and time. A drawback, however, is that it doesn't create precedent to guide other judicial decisions. Another possible resource may be the creation of an international science and technology reference body that would render neutral advisory opinions to resolve complex disputes involving genetics and biotechnology.

As proposed by EINSHAC, the decisions of such a specialty court would be nonbinding but could serve as written precedents for evaluation by potential litigants and traditional courts dealing with similar problems. This forum could serve as a useful alternative to litigation, arbitration, or mediation, where multiple parties and complex issues require knowledgeable judges. The next international meeting, scheduled for June in Ottawa, will explore this proposition in more detail.

Full meeting proceedings

- www.einshac.org (click on Kona Round)

As the conference drew to a close, Zweig observed, "In most countries, the judiciary is still looked to for the preservation of human rights. At this conference we truly have come to work together on that score. Our objective is to create an invested global judicial core who can take ideas back to their courts and build bridges with their scientists. This Hawaii conversation has merely initiated a discourse and suggested the agenda for further deliberation." A meeting is planned for Melbourne in 2003. *[Reported by Denise K. Casey, HGMIS, with contributions from Laurie Gordon, LLNL]* ♦

Beryllium Symposium in June

A Beryllium Symposium will be held June 25–26 at the National Library of Medicine in Bethesda, Maryland (*www.ornl.gov/meetings/beryllium/*). It is sponsored by DOE in cooperation with the National Jewish Medical and Research Center and the Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health. ♦

Exceptional Chromosome Region Abstracts

Abstracts from the second meeting on Exceptional Chromosome Regions (ECR2), along with abstracts and references from ECR1, are at *www.ornl.gov/meetings/ecr2*. ECR2 was held in Oakland, California, in May 2001 to continue consideration of special resources and technologies for clarifying chromosome structure and DNA sequence in particularly difficult regions, including near telomeres and centromeres and at large duplications. ♦

Allergies. Another often-cited area of concern is the chance that genetic technologies may trigger allergies, either by introducing or creating new allergenic proteins in foods.

In the widely publicized StarLink corn case, the GM corn produced a protein that was not approved for human use because of its possible allergenicity as determined by standard tests. Controversy arose when GM corn was inadvertently mixed with non-GM corn and ended up in several human food products such as taco shells. Some people claimed adverse reactions to the products, but a subsequent investigation in which standard tests were used by the Centers for Disease Control and Prevention (at the request of the Food and Drug Administration) found no evidence of hypersensitivity to the protein.

The science community continues to debate whether GM foods are being tested adequately for these substances. Rebecca Goldburg (Environmental Defense Council) referred to a recommendation by the National Academy of Sciences that priority be given to improving tests to identify potential allergens. Goldburg was a member of a consortium between the United States and the European Union that delivered a consensus on the benefits and risks of biotechnology (*www.useu.be/issues/biotreport2000.htm*).

Environment. Goldburg also described how a GM food might pose a risk to the environment, giving the example of transgenic salmon that probably will be the first GM animal food to come to market. These animals carry a growth-hormone gene from a different salmon species that enables them to grow faster and larger than wild-type salmon. The concern is that they will escape from their pools into the wild, interbreed with other salmon, and outcompete them for resources. Unanticipated effects may harm other organisms as well.

Progress. Research on gene function will provide a better understanding of the effects of particular genes and their products. Preuss said such a project is under way for the genome of the model organism *Arabidopsis thaliana*, the common mustard weed; much knowledge gained from the project will be applicable to other plants. She suggested that by 2010 there would be many improvements in biotech foods, including the elimination of food allergens; rotating use of many biological pesticides to avoid resistance; construction of artificial chromosomes; and control of cross-pollination problems.

GM Microbes

On the microbial level, Ken Nealson (Jet Propulsion Laboratory) and Ananda Chakrabarty (University of Illinois) discussed some scientific uncertainties regarding the use and environmental impact of GM bacteria to perform such tasks as cleaning up toxic spills, generating cleaner fuels, and sequestering excess carbon. How can risk for releasing GM organisms be assessed when scientists don't know what 99.9% of the microbes present in a community are, not to mention the results of their interactions? Although science is beginning to be data rich from microbial genome sequencing projects, it remains knowledge poor in the ways these communities work. However, Nealson pointed out, the technology is there to find out and efforts are being made.

What are Genetically Modified (GM) Organisms and Foods?

Although *biotechnology* and *genetic modification* commonly are used interchangeably, GM is a special set of technologies that alter the DNA of such living organisms as animals, plants, or bacteria. Biotechnology, a more general term, refers to using natural living organisms or their components.

Combining DNA from different organisms is known as recombinant DNA technology, and the resulting organism is said to be "genetically modified," "genetically engineered," or "transgenic." GM products (current or in the pipeline) include medicines and vaccines, foods and food ingredients, feeds, and fibers.

Locating genes for important traits—such as those conferring insect resistance or desired nutrients—is one of the most limiting steps in the GM process. However, genome sequencing and discovery programs for hundreds of different organisms are generating detailed maps along with data-analyzing technologies to understand and use them.

GM crops are grown commercially or in field trials in over 40 countries and on 6 continents. In 2000, about 109.2 million acres were planted with transgenic crops, the principal ones being herbicide- and insecticide-resistant soybeans, corn, cotton, and canola. Other crops grown commercially or field tested are a sweet potato resistant to a virus that could decimate most of the African harvest, rice with increased iron and vitamins that may alleviate chronic malnutrition in Asian countries, and a variety of plants able to survive weather extremes.

Link to GM Sites:

- *www.ornl.gov/hgmis/elsi/gmfood.html*

On the horizon are bananas that produce human vaccines against infectious diseases such as hepatitis B, fish that mature more quickly, fruit and nut trees that yield years earlier, and plants that produce new plastics with unique properties.

In 2000, countries that grew 99% of the global transgenic crops were the United States (68%), Argentina (23%), Canada (7%), and China (1%). Although growth is expected to plateau in industrialized countries, it is increasing in developing countries. The next decade will see exponential progress in GM product development as researchers gain increasing and unprecedented access to genomic resources that are applicable to organisms beyond the scope of individual projects. ♦

Meeting Report

Feeding the World

One of the most critical problems facing the majority of the world's population is food security. In 2000, the global population exceeded 6 billion and is expected to reach some 9 billion by 2050, when about 90% of the population will live in Asia, Africa, and Latin America. Most arable land already is in use, and producers need access to technological advances and the ability to apply them. GM plants and animals offer great promise for meeting these needs and achieving sustainable agriculture.

In a sense, China is both a rich and poor country, said Huanming Yang (Beijing Genome Institute), member of the Chinese group that participated in the draft sequencing of the human genome. While acknowledging the needs of his country's huge population, he also talked about a thriving scientific community and ongoing rice genome project. New genome centers are developing GM foods, and an international research collaboration on the pig genome is being conducted with Denmark. The goal in the pig project is to create less aggressive and more disease-resistant animals better adapted to the close confines of industrialized farming.

India is looking to GM technologies to meet the challenge of doubling crop productivity within two decades. Asis Datta (Jawaharlal Nehru University) presented some examples that illustrate the possibilities of responsibly engineering GM foods, which include a potato with enhanced nutrients and increased yield. Datta emphasized the necessity for rigid adherence to stringent safety protocols.

Sharing Benefits with Developing Countries

Peter Lillford (Unilever Corporation) expressed concern that control of GM technologies may be concentrated in the hands of a few players in the West. Large corporations could monopolize knowledge, access, and research to skew markets and cater to rich nations interested more in obesity than malnutrition. He stressed the importance of international laws to ensure that benefits are shared with the developing world, where food shortages and nutritional deficiencies remain a daunting problem.

A United Nations report released in July 2001 essentially agrees, noting that the current debate in Europe and the United States largely ignores the concerns and needs of the developing world (*Human Development Report 2001; www.undp.org/hdr2001/*). The report compares the current debate with Western efforts to ban the use of the pesticide DDT, which resulted in the explosion of malaria-carrying mosquitoes in some tropical countries.

Inequalities in the means to develop or own technologies can perpetuate societal imbalances. Decio Ripandelli (International Center for Genetic Engineering and Biotechnology) described the work of the intergovernmental organization, International Centre for Genetic Engineering and Biotechnology. The center provides developing countries with access to advanced technologies through training and obtaining patents for subsequent work. He noted that for economic and political reasons, most of the world population is represented as member countries in this organization, but most of the rich population is not.

The rights of developing countries to share in new scientific advances were emphasized further by A. S. Daar (University of Toronto), who described related declarations by the World Health Organization; the international Human Genome Organisation; and the United Nations Educational, Scientific, and Cultural Organization. Noting that 20% of the world's population controls 82% of the income, he spoke of the imperative to ensure that technological advances do not increase inequities and create a "genomic divide." The existing technology divide, he acknowledged, needs to be remedied first. Technological risks, he said, probably will be borne by developing countries.

A common refrain running through meeting presentations was the importance of educating communities worldwide on key ethical and scientific issues surrounding genomic advances. Only then can there be an informed discourse about the level of acceptable risk for this emerging set of technologies that hold unprecedented promise for the future. *[Reported by Denise Casey, HGMIS]* ♦

In the News

Patrinos Wins Award as Distinguished Executive

Aristides Patrinos, DOE Associate Director for Biological and Environmental Research, Office of Science, received a 2001 Presidential Rank Award in the Distinguished Executives category (*www.opm.gov/ses/presrankaward.html*). He was cited for exemplary leadership of the Human Genome and Global Climate Change programs, which have resulted in extraordinary public benefits and international recognition.

This prestigious and unique award is granted to only 1% of career members of the government's Senior Executive Services who have provided exceptional service to the American people over an extended period of time. Through a rigorous selection process focused on leadership in producing results, winners are nominated by their agency heads, evaluated by boards of private citizens, and approved by the President. Distinguished Executives receive a lump-sum payment of 35% of their base pay, a silver pin, and a framed certificate signed by the President. ♦

Protein Data Bank Annual Report

The *Protein Data Bank Annual Report: July 2000–June 2001* is on the Web (*www.rcsb.org/pdb/annual_report01.pdf*). It contains information on the purpose, mission, and history of PDB; data distribution and access; outreach and education; administration; progress and achievements; collaborations with other organizations; future of PDB; and selected references. ♦

Meeting Report

Analyzing Genetic Discrimination in the Workplace

Following are remarks of EEOC Commissioner Paul Miller at the EINSHAC International Working Conversation on Enviro/Genetics Disputes and Issues in July 2001. They have been adapted for use in HGN *(see other meeting articles, pp. 5–8).*

"I believe that the notion of private genetic information is a quaint misnomer."

