Lawrence Berkeley National Laboratory

PROGRAM DIRECTOR'S REPORT FOR THE OFFICE OF

Health & Environmental Research

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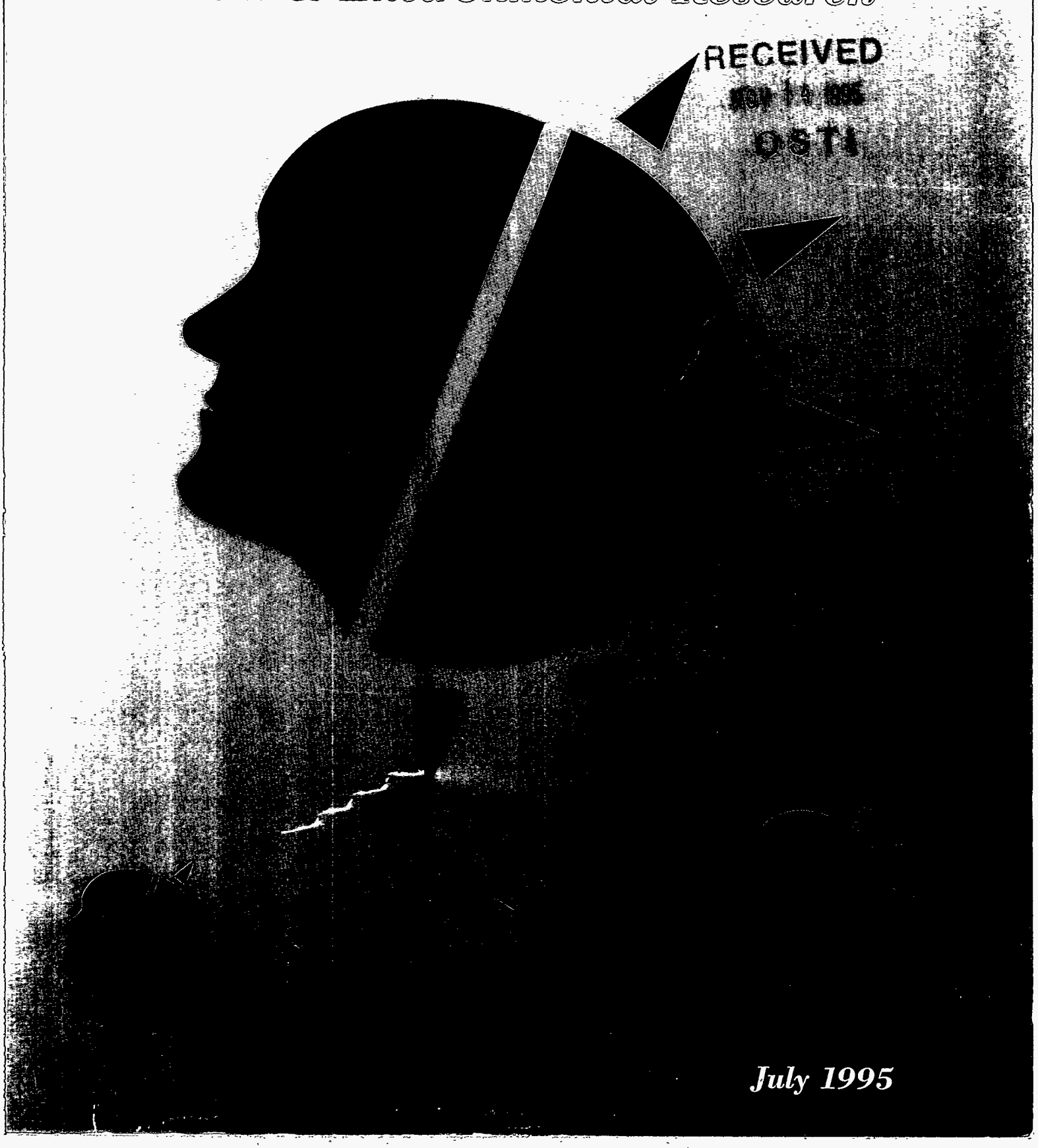

July 1995

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Program Director's Overview Report for the Office of Health and Environmental Research

July 1995

Prepared for
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UC REGENTS APPROVE NEW OFFICIAL NAME FOR LBL: "ERNEST ORLANDO LAWRENCE BERKELEY NATIONAL LABORATORY"

June 16, 1995—Lawrence Berkeley Laboratory, home of nine Nobel Prize winners over its 65 years of existence, will add "national" to its name to more accurately reflect the scope of work conducted at the East Bay research facility.

Laboratory Director Charles V. Shank received approval from the University of California Board of Regents for the laboratory, managed by UC for the U.S. Department of Energy, to be officially known as the "Ernest Orlando Lawrence Berkeley National Laboratory." The new name continues to acknowledge the lifelong achievements of the late Nobel Prize winner Ernest O. Lawrence, who invented the cyclotron that spawned modern high energy physics research, the discovery of many new elements, and nuclear medicine.

"At a time when government leaders and funding sources are demanding more relevance in scientific research, a laboratory's future depends upon its ability to convey distinction and quality," Shank said. "The 'Ernest Orlando Lawrence Berkeley National Laboratory' projects excellence and scope in its identity. It continues to reflect its academic heritage and partnership with the Berkeley campus, and now it will highlight its commitment to respond to national needs—a reality that can be lost if not stated specifically.".

Shank pointed out that the Regents' action is a modest change, but one that clarifies the mission of the Berkeley laboratory, which has over the years evolved from a single-purpose high-energy physics center to a multi-program laboratory seeking answers to the country's most difficult scientific and technological challenges. He said its programs in detectors and accelerators (for example, the Advanced Light Source), advanced materials, biosciences and energy efficiency are focusing on today's national problems in technology and environmental quality.

Seven of the eight other multi-program laboratories in the DOE's network include "national" in their names (Pacific Northwest Laboratory is the only exception). Shank described the action as a first step in the Laboratory's continuing process to establish and project a more distinctive identity to the general public and to decision-makers in Washington.

He said the lab will next explore various options for a short common reference that will attempt to clarify public confusion about the three East Bay science facilities named after Lawrence—Lawrence Berkeley Laboratory, Lawrence Livermore National Laboratory, and the Lawrence Hall of Science.

Over the next several months, Lawrence Berkeley National Laboratory will replace the familiar "LBL" acronym with "LBNL," to be used in text references and electronic mail addresses. However, Shank said he wants to ultimately replace the identity currently defined by initials with a simple title that distinguishes the Berkeley national laboratory from its scientific neighbors.

"Our laboratory is internationally known within the scientific community," the Director said, "but we have a confusing identity among the general public."

Lawrence was the founder of the "radiation laboratory" in 1931, a University-affiliated precursor to the present-day laboratory. Before that decade was over, he had developed a large-scale team approach to scientific research and technology development that is the hallmark of today's laboratory.

By 1936, the UC Regents recognized the laboratory's size and complexity as a discrete administrative unit of the physics department at Berkeley and officially named it "The Radiation Laboratory," with Lawrence as director. With the death of Lawrence in 1958, The Regents changed the "Rad Lab's" name to the "Lawrence Radiation Laboratory" in the founder's honor. And in 1971 they approved "Ernest Orlando Lawrence Berkeley Laboratory" and "Ernest Orlando Lawrence Livermore Laboratory" when the two units were separated administratively from the Berkeley campus.

The Berkeley laboratory's current examination of its identity is an outgrowth of its 1992 Strategic Plan and a subsequent year-long effort which produced a comprehensive Laboratory Communications Plan. The priority goal of that latter plan was to develop and promote "a distinctive identity and visibility," especially with local and national public constituents.

The Berkeley lab is a U.S. Department of Energy national laboratory located in Berkeley, California. It conducts unclassified scientific research and is managed by the University of California.

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David Gilbert—Editor
(please call 510-486-6096 with your questions or comments, or contact me through the Internet at degilbert@lbl.gov)

1. OHER Program Summary

1.1 Director's Overview:

"Today, we take an historic step in restructuring the Department of Energy for its vital post-Cold War missions. Our downsizing and alignment commitments will enable us to do our work better and at lower cost. Our legislative package puts the ball squarely in Congress' court. With their cooperation, we can deliver $5.3 billion more of our $14.1 billion commitment made to President Clinton and American taxpayers in December.

We know DOE can operate more efficiently. We also know how difficult change can be. Our implementation team will ensure quick progress and that workforce reductions are handled with sensitivity. Reductions In Force will be our last choice."

—Energy Secretary Hazel O'Leary, May 3rd, 1995

In spite of the climate of uncertainty with respect to DOE funding of basic research, the Ernest Orlando Lawrence Berkeley National Laboratory legacy of quality, innovative science—one built over the last sixty years—will endure.

LBNL performs basic and applied research and develops technologies in support of the Department of Energy Office of Health and Environmental Research's mission to explore and mitigate the long-term health and environmental consequences of energy use and to advance solutions to major medical challenges. The ability of the Laboratory to engage in this mission depends upon the strength of its *core competencies*. In addition, there are several key capabilities that are cross-cutting, or underlie, many of the core competencies. We refer to these as *foundations* in order to distinguish them from the core competencies.

Bioscience and Biotechnology: Structural biology; Genome research; Bioinstrumentation; Cell and molecular biology, Molecular cytogenetics; Medical imaging; Biology of human diseases; Biomolecular design.

Environmental Assessment and Remediation: Advanced instrumentation and methods for environmental characterization and monitoring; Human health and ecological risk assessment; Indoor air quality; Subsurface remediation of contaminants; Geologic isolation of high-level nuclear waste; Actinide chemistry.

Advanced Detector Systems: Major detectors for high-energy physics, nuclear science, and astrophysics; Scientific conception and project leadership; Advances in particle and photon detection; Implementation of new concepts in detector technology.

Materials Characterization and Synthesis: Advanced spectroscopies and microscopies based on photons, electrons, and scanning probes; Ceramics; Alloys; Heterostructures; Superconducting, magnetic, and atomically structured materials; Bio-organic synthesis.

Chemical Dynamics, Catalysis, and Surface Science: Reaction dynamics; Photochemistry of molecules and free radicals; Surface structures and functions; Heterogeneous, homogeneous, and enzymatic catalysis.

Advanced Technologies for Energy Supply and Energy Efficiency: Subsurface resources and processes; Building technologies; Electrochemistry; Fossil-fuel technologies; Energy analysis.

Particle and Photon Beams: Analysis and design of accelerators; Beam dynamics; High-brightness ion and photon sources; Advanced magnet design and R&D; High-frequency rf technology; X-ray optics and lithography; Induction linacs and neutral beams for fusion energy.

LBNL has identified four foundations; they are listed below together with descriptive subheadings.

National Research Facilities: The development, construction and operation of major research facilities: Advanced Light Source; National Center for Electron Microscopy; 88-Inch Cyclotron; National Tritium Labeling Facility.

Computation and Information Management: High-speed networking and distributed computing; Processing and analysis of scientific images; Data-acquisition and -analysis systems; Scientific-information systems; Database technology.

Engineering Design and Fabrication Technologies: Custom integrated circuits; Integrated accelerator systems; Superconducting-magnet assemblies; Insertion devices for synchrotron radiation; Large-volume semiconductor-detector technology; Laboratory automation; Advanced CAD/CAM facilities for large systems; Facilities for materials processing and fabrication.

Education of Future Scientists and Engineers: Undergraduate, graduate, postdoctoral, and faculty involvement in scientific and engineering research through close ties with the University of California (UC) system; Educational programs for elementary schools, high schools, and colleges.

The following areas bear most significantly on the role that LBNL plays in furthering the mission of OHER.