We have entered an age in which mankind wields increasing power to alter and dictate the course of nature. The mysteries of our genetic code have been unveiled, providing remarkable new insights into our unique human characteristics. Indeed, the information age has taken hold and the genetic revolution is upon us, and, with apologies to Aldous Huxley, we stand at the precipice of a brave new world.

Genetic discrimination is an issue that interests me greatly, for both professional and personal reasons. In my work at the Equal Employment Opportunity Commission (EEOC), the federal agency charged with enforcing workplace antidiscrimination laws, I am concerned about all forms of workplace discrimination, and I struggle with ways to decrease its incidence and to fight for those who have been victimized.

The policy question is, Should employers have access to genetic information? Moreover, should they know my genetic information even if I chose not to know it? Should they be able to participate in or influence these most personal questions and issues? What protections do I have to ensure that my genetic information will not be misused?

Exploding Genetic Technology

We constantly are learning of the discovery of new genes. As the science of genetics explodes and the technology becomes more accessible, the issue of how society protects its workers against the misuse of genetic information will become more important and legal and policy development in the area, more compelling. My concern, and a concern shared by many, is that if employers are permitted to consider genetic information in making personnel decisions, people may be unfairly barred or removed from employment for reasons that are wholly unrelated to their ability to perform their jobs.

By 2010, scientists predict, the modest sum of $100 will buy a test that effectively identifies genetic markers for a myriad of conditions and diseases. Think about whether you want your employer to know your genetic predispositions and your genetic potential or lack thereof.

Genetic Discrimination in the Workplace

For purposes of this talk, I am calling it genetic discrimination when an employer takes an adverse employment action based on an applicant's or employee's asymptomatic genetic predisposition to or probability of having a disease or medical condition. The potential for genetic discrimination is real and no longer just the stuff of science fiction. Employers can learn an employee's genetic information through genetic testing, company medical exams, family history, or medical records. In addition, employers who self-insure have unique access to medical information. I believe that the notion of private genetic information is a quaint misnomer.

Studies show both empirical and anecdotal evidence of genetic discrimination in the workplace. Moreover, the fear of discrimination may in turn make people reluctant to take advantage of the growing array of genetic tests that can identify vulnerability to specific diseases. Legal protections are essential so that scientific breakthroughs are realized, privacy is preserved, and the workplace remains free from discrimination. Moreover, law can provide a uniform standard of conduct regarding the use of genetic information in the workplace.

It is important to note as we begin our discussion that the entire body of American workplace antidiscrimination law is built upon the premise that applicants and employees must be selected based on their ability to do the job and not on myths, fears, and stereotypes regarding race, ethnicity, gender, age, religion, or disability. As it becomes possible to learn more about genetic predispositions, society faces the questions of whether employers should be able to consider such information in making employment decisions and, if not, how the law should protect workers from its misuse.

Civil Rights Model

There are several different approaches in the United States for analyzing genetic discrimination. The Americans with Disabilities Act, the ADA, prohibits discrimination against a qualified individual with a disability. A person with a disability is defined by the law as one who either has a physical or mental impairment that substantially limits a major life activity, has a record of such an impairment, or is regarded as having such an impairment. In short, an individual can be covered by the ADA under any of these three different prongs of the law. It is important to note that the ADA does not explicitly address or define genetic discrimination.

Clearly, the ADA covers people who have a manifested genetically related illness or disability that impairs a major life activity as well as those who have a record of a genetically related disability (e.g., someone who has recovered from cancer). The more challenging question is whether the ADA prohibits discrimination based on a diagnosed but asymptomatic genetic condition that does not substantially limit a major life activity.

EEOC Guidance

In 1995, the EEOC adopted the view that the ADA prohibits discrimination against workers based on their genetic makeup. Though lacking the force of law, the EEOC's policy explicitly states that discrimination on the basis of genetic information is covered under the third prong of the statutory definition of "disability," which covers

people who are regarded as having impairments. This part of the statute is designed to protect against prejudices and misconceptions about disability and reflects a recognition by the U.S. Congress that the reactions of others to a perceived impairment can be just as disabling as limitations caused by an actual impairment. In my opinion, genetic-predisposition discrimination is exactly the kind of situation Congress intended to be covered by the "regarded as" prong.

Bragdon v. Abbott

In the first U.S. Supreme Court decision interpreting the ADA, *Bragdon v. Abbott*, the Supreme Court crafted a decision that supports an alternative model for analyzing genetic-predisposition discrimination. In *Bragdon*, the Supreme Court held that a person with asymptomatic HIV is a covered individual with a disability under the ADA. The court found that HIV infection is an actual physical impairment that substantially limits the major life activity of reproduction, even when the individual is not exhibiting any visible symptoms of illness.

In its opinion, the court meticulously described the cellular impact of HIV infection on blood and other body tissues. Looking beyond any visible symptoms or easily detectable manifestations of the disease, the court found a physical impairment based on the cellular and molecular changes that take place in the body due to the infection. Similar reasoning might support the argument that the ADA covers individuals with asymptomatic genetic predispositions under the "actual" prong of the ADA's definition.

Ominously, however, in his dissent Chief Justice Rehnquist, joined by Justices Scalia and Thomas, seemed to reject the notion that the ADA covers genetic discrimination.

Pending Genetic-Discrimination Legislation

Notwithstanding these strong arguments for ADA coverage, some are concerned that courts will find that the ADA does not cover genetic-predisposition discrimination. Others believe that genetic discrimination is so different from traditional disability discrimination that the ADA does not provide a satisfactory framework. Thus, legislation introduced in the U.S. Congress by Senate Majority Leader Tom Daschle and Senator Edward Kennedy specifically prohibits discrimination by private-sector employers on the basis of genetic information. Moreover, just last week President Bush spoke in support of genetic-discrimination legislation.

Genetic Executive Order

The Daschle-Kennedy bill is based on a presidential executive order signed by Bill Clinton that prohibits the federal government from considering genetic information in hiring, promoting, discharging, and all other employment decisions. As an executive order and not legislation, it applies only to employees, former employees, and applicants to the federal government.

Is Genetic Testing Ever Appropriate?

As it appears that genetic discrimination will be prohibited by either the ADA or specific legislation, the question arises whether genetic testing is ever appropriate in the employment context. Again, the ADA and the pending genetic legislation provide two different frameworks.

The ADA permits disability-related inquiries and medical examinations of employees when they are job related and consistent with business necessity. The historical antecedent for this standard is that employers often used information about the physical or mental condition of employees to exclude or otherwise discriminate against those with disabilities, despite their ability to do the job. The "job-related" standard provides the employer with the opportunity to demonstrate that the existence of a genetic predisposition is a relevant and appropriate subject for inquiry.

Pending genetic-discrimination legislation analyzes the issue differently. Rather than containing a job-related test, the bill establishes a much more restrictive standard. An employer would not be permitted to request or collect genetic information except where used to monitor the biological effects of toxic substances in the workplace and then only with knowing and voluntary consent. The genetic testing must conform with regulations promulgated pursuant to OSHA, the Occupational Safety and Health Act.[1]

Burlington Northern

It is important to note that no genetic-employment discrimination case has ever been decided, in either U.S. federal or state court. However, recently the EEOC settled the first lawsuit alleging such discrimination.

The facts of the case are simple. The EEOC alleged that the Burlington Northern Sante Fe (BNSF) Railroad subjected its employees to surreptitious testing for a genetic marker linked to carpal tunnel syndrome. BNSF was attempting to address its high incidence of repetitive stress injuries—and the resulting payment of compensation—among its employees. Moreover, at least one employee was threatened with discipline and possible termination for refusing to take the genetic test.

The genetic-testing program was revealed when one of the workers diagnosed with carpal tunnel syndrome went to the company doctor with his wife for a mandatory exam. His wife, who is a nurse and the Erin Brockovich of the story, became suspicious when the doctor drew seven vials of blood during the examination of the worker's wrist.

Because the possibility of termination was imminent, the EEOC acted swiftly and sought an emergency injunction in federal court in Iowa. In the motion for the injunction, the EEOC alleged that the tests themselves were unlawful under the ADA because they were not job related and consistent with any business necessity. To condition any employment action on the results of such tests would be to engage in unlawful discrimination based on disability. Just 2 months after the suit was filed, the EEOC and BNSF

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[1]The Supreme Court decision in *International Union v. Johnson Controls*, 499 U.S. 187 (1991), also may be instructive on this issue. In *Johnson Controls*, the court held that a chemical company's policy barring opportunities to women who had the ability to bear children due to concerns over harmful lead exposure violated Title VII as gender discrimination. The beneficence of the employer's purpose did not remedy the facially discriminatory practice.

reached a settlement in which the EEOC achieved everything it sought.

What was particularly reassuring to me about the Burlington Northern case was that no one, not the business community, the employer groups, the scientists, the press, the politicians, nor even the talking heads on MSNBC thought that surreptitious genetic testing of employees and adverse actions against those who have the "wrong" genetic marker should be allowed.

Conclusion

In closing, I think that while genetics may be good science and suitable for determining paternity or finding out who the bad guy is in a criminal case, genetic information should not be used to exclude qualified workers from the workplace. Genotype is no substitute for qualifications, and no employer should ever review your genetic records along with your resume.

Although I practice law here in the United States, these issues are equally relevant throughout the world. The genetic revolution in science and medicine does not end at the U.S. border, and its implications for privacy and potential abuse are as likely to arise wherever the technology exists, regardless of the legal or cultural environment or tradition.