BIOSCIENCES AND BIOTECHNOLOGY

LBNL's bioscience and biotechnology competency focuses on structural biology; genome research; bioinstrumentation; molecular cytogenetics; medical imaging; the biology of human diseases, and biomolecular design. New recruits in the areas of genomic instability and chromatin structure will provide added cohesiveness to the on-going studies under the newly established Department of Cancer Biology. LBNL has pioneered the use of such techniques as electron crystallography, and has strong programs in x-ray crystallography, NMR spectroscopy, and chemical probe analysis to study biological problems. Recent programs have accelerated the capability to evaluate molecular complexes and the function of biological molecules, particularly proteins and DNA, from the perspective of their specific three-dimensional structure. The LBNL Human Genome Center excels in genomic DNA sequencing, genetic mapping, physical mapping, and DNA isolation and analysis. Several major facilities serve as areas of intellectual and technical cross-fertilization. The Advanced Light Source will offer dramatic new scientific opportunities for biosciences, especially in the fields of x-ray crystallography, x-ray spectroscopy, and x-ray microscopy.

One of LBNL's most ambitious partnerships, the Resource for Molecular Cytogenetics, a joint a venture with the University of California, San Francisco funded by the Department of Energy and Vysis, a diagnotics subsidiary of Amoco, was created to facilitate the application of molecular cytogenetics in clinical and biological studies. Work is being pursued in three areas: Development and application of improved hybridization technology, selection of probes optimized for the use *in situ* hybridization (FISH), and development of digital imaging microscopy. One of the missions of the Resource is the generation of physically mapped probes for use in

in situ hybridization cancer studies. The probes have large inserts (50-100 kb), are cloned in a stable vector (typically cosmids, P1s or PACs) and will be distributed at ~5 Mb intervals over the entire human genome. A public data base showing the currently available probes, along with a request form, can be accessed on the world wide web (WWW) using the Mosaic interface at (http://rmc-www.LBNL.gov/).

Environmental Science and Remediation Technologies

LBNL scientists and engineers working in a range of disciplines from life sciences to geosciences address the breadth of issues outlined above and form a core competency in environmental assessment and remediation. This competency at LBNL includes: advanced instrumentation and methods for environmental characterization and monitoring; human health and ecological risk assessment; indoor air quality; subsurface remediation of contaminants; geologic isolation of high-level nuclear waste; and actinide chemistry. LBNL researchers study non-invasive methods for describing the subsurface, new methods for collecting samples and for measuring contaminant concentrations and identifying their chemical form, methods for assessing the biological toxicity of mixtures of contaminants, and methods to eliminate the exposure pathways and monitor the effectiveness of remediation schemes. LBNL has defined key scientific issues for locating a nuclear waste repository and provided the computational tools used throughout the DOE laboratory system, and is developing techniques and characterizations of complex agents that specifically and effectively sequester actinide ions (e.g., plutonium). LBNL has capabilities for improving risk assessments by developing assays to elucidate the chronic and sublethal effects of exposure to toxic substances in aquatic and terrestrial ecosystems. LBNL is preeminent in conducting research on the nature, sources, transport, transformation, and deposition of indoor air pollutants including radon and combustion emissions. In addition, LBNL's strengths in reaction dynamics, combustion chemistry, and photochemistry of molecules and free radicals will lead to better understanding and control of the environmental impact of fossil fuel combustion.

Advanced Materials Synthesis, Characterization, and Processing—This competency draws its strength at LBNL from expertise in physics, chemistry, traditional materials science and theoretical modeling, combined with the development and application of many types of advanced spectroscopies and microscopies. An important aspect of LBNL's strength in materials research is its expertise in the synthesis of advanced ceramics and alloys; multilayer heterostructures; superconducting, magnetic, and atomically structured materials and devices; and materials made by bioorganic synthesis. In addition, new materials for heterogeneous catalysis and surface structures and functions are also an integral part of the materials research effort, which are in the LBNL competency on Chemical Dynamics, Catalysis, and Surface Science. LBNL employs and develops a unique array of techniques for spectroscopy and microscopy based on photons, electrons, and scanning probes. The Laboratory draws upon two national research facilities—the Advanced Light Source and the National Center for Electron Microscopy, as well as a broad spectrum of novel and unique characterization techniques.

Advanced Computing, Modeling, and Simulation of Complex Systems

Computation, modeling simulation and information management are basic capabilities that contribute directly to the LBNL and DOE science programs. LBNL has developed capabilities in high-speed networking and distributed computing; processing and analysis of scientific images; data-acquisition and -analysis systems; scientific-information systems; and database technology. LBNL contributions to an advanced high speed networking technology through basic research has contributed to the development of nationally enhanced network protocols and improved interprocess communication. The LBNL data management group maintains an underlying capability on ways to model large database systems and to optimize that storage of these databases on mass storage systems. As an example of LBNL competency, the Laboratory is contributing to the national initiative in High Performance Computing and Communications (HPCCI) in three areas: database tools and interprocess communication mechanisms, high speed networking and distributed computing, and data management. Another example is the application of advanced modeling techniques in the study of reaction dynamics, particularly combustion research.

Advanced Manufacturing and Process Technology

LBNL maintains unique capabilities in the area of engineering design and fabrication technologies. The integrated capability at LBNL includes: Laboratory and process automation; Advanced CAD/CAM facilities for large systems; Facilities for materials processing and fabrication; and Engineering Design and Fabrication Technologies. The laboratory and process automation is

represented by engineering in the Human Genome Project while the advanced CAD/CAM capability is represented through design efforts of major facilities such as the Keck Telescope, Advanced Light Source, and Sudbury Neutrino Observatory detector assembly. The Laboratory is also coordinating the process automation team for the AMTEX (American Textile Industry) partnership.

LARGE SCALE RESEARCH AND DEVELOPMENT FACILITIES

LBNL has an underlying capability for designing, building and operating large shared research facilities for the benefit of the national scientific community. LBNL operates four such research facilities: the Advanced Light Source (ALS), the 88-Inch Cyclotron, the National Center for Electron Microscopy (NCEM), and the National Tritium Labeling Facility. The ALS is a third-generation synchrotron radiation source optimized for the use of insertion devices to produce very high brightness tunable radiation in the XUV spectral range. The 88-Inch Cyclotron is a versatile and reliable accelerator of beams from hydrogen to uranium which has unique capabilities for multiply-charged ion beams with variable energy for research in nuclear structure, nuclear astrophysics and fundamental symmetries. The NCEM is an electron microscopy user facility for materials characterization at high spatial resolution which operates advanced high voltage and high resolution transmission electron microscopes for atomic resolution imaging and electron beam microanalysis of materials. The National Tritium Labeling Facility produces compounds with high specific activities of tritium to serve as tracers in chemical and biomedical research, and provides them to biomedical researchers in North America. The resource contains a unique combination of labeling equipment alongside analytical equipment dedicated to radiochemical analyses.

SCIENCE AND ENGINEERING EXCELLENCE AND INTEGRATION

LBNL maintains an underlying capability for engineering excellence and systems integration in many technical areas. Examples include: custom integrated circuits, integrated accelerator systems; integrated superconducting-magnet assemblies; insertion devices for synchrotron radiation; and large-volume semiconductor-detector technology. In addition to an extensive infrastructure of engineering and technical staff together with supporting mechanical and electronic shop facilities, the Laboratory maintains a number of unique, excellent and highly specialized functions that constitute the fundamental capability. These include facilities for the design of custom integrated circuits together with a capability to fabricate and test prototype devices, experience in the design and evaluation of superconducting magnet assemblies, a unique collection of experts in the area of permanent magnet insertion devices for synchrotron applications, and advanced CAD/CAM facilities for the design of large systems and corresponding expertise in managing large engineering projects.

ADVANCED ENERGY TECHNOLOGIES AND END USE APPLICATIONS

LBNL has a strong core competency in energy technologies that integrates scientific, engineering, architectural, information and other capabilities for end use applications to address national needs in building technology, electrochemical energy storage, combustion, and energy use and subsurface energy resources.

1.2 Program Integration:

Biosciences at LBNL, and the Health Effects area in particular, are being transformed into a more integrated program that emphasizes the links between genome, structural biology, and health effects research. Complex biological problems are indeed appropriate for National Laboratories where multifaceted teams could be brought together to address them. Such problems will require massive knowledge about the genes, protein structure, subcellular structure, tissue structure and gene expression and will require the collaboration and cooperation between biologists, biophysicists, engineers, chemists and computer scientists.

Vertical support of a given problem should be encouraged. Protein structure should be used to understand function in the context of the higher organization of the cells and tissues. Functional human culture models should be developed in parallel with transgenic and knockout studies. Support for higher order structures should be considered. As such, molecular and nuclear medicine and imaging need to be integrated with cell and molecular biology and informatics.

The genome and structural biology programs will continue to emphasize technology development and support of user facilities, respectively, aimed at the rapid and cost-effective determination of DNA sequences and protein structures. Priority will be given, when choices need to be made, to the analysis of gene sequences and protein structures of interest to DOE, including genes and proteins that affect individual susceptibility to

energy-related materials or that are related to energy production, energy utilization, and environmental cleanup.

Technologies and information developed in the genome and structural biology programs will be utilized in the health effects research program to understand the function of genes of interest to DOE. In particular, efforts will be focused on:

- determining the relationship between susceptibility genes and adverse health effects from exposure to energy-related materials;
- developing diagnostic tools that can be used to characterize low level environmental and occupational exposures to energy-related agents and to identify at-risk individuals;
- characterizing the relationship between the structure and function of proteins with potential uses in energy production, energy utilization and environmental cleanup; and re-engineering these proteins to improve their utility for achieving DOE's mission in these areas;
- understanding the function of these proteins and their regulation in the context of tissues and organisms, with additional emphasis on human cells and systems.

Scientific Strategy

The major focus of the health effects research program at LBNL is directed to understanding, at the molecular and structural levels, the function of genes of interest to DOE. This effort will require a coordinated, multidisciplinary research effort that takes advantage of the resources and technologies emanating from OHER's genome, structural biology, and health effects programs.

The components of a scientific program to understand the function of a particular gene of interest to DOE might include:

Gene Isolation
Human or microbial genome program libraries and technologies will be used to isolate clones containing genes of interest.

Clone physical characterization
Fine structure maps and sequence information useful in studying gene expression and genetic variability will be developed.

Interspecies comparison
Homologous genes will be identified, isolated, and characterized from different species enabling more rapid determination of gene function. Specific examples could include human and mouse genes for susceptibility or genes from different microbial species that could be useful in bioremediation.

Genetic variation at the sequence level
Genetic variation will be determined on the genes of interest enabling an association of known sequence variation with biological effects. Specific genes/effects of interest will include susceptibility to disease in people or effectiveness of waste degradation in microbes.

Protein structure and function
The function and/or structure of proteins will be characterized using both biological and molecular approaches. For example, the function of potential human susceptibility genes could be characterized in vivo using transgenic mice and antisense methods. In contrast, the structure of microbial genes of potential use in bioremediation could be characterized using structural biology approaches.

Development of biological tools
Biological tools that address critical DOE needs will be developed from understanding gene function. Functional culture models will be created and studied. Additional examples would include cost-effective diagnostic tools for identifying at-risk individuals working at DOE clean-up sites. Similarly, microbial enzymes could be re-engineered for use in bioremediation or energy production.

This approach represents large scale, multidisciplinary science that would require appropriate coordination among DOE-funded research programs at national laboratories and universities. It makes use of the unique laboratory strengths in genomics, structural biology, cell and molecular biology, mouse genetics, and biomedical engineering. As outlined above, this approach is applicable to Departmental issues in health and the environment. These range from the identification of individuals with increased susceptibility to health risks from exposure to energy-related materials to the development of more efficient and cost-effective methods for cleaning up DOE waste sites or producing energy.

A Major New Initiative in FY 1996

In the course of LBNL's strategic planning effort, Environmental Biotechnology has been identified as a major area for growth and development. While LBNL has several core competencies in this area, we now need to integreate these areas and add additional expertise. We therefore have embarked on the creation of a center and have attracted a highly productive and imaginative coordinator.

Lawrence Berkeley National Laboratory Center for Environmental Biotechnology

Ongoing environmental research at the Lawrence Berkeley National Laboratory is focused in several key areas:

- pollution prevention
- bioremediation
- waste site characterization and monitoring
- hazardous waste treatment
- human exposure and comparative risk assessment
- ecological risk assessment and restoration

The research approach is multi-disciplined and involves diverse departments within several divisions. Hence, it was decided to establish a Center for Environmental Biotechnology under a Director's Initiative. Programs are being established under key focus areas that bring together not only the capabilities at LBNL, but also link those established within academic institutions, industry, and government research institutions. Our goal is not to be redundant but rather to build bridges that link different capabilities within the DOE laboratories and associated universities and to focus on key environmental clean-up problems by enhancing synergy rather than competition between the various institutions.