I think it is important to note that genetic mutations are not themselves all bad—even those that cause a disorder. A genetic mutation created my achondroplasia, but I do not think having that gene is bad or something that needs to be cured. Most people with nonlife-threatening genetic disabilities, mental retardation, deafness, and so on feel this same way. Society imparts value to one's mutation and, until now, has imparted a negative value on mutations that are expressed, those that one can see or be aware of. What about the hidden markers that we now will learn each of us harbors? Will we be willing to allow employers to assign a negative value to such genetic markers even if they have no effect on one's ability to do a job? I hope not. I hope we call that illegal discrimination. *[Paul Miller, EEOC, www.eeoc.gov]* ♦

Spinach Protein Offers New Hope for the Blind

This article, written by Carolyn Krause, appeared in the ORNL Review ***34****(2), 14 (2001). It has been adapted for use in* HGN.

Spinach may make Popeye the Sailor Man strong, but a protein from spinach may someday strengthen the vision of people who can barely see. Researchers at Oak Ridge National Laboratory (ORNL) and the University of Southern California (USC) are investigating whether this chlorophyll-containing protein might be useful in restoring sight by replacing a key light-receiving part of the human eye that has lost its ability to function. People who suffer from age-related macular degeneration (AMD) or retinitis pigmentosa (RP), diseases that are the leading causes of blindness worldwide, may find hope in this research.

Although the neural wiring from eye to brain is intact in patients with these diseases, their eyes lack photoreceptor activity. A team led by Elias Greenbaum at ORNL is replacing these inactive photoreceptors with a spinach protein that gives off a small electrical voltage after capturing the energy of incoming photons of light. Called Photosystem I, or PSI (pronounced PS One), the main function of this "photosynthetic reaction center" protein is to perform photosynthesis, using the energy of the sun to make plant tissue (see image, next page).

Mark Humayun of the Doheny Retina Institute at USC (*www.usc.edu/hsc/doheny/*) and his research team showed that if retinal tissue is stimulated electrically using pinhead-sized electrodes implanted in the eye, many patients can perceive image patterns that mimic the effects of stimulation by light. Teaming with Humayun, Greenbaum suggested the possibility of using PSI proteins to restore photoreceptor activity. ORNL experiments showed that PSI proteins can capture photon energy and generate electric voltages (about 1 V).

This project, Greenbaum stated, is based on recent original discoveries.

FYI Currently, in the United States, degeneration of the retina—the light-sensitive layer of tissue at the back of the eye—has left 20,000 people totally blind and 500,000 people visually impaired. RP is an inherited condition of the retina in which specific photoreceptor cells, called rods, degenerate. The loss of function of these rod cells diminishes a person's ability to see in dim light and gradually can reduce peripheral vision as well.

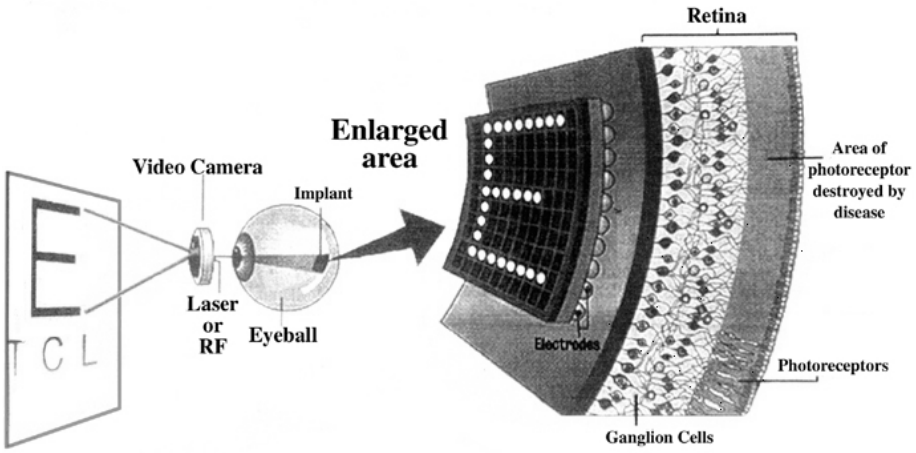
AMD is a disease that affects the center of vision; people rarely go blind from the disease but may have great difficulty reading, driving, and performing other activities that require fine, sharp, straight-ahead vision. AMD affects the macula, the center of the retina. When light is focused onto the macula, millions of cells change the light into an electrical current for the benefit of the neural wiring that tells the brain what the eye is seeing.

"Using the technique of Kelvin force microscopy, we have performed the first measurements of voltages induced by photons of light from single photosynthetic reaction centers. This work was published in 2000 in an issue of the *Journal of Physical Chemistry B (http://pubs.acs.org/journals/jpcbfk/)*. The measured photovoltage values, typically 1 V or more, are sufficiently large to trigger a neural response. We are inserting purified PSI reaction centers into retinal cells to determine whether they will restore photo-receptor function in persons who have AMD or RP. Once we demonstrate that this is possible, USC researchers will test the technique in the laboratory, and, if feasible, later in humans in clinical trials."

In recent research, the collaborators showed that PSI reaction centers could be incorporated into a liposome, an artificial membrane made of lipids that mimics the composition of a ➠

membrane of a living cell. They also demonstrated that PSI can be functional inside a liposome—that is, it produces the experimental equivalent of a voltage when light is shined on it. A liposome probably will be used to deliver PSI to a retinal cell.

Also, in work published in *Photochemistry and Photobiology* in June 2001, the collaborators showed that isolated PSI reaction centers can photo-evolve hydrogen, indicating that PSI maintains its voltage-generating properties under conditions of current flow. ♦



Researchers are developing a small optical sensor device to improve the vision of patients with macular degeneration and retinitis pigmentosa.

Advanced Medical Instrumentation Program

The DOE Biological and Environmental Research Advanced Medical Instrumentation (AMI) program supports basic biomedical engineering research. Using the unique resources and expertise of the DOE national laboratories, as well as synergies with universities and other organizations, AMI research develops innovative medical technologies. While the most recent AMI projects focus on biomedical imaging (see "Spinach Protein," p. 11), other research areas in medical sensors, medical photonics, and smart medical instruments also are supported. The following projects, listed with lead institutions, are funded for FY 2002 (*www.er.doe.gov/production/ober/msd_bio_eng2.html*).

- **Enabling the Blind to See:** Elias Greenbaum (Oak Ridge National Laboratory, ORNL). **Goal:** Develop a small optical sensor device to improve the vision of patients with macular degeneration and retinitis pigmentosa.
- **Monitoring the Human Circulation at a Distance:** M. Nance Ericson (ORNL). **Goal:** Develop technology to assess circulation in real time using an implantable sensory device and remote monitoring.
- **Using Astronomy to Diagnose Eye Diseases and Correct Human Vision:** Scot Olivier (Lawrence Livermore National Laboratory, LLNL). **Goal:** Use adaptive optics from astronomy to correct high-order aberrations in the eye and provide high-resolution imaging looking both into and out of the eye.
- **Imaging Brain Function Without Anesthesia:** Thomas Ernst (Brookhaven National Laboratory). **Goal:** Develop novel positron emission tomography (PET) and magnetic resonance imaging (MRI) technologies to image the awake animal brain in natural physiological conditions.
- **Imaging the Moving Patient:** Andrew Weisenberger (Thomas Jefferson National Accelerator Facility). **Goal:** Develop technologies, especially an optical tracking system, to permit the use of restraint-free PET and single photon emission computed tomography (SPECT) in unanesthetized subjects.
- **Optical Sensors for the Diagnosis of Tuberculosis:** Basil Swanson (Los Alamos National Laboratory). **Goal:** Develop a compact multielement sensor device for use in the field to diagnose tuberculosis and other pathogens.
- **Using Astronomy to Improve Medical Imaging:** Klaus Ziock (LLNL). **Goal:** Develop an X-ray detection system for high-resolution imaging of small animals using radioactive tracer techniques.
- **Development of Long-Term Implantable Biosensors for Diagnostics:** Thomas Thundat (ORNL). **Goal:** Develop a technique for detecting molecules (e.g., prostate-specific antigens in cancer testing) using lasers that can detect biochemical reactions on microscopic cantilevers (see p. 18).
- **Precise Eradication of Cancer with Radiotherapy:** Christine Hartman-Siantar (LLNL). **Goal:** Use the improved computational program Peregrine to direct high doses of radiation to cancer and avoid damage to normal tissue.
- **Radioisotope Production Using Compact Laser Accelerators:** W. P. Leemans (LBNL). **Goal:** Investigate the production and application of radioisotopes using new, rapidly evolving technology. ♦

TIGR to Establish Functional Genomics Center

The NIH National Institute of Allergy and Infectious Diseases has awarded a $25-million, 5-year contract to The Institute for Genomic Research (TIGR) to establish a center for functional genomics. The Pathogen Functional Genomics Resource Center (PFGRC) will centralize production, access, and training in the use of a variety of resources for exploring the roles of genes and gene products (including proteins) in a significant number of microbes known to cause disease. PFGRC, also funded by the DOE Microbial Genome Program, will be a multidisciplinary laboratory, resource, and teaching facility (*http://pfgrc.tigr.org*). ♦

In the News

The Protein Trinity: Importance of Intrinsic Disorder for Protein Function

Keith Dunker, Washington State University, and Zoran Obradovic, Temple University

Protein function generally is thought to follow from, indeed to require, a specific three-dimensional (3-D) structure. This view arose 100 years ago in Fischer's lock-and-key proposal. About 70 years ago Wu and, independently, Mirsky and Pauling proposed that proteins assume particular 3-D structures as the result of weak interactions and that denaturation results from disruption of these weak forces accompanied by loss of specific 3-D structure. This dependence of function on 3-D structure was largely accepted by the time of Anfinsen's protein-folding studies. The flood of 3-D structures determined by X-ray diffraction and nuclear magnetic resonance (NMR) has largely drowned out alternative views.

In contrast to the dominant sequence-to-structure-to-function view given above, a few reports on proteins whose functions require disorder* have trickled through the literature for the past 50 years. For example, as early as 1950, Karush provided evidence that serum albumins' binding site exists as a structural ensemble with different members in equilibrium with each other. The promiscuity of ligand binding by the albumins is explained by selection of the ensemble member that fits the ligand shape—a process Karush called configurational adaptability.

To provide a more recent example, the calmodulin binding site in calcineurin (Fig. 1) was shown by Klee to be extremely sensitive to protease digestion and thus to be a disordered ensemble; this disorderliness was confirmed in Kissinger's X-ray diffraction structure as indicated by missing coordinates in the same region. The disorder is likely to be essential to provide calmodulin (Fig. 2) with the space it needs to completely surround its target helix as observed in a calmodulin-target helix cocrystal, the structure of which was determined by Quiocho and colleagues. After these many years, general reviews on intrinsically disordered proteins are just now beginning to appear. In one of these reviews, Wright and Dyson suggested that the existence and commonness of proteins with intrinsic disorder call for a reassessment of the structure-function paradigm.[1]

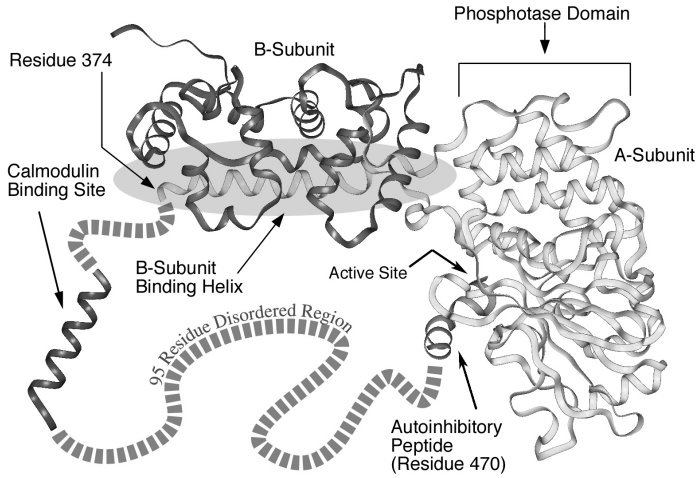


Fig. 1. Disorder in Calcineurin. Calcineurin's a-subunit contains a globular phosphatase domain, a helical extension that binds the b-subunit, a disordered region not observed in the crystal structure, and an autoinhibitory peptide that binds in the phosphatase domain's active site. The a-subunit's intrinsically disordered region, containing 95 amino acids, connects the ends of the helical extension (residue 374) and the autoinhibitory peptide (residue 470) and includes a calmodulin binding site. This region probably is disordered at least in part to allow calmodulin to bind (see Fig. 2).

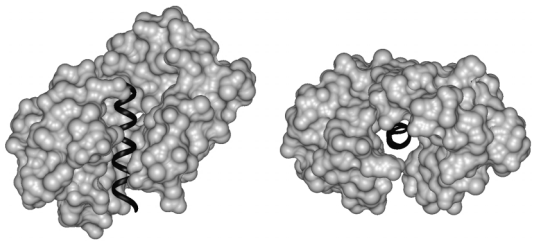


Fig. 2. Disorder Necessary for Calmodulin Binding. Calmodulin (light) bound to the target helix from calmodulin-dependent protein kinase II (dark) is shown in two orientations: (left) from the side and (right) looking down the target helix. Calmodulin completely surrounds the target helix, indicating that calmodulin cannot bind a target helix if the helix is interacting closely with its parent protein.

In our work we hypothesized that, since amino acid sequence determines 3-D structure, sequence should determine lack of 3-D structure as well. If this were true, the accuracies of disorder predictions using amino acid sequence information would exceed the accuracies expected by chance. From literature and database searches, we collected a set of proteins that were structurally characterized to have regions of disorder under physiological conditions, including a few proteins indicated by NMR to be wholly disordered. Once a set of disordered proteins was assembled, we constructed predictors to test the hypothesis.

For datasets with equal numbers of ordered and disordered residues, our predictors of natural disordered regions (PONDRs) initially were about 70% accurate. The latest PONDR was

*Disordered regions are amino acid sequences within proteins that fail to fold into a fixed structure and are involved in a variety of biological functions.

trained using 16,785 putatively disordered residues from 145 nonhomologous proteins, balanced by an equal number of ordered residues, and gave an accuracy of about 83%.[2]

These accuracies are far above the 50% expected by chance. Thus, the hypothesis that intrinsic disorder is encoded by the sequence is strongly supported. Furthermore, the intrinsically disordered regions have amino acid compositions that are very different from those of ordered proteins in just exactly the way a biochemist would expect. Compared to ordered proteins, disordered proteins are depleted in hydrophobic and, especially, aromatic amino acids. Further, disordered proteins are necessarily enriched in hydrophilic amino acids, often with charge imbalance.

In addition, we have PONDRed the proteomes of more than 30 organisms. The findings were summarized as percentages of the proteins in each proteome predicted to contain long disordered regions (LDRs), where an LDR is a disorder prediction of 40 or more consecutive residues. By this measure, the percentages of proteins with predicted LDRs ranged from 7% to 33% in 22 bacteria, 9% to 37% in 7 archaea, and 36% to 63% in 5 eukaryota. The large jump in LDRs in the multicellular organisms was completely unexpected.

Why such a large jump in LDRs for the eukaryota? We are unsure, but there are some interesting possibilities. We noticed that most of the disordered training examples use their disordered regions for cell signaling or regulation, just as in the calcineurin example cited above. The association between regulatory function or signaling and intrinsic disorder appears, furthermore, to be conserved across all three kingdoms. Qualitatively, it seems reasonable for highly flexible disordered proteins, rather than rigid ones, to be used to respond to environmental changes.

In more detail, Schulz showed that disordered proteins can bind to partners with both high specificity and low affinity because a large fraction of the contact energy has to be used for folding rather than for affinity. Thus, regulatory interactions can be both specific and easily dispersed. This is a major advantage because turning a signal off is as important as turning it on. Karush, Quiocho, and Wright, furthermore, all have pointed out that conformational disorder mediates binding diversity because a flexible chain can adopt different conformations to fit with different ligands. Thus, a significant advantage of intrinsic disorder is to allow one regulatory region or protein to bind to many different partners. The ability to partner with many ligands, potentially including both proteins and nucleic acids, is likely to be of central importance in the development of information networks across the cell membrane as well as inside the cell. Indeed, a recent observation is that the more interactions a given protein makes with other proteins, the more likely that a deletion will lead to lethality.[3]

While attempting to organize our thoughts about the various relationships between intrinsic disorder and protein function, we created the Protein Trinity Hypothesis (Fig. 3). In this view, native proteins can be in one of three states: the solid-like ordered state, the liquid-like collapsed-disordered state, or the gas-like extended-disordered state. Function is then viewed to arise from any one of the three states or from transitions among them.

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2. Vucetic et al., *Proc. Int. Joint INNS-IEEE Conf. Neural Networks* **4**, 2718–23 (2001).

3. H. Jeong et al., *Nature* **411**, 41–42 (2001). ♦

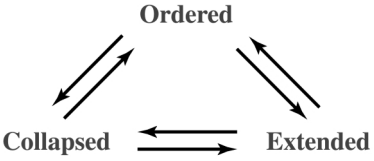

Fig. 3. The Protein Trinity. Protein structure and function can depend on any of the three states and their transitions.

Minireviews on Gene p53 Published

The p53 gene, found on chromosome 17, is a tumor-suppressor gene. In the cell, the p53 protein binds DNA at specific locations and stimulates another gene to produce a protein called p21. In turn, p21 suppresses a division-stimulating protein (cdk2) to prevent the cell from passing through to the next stage of cell division. When p53 is mutant and can no longer bind DNA effectively, the p21 protein is not available to act as the "stop signal" for cell division. Thus cells may divide uncontrollably and form tumors. The p53 gene plays a key role in the pathogenesis or etiology of human cancers and clearly is an important component in a network of events that culminate in tumor formation. Mutations in p53 are found in most tumor types.

Some articles on p53 appear in the May 18, 2001, issue of *European Journal of Biochemistry* **268**(10). The journal contains several minireviews commissioned and organized by Ettore Appella (NIH National Cancer Institute) and Carl Anderson (Brookhaven National Laboratory). The articles are as follows:

- "Modulation of p53 Function in Cellular Regulation," introduction by Appella.
- "Post-Translational Modifications and Activation of p53 by Genotoxic Stresses," by Appella and Anderson.
- "Regulation of p53 Localization," by Shun-Hsin Liang (Pennsylvania State University) and Michael Clarke (University of Michigan Medical Center).
- "P300/CBP/p53 Interaction and Regulation of the p53 Response," by Steven Grossman (Brigham and Women's Hospital and Harvard Medical School).
- "Regulation of Cellular Senescence by 53," by Koji Itahan, Goberdhan Dimri, and Judith Campisi (all at Lawrence Berkeley National Laboratory). ♦

In the News

PROSPECT for Protein Structure Predictions

Wins 2001 R&D 100 Award

Explorations into the 3-D structures of proteins hold the key to understanding their biological functions and thus their roles in a living system. Proteins fold into complex shapes, creating active areas that enable them to interact with other proteins to accomplish a complex biological function in much the same way that gears in a watch mesh into a functioning machine. A broad collection of protein structural data will have an abundance of applications in the life sciences, biotechnology, and medicine. [This goal is the focus of an international structural genomics effort reported in *HGN* (*www.ornl.gov/hgmis/publicat/hgn/v11n3/07struct.html*).]

Revealing these structures, however, is not easily accomplished (see box below). Typically, a protein's 3-D structure is determined through such experimental methods as X-ray crystallography or nuclear magnetic resonance (NMR). The whole process, including protein expression and sample preparation, data collection, and structure-model construction, may take months or even years. This pace clearly cannot keep up with the rate at which protein-encoding genes are being identified worldwide. Nor can it satisfy increasing demands by drug companies hoping to use these data to custom-design drugs that fit precisely in the proteins like hands in gloves, blocking or enhancing their activities and minimizing side effects.