There is also an educational component to the Center in that we are directly linked to the BEST (Bioremediation, Education, Science and Technology) program and offer the opportunity for academic students to obtain hands on experience in the laboratory and field operations.

Another important link is to the community within the Bay Area. The Alameda Center for Environmental Technology (ACET) is being established and the Center for Environmental Biotechnology will help facilitate collaborative CRADAs with ACET.

The Center for Environmental Biotechnology will also collaborate with CAL-EPA in setting up a program to validate and certify new environmental biotechnologies for field application. In addition an Advisory Council has been organized to guide our research efforts to meet the demands of the market. The members of the Council are well known in the area of environmental technology and are representative of industry, academia and government sectors.

Currently, funds are being sought for establishing a central microbiology working laboratory and several proposals are being written in collaboration with UC Berkeley, Oregon State, CYTEC, General Electric, Idaho National Engineering Laboratory, Oak Ridge National Laboratory, and Los Alamos National Laboratory.

Both basic and applied research programs are being established within the Center for Environmental Biotechnology. These programs are:

Ecotoxicity:

This program focuses on determining the effect of pollution on small animals such as nematodes, worms, shellfish and amphibians. Studies include the monitoring of reproductive cycles and DNA damage as a result of being exposed to different levels and types of pollution in the Bay Area. This program is linked to the Health Risk Assessment program by working with the cell biologists on developing more sensitive assays and a better understanding of the regulation system involved in having DNA damage and a negative cascade effect on normal metabolic functions.

Health Risk Assessment:

Research is concentrating on the development of assays using primary human cells and measurement of enzyme levels to determine how pollutants are actively metabolized. The EPA has a list of potential carcinogenic pollutants. However, most of the work done in this area has been done on rats and mice. We are assessing the health risk to humans by determining which pollutants, if ingested, inhaled, or absorbed, can be deleterious to our body cells by causing abnormalities in cell structure and function.

Molecular Evolution and Ecology:

California has many contaminated sites due to activity from the armed forces and industries such as oil refineries, metal electroplate shops, mining industry and agriculture. Nature has the ability to deal with

some of the contamination by genetically altering certain microbial and plant species to either completely mineralize the contaminant or detoxify the pollutant. Our research will focus on how the microorganisms and plants evolve through genetic mutations either spontaneous or by induction to "deal" or "handle" the pollutant in a toxic environment. By better understanding the regulation and expression systems of specific organisms, we can metabolically or genetically engineer "super organisms" such as microorganisms with more active enzymes or plants with better absorption capacity that can be used in bioremediation efforts.

Structure-Function Relationships:

One of the barriers to using microorganisms more effectively in the field for remediation has been the lack of understanding the cells physiology in a natural environment. Microorganisms grown under laboratory conditions such as on agar (solid) media or in submerged culture (fermentors) exhibit a different structure and function than what is found in nature in soil, in water, and on plant surfaces. The research focus will be on linking engineering with microbiology to design bioreactors that would function more closely to what goes on within the subsurface or water column. This technology would improve soil treatment methods for field applications. In addition, physiological studies would yield information in regards to what are the limits for optimization in field treatments. For example, microorganisms are notorious for developing biofilms that stick and clog soil pores. Finding a way to inhibit that formation yet enable the microorganism to bind to soil particles would improve the bioremediation treatment for certain soil matrixes.

Natural Augmentation:

Since, in many instances, microbial populations have been exposed to pollutants over time within a given site,. natural selection occurs and enables these microorganisms to use the pollutant as a food substrate. Augmenting this population would have an advantage in that more biomass should theoretically have to have more food in order to survive. However, in some cases, this approach backfires, and the population dies out or no longer desires to eat the pollutant. Studies will center on optimizing natural augmentation by incorporating biophysical parameters such as moisture, pH, salinity, redox potential that would improve and stabilize microbial metabolic processes occurring in soil or water systems and evaluate competition factors, such as waste products, soil binding properties, gas-water interface effect on nutrient transport, that may affect establishment of the desired population.

In situ Microbial Monitoring:

Bioremediation has been described as a black box by engineers since we do not have the technology to verify "balancing the equation". Therefore, improved systems to monitor microbial activity in the soil is needed. Research has begun on use of cold radio-isotopes to monitor carbon metabolism and applications of this technology is being studied for metal uptake and detoxification. We will need to focus on developing improved systems of biomarkers and biosensors for use in the field. One possibility is to integrate biopolymers with engineering knowledge to create semi-organic sensors.

From the descriptions above, it should be clear that the Center for Environmental Biotechnology is integrating all of the existing capabilities related to environmental biotechnology within LBNL and establishing key synergistic links with outside laboratories. We feel that by using this approach, resources will be saved in solving several of the key environmental pollution problems in DOE, DOD, and in California, including those critical pollution problems created by the agriculture, oil, and mining industries.

PARTNERSHIP INTEGRATION FOR TECHNOLOGY

LBNL maintains an underlying capability for utilizing a multiprogram energy research laboratory as a national resource in partnership with industry, universities and other government agencies. An excellent example of interagency partnership for the advancement of technology is a new Cooperative Research and Development Agreement (CRADA), funded by the National Institute of Standards and Technology. Led by Dr. Daniel Pinkel of the LBNL/UC San Francisco Resource for Molecular Cytogenetics, this collaboration with Vysis, (a subsidiary of AMOCO) entails the development and evaluation of techniques and DNA reagents for performing comparative genomic hybridization (CGH) to arrays of DNA on a microchip. The resulting technology will be configured so that it will be applicable to clinical genetic testing in a wide range of health problems including cancer and developmental abnormalities.

One of the most productive forms of technology partnerships, the direct exchange of personnel and ideas through the educational process, is strongly supported through LBNL's fundamental capability of Education of Future Scientists and Engineers. LBNL supports undergraduate, graduate, postdoctoral, and faculty

involvement in scientific and engineering research through close ties with the University of California system and with educational programs for elementary schools, high schools, and colleges.

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CENTER FOR SCIENCE AND ENGINEERING EDUCATION

Lawrence Berkeley Laboratory's Center for Science and Engineering Education (CSEE) was established in 1987 to bring together many of the laboratory's educational programs. Since its inception, CSEE programs have grown to include new pre-college, undergraduate, graduate, and faculty programs that support the Center's goals to: (i) promote equal access to scientific and technical careers for all students, including women, minorities, the handicapped, and the economically disadvantaged; (ii) improve the quality of science and engineering teaching by supporting increased classroom emphasis on the scientific process and exposure to frontier science and technology; (iii) increase the number of U.S. students who become scientists and engineers by developing and implementing strategies to provide continuity of opportunity from elementary through graduate school; and (iv) promote scientific literacy, including an understanding of relationships among frontier science and technology.

Undergraduate Student Research Participation Programs enable junior and senior level college and university students to get hands-on research experience at a national laboratory. Summer and Academic-Term Residential programs brought 84 students to the laboratory in FY 1994. Because the Lawrence Berkeley National Laboratory has one of the highest fractions of Life Sciences research of the national laboratories, a significant number of the CSEE undergraduate student participants are majoring in the biological sciences and are assigned to Life Sciences departments. During FY 1994, over 20% of the summer and academic-term undergraduate students were assigned to scientist-mentors in the Life Sciences. OHER investigators play an active and significant role in mentoring these students. Not only is a student assigned to work with an LBNL staff scientist who is the principal investigator of her/his group, but junior staff, post-doctoral staff, and even graduate students play a role in mentoring and guiding the undergraduate. Several of CSEE's summer and academic programs target women and underrepresented minorities for opportunities to work and learn at the laboratory. In the summer of 1994, CSEE's College and University Programs and Life Sciences Division's Human Genome Project cooperated to pilot a program for students in the City College of San Francisco (CCSF) Biotechnology Training program. Three students were selected to participate fully in CSEE's summer research program. Mentors, carefully selected from among Life Sciences researchers, assisted in assessing the students' skills and provide input to CCSF regarding curriculum needs. Though a small initial offering, the pilot program was successful. Plans are underway to expand it both scope and size. Life Sciences scientists are major contributors to increasing the diversity of the next generation of scientists.

In the pre-college area, CSEE participates in the national Department of Energy Teacher Research Associates Program (TRAC) for middle and secondary science teachers. TRAC teachers spend eight weeks at the laboratory participating in hands-on research experiences. During the summer of 94, thirty-four teachers participated at the laboratory, over 22% of them working directly with Life Sciences Division scientific staff.

LBNL is host to the Department of Energy's High School Honors Life Sciences Program. This two-week program brings sixty eight outstanding high school students from around the country to participate in a series of lectures and hands-on experiences in the biological sciences. OHER scientists contribute their time to present lectures and teach the students skills in such areas as recombinant DNA technology.

The Education Outreach Program provides LBNL volunteers to assist local schools and community organizations in education enhancement activities. Volunteer activities range from guest speaking, tutoring, serving as a mentor, assisting teachers with curriculum development and serving as a tour guide at the laboratory for school groups. Approximately one-fifth of the LBNL volunteers were from the Life Sciences Division. Of the twenty-six students who participated in the eight week summer high school student research program, nine worked in the Life Sciences Division.

Center for Science and Engineering Education *In Action*

Carmina Catuar of City College of San Francisco. Carmina's mentor for the Summer of 1994 was Life Science's investigator Amy Kronenberg.

Jerry Lee of City College of San Francisco. Priscilla Cooper of Life Sciences Division was Jerry's mentor during the Summer of 1994.

Michael Wong of City College of San Francisco. Jabob Bastacky of Life Sciences Division was Michael's mentor during the Summer of 1994.

2.0 Program Organization, Facilities, and Resources

Laboratory Facilities—Table 3

ADVANCED LIGHT SOURCE

The Advanced Light Source (ALS), America's first third-generation synchrotron, offers dramatic new scientific opportunities for the life sciences, especially in the fields of x-ray microscopy, x-ray spectroscopy, and x-ray crystallography. In particular, the brightness of the facility's soft x-rays and the advanced design of its insertion devices and beamlines are unmatched resources that are already opening up new horizons in biological research.

Life sciences research at the ALS will ultimately use several insertion device and bend-magnet beamlines, each the source of radiation for a different "resource center." In x-ray microscopy, our current vision includes two complementary x-ray microscopes, each offering its own advantages. The first one, called XM-1, became operational in August 1994 and is illuminated by a bend magnet source for full field imaging. The XM-1 operates in transmission, providing information about material within a sample, not just on its surface. Researchers can analyze samples with thicknesses on the order of 1 to 10 microns with this x-ray microscope, making it an ideal complement to optical and electron microscopes. The microscope was designed as an easy to use instrument and is attracting a wide range of interest from the biological community. The light source for the second microscope would be an undulator producing ultrabright soft x-rays for scanning imaging.

A second resource center, devoted to x-ray spectroscopy using both hard and soft x-rays, will be based on an elliptical wiggler of advanced design producing circularly polarized radiation. Use of this wiggler will be shared with materials science users; accordingly,

funds to begin its fabrication have been provided by both the Office of High Energy Research (OHER) and the Office of Basic Energy Sciences. The resource center will consist of two beamlines—a soft x-ray grating monochromator beamline and a hard x-ray crystal monochromator beamline—with the optics arranged so that either can intercept the central radiation fan. This project is now well underway: the elliptical wiggler is under construction, the optical design is well advanced, and the engineering design for the beamlines has started.

A third resource center devoted to macromolecular crystallography, the primary technique of structural biology, is also under construction—one that will match or exceed the performance of any synchrotron facility currently in operation. The heart of the crystallography facility will be a beamline delivering x-rays from a 38-pole wiggler source to three automated endstations for high-quality data collection with rapid sample turnaround. The first endstation is scheduled for availability in May 1996. The protein crystallography facility project was started in FY 94 with funding through OHER and the University of California.