Predicting Structures with PROSPECT

In response to this need, Ying Xu and Dong Xu (Oak Ridge National Laboratory, ORNL) have developed PROSPECT (PROtein Structure Prediction and Evaluation Computer Toolkit), a threading-based protein structure-prediction program. PROSPECT uses an algorithm (see box at left) that mathematically guarantees finding the globally optimal sequence-structure placement and doing so in a computationally efficient manner—a feature unique to PROSPECT. This algorithm was achieved through the discovery that 3-D protein structures generally have topologically simple arrangements between key components (α-helices and β-strands). The prediction capability made PROSPECT one of the top six performers in the threading category in the last CASP contest (see box at lower left).

Another of PROSPECT's unique capabilities allows users to enter any known structural data as constraints on the prediction. That structural information could be disulfide bonds between certain cysteines, geometrical relationships among residues identified as involved in the active site, and experimentally verified or predicted secondary structures—just to name a few. This use of additional structural data as prediction constraints has greatly increased PROSPECT's accuracy.

By further extending the data-constrained prediction paradigm, ORNL researchers have developed a hybrid technique for protein-structure determination, using PROSPECT and large-scale experimental data from

Predicting 3-D Protein Structure

In theory, a protein's 3-D structure could be solved computationally. Using the first principle of physics, investigators could determine the interacting potential energy's exact formula among the atoms of a protein's amino acids with their environment in solution. Many years of research into protein structures have revealed that a protein folds its peptide chain into a "unique" 3-D conformation that minimizes potential energy. So scientists could search for its folded conformation with the minimum energy state. However, this search method, called ab initio folding, is impractical because it requires many times more computing power than the current industry can offer.

Protein threading is a computational method for predicting a protein's backbone structure or fold by comparing its amino acid sequence with solved structures already in the international depository Protein Data Bank and then assessing how well it fits from the potential energy point of view. Within hours to a couple of days of computing time, the method can predict a backbone structure by selecting the placement with the best assessment score. Existing threading techniques are thought to be capable of solving 60% to 70% of proteins identified through the genome projects.

Although there could be millions of proteins in nature, the number of unique structural folds could be as few as 1000, as many structural biologists believe. Up to now, more than 12,000 protein structures have been determined experimentally and deposited into PDB. Among these proteins, about 700 have unique structural folds. If estimates are correct, about 70% of all structural folds have been calculated. Statistics from PDB submissions are consistent with this hypothesis; over 90% of protein structures solved in the past 3 years have similar structures in PDB. Scientists have found more efficient ways to calculate a protein structure by making use of this information. ♦

CASP Competition for Protein Structure Prediction

To assess objectively the state of the art in prediction tools for protein structures, the computational structural biology community agreed on an evaluation system called CASP (Community Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction). CASP was initiated in 1994 by John Moult (National Institute of Standards and Technology and University of Maryland). CASP has been a biannual event since its inception, with each event lasting about 4 months. In each CASP, participants were given a few dozen protein sequences whose structures had been solved experimentally and not published; participants could select targets to predict by a certain date. A group of invited assessors evaluated how well each predicted structure matched the experimental structure. At the end of the prediction season, the performance of each team was ranked, and the results were announced at a meeting in Asilomar Center, California. More than 160 international teams participated in CASP4, which ended in December 2000 (*http://predictioncenter.llnl.gov*). CASP5's prediction season is expected to begin in May and end in August. ♦

NMR or mass spectrometry (MS) in conjunction with chemical cross-linkers. The basic idea is to systematically obtain a large number of distances across amino acid residues and use them as constraints to threading and detailed atomic structure modeling through energy minimization. The investigators have demonstrated that structural information from fold recognition by threading is complementary to that from NMR or MS. Effectively combining these multiple sources of information makes it possible to solve protein structures or structure complexes that cannot be identified by existing methods. This series of developments led *R&D Magazine* to designate PROSPECT as winner of a 2001 R&D 100 award, presented for the year's most significant technological innovations (see box).

The hybrid technique could have significant implications for structural genomics projects, where the goal is to solve protein structures on a genome scale through the development and application of new and improved technologies. NMR methods generally work well for small proteins, but their effectiveness drops quickly as protein weight increases beyond 30 kD. The problem is in assigning enough NMR spectral peaks for an accurate structure determination of a large protein. Typically, when this problem is solved, valuable information can be retrieved in identifying the correct structural folds and providing accurate backbone and even detailed side-chain conformation predictions, as the ORNL researchers have demonstrated. As it matures, this capability should allow at least a good approximation of a protein's actual structure for which existing NMR methods may not work well, due either to the protein's size or its structural stability under NMR experimental conditions.

PROSPECT is the second biological analysis system from ORNL to receive an R&D 100 award. The first was GRAIL, an online automated gene-finding tool that won in 1992. Detailed information about PROSPECT and related projects can be found at *http://compbio.ornl.gov/structure/*. ♦

FYI **R&D Awards.** DOE-supported laboratories, facilities, and small businesses claimed more than a fourth (26) of the 2001 R&D 100 awards (*www.rdmag.com/scripts/awards.asp/*). Investigators at Oak Ridge National Laboratory have won a total of 109, placing second only to General Electric. Conducted annually by *R&D Magazine*, the competition honors the 100 most outstanding new technologies, processes, materials, and software with commercial potential. Entries are judged on technical significance, uniqueness, and promise of real-world application. ♦

Human Genome news

This newsletter is intended to facilitate communication and collaboration, help prevent duplication of research effort, and inform persons interested in genome research. Views expressed are not necessarily those of the Department of Energy Office of Biological and Environmental Research. Suggestions are invited.

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Understanding Health Risks from Low Doses of Ionizing Radiation

The Low Dose Radiation Research Program (*www.lowdose.org*) supports basic research to help characterize risk from exposure to low levels of ionizing radiation. This program is possible because of scientific advances in both genomics and technology over the past 10 years. Recognizing the importance of using these new and exciting tools and techniques, Congress requested in 1998 that DOE initiate a 10-year basic research program to support science that will underpin future risk-assessment standards and guidelines.

Epidemiological and toxicological research has long been used to characterize health responses by populations and individuals to high radiation doses and to set exposure standards that protect the public and the workforce. Standards for low radiation doses are determined from the number of cancers observed after high dose exposure. Models extrapolate this number to predict unmeasurable and unvalidated cancers following low radiation doses.

Recent advances, however, allow direct measurements of biological changes in cells and molecules after environmentally relevant radiation exposures. Such capabilities will enable regulators and policymakers to develop radiation-exposure standards based on a strong scientific and mechanistic foundation rather than on extrapolation.

One new tool is microbeam technology, which is being used to expose individual cells or cell parts—the nucleus, cytoplasm, or their specific regions—to a wide range of radiation energies (from heavy ions to electrons) and doses (including the ultimate low dose, a single ion).

(See Low Dose, p. 17)

In the News

Discover Magazine Honors PNNL Inventor

New Microscope Offers First Views of Living Cells in Action

In July 2001, researcher Robert Wind (Pacific Northwest National Laboratory, PNNL) accepted the top innovation award in *Discover Magazine*'s health category. The award was for inventing a combined optical and magnetic resonance imaging (MRI) microscope that provides a more accurate and complete picture of cellular activity (*www.biomolecular.org/research/cellob/highlights/mrioptical.html*).

The combined microscope takes advantage of confocal optical microscopy's extremely high resolution imaging and MRI's ability to capture physical and chemical cell information such as water properties and the presence of certain chemical compounds. "This unique combination provides scientists with more information about cells than either technique offers individually," said Wind.

Current approaches for studying cellular activities include breaking up cells, which usually results in the loss of cell integrity and thus limits what can be learned from them. The new microscope enables scientists to monitor the response of living cells to such environmental stresses as heat, chemicals, and radiation. This technique provides a necessary link between cellular response and molecular information on proteins and other biochemicals involved in cellular events.

The technology will greatly enhance understanding of the connection between environmental exposures and human health problems. Studies of cellular changes in real time also will help explain how cells succeed or fail in fighting off diseases and will enable practitioners to track healthy cells that become cancerous or diseased cells undergoing treatment.

The new microscope will allow researchers to obtain more accurate and early physiological "signatures" of cell death by providing data pertaining to such changes as cell volume, water mobility, and metabolism. In cancer patients, these changes are telltale signs that diseased cells have died as a result of chemotherapy. This would be a great improvement over current clinical practice because doctors currently are unable to gauge chemotherapy's effectiveness until they see tumors regress.

The work is part of PNNL's Cellular Observatory, whose goal is to visualize the inner workings of living cells by understanding the mechanisms that control a cell's response to its environment (*www.biomolecular.org*). This knowledge will help scientists predict how cells respond to injury and what goes wrong in diseases such as cancer.

PNNL, the Advanced Medical Technology Program in the DOE Office of Biological and Environmental Research, and the NIH National Cancer Institute supported the initial research. A multidisciplinary team of scientists working at the Environmental Molecular Sciences Laboratory (EMSL), a DOE user facility at PNNL, supported the development of the combined microscope for the last 3 years. EMSL provides advanced resources to scientists engaged in fundamental research on the physical, chemical, and biological processes that underpin critical scientific issues. *[Robert.Wind@pnl.gov; PNNL: LAUR-01-4757]* ♦

Low Dose *(from p. 16)*

New genomic technologies and data now allow scientists to measure the biological effects of radiation at the level of individual genes. This capability will enable the identification of genes critical for cancer development and the determination of their activities during radiation-induced carcinogenesis. The magnitude and spectrum of gene-expression changes hold important clues for understanding the mechanisms of radiation-induced cancer. Expression changes induced by high radiation doses in a specific subset of 10,000 to 15,000 genes can be compared quickly with those induced by low doses.

Biological effects also are being studied using new proteomics approaches. These techniques evaluate the types, activities, and configuration of proteins produced in cells in response to both high and low doses of radiation.

Some important questions to be addressed with these tools include the following: Are the cellular effects induced by different radiation doses identical at the molecular level, varying only quantitatively in proportion to dose? Do cells recognize changes induced by low radiation doses the same way they recognize changes induced by high doses? Do cells, tissues, and whole organisms each respond in a qualitatively similar way to different levels of radiation?

FYI Ionizing radiation has enough energy to remove one or more electrons from atoms it encounters, creating charged particles (ions) inside living cells. These ions can damage key substances in cells, including DNA, and can lead to cancer or other defects. People may be exposed to radiation through their occupations; such medical procedures as X rays and radiotracers; and natural background radiation from cosmic rays, radon, radium, and other radioactive materials.