To provide the facilities and services the biological community will require to make full use of the ALS, construction has begun on an ALS Structural Biology Support Facility which will offer a full range of highly automated instrumentation and support laboratories, including high-performance computer workstations networked to beamline computers for on-site data reduction. The $7.9M facility is funded by the Department of Energy and scheduled to open in October 1996.

The Structural Biology Support Facility, which contains over 1000 sq. meters of laboratory and office space, is adjacent to the ALS experiment floor, making it easily accessible. Crystallographers will be able to check their crystals' quality and pre-align the crystals before their scheduled beamtime, and numerous microscopy and spectroscopy resources will be available for parallel investigations—laboratory space for cell and tissue culture, light and electron microscopy equipment, and facilities for EPR and Fourier transform IR spectrometry. Finally, high-performance computers will offer full graphics capabilities for visualizing experimental results.

Imaging Facilities

The Donner 600-Crystal Positron Tomograph was designed and built at LBNL to provide better than twice the spatial resolution of any previous tomograph. The very high resolution is achieved by six hundred 3-mm-wide bismuth germanate crystals, coupled individually to 14-mm phototubes. A commercial single-photon emission tomograph has been provided by the manufacturer. Magnetic resonance imaging facilities are also available for medical use; in particular, facilities for safety research into extending the use of NMR for noninvasive human studies are centered at LBNL. The Laboratory is also home to a unique array of electron microscope facilities, whose purchase and operation are funded from several sources. These facilities include the Atomic-Resolution Microscope and the High-Voltage Electron Microscope at the National Center for Electron Microscopy, as well as an Intermediate-Voltage Electron Microscope, funded by NIH, for high-resolution electron crystallography. In collaboration with the Lawrence Livermore National Laboratory, LBNL has also developed the capability of imaging biological macromolecules by scanning tunneling microscopy. Several STMs are operational at LBNL.

National Tritium Labeling Facility

This national facility, supported by the NIH, carries out research into the labeling of compounds to high specific activity with tritium and provides a tritium-labeling service for investigators throughout the country. A primary direction for this facility is now the development of tritiated reagents and techniques for NMR studies of biological macromolecules.

Biomedical Isotope Facility

Nuclear and organic chemical synthesis instrumentation and laboratory facilities at LBNL include the 88-Inch Cyclotron and radiation-containment devices for rapid synthesis of radiotracers.

With the completion of the Biomedical Isotope Facility in June 1995 and the commissioning of the medical cyclotron by September 1995, LBNL will have the wherewithal to expand its efforts on the development of labeling strategies for the incorporation of short-lived isotopes into medicinally useful compounds.

RESOURCE FOR MOLECULAR CYTOGENETICS

The mission of the Resource is to stimulate progress in molecular cytogenetics through (1) development of improved hybridization technology, (2) design and development of digital imaging microscopy (3) production of probes optimized for molecular cytogenetic studies, (4) support of the molecular cytogenetic community through collaboration, distribution of probes and analysis technology and education and (5) by transfer of useful reagents, processes and instruments to the private sector for commercialization. This is accomplished in cooperation with LBNL's Human Genome Center and Engineering Division, the chromosome microdissection and cancer genetics expertise from the National Institutes of Health , and clinical and genetic investigators at the University of California, San Francisco (UCSF) , the Lawrence Berkeley National Laboratory, University of California, Berkeley and elsewhere throughout the scientific community. These activities will serve the research and clinical communities indirectly through general advances in technology, and directly thorough the production and distribution of probe reagents of clinical and biological utility, development of instrumentation, initiation of training programs, and technology transfer. The Resource receives funding by DOE's Office of Health and Environmental Research and Vysis, a subsidiary of AMOCO. For more information, the Resources' HomePage can be accessed through the World Wide Web at http://rmc-www.LBNL.gov/.

LBNL TRANSGENIC RESOURCE

Lawrence Berkeley National Laboratory has a well established infrastructure in place at the development of transgenic mice. This includes two microinjection setups, three trained staff members skilled in microinjection and embryo transfer techniques, and two trained animal technicians to assist with screening and breeding regimens. In addition, there are plans to add a third microinjection setup in the near future to further expand our injection capabilities. Currently, each investigator is asked to provide DNA which has been prepared according to our instructions. FVB mice are used primarily as these provide an excellent substrate for developing transgenics in an inbred background. Investigators are given tails from potential transgenics at weaning age along with detailed instructions in the preparation of tail DNA and screening techniques. Positive founders are then shopped. The inclusion of the investigator in the preparative and screening phases has been found to keep them more involved in the process. LBNL's transgenic expertise extends beyond the mere production of transgenic animals to providing detailed advice in construct design and preparation as well as advice regarding screening and maintenance of the transgenic lines. LBNL has extensive expertise in all areas of transgenic production and can provide assistance in designing experiments which utilize a minimum number of DNA constructs and a minimal number of transgenic animals. Discussions with the investigators provides an essential component in planning a well-designed experiment that will efficiently and economically enable the less experienced investigator (with regard to transgenic experiments) design good experiments.

Presently, the Resource creates transgenic animals for two additional DNA constructs per week for DOE investigators without taxing our capabilities at LBNL.

LIFE SCIENCES MICROSCOPE RESOURCE

The LBNL Life Sciences Microscope Resource (LSMR) is in the final stages of development. A wide range of users are currently obtaining quantitative digital images, color hard copy, and training at the Resource. The LSMR will provide access to a variety of inverted and upright microscopes with a range of experimental capabilities including real-time intensified video microscopy and micromanipulation, multicolor quantitative CCD digital imaging, and multicolor 3D confocal microscope imaging. Access to computer workstations for automated data acquisition, image analysis, data archiving, video digitization, and preparation of color hard copy (paper, transparencies, and 35 mm slides or prints) will also be provided. A cooled CCD camera for digital photography (such as digitization of autoradiograms) is also available. The resource contains four fluorescence microscopes: a Zeiss Axiovert 135 H/DIC; a Bio-Rad MRC 1000 Laser Scanning Confocal Microscope; a Zeiss Axioskop; and a Zeiss Standard.

YEAST GENETIC STOCK CENTER

The Yeast Genetic Stock Center maintains a collection of approximately 1000 genetically defined strains of the yeast Saccharomyces cerevisiae. These strains are stored at 4°C in "milk-paper replicas" for ease of dissemination and at –75°C in 20% glycerol as a back-up supply. The Center is located in Donner Laboratory in the Department of Molecular and Cell biology at the University of

California, Berkeley. The YGSC collects, maintains, monitors, and provides these strains for basic and applied research and for teaching in the biological sciences. Thus the Stock Center acts as a depository for yeast mutants of general interest, making them available to a large number of investigators and educators. The Stock Center responds to nearly 1500 requests a year from scientists all over the world. At intervals of two to three years the YGSC publishes an updated catalogue of strains listing new acquisitions and revisions. Since 1982, NIH grant funding has been supplemented by charging users of the Stock Center a fee for strains.

OTHER FACILITIES

Complex biological phenomena require sensitive and specific analytical techniques for analysis. OHER programs at LBNL have pioneered the use of such techniques as x-ray and electron crystallography, NMR spectroscopy, flow cytometry, and chemical probe analysis to study biological problems. Instrumental techniques such as analytical electron and x-ray microscopy, gas chromatography/mass spectrophotometry, confocal microscopy, fluorescent-detected circular dichroism, and differential polarization microscopy are also utilized for analytical purposes in the life sciences program. X-ray photoelectron and laser Raman spectroscopies are used to study atmospheric processes, and equipment and techniques have been developed to provide near real-time measurements of environmental concentrations of absorbing aerosols and of radon and its decay products.

Standard laboratory facilities and instrumentation include laminar flow biosafety cabinets, incubators, warm rooms, fermenters, cold rooms and ultracold storage units, fluorescence microscopes, all types of gel electrophoresis apparatus, cryostats, scanning densitometers, ultracentrifuges (analytical as well as preparative), high-pressure liquid chromatographs, dark rooms, automated DNA and peptide synthesizers and sequencers, and polymerase chain reaction (PCR) apparatus. We also maintain an AALAC-accredited animal colony, which has facilities for housing rodents (including transgenic mice), dogs, cats, rabbits, guinea pigs, goats, and monkeys.

Engineering facilities include: (i) a fully equipped semiconductor materials/detector fabrication facility with extensive diagnostics capabilities; (ii) a Van de Graaff accelerator (2-MeV protons) equipped for backscatter and x-ray fluorescence studies; (iii) extensive electronic design and development facilities, with particular emphasis on very low-noise measurements required for high-resolution spectroscopy; (iv) an ultralow-background radiation-counting facility equipped with high-resolution gamma-ray spectrometers; and (v) a clean-room facility devoted to the fabrication of microsystems such as detectors and electronic devices with micrometer-size structures. In addition, LBNL's Information and Computing Sciences Division maintains state-of-the-art information and computing resources. These resources include SEEDIS, the LBNL Socioeconomic Environmental Demographic Information System, which contains the country's most complete computerized archive of U.S. health, demographic, and socioeconomic data. This archive is now being integrated with the OHER Comprehensive Epidemiologic Data Resource (CEDR) for use in OHER epidemiologic research activities.

Laboratory facilities of the Indoor Environment Program include the Indoor Air Quality Research House, which contains a three-room test space instrumented for studies of radon decay product behavior and for investigations of other specific pollutants, such as environmental tobacco smoke. A controlled 20 m^3 chamber is also available for detailed studies of pollutant emissions and behavior. A new facility, consisting of two intensively instrumented, basement-like concrete structures in the Santa Cruz mountains, has been built for a detailed examination of radon transport and entry processes, including a more rigorous experimental validation of theoretical predictions based on complex numerical codes than has been possible previously. Modeling is conducted on a Sun 4-280 and a Hewlett Packard series 7000 computer operated by the Indoor Environment Program and on Energy Research-sponsored CRAY supercomputers.

Additional facilities for environmental research include our fully functional cloud/aerosol observatory on El Yunque Peak, Puerto Rico. Measurements of cloud microphysical and optical properties, as well as aerosol physical and chemical properties will be complemented by NASA's Goddard Research Center satellite observations and a cloud chamber for studying cloud chemistry and physics.

Table 1: Budget Data FY 1993, 1994, & 1995

Laboratory Funding (millions of dollars)

Source	1993	1994	1995*
OHER			
Operating	17.9	21.2	20.1
Capital equipment	1.4	2.6	1.3
Construction	1.2	2.7	20.5
TOTAL	20.5	26.5	41.2
Other OER			
Operating	115.1	104.2	115.0
Capital equipment	15.0	20.8	15.0
Construction	24.8	19.9	15.4
TOTAL	154.9	144.9	145.4
Other DOE			
Operating	49.2	59.5	40.1
Capital equipment	0.5	1.6	1.0
Construction	1.2	0.0	0.0
TOTAL	50.9	61.1	41.1
WFO	35.4	44.1	49.0
Total Lab Funding	**261.7**	**276.6**	**276.7**

*Figures for 1995 are estimates.

Table 2: Staff Working on OHER-Sponsored Projects

Laboratory Personnel (FTEs)			
OHER direct	115	126	140
Other DOE direct	1415	1323	1300
WFO	340	386	400
Indirect	780	760	760
Total Lab Personnel	**2650**	**2595**	**2600**

On the following pages are charts listing the OHER-funded principal investigators at LBNL and tables summarizing OHER funding and personnel levels, in the context of the Lawrence Berkeley National Laboratory as a whole. More detailed information on personnel involved in OHER activities is tabulated in Section I. Also reproduced on the following pages are organization charts for the Laboratory and for Life Sciences Division, Structural Biology Division and Energy and Enviroment Division, where the bulk of the OHER-sponsored research is conducted.

Figure 1: LBNL OHER-Funded Principal Investigators

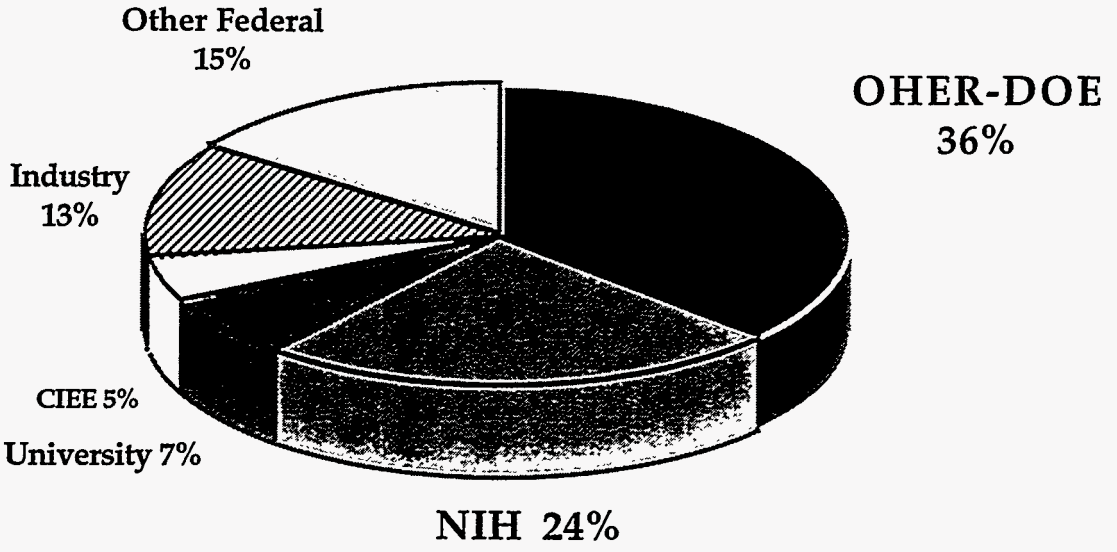
B&R	Project Title	Investigator	Div	Phone	Fax	Mailstop
KP0101	Char. of Airborne Radon Concent.	Gadgil, AJ	EED	4651	6658	90-3058
		Sextro, RG	EED	6295	6658	90-3058
KP0102	Detector Materials, Defect, Instru.	Jaklevic, JM	EG	5647	5857	70A-3363
KP02	**Environmental Research**					
KP0201	Aerosol & Cloud Chemistry	Novakov, T	EED	5319	5172	73-101
KP0203	Exper. & Theor. Invest. of Radon	Sextro, RG	EED	6295	6658	90-3058
		Fisk, WJ	EED	5910	6658	90-3058
KP0203	Therm. Prop. of Chem. Species	Al Mahamid Al Rifai, I	ES	7001	5799	70A-1150
KP0203	Field Heterogeneity	Majer, EL	ES	6709	5686	50E
KP03	**Health Effects**					
KP0302	Air Poll. Exp. in Bldgs.	Daisey, JM	EED	7491/6591	7202/6658	90-3058
KP0302	Mole. Mech. of Cell Effect	Blakely, EA	LS	6595	4475	70A-1118
KP0302	Mech. of Tumor Promotion	Bissell, MJ	LS	4365/6890	5586	83-101
KP0302	*In Vitro* Transformation of Human Mammary Epithelial Cells	Stampfer, MR	LS	7273	5735	934-47A
KP0302	Radiation Transcription	Campisi, J	LS	4416/4417	4475	70A-1118
KP0302	Interspec. Extrap. & Risk Assess.	Gold, LS	LS	7080	6773	Barker
KP0302	Bone Marrow Stem Cells	Narla, M	LS	7029	6746	74-157
KP0302	Molecular Cytogenetics Resource	Gray, JW	LS	5812	5343	74-157
KP0302	Bio. Effects of Magnetic Fields	Liburdy, RP	LS	6240	6644	934-47A
KP0303	Radiological Physics and Chemistry	Chatterjee, A	LS	5415	6949	29-100

All telephone and facsimile numbers should include the 510 area code and the 486- prefix unless otherwise stated. Internet E-mail addresses are, in most cases, the investigators first two initials, last name (no spaces) @LBNL.gov (for example vmmarkowitz@LBNL.gov).

For other important information, LBNL's WWW Home Page can be accessed at http://www.LBNL.gov/

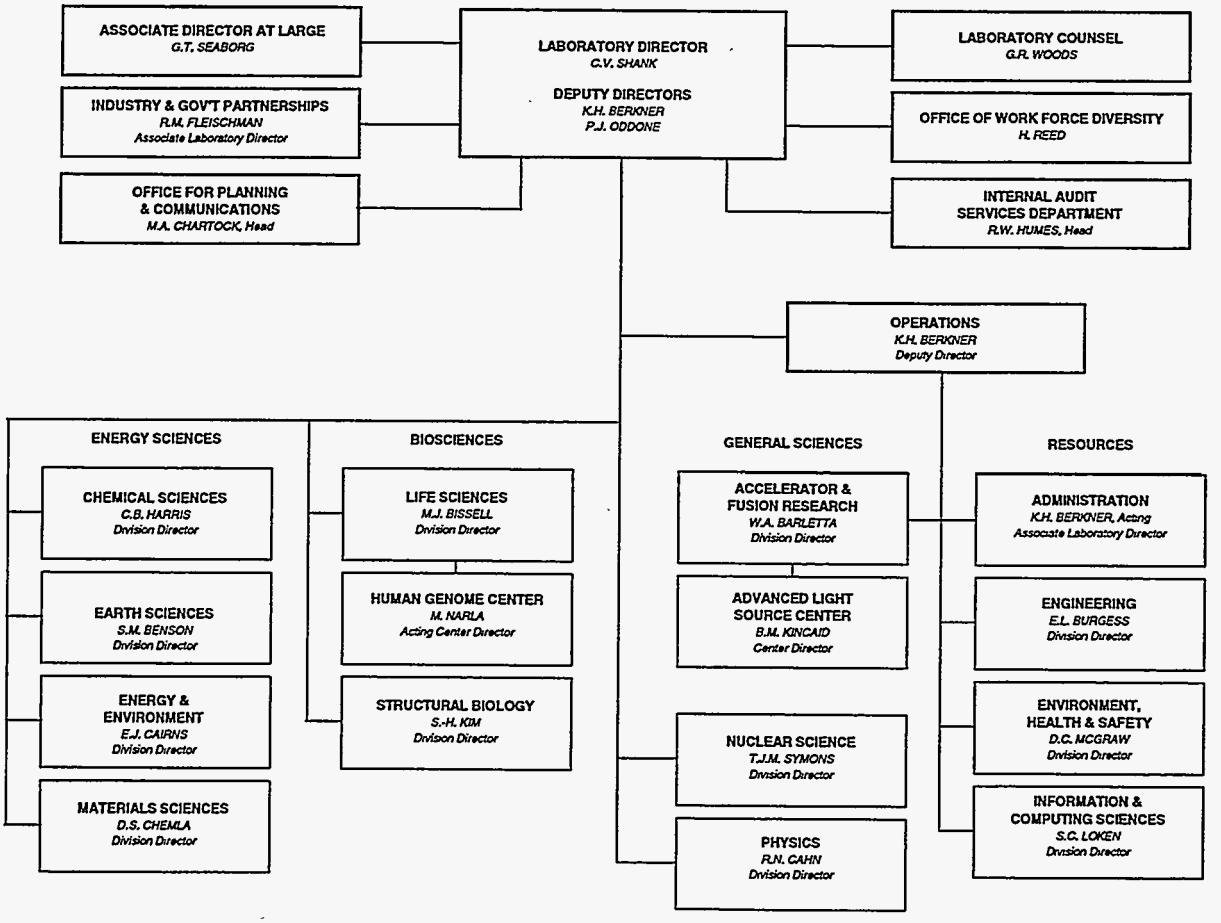
B&R	Project Title	Investigator	Div	Phone	Fax	Mailstop
KP04	**General Life Sciences**					
KP0401	Mole Structure of Membrane Trans Sys.	Jap, BK	LS	7104/7990	6488	Donner
KP0401	Biophysical Chemistry	Klein, MP	SB	4331	6059	Calvin
KP0401	Structural Biophysics	Kim, SH	SB	4333	5272	Calvin
KP0401	Biomolecular Structure Analyses NMR	Wemmer, DE Tinoco, IN	SB	4318 642-3038	6059	Calvin
KP0401	3-D Structure of Catalytic RNA	Kim, SH Wemmer, DE	SB	4333 4318	5272	Calvin
KP0401	Artificial Enzymes	Schultz, PG Wemmer, DE	SB SB	642-9277 4318	643-6890 6059	Latimer Calvin
KP0401	ALS Microscopy	Glaeser, RM	LS	642-2905	6488	Donner
KP0401	ALS Spectroscopy	Cramer, SP	EED	4720	4550	2-300
KP0401	ALS Crystallography	Kim, SH	SB	4333	5272	Calvin
KP0401	Semi-Conductors X-Ray Detectors	Jaklevic, JM	EG	5647 643-8877	5857 5342	70A-3363
KP0402	Genetic Study on Yeast	Mortimer, RK	LS	642-0815	642-8985	Donner
KP0402	Molecular Carcinogenesis	Bissell, MJ	LS	4365/6890	5586	83-101
KP0402	Center for Biomolecular Engineering	Schultz, PG Kim, SH	SB SB	642-9277 4333	643-6890 5272	Latimer Calvin
KP0404	Human Genome Center	Narla, M	LS	7029	6746	74-157
KP0404	Human Genome Field Operation	Spengler, SJ	LS	4879	5717	Donner
KP0404	Automated Users Interface	Zorn, MD	ICSD	5041	4004	50B-3238
KP0404	Molecular Cytogenic Tech	Gray, JW	LS	5812	6746	74-157
KP0404	Advanced Detectors for Mass Spect	Jaklevic, JM	EG	5647	5857	70A-3363
KP0404	Lab Info Mgnt Sys for Megabase Seq.	Markowitz, VM	ICSD	6835		50B-3238
KP05	**Carbon Dioxide Research**					
KP05	Modification of Cloud Optical Prop.	Novakov, T	EED	5319	5172	73-101
KP06	**Medical Applications**					
KP0601	Vascular & Blood Diseases	Ebbe, SN Taylor, SE	LS LS	6262 4103	6746 4768	74-157 55-121
KP0601	Exper. Med. Dev. of Radionuclide	Van Brocklin, HF	LS	4083	4768	55-121
KP0601	Exper. Med. Clinical Diag.	Budinger, TF	LS	5435	4768	55121
KP0601	Positron 3D Imaging Instrument	Derenzo, SE	LS	4097	4768	55-121
KP0601	Imaging of Apolipoprotein E-binding	Krauss, RM	LS	4277	5342	Donner