What biological mechanisms are responsible for the bystander effect, in which unirradiated neighbors of exposed cells can exhibit biological responses as if they too had been irradiated? Similarly, what mechanisms lead to other phenomena such as the adaptive response and genomic instability?

Using emerging biological data to provide answers to these questions and help predict health risks is itself a difficult but crucial challenge requiring the development of appropriate mathematical models. The scientific foundation built upon these new data will be critical in adequately and appropriately protecting people from radiation

(See Low Dose, p. 18)

In the News

Bio-Science News From the National Laboratories

Cancer-Detecting Microchip May Work as Assay

A new technique for detecting proteins associated with prostate cancer may serve as a sensitive assay for this common killer and have wide applications beyond diagnostics as well. The work was reported in the September 2001 issue of *Nature Biotechnology* by a team of researchers led by Thomas Thundat (Oak Ridge National Laboratory), Arun Majumdar (University of California, Berkeley), and Richard Cote (University of Southern California).

The new instrument induces cancer-associated proteins known as prostate-specific antigens (PSAs) to stick to and ultimately bend a cantilever that measures one-hundredth the width of a human hair and looks like a tiny diving board. When proteins bind to the surface, the microcantilever bends and a sensitive laser detects and measures the minute movement, thus signaling the presence and amount of increased levels of PSA. Although the cantilever moves only about 10 to 20 nm, lasers can detect a deflection as small as a fraction of a nanometer. The researchers report that the instrument is sensitive enough to detect PSA levels at 5% of the clinically relevant threshold and at potentially much lower cost than the conventional assay. A commercial application may be available within 3 to 5 years.

Thundat won a *Discover Magazine* award for the use of microcantilever sensors in detecting land mines. This technology can be applied to the detection of chemical and biological threat agents, environmental pollution (volatile organic compounds and groundwater contamination), and cations such as calcium in biological fluids, proteins, DNA, and DNA SNPs. It also can be used for night-vision cameras, thermal spectrometers, viscosity meters, and flow-rate meters.

The many and varied applications of this technology illustrate the cross-disciplinary nature of the national laboratories, including their unanticipated value to bioscience and human health. The research is supported by the DOE Office of Biological and Environmental Research and Office of Basic Energy Sciences and the NIH National Cancer Institute (*www.sc.doe.gov/feature_articles_2001/october/Cancer-detecting/Cancer-detecting-microchip.htm*).

Protective Mechanism in Young Could Promote Cancer in Aged

Cells can respond to stresses by entering a state of arrested growth and altered function known as senescence, which may have evolved as a check against tumor growth. New findings by researchers at Lawrence Berkeley National Laboratory (LBNL) indicate that in older organisms, senescence promotes rather than inhibits cell proliferation and may contribute to the much higher rates of cancer in older people.

In a paper published in the October 9, 2001, issue of the *Proceedings of the National Academy of Science*, Judith Campisi and colleagues report that injecting mice with senescent human fibroblast cells and mutated epithelial cells results in tumor formation in the animal. The LBNL researchers propose that interactions between epithelial cells lining the ducts, glands, and surfaces of organs and the stromal fibroblast cells that support and maintain them are key to the progression from cancer-prone, mutated cell to full-blown tumor. Senescent fibroblasts exhibit dramatic changes in gene expression and secrete many molecules that can alter the environment surrounding cells. As senescent cells accumulate with age, the altered microenvironment may facilitate the progression and proliferation of mutant cells to tumors. Although other events contribute to late-life cancers, they note, stromal changes may be of particular importance to humans because most age-related cancers such as breast and prostate arise from epithelial cells (*www.lbl.gov/Science-Articles/archive/senescence.html*). The work was supported by a grant from the National Institutes of Health.

Microenvironment Important in New Cancer Therapies

Cells with the same genetic material are observed to behave very differently when confronted with different microenvironments. In an article appearing in the October 2001 issue of *Nature Reviews Cancer*, Mina Bissell and Derek Radisky (LBNL) note that the association of cancer cells with their surrounding tissues forms a new tumor context that changes to maintain the functional disorder as malignancy progresses (*www.nature.com/nrc/*). The microenvironments of cancer cells thus are "powerful and insidious carcinogens" that also must be targeted in therapeutic regimens. Investigation into the mechanisms of tumor formation (tumorigenesis) can lead to next-generation combination therapies that not only inhibit and destroy tumor cells but also normalize the microenvironment (*www.lbl.gov/lifesciences/BissellLab/main.html*).

First 3-D Structure of Integrin Protein Solved

Expected to Guide Drug Development

Understanding the 3-D structures of proteins can provide clues to the roles they play in living organisms. A landmark study by an international team using the Advanced Photon Source (APS) at Argonne National Laboratory has solved for the first time the structure of a protein thought to have a significant function in tumor growth, bone maintenance, and

Low Dose *(from p. 17)*

and in making the most effective use of the nation's resources.

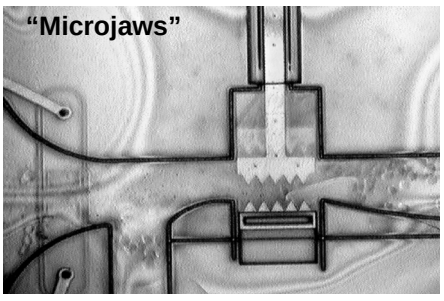
Program funding was $18.5 million for FY 2001. The program's home page includes solicitations for new research, frequently asked questions, and project descriptions. *[Antone Brooks, Washington State University Tricities(tbrooks@tricity.swu.edu) and David Thomassen, DOE (david.thomassen@science.doe.gov)]* ♦

inflammation. It may even be involved in enabling infection by the viruses responsible for AIDS and foot-and-mouth disease (*Science* **294**, 339–45).

Integrins are proteins found in cell membranes that control many cellular processes and also serve as a channel through which viruses can enter and infect cells. These proteins have proven extremely difficult to isolate and crystallize in preparation for determining their 3-D structures. The structure revealed by the APS is a complicated one, with 12 distinct regions (domains) arranged in the shape of a propeller. Researchers hope this new information will help biologists understand how integrin transmits its signals and will guide drug design.

"Microjaws" Trap Individual Cells for Genetic Engineering, Therapeutics

Researchers at Sandia National Laboratories announced the creation of a micromachine that operates on the minute scale of cells. The new device features silicon teeth that open and close like jaws to capture and release human cells, which appear unharmed by the process. The machine fits into a microchannel about one-third the width of a human hair and can be mass-produced easily and cheaply through computer-chip production techniques. The ultimate goal of the device is to puncture cells and inject them with DNA, proteins, or pharmaceuticals to counter biological or chemical attacks, gene imbalances, and natural bacterial or viral invasions. Although the prototype tool traps red blood cells, the machine also may be used to hold stem cells for possible gene implantation or other alterations. Developers note that the ability to implant materials in cells has important implications for the multibillion-dollar microfluidic device industry, currently capable of analyzing biofluids but not altering them (*www.sandia.gov/media/NewsRel/NR2001/gobbler.htm*).



"Microjaws"

New Heart Disease Risk Factor Found

LBNL researchers report the discovery of a new human gene that affects triglyceride levels in the blood and may influence an individual's risk of developing cardiovascular disease. The gene encodes a member of the apolipoprotein (APO) family of proteins that transport cholesterol, triglycerides, and other blood lipids in the body. The findings may lead to the development of tests to detect susceptibility to hypertriglyceridemia and to new methods to reduce risk.

The team scanned the region of human chromosome 11 known to contain a cluster of APO genes to find comparable regions in the mouse genome. Results surprised the researchers, who thought all members of the APO family had been identified. "By comparing the sequence of the genomes of humans and mice, we have found a genetic jewel that had been missed when the sequence of the human genome alone was analyzed," said Edward Rubin, leader of the study that was reported in the October 5, 2001, issue of *Science*. Mutations in the gene were shown to influence triglyceride levels in both mice and humans. The next step, he said, will be to look into the mechanism by which gene polymorphisms influence triglyceride levels and try to identify specific sequence changes leading to lower APO protein

(See Bio-Science News, p. 20)

Online Sources of Bio-Science Information

U.S. Department of Energy

- ***DOE Pulse*** (*www.ornl.gov/news/pulse/pulse_home.htm*)
- ***Energy Science News*** (*www.pnl.gov/energyscience/*)

DOE Office of Science

- ***DOE Science News*** (*www.sc.doe.gov*)

DOE National Laboratories

- **Ames National Laboratory** ***Inquiry*** (*www.external.ameslab.gov/news/*)
- **Argonne National Laboratory** ***Frontiers*** (*www.anl.gov/OPA/frontiers/*) ***logos*** (*www.anl.gov/OPA/logos/*)
- **Lawrence Berkeley National Laboratory** ***Research Review Magazine*** (*www.lbl.gov/Science-Articles/Research-Review/*)
- **Lawrence Livermore National Laboratory** ***Science & Technology Review*** (*www.llnl.gov/str/*)
- **Los Alamos National Laboratory** ***Dateline Los Alamos*** (*www.lanl.gov/worldview/news/publications.shtml*) ***Los Alamos Science*** (*http://lib-www.lanl.gov/pubs/Science.htm*)
- **Oak Ridge National Laboratory** ***ORNL Review*** (*www.ornl.gov/ORNLReview/*)
- **Pacific Northwest National Laboratory** ***Breakthroughs*** (*www.pnl.gov/breakthroughs/*)

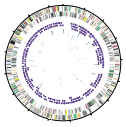
Online Science Magazines

- ***Scicentral News Alert*** (*http://newsletter.scicentral.com*)
- ***Science News*** (*www.sciencenews.org*)
- ***Scientific American*** (*www.sciam.com*)
- ***The Scientist*** (*www.the-scientist.com*)
- ***Wired*** (*www.wired.com*)
- ***Discover*** (*www.discover.com*)
- ***Signals*** (*www.signalsmag.com*)

Nonprofit Organizations

- **Howard Hughes Medical Institute** (*www.hhmi.org*)
- **Wellcome Trust** (*www.wellcome.ac.uk*)

In the News



DOE Microbial Genome Program Evolves

Rapid DNA Sequencing Generates Abundant Data for Gene Discovery, Insights

From its beginning in 1994, the DOE Microbial Genome Program (MGP) sparked a revolution in microbiology. Since the complete genome sequence of *Haemophilus influenza* was published by Craig Venter's group (then at The Institute for Genomic Research, TIGR), genome sequences of some 51 microbes have been completed and published. Sequencing of at least a dozen more is complete but not yet published, and about 140 additional microbes are in varying stages of progress. Activity in the private sector also has been intense. Sequencing technologies have progressed to the point where a high-throughput facility such as the DOE Joint Genome Institute (JGI) can draft the sequence of a 2.5-Mb microbe in a day and 65 Mb of microbial sequence (about 17 to 20 microbes of different-sized genomes) in a month.