Proposed FY 1995 Funding

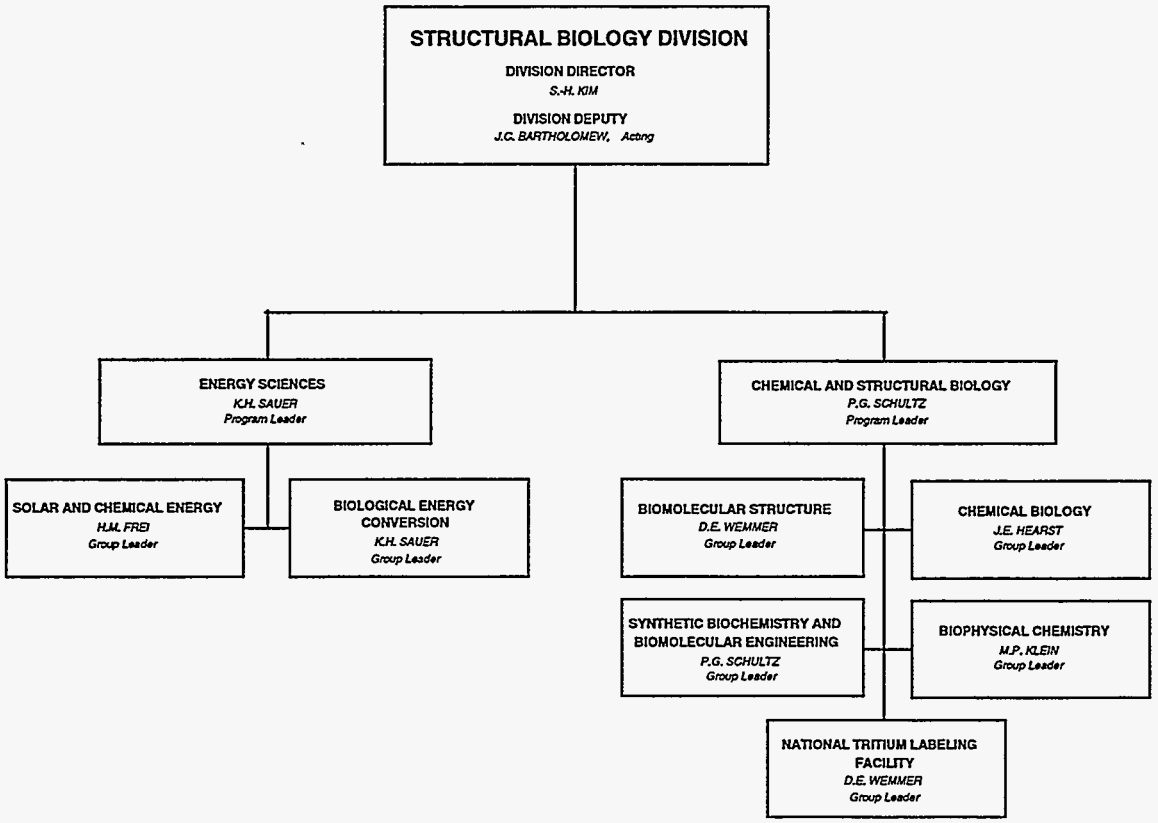


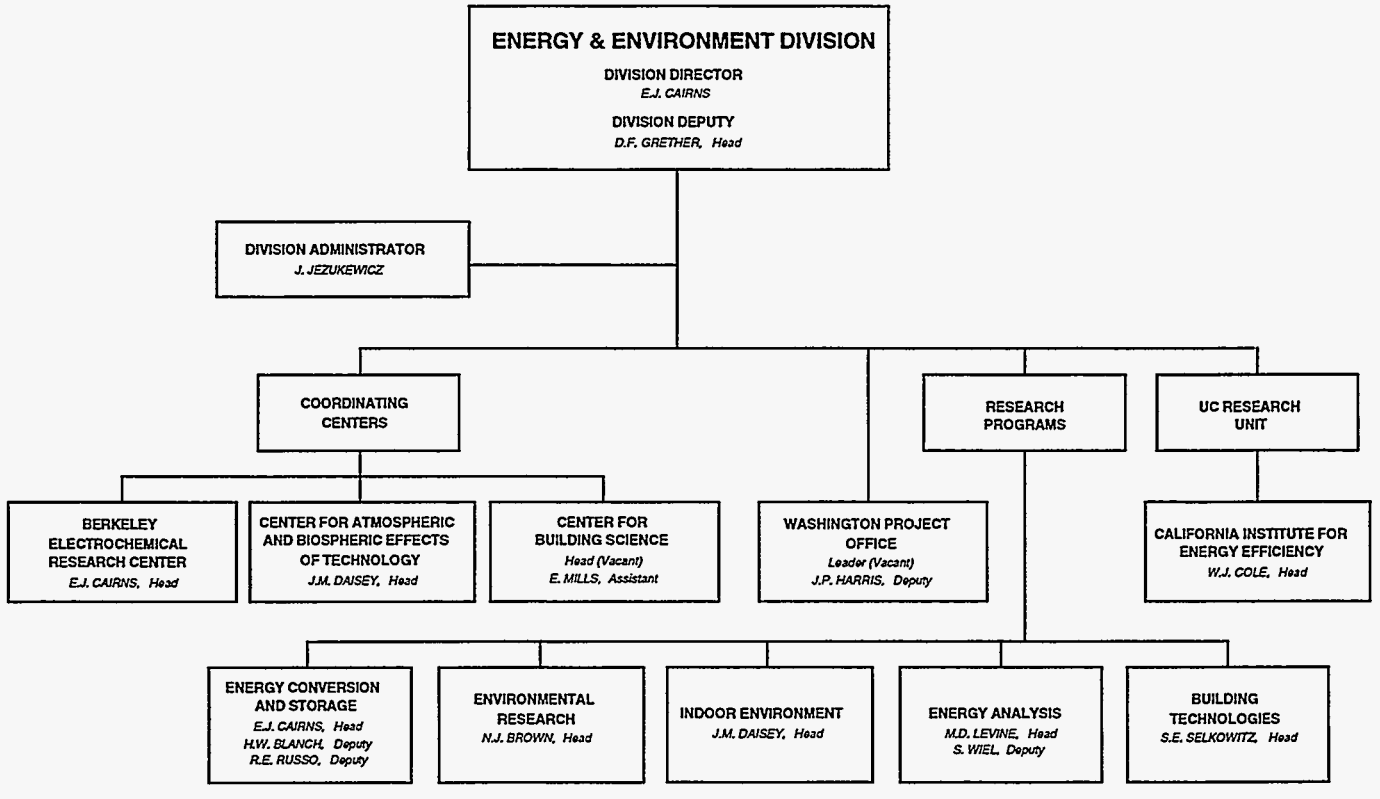
Sponsors of biosciences and environmental sciences research programs at Lawrence Berkeley National Laboratory. CIEE—California Institute for Energy Efficiency; NIH—National Institutes of Health

LAWRENCE BERKELEY LABORATORY • UNIVERSITY OF CALIFORNIA



LAWRENCE BERKELEY LABORATORY • UNIVERSITY OF CALIFORNIA





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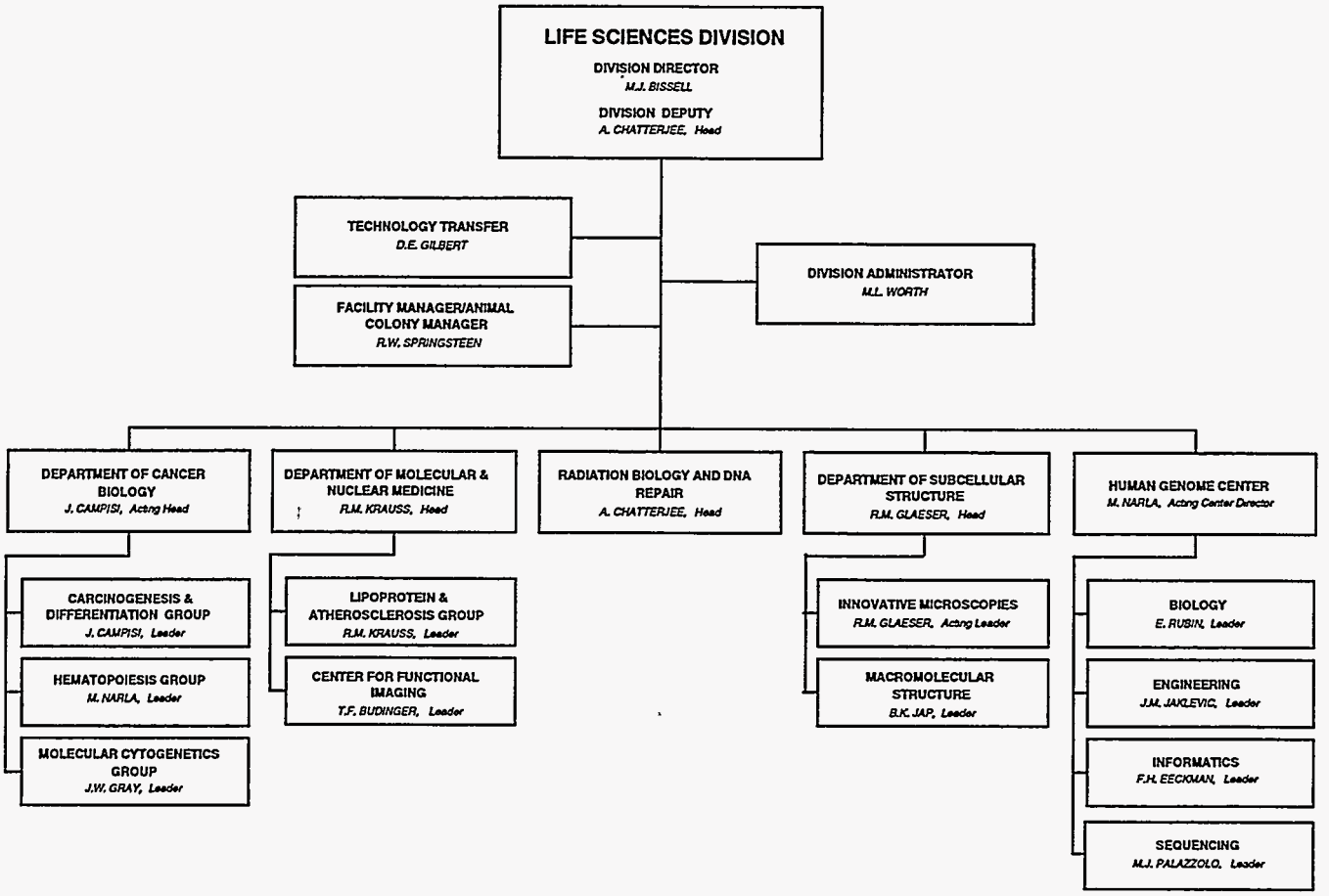


3.0 Research Management Practices

3.1 Advisory Committees and Program Reviews

The quality and direction of research in biosciences and environmental sciences at LBNL are evaluated within each of the four divisions that have a major role in OHER research. In particular, an annual "Director's Review" is mandated for each Division. This is an opportunity for eminent scientists from around the country to review the Division's programs and report their findings directly to the Laboratory Director. The current committee membership for Life Sciences, Structural Biology, and Energy and Environment Divisions follows:

LIFE SCIENCES DIVISIONDIRECTOR'S REVIEW
OCTOBER 27-28, 1995

Franklin H. Bunn
Research Director, Hematology/Oncology
Brigham and Womens Hospital
Harvard Medical School

Charles Daniel,
Professor, Department of Biology
University of California at Santa Cruz

Harold F. Dvorak
Professor and Chief, Department of Pathology
Beth Israel Hospital/Harvard Medical School

Uta Francke
Howard Hughes Medical Institute
Stanford University Medical Center

Carmen A. Mannella
Associate Chairperson of Biomedical Sciences
Wadsworth Center for Labs and Research

Phil Hanawalt
Department of Biological Sciences
Stanford University

Human Genome Advisory Committee

David Botstein
Professor and Chair, Department of Genetics
Stanford University School of Medicine

David R. Cox (Chair)
Professor, Department of Genetics
Stanford University School of Medicine

Raymond Gesteland
Human Genetics Department
University of Utah

Steven J. Gordon
Intelligent Automation Systems
Cambridge, MA

Joe Gray
Division of Molecular Cytology
University of California at San Francisco

Thomas Marr
Cold Spring Harbor Laboratory

Maynard Olson
Molecular Biotechnology & Medicine Department
University of Washington

Edward Rubin
Life Sciences Division
Lawrence Berkeley National Laboratory

The Center for Functional Imaging

CFI has three major reviews by external committees of peers. The OHER review which has occurred every 3–5 years, the LBNL Directors review which was last held in July 1993 with emphasis on the molecular and nuclear medicine programs of the Life Sciences Division, and the NIH program project external advisory committee review of the major NIH program within CFI.

NIH Program Project External Advisory Committee:

James Bassingthwaighte, M.D., Ph.D.

Craig Malloy, M.D.

Albert Macovski, Ph.D.

Ronald Nutt, Ph.D.

H. William Strauss, M.D.

The Molecular Cytogenetic Resource

Resource directions are set during meetings of the Resource Project leaders. Resource staff presented progress and plans to the Advisory committee in September, 1994.

ENERGY AND ENVIRONMENT DIVISION

Arnold P. Fickett (chair)
Vice President, Department of Customer Systems
Electric Power Research Institute

William Fulkerson
Associate Director for Advanced Energy Systems
Oak Ridge National Laboratory

Adel Sarofim
Massachusetts Institute of Technology

John Spengler
Harvard University
Exposure Assessment and Engineering Prg.
Department of Environmental Health

Donald Watson
School of Architecture
Rensselaer Polytechnic Institute

STRUCTURAL BIOLOGY DIVISION

Chemical and Structural Biology Program

James Wells, Chairman
Department of Protein Engineering
Genentech, Inc.

Charles Craik
Pharmaceutical Chemistry
University of California, San Francisco

Harry F. Noller, Jr.
University of California, Santa Cruz

Energy Sciences Program

James Norris
Chemistry Division
Argonne National Laboratory

Peter R. Ogilby
Department of Chemistry
University of New Mexico, Albuquerque

President's Council

The University of California President's Council on the National Laboratories advises the President on matters related to the management and operation of the three DOE Laboratories: LBNL; Lawrence Livermore, and Los Alamos. The Council also serves as a source of advice and counsel to the Laboratory Directors, who have the opportunity to call upon the scientific and managerial expertise of the Council as a whole and that of its individual members.

The Council has three major functions. One function is to review the strategic plans of the Laboratories as they change their research agendas. The Council is to be especially attentive to opportunities for the Laboratories to apply their capabilities to issues of increasing national importance. A second function of the Council will be to review the scientific and technical quality of work undertaken at the three DOE Laboratories. The contracts between the University and the DOE require the University to carry out an annual performance evaluation of the management and mayor programs at the Laboratories. The Council will assist the University in these evaluations. The Council will provide the President an annual written report of its findings and recommendations, and it also will prepare other written and oral reports at the request of the President or as the Council deems appropriate. The third function of the Council is to monitor the effectiveness of the Laboratories in fostering an atmosphere conducive to scientific inquiry and the development of new knowledge.

President's Council on the National Laboratories—Current Membership

Sidney D. Drell, Chairman
Deputy Director
Stanford Linear Accelerator Center

William R. Frazer, Vice Chairman
Department of Physics
University of California, Berkeley

John F. Ahearne
Executive Director, Sigma Xi
Research Triangle Park, North Carolina

Lew Allen, Director (retired)
Jet Propulsion Laboratory

Patricia Buffler
Dean, School of Public Health
University of California, Berkeley

Robert C. Dynes
Professor, Physics Department
University of California, San Diego

Edward Frieman
Vice Chancellor, University of California, San Diego
Director, Scripps Institution of Oceanography

Andrew J. Goodpaster
General, U.S. Army (retired)

Judson C. King
Provost, Professional Schools and Colleges
University of California, Berkeley

John W. Landis
Senior Vice President and Director
Stone & Webster Engineering Corporation

John McTague
Vice President, Technology
Ford Motor Company

Venky Narayanamurti Dean,
College of Engineering
UC Santa Barbara

Malcolm Nicol
Professor of Chemistry and Biochemistry
UCLA

Gilbert Omenn
Dean, School of Public Health
University of Washington

Raymond Orbach
Chancellor
University of California, Riverside

Thomas A. Page
Chairman and Chief Executive Officer
San Diego Gas & Electric Company

Lucille Shapiro
Professor and Chairman
Department of Developmental Biology
Stanford Medical Center

Neil S. Smelser
Professor of Sociology, University of California, Berkeley

Rochus E. Vogt
Professor of Physics, California Institute of Technology

Albert D. Wheelon
Former Chief Executive Officer, Hughes Aircraft

Herbert F. York
Director Emeritus,
Institute on Global Conflict and Cooperation
University of California, San Diego

Ex Officio

Arnold Binder
Professor, Social Ecology
Vice Chairman, Academic Council
University of California

Wayne Kennedy
Senior Vice President, Business and Finance
University of California

Siegfried S. Hecker
Director
Los Alamos National Laboratory

Daniel L. Simmons
Vice Chairman Academic Council
School of Law
University of California at Davis

Walter E. Massey
Provost, Senior Vice President of AcademicAffairs
University of California

Charles V. Shank
Director
Lawrence Berkeley National Laboratory

C. Bruce Tarter
Director
Lawrence Livermore National Laboratory

To advise the Division Directors on a more frequent basis, both with regard to scientific priorities and operational matters, a standing advisory committee comprising senior scientists from within the division meets periodically and discusses divisional issues. In several divisions, these committees (or councils) serve as the review panels for new Field Task Proposals and for proposals for Laboratory-Directed Research and Development funds. Group leaders' or project leaders' meetings also serve as forums for discussion, though in some cases the group leaders may constitute the advisory committee.

The Directors and Department Heads of Life Sciences and Structural Biology Divisions also meet regularly as the Biosciences Advisory Committee. Out of the three Divisions (including Energy and Environment) receiving the bulk OHER funding, one Director is designated as the coordinator for communication among the three divisions and between the Laboratory and OHER, as well as coordinator of other multidivisional administrative functions. The coordinator is a member of the Laboratory Director's Action Committee, which meets weekly.

Finally, mention should be made of the several specialized committees in each division, which support but do not influence directly, the conduct of scientific research. These include, a professional staff committee, a salary committee, safety and space committees, and others.

Laboratory- Directed Research and Development Program (LDRD)

LDRD funding provides initial support for innovative research projects and encourages pursuit of promising new institutional directions in ongoing research. The goal of support for new initiatives is to produce preliminary results that will provide a credible basis for seeking long-term funding from DOE or another agency. In the case of ongoing projects, the objective is to test the feasibility of a new research direction quickly and, if truly promising, to provide documentation for future proposals to support those new aspects of the program.

LDRD proposals originating in a given division are reviewed each year by the Division Director, who prepares a prioritized list, typically based on the recommendations of a divisional advisory committee. This list is submitted to a Laboratory-wide review panel, which makes a final recommendation to the Laboratory Director.

TABLE 5: Summary of Major FY 1995 OHER-Related Discretionary Funding Investments

EARTH SCIENCES

Bodvarsson:
Studies in the Geologic Disposal of Nuclear Waste—$33,000

DePaolo:
Characterization and Monitoring of Subsurface Biologic Activity Using Stable Isotope Soil Gas Analysis—$81,000

Doner:
Soil Carbonate Sorptive Properties for Trace Elements: Advanced Methods in Determination

of Microscopic and Molecular Level Associations—$31,000/$9,000 equipment

Holman:
Laboratory Studies of Microbial Transformation of Petroleum Hydrocarbons in Transient Subsurface Environment—$113,000

Wan:
Fate and Transport of Bacteria in Vadose Environments: New Studies of In-Situ Microbial Behavior and Bioremediation—$79,000

ENERGY AND ENVIRONMENT

Anderson:
Sediment Quality and Wetland Restoration at Mare Island Naval Shipyard—$78,000

Levine et al.:
Research to Improve the Estimation of In-Use Pollutant Emissions from Motor Vehicles—$138,000

Cramer:
Broad Band High Resolution Microcalorimetry for Biological and Materials Science Applications on the ALS—$83,000/$20,000 equipment

ENGINEERING

Jaklevic:
Development of Microchemical Methods for Biological Assays—$70,000

ENGINEERING/ACCELERATOR AND FUSION RESEARCH

Millaud /Padmore /Nygren
Advance Towards the Next Generation of Pixellated Detectors for Protein Crystallography $258,000/$50,000 equipment

Gray:
Quantitative Molecular Cytogenetics Workstation—$58,000

Jap:
Electron Crystallography of Selected Membrane Proteins—$148,000

Pallavicini:
Genetically-Damaged Hematopoietic Stem Cells: Biological Dosimetry—$49,000

Rubin:
Creation of Transgenic Mice Containing a Library of P1 Clones Encompassing the Down's Syndrome Region from Chromosome 21—$266,000

Shyamala:
A Transgenic Model for Clinical Testing of Progestins and Analysis of Progesterone Receptor Function—$70,000

Tribble:
Variations in Susceptibility to Environmental Oxidants as Studied using Transgenic Mice Models—$110,000

Yaswen:
Isolation of Genetic Supressor Elements in Human Mammary Epithelial Cells—$67,000

STRUCTURAL BIOLOGY

Kim:
Genomes, Enzymology and Biology of Hyperthermophilic Microorganisms—$109,000

MULTI-DIVISIONAL, EARTH SCIENCES

Benson, Brown, Daisey, Gold:
An Interdisciplinary Environmental Research Center—$448,000

Selected FY 1995 LDRD Project Summaries

Studies in the Geologic Disposal of Nuclear Waste—Gudmundur S. Bodvarsson

To identify and address technical problems in geologic disposal of nuclear waste, including multiphase flow of water and radionuclides in fractured rocks, thermal loading strategies and constraints, and repository stability, in collaboration with researchers in France.

Fate and Transport of Bacteria in Vadose Environments: New Studies of In-Situ Microbial Behavior and Bioremediation—Jiamin M. Wan

To study bacterial fate and transport in soils and vadose environments so that microbially based bioremediation strategies in such settings can be better designed. We will quantify bacterial transport in vadose environments under hydrostatic and hydrodynamic conditions; and study bacterial transport mechanisms in relation to changes of water-film thickness, pore geometry, flow rate, and surface hydrophobicity.

Sediment Quality and Wetland Restoration at Mare Island Naval Shipyard—Susan L. Anderson

To assess the potential for remediation of three contaminated wetland sites at Mare Island Naval Shipyard using toxicity-based methods, thereby advancing the application of ecological hazard assessment techniques to the important problems of wetland restoration and military base closure. We will be lead ecotoxicologists in an effort with UC Davis to develop a wetlands research consortium at Mare Island.

Broad Band High Resolution Microcalorimetry for Biological and Materials Science Applications on the ALS—Stephen P. Cramer

To carry out proof-of-principle experiments on the ALS using a microcalorimeter in lieu of existing detectors for x-ray spectroscopy. We will replace the solid-state detectors and crystal or grating spectrographs with a microcalorimeter. Work will be based on prototypes of a stand-alone, compact, robust, and portable microcalorimeter that have already been developed with energy resolutions of 18 eV, and we believe a resolution of ~1 eV is theoretically possible.

Research to Improve the Estimation of In-Use Pollutant Emissions from Motor Vehicles—Mark D. Levine

To analyze existing motor vehicle emissions data and incorporate our findings into existing or new emission and air quality models. To develop new instruments to collect data from in-use vehicles to more accurately represent their operating conditions and emissions.

Advance Towards the Next Generation of Pixellated Detectors for Protein Crystallography—Jacques E. Millaud, Howard A. Padmore, and David R. Nygren

To develop and build a new pixel detector for the detection of x-rays This detector can be used in a number of applications of synchrotron radiation. It will offer pulse counting statistics, reduce the point-spread function of the phosphor conversion CCD approach, and provide fast, adjustable electronic shutter and high and variable frame repetition rate while maintaining or improving the signal-to-noise ratio.

Isolation of Genetic Suppressor Elements in Human Mammary Epithelial Cells—Paul Yaswen

To develop a method to isolate and identify genes whose normal functions are suppressed during malignant transformation of human mammary epithelial cells (HMECs). We will construct a library of random cDNA fragments from normal HMECs in a retroviral vector containing a powerful eukaryotic promoter, a translation start site, and translation termination signals. This library will then be used to infect HMEC cultures. Cells that continue to grow under selective conditions will be harvested, and inserted retroviral DNA rescued by PCR. After additional screening, the inserted cDNA fragments will be sequenced and used to characterize biochemical pathways disrupted during malignant transformation.

Animal Welfare and Human Use

The LBNL Animal Welfare and Research Committee (AWRC) oversees procedures that ensure compliance with policies of the U.S. Department of Agriculture and the NIH's Office for Protection from Research Risks (OPRR). The AWRC has the responsibility and the authority to deny animal use privileges to investigators found in noncompliance. Once approved, each research protocol is reviewed annually and prior to implementation of any changes in the protocol. The annual review verifies past and continued compliance with LBNL and OPRR policies and evaluates research progress derived from animal use. Members of the AWRC are selected in strict compliance with NIH principles.