These sequences are enabling the discovery of new genes and pathways, as well as the insight that the horizontal transfer of genetic information may have been remarkably frequent in microbial evolution. Even in the 470-gene sequence of the smallest known free-living microbe *Mycoplasma genitalium*, perhaps as many as 100 to 150 genes are not required for life. With many microbial genomes finished or nearly finished (and thus sufficient for gene analyses), scientists have found that about 50% of each genome characteristically comprises genes of unknown function. Microbes have been isolated (and their genomes sequenced) from many different environments characterized by extremes: low pH, temperatures above that of boiling water, pressures more than 200 atmospheres, high toxic-metal concentrations, high-radiation fluxes, high salinity, and just about every other inhospitable condition imaginable. Most microbes do not cause diseases and, in fact, increasingly are thought to play important roles in maintaining the earth's ecology. DOE hopes to use the unique capabilities of microbes to fulfill its missions in carbon sequestration, bioremediation, cellulose degradation, energy ➟

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concentrations in the blood (*www.Science.doe.gov/feature_articles_2001/october/genetic_research/genetic_research.htm*).

Structures Reveal How Viruses Attach to Cells

Possible Vehicles for Gene Therapy

Coxsackie viruses can evade detection by human immune systems to infect cells of the heart, brain, pancreas, and other organs. New details of how they gain entry into cells were reported in the October 2001 issue of *Nature Structural Biology* by investigators from Brookhaven National Laboratory and Purdue University.

Structures of the virus generated by cryoelectron microscopy of frozen samples reveal molecular-level interactions between virus and receptors on the cell's surface. Studies show that viral receptor proteins form pairs on the surface of human cells, with two adjacent proteins attached to each other below the cell membrane's surface. When the virus binds to the human cell, it forms bonds with both the pair's receptors. Structural studies also reveal that the binding sites on the virus are hidden from the body's immune system, which produces antibodies to fight infections. These features are shared with other viruses in the same family. The work may lead to improved ways to thwart viral infections and design virus-based vehicles for gene therapy (*www.science.doe.gov/feature_articles_2001/September/How_Some_Viruses/How_Some_Viruses.htm*).

Powerful Molecular Motor Packs Virus DNA with Explosive Pressure

In a cover story appearing in the October 18, 2001, issue of *Nature*, Carlos Bustamente and colleagues at LBNL reported that the DNA inside some viruses is packed so tightly that the internal pressure reaches 10 times that in a champagne bottle. They speculate that this high pressure helps the virus inject its DNA into a cell after it has latched onto the surface. Once inside the cell, the DNA begins retooling the cell to manufacture new copies of the virus.

Such tight packing is achieved by one of the most powerful molecular motors ever observed, stronger than the motors that move human muscles or the nanoscale molecular motors that duplicate DNA or transcribe it into RNA. Measurements were taken using a specialized optical tweezer microscope that allowed researchers to pull on single DNA molecules as they were packaged to determine their resistance to stretching.

A collaboration with scientists from the University of Minnesota, the research was funded by the DOE Biological and Environmental Research program, the National Institutes of Health, and the National Science Foundation.

Bustamente's work has been critical for understanding how biological machines made up of complex parts assemble and control key cellular activities. To recognize his achievements, the American Physical Society awarded him its 2002 Prize in Biological Physics Research, citing "his pioneering work in single molecular biophysics and the elucidation of the fundamental physics principles underlying the mechanical properties and forces involved in DNA replication and transcription." The society established the prize in 1982 to recognize and encourage outstanding achievement in biological physics research. ♦

production, biothreat reduction, and biotechnology.

The new awards represent a departure from the past. Instead of focusing on the high-throughput production of microbial genome sequence data (a mission now given over to JGI), MGP has evolved towards sequence analyses. MGP goals include functional analyses of microbial genomes; bioinformatics tools for microbial genome analyses; studies of lateral gene transfer (LGT), including its frequency and biological constraints; novel technologies for genomic characterization; and studies of microbial consortia and communities. Of more than 70 applications to the program, 28 awards were made in 2001.

Functional Studies

- John Battista (Louisiana State University): Enhance understanding of *Deinococcus radiodurans* and define the novel protein family responsible for DNA damage repair; identify genes required for radiation resistance as well as those whose expression changes after exposure, and compare them with genes in other radiation-resistant bacteria.
- Ray Gesteland (University of Utah): Use computation and experimentation to understand the phenomenon of "genome recoding," the determination of additional expressed proteins from a known gene.
- David Wilson (Cornell University): Study genes coding for plant polysaccharide-degrading enzymes in the virtually complete *Thermobifida fusca* genome sequence; use the sequence to study the expression regulation of genes involved in cellulose digestion.
- Derek Lovley (University of Massachusetts, Amherst): Identify genes and proteins involved in the electron transfer system matrix of the environmentally significant bacterium *Geobacter sulfurreducens*.
- Brian Palenik (Scripps Institute of Oceanography): Explore transport proteins and their regulation in *Synechococcus WH8102;* add to the knowledge of nutrient transport in an environmentally important photosynthesizer.
- Caroline Harwood (University of Iowa): Use new microarray technologies to determine genes expressed during carbon dioxide and nitrogen fixation by the photosynthetic bacterium *Rhodopseudomonas palustris*.
- Daniel Arp (Oregon State University): Describe in *Nitrosomonas europaea* the gene regulatory networks that respond to changes in nutrients and other conditions; infer gene function using microarrays.
- Jizhong Zhou (Oak Ridge National Laboratory) in collaboration with James Tiedje (Michigan State University, MSU), Kenneth Nealson (University of Southern California), Harwood, and Arp: Construct whole-genome microarrays for several key microbes relevant to DOE's missions to elucidate gene-expression profiles under various growth conditions and to characterize different genetic mutants.

Bioinformatics Tools

- Owen White (TIGR): Continue the Comprehensive Microbial Resource, a coherent collection of careful, consistent annotations of microbial genome data that integrates all of TIGR's microbial sequencing and bioinformatics expertise in an easy-to-use Web interface.
- Mark Borodovsky (University of Georgia): Enhance gene-finding algorithms to better detect atypical genes in microbial genomes; analyze these genes to identify those that might have been laterally transferred.
- Jeremy Edwards (University of Delaware): Develop novel computational and high-throughput experimental tools to analyze DNA-repair pathways in *D. radiodurans* and the influence of metabolic flux distribution on DNA repair.
- Charles Lawrence (Wadsworth Center): Develop technologies for identifying transcription regulation networks in genomes of microbes sequenced by DOE-funded projects.
- Gary Olsen (University of Illinois, UI): Use tools and concepts of molecular phylogenetics with those of molecular biology to build an integrated computational tool for constructing and editing multiple sequence alignments.
- Monica Riley (Marine Biological Laboratory): Construct a metabolic database for *Shewanella oneidensis*; examine proposed pathways for missing functionality, experimentally validate the pathways, and attempt to identify candidates for the missing functions.
- Larry Wackett (University of Minnesota): Extend the University of Minnesota Biocatalysis/Biodegradation Database (called UM-BDD) to incorporate data on microbial metabolism relating to metals, metalloid, and organometallics.
- Diane Makowski (Argonne National Laboratory, ANL): Identify small molecule binding sites on microbial proteins genome wide.
- George Garrity (MSU) and *Bergey's Manual* editors: Use a variety of statistical analyses to view information on bacterial species so the species can be clustered functionally and evolutionarily.
- William Cannon (Pacific Northwest National Laboratory, PNNL): In association with Richard Smith, develop computatonal tools for analyzing the output from high-throughput mass spectroscopy of microbial proteins.

Horizontal Gene Transfer

- Gary Olsen with Carl Woese (both at UI): Explore LGT to estimate the frequency of recent gene transfers to diverse microbial lineages; determine traits that correlate with high acquisition rates of external genetic information; identify donor lineages of ancient gene transfers; determine any relationship between gene transfer and biologically important events.
- Karen Nelson (TIGR): Substantiate and expand recent findings from whole-genome sequencing of *Thermotoga maritima* and other high-temperature archaea showing extensive genomic homologies that could have arisen only by LGT; apply biochemical methods to identify common and unique regions among the genomes of some 50 high-temperature bacteria and archaea.
- Terry Marsh (MSU): Investigate genomic plasticity in *Ralstonia eutropha* and *R. pickettii,*environmental isolates important in such processes as hydrocarbon degradation and resistance to high metal concentrations.
- Howard Ochman (University of Arizona): Investigate a set of 30 conserved and universally distributed genes to assess their involvement in LGT; establish LGT's effects on shaping bacterial genomes.

See the HGPI Web site for a Calendar of Genome and Biotechnology Meetings: *www.ornl.gov/hgmis/*

Novel Technologies

- David Schwartz (University of Wisconsin, Madison): Characterize some 12 widely diverse microbial genomes by optical mapping over a 3-year period; coordinate microbe selection with JGI.
- Jay Keasling (University of California, Berkeley, UCB): Develop chip microfabrication arrays that will enable the automated testing of a large range of culture conditions; expose difficult-to-grow organisms to different environmental conditions to discover their growth requirements; test the effects of different metabolic substances on the physiology of established cultures.

Microbial Consortia and Hard-to-Culture Species

- Jill Banfield (UCB): In association with JGI, characterize the genetics and biochemistry of a community of acid-tolerant microbes; study LGT events between community members by utilizing the BAC libraries made by Ed DeLong (Monterey Bay Aquarium Research Institute).
- Fred Brockman (PNNL): Explore microbial subgenomes from undefined microbial consortia in contaminated subsurface sediments; characterize a genomic "signature" for the microbes in highly radioactive aquifer sediments.

MCP Online

Microbial Cell Project Home Page:

- *http://microbialcellproject.org/index.html* ♦

- Cheryl Kuske (Los Alamos National Laboratory): Further the characterization of microbial backgrounds and isolate useful genes by determining the abundant and active microbial species in soils and sediments contaminated by radionuclides and metals, flow-sorting such cells, and developing methods to simplify microbial DNAs to generate libraries for future sequencing analyses.
- David Kirchman (University of Delaware): Address a key aspect of carbon cycling in the biosphere by studying how insoluble biopolymers are broken down to enter the food chain, especially in the marine bacterium *Cytophagales*.

[Daniel Drell, with contributions from John Houghton and Anna Palmisano, DOE]

A more detailed version of this article is on the Web (*www.ornl.gov/microbialgenomes/2001awards.html*). ♦

New *DNA Files* Aired

Five new one-hour programs in *The DNA Files* series of documentaries have been broadcast on 200 National Public Radio stations across the country since November 2001. These shows explore recent progress in genetics research and related ethical, legal, and social issues, all in easy-to-understand language. Produced by SoundVision and hosted by John Hockenberry, the new programs are

- *Planet of the Bugs: The Never-Ending Tale of DNA and Infectious Disease*;
- *Life: How to Make a Cosmic Omelet*;
- *Genetic Medicine: Prescription for Conflict*;
- *DNA: Code of the Wild*; and
- *Search for the Fountain of Youth: The Genetics of Aging and Longevity*.

Tapes and transcripts of the new series are available from 510/486-1185 or *www.dnafiles.org/about/tapes.html*. Transcripts of the first nine award-winning programs, featured on NPR stations in 1998, can be downloaded free from the same site. Five segments from the earlier series can be heard at *www.ornl.gov/hgmis/education/audio.html*. ♦

DOE-Sponsored CD-ROM Wins Rave Review

The New Genetics: Courseware for Physicians, a CD-ROM that offers Continuing Medical Education (CME) credits for medical doctors, received a glowing review in the *Journal of the American Medical Association*. With the support of the DOE Human Genome Program Ethical, Legal, and Social Issues component, Sara Tobin (Stanford University) and Ann Boughton (Twisted Ladder Media) produced the CD-ROM for physicans who wish to update their knowledge about genetics and genomics. The *JAMA* reviewer noted that "a thick and detailed syllabus with slides from a CME conference in a cruise ship or resort hotel cannot hold a candle to [this] CD that can be accessed in its entirety at any time."

A second CD-ROM, *The New Genetics: Medicine and the Human Genome*, presents the same content without CME credits for college students, researchers, nurses, policymakers, attorneys, and others who are interested in the impact of genetics and genomics on healthcare and society. Both can be ordered through the Web site (*www.twistedladdermedia.com*). ♦

Transcribed Sequences Meeting on Web

A meeting report and abstracts from the 11th annual Beyond the Identification of Transcribed Sequences: Functional and Expression Analysis workshop held November 9–12, 2001, in Washington, D.C., are on the Web (*www.ornl.gov/meetings/bits2001/*). ♦

Free Primer Covers DNA Basics

The new DOE primer on molecular genetics is accompanied by PowerPoint slides (*www.ornl.gov/hgmis/publicat/primer2001/*). Prepared by Denise Casey and Marissa Mills of HGMIS, *Genomics and Its Impact on Medicine and Society: A 2001 Primer* includes brief discussions about basic genomic science, Human Genome Project history and goals, the significance and meaning of the draft human genome sequence, next steps after HGP, medical and other anticipated benefits and societal concerns arising from the new genetics, and a dictionary of related terms. Individual or multiple print copies are available from HGMIS (865/574-7582, *yustln@ornl.gov*). A longer version of the primer is planned. ♦

For Your Information

NABIR Strategic Plan on Web

The NABIR Strategic Plan (2001) is on the Web *(www.lbl.gov/nabir/researchprogram/strategicplan/NABIR_strat_plan.pdf)*. The Natural and Accelerated Bioremediation Research program focuses on radionuclides and metals that are of great concern at DOE sites and are tractable through bioremediation. The current NABIR program announcement is at *www.er.doe.gov/production/grants/LAB02_12.html*. ♦

GTL Program Announcements

- **Program Notice 02-13 *www.sc.doe.gov/production/grants/fr02-13.html***

The DOE Office of Biological and Environmental Research and the Office of Advanced Scientific Computing Research have issued an RFA for large multidisciplinary teams to support the Genomes to Life program (*www.doegenomestolife.org*). A central theme of GTL is to develop capabilities for predicting the behavior of microbes and microbial communities of interest to DOE (see p. 1 for more details).

- **Applications due**: May 7

Contacts: *david.thomassen@science.doe.gov,* 301/903-9817 and *walt.polansky@science.doe.gov*, 301/903-5800

A complementary request for proposals from teams led by DOE national laboratories was issued earlier (*www.sc.doe.gov/production/grants/LAB02_13.html*). ♦

New NHGRI System for Sequencing Targets

The NIH National Human Genome Research Institute (NHGRI) has developed a new competitive process for selecting genomes to be sequenced with NHGRI support. Based on the submission and review of white papers, the new procedure will apply to all organisms except eubacteria, archaea, and plants, which are more appropriate to the missions of other NIH components or other agencies.

White papers will be accepted three times a year: February 10, June 10, and October 10. More information is online (*www.nhgri.nih.gov/About_NHGRI/Der/org_request/seq_target_genome_html*). ♦

DOE Human Genome Program

Ethical, Legal, and Social Implications

- **Program Notice 02-14 *www.er.doe.gov/production/grants/fr02-14.html***

Topic: Issues of genetics and the workplace, storage of genetic information and tissue samples, education, and complex or multigenic traits.

- **Applications due:** March 28

Contact: See DOE OBER Human Genome Program, at right.

Latest ELSI awards: *www.ornl.gov/hgmis/resource/elsi2001.html*♦

DOE Seeking Microbial Sequencing Candidates

DOE is seeking nominations for candidate microbes and microbial communities to sequence in support of the Microbial Genome and Genomes to Life programs (*http://microbialgenome.org/funding/seqtargets.htm*). Candidate microbes should be relevant to DOE missions including waste remediation, carbon management, energy production, and biodefense. Sequencing will be carried out at the DOE Production Genomics Facility of the Joint Genome Institute (*www.jgi.doe.gov*).

Nominations are due March 28, and review will be completed early in the summer. Draft sequencing will begin later in the year after high-quality DNA has been provided. ♦

Medicine and the New Genetics

- ***www.ornl.gov/hgmis/medicine/medicine.html***

U.S. Genome-Related Research Funding

Investigators wishing to apply for funding are urged to discuss projects with agency staff before submitting proposals.

DOE Office of Biological and Environmental Research (OBER) Human and Microbial Genome Programs

- Funding opportunities: *www.sc.doe.gov/production/grants/grants.html*
- Life Sciences Division: 301/903-6488, *genome@science.doe.gov*
- Medical Sciences Division: 301/903-3213, *sharon.betson@science.doe.gov*

Computational Molecular Biology Postdoctoral Fellowships

Support career transitions into computational molecular biology from other scientific fields. Funded by DOE and the Alfred P. Sloan Foundation.

- Contact: Pat Stanley, Sloan Foundation; 212/649-1628, *stanley@sloan.org*, *www.sloan.org/programs/scitech_fellowships.shtml*

NIH National Human Genome Research Institute (NHGRI)

- NHGRI program: 301/496-7531, *www.nhgri.nih.gov/About_NHGRI*
- Funding opportunities: *www.nhgri.nih.gov/Grant_info*
- ELSI: 301/402-4997

Small Business Innovation Research Grants

DOE and NIH invite small business firms (under 500 employees) to submit grant applications addressing the human genome topic. The two agencies also support the Small Business Technology Transfer (STTR) program to foster transfers between research institutions and small businesses.

Contacts:

- DOE SBIR/STTR office: 301/903-1414 or -0569, Fax: -5488, *sbir-sttr@science.doe.gov*, http://*sbir.er.doe.gov/sbir*; DOE SBIR and STRR due January 2003.
- Bettie Graham (see ELSI contact, NHGRI). NIH SBIR and STTR due April 1, August 1, and December 1.
- National resources, calendar: *www.zyn.com/sbir*
- National SBIR/STTR conferences: 360/683-5742, Fax: -5391, *sbir@zyn.com*
- Alerting service: *http://lyris.pnl.gov/cgi-bin/?enter=sbir-alert* ♦

Mouse Genome Newsletter

Mouse Genome Monthly, being produced for several months by the Mouse Sequencing Liaison Group, is designed to keep the research community abreast of sequencing progress. The newsletter and related information are available on the Web (*www.nih.gov/science/models/mouse/genomics/*). ♦

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- *www.ornl.gov/hgmis/publicat/publications.html*

Publications Available Online Only

- *Primer on Molecular Genetic*s (1992)
- HGP Five-Year Plan
- DOE HGP ELSI Grants and Their Products

SELECTED ACRONYMS

BAC bacterial artificial chromosome
bp base pair
BERAC Biological and Environmental Research Advisory Committee
DOE Dept. of Energy
ELSI ethical, legal, and social issues
EST expressed sequence tag
HGMIS Human Genome Management Information System
HGN Human Genome News
HGP Human Genome Project or Human Genome Program
HUGO Human Genome Organisation
kb kilobase
Mb megabase
NIH Natl. Institutes of Health
NHGRI National Human Genome Research Institute
OBER Office of Biological and Environmental Research
PCR polymerase chain reaction
PDF portable document format
RFA request for applications
SBIR Small Bus. Innovation Research
SNP single-nucleotide polymorphism
STC sequence tag connector
STS sequence tagged site
TIGR The Inst. for Genomic Res.
YAC yeast artificial chromosome

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