In October 1975, following the recommendations of the Director, a Memorandum of Understanding between the Chancellor of the University of California at Berkeley and the Director of LBNL was issued regarding the use of human subjects in clinical research. The campus Committee for the Protection of Human Subjects (CPHS) assumed official responsibility for reviewing and approving all LBNL activities involving human subjects, pursuant to Office of Science and Technology Policy (OSTP), UC Systemwide, and campus directives. An LBNL advisory committee assists the CPHS in their review of LBNL projects, and LBNL research scientists serve as committee members. LBNL protocols are submitted to the LBNL Human Use Committee (HUC), which reviews all submissions and approves them before submission to the CPHS for review. The LBNL

HUC keeps LBNL Administration advised of all actions taken with respect to human use approvals, maintains a permanent file of all documents pertaining to human subjects, and serves as an advisory group for investigators on matters concerning human use.

Waste Minimization and Pollution Prevention

LBNL has made a strong commitment to waste minimization—to substantially reduce waste generation and to increase recycling. Our goal is to achieve an overall reduction in the generation of hazardous, radioactive, and mixed waste streams through reduced generation at the source, process chances, employee awareness, administrative controls, and increased recycling.

Through the Waste Minimization and Pollution Prevention Awareness Program we will systematically eliminate or reduce the generation of waste from site operations to prevent or minimize the release of pollution in any environmental medium. The program seeks to make source reduction and environmentally sound recycling integral parts of the philosophy at LBNL.

The Waste Minimization and Pollution Prevention Awareness Program meets the requirements of DOE Order 5400.1 and other federal, state, and local waste minimization and pollution prevention requirements.

Safety Committees

Each of the LBNL divisions has a safety committee that includes staff members from all levels within the division. Committee members receive specialized training in federal, state, and local safety, environmental, and operational laws and regulations. The committees are appointed by the Division Directors and charged with the responsibility of conducting annual inspections of all divisional laboratories, offices and shops to uncover any safety violations or lack of compliance with environmental requirements. The primary activity of the safety committees is to carry out the self-assessment of divisional compliance with safety and environmental regulations on a pro-active, continuous basis, including maintaining documentation of reports from walk-through and follow-up inspections and alerting Division Directors to problem areas.

In addition, LBNL is using the safety committee network as a resource to assist in the installation of recommended operational procedures required of management by specific DOE orders and federal and state laws. The Environment, Health and Safety Division and the Office of Assessment and Assurance are prime resources in the implementation of DOE, state and other federal regulations. For example, conduct of operations requirements include detailed documentation of compliance with specific procedures related to record-keeping of visitors to controlled areas where radiation hazards exist. DOE orders also require detailed records of laboratory procedures and employee safety training. The safety committee network has been used to assist supervisors in complying with these responsibilities. Federal and state laws also require documentation of appropriate disposal of medical and biohazardous waste.

Federal and state laws are in effect that require individual laboratories to retain documentation of annual reporting of hazardous chemical inventories and of compliance with measures to ensure that the atmosphere and the water supply are not fouled by effluents from laboratories and shops. In some cases, permits are required to assure adequate monitoring of waste streams. LBNL administration has chosen a "notebook" approach to consolidate documentation required by regulations related to environment, health, safety, and operations. Project, Facility and Function Notebooks have been formatted to assist LBNL staff. The safety committee network will provide assistance to responsible supervisors in meeting the requirements.

The Life Sciences Division Environmental Safety and Health program structure consists of the Division Director, the Division Deputy Director, the ES&H Team, the ES&H Committee, Building Managers, Division Facility Supervisors and embraces the Departments Heads, Group Leaders, Supervisors and Staff of the Division. The ES&H team consists of lead individuals in specialty areas as identified, outlined and detailed below:

The Division E.S.&H. Team routinely meets twice a month (first and third Wednesday), and more frequently when necessary, to discuss urgent action items and ongoing ES&H activities, e.g., planning the ES&H Committee agenda; developing action plans for the introduction and implementation of new programs, policies, procedures and regulations. In addition, telephone, FAX and face-to-face communication on EH&S issues and concerns is an on-going, daily process for the members of the Life Sciences Division EH&S Team.

Life Sciences Division ES&H Committee

- The Division Safety Committee is comprised 25 members from various areas of the Division, the Division Safety Team (4), as well as representatives of the LBNL ES&H Division (5). This committee has refocused on a collaborative exchange of concerns, ideas and solutions. Attendance is up, and interest and involvement have significantly increase during the latter half of FY94.
- Members appointed by the Division Director at the beginning of each calendar year for a one-year term.
- Membership is rotated among Division scientists, research associates, technicians and administrative staff, as appropriate, to maximize the number trained in ES&H regulatory compliance and the Division's self-assessment space inspection program.
- Members act as a resource for the Division personnel in their area and post or distribute information about new regulations and requirements as presented in the ES&H committee meetings and minutes.
- Members receive ongoing training during committee meetings in ES&H policies, procedures, laws and regulations that must be followed at LBNL.
- Members receive ongoing self-assessment training at ES&H Committee meetings. Members serve on the Division's space self-assessment inspection teams. Time committee is 2-3 days per calendar year.
- Members review self assessment corrective action items in their area and work with scientists and technical staff for completion.
- Members communicate to the Division personnel in their area any new regulations and requirements described in LBNL EH&S Safety Bulletins, as well as memos sent by the Division ES&H Specialist, Division ES&H Coordinator or Division Director.

Division ES&H Self-Assessment Space Inspection Approach

- The Division has moved to a Graded Approach to Self-Assessment, incorporating Division-wide inspections on a two-year rotation, with more frequent inspections of selected areas.
- Supplemental inspections are routinely conducted by the Division ES&H Coordinator and the EH&S Facilities Manager/Compliance Resource on an as-needed basis. These are routinely applied to Division facilities in order to focus and direct further attention in application of a Graded Approach to Self-Assessment.
- The Inspection Teams are chosen from the ES&H Committee and the Safety Committee, by the ES&H Coordinator, in collaboration with the Safety Team, to inspect Division space in buildings other than their own. They are frequently supported by representatives of the EH&S Division, selected for expertise specific to the inspection target area.
- The Safety Committee review the checklist provided by the Office of Assessment and Assurance (OAA), and provide this insight to the Inspection Teams.
- The Inspection Teams provide observations only. These observations are reviewed by the Division ES&H Coordinator and the EH&S Facilities Manager/Compliance Resource to determine which are legitimate findings that require tracking and trending in the LSAD file, as well as to determine appropriate corrective action.
- The Division ES&H Coordinator and the Facilities Manager/Compliance Resource provide advice to the responsible investigator or individual (or their representative) for resolutions of ES&H Compliance requirements and development of corrective action plans.
- Items where the corrective action can not be taken immediately, and therefore require tracking are entered into the Division LSAD file.

Division ES&H Self-Assessment Training Evaluation Approach

- Lists of training received (FOCUS Database printouts) are sent to principal investigators annually, with a cover memo asking them to go over the workplace hazards with their staff and schedule courses as needed.
- Presently the Jobs Hazard Questionnaires are pending input, and will then be transmitted to the LBNL EH&S Training Unit. This information will provide a profile record with a matrix that includes flagging courses which require retraining. Training profiles will be reviewed by the Division ES&H Team for deficiencies and corrective action will be tracked in the LSAD file and/or referred to the Division Director.

- Training records from the LBNL EH&S FOCUS Training Database are posted on each floor of each Life Sciences Division building. Listings are updated quarterly, and distributed for posting via the Division Safety Committee.

- Jobs Hazards Questionnaires to P2R-reviewed staff were distributed with the review forms and are on hand. Jobs Hazards Questionnaires to non-reviewed staff were distributed for completion 9/7/94.

4.0 Research in Progress & Major Accomplishments

D. Research in Progress

Analytical Technology

KP-01-01

AIRBORNE RADON CONCENTRATIONS (GADGIL, SEXTRO)

Radon, a radioactive gas that arises from the radioactive decay of radium that is naturally present in all soil and rock, is the largest single source of radiation exposure to the general public. Exposure to the short-lived radon decay products-which are themselves radioactive-is estimated to be a significant cause of lung cancer in the U.S. population, particularly among non-smokers.

The Indoor Environment Program at LBNL has developed a multifaceted research effort to investigate and characterize the sources of indoor radon, and the factors determining the concentrations on indoor radon and indoor radon decay products. The program is paying particular attention to the driving forces for radon transport through soil, its entry into buildings, and the build up and removal processes for radon progeny in indoor air. Another focus is on the effects on the transport and entry of radon, of the interaction of wind with the structure and the surrounding ground. These effects appear to significantly influence radon transport and entry into buildings, in either direction (i.e. increasing or decreasing the entry rate) depending on the soil permeability, wind speed and other factors. Improving our understanding of these effects will improve the ability to predict when, where and why different concentrations of radon are found in the indoor air.

Investigations are also targeting the discrepancy between the predicted and experimentally estimated

rates of deposition of unattached radon progeny on to indoor surfaces, in the presence of natural convection. This research has bearing on understanding one of the important removal processes for indoor unattached progeny, and its dose to the lung. Both of these research areas are being addressed with experimental and theoretical investigations. These efforts are coordinated with other radon-related OHER-sponsored research tasks discussed in other sections of this report.

KP-01-02

SEMICONDUCTOR RADIATION DETECTOR TECHNOLOGY (JAKLEVIC, HALLER)

The program to develop semiconductor detector technology has, in the past fiscal year, focused on the following areas : (1) room temperature semiconductor gamma ray detectors; (2) germanium detectors with amorphous contacts; (3) defect gettering in floating-zone silicon; (4) p-type silicon drift detectors; (5) epitaxial gallium arsenide crystal growth. They are described below.

Room temperature semiconductor gamma ray detectors.
We have initiated a program to develop high resolution gamma-ray detectors using wide band-gap compound semiconductors. These detectors can be operated at room temperature and have the potential to achieve energy resolution comparable to liquid-nitrogen-cooled Ge detectors. A major obstacle in their development has been the poor collection of holes in these compound semiconductor materials, which prevents good energy resolution to be achieved. We have developed a novel charge sensing technique that provides an effective solution to this problem, allowing high resolution room temperature gamma-ray detectors to be realized. A vast improvement in spectral performance has been observed when this technique was applied to a CdZnTe detector. While the energy resolution achieved is already a factor of two better than scintillation detectors, considerably better performance should be possible. We are currently investigating possible resolution-limiting factors so that further improvements can be made. In addition, we have established a CRADA with a commercial supplier of CdTe and CdZnTe detectors (eV Products) to investigate methods to improve the material and to commercialize detectors based on this new detection technique.

Germanium detectors with amorphous contacts.
The development of amorphous semiconductor contacts on Ge detectors during the past several years has culminated in their successful application in a variety of high performance detection systems. Transmission detectors for charged particle telescopes have been fabricated using this contact technology in place of previous methods that are time consuming, have low yield and result in low-quality contacts. The flexibility of the amorphous contacts has also enabled the development of monolithic, low-capacitance and thin-window germanium arrays. A 4-element detector array has been fabricated and preliminary measurements have shown that it outperforms, by a wide margin, a conventional 13-element discrete array in high-count-rate synchrotron radiation applications.

Defect gettering in floating-zone (FZ) silicon.
As a result of our earlier publications of defect gettering results, we have entered into extended discussions with a major silicon crystal supplier on possible modifications to their FZ crystal growth process to produce crystals free from the defects that we have discovered. To date we have received wafer samples from two crystals that they have grown with radically different growth conditions. We intend to begin evaluation of these crystals shortly.

P-type silicon drift detectors.
During the past year we have fabricated a series of p-type drift detectors and test diode structures that culminated in our successful fabrication of several 4 cm X 4 cm p-type drift detectors. These detectors are presently under evaluation for linearity, two-track resolution and long term stability.

Epitaxial Gallium Arsenide Crystal Growth.
High purity GaAs epilayers, grown on GaAs substrates, are now being routinely produced for future use in new radiation detector technologies. GaAs is an attractive alternative to conventional Si and Ge materials used in x- and gamma-ray spectrometers, because it can be used at room temperature and does not require cryogenic cooling as do Si and Ge. The GaAs epilayers are of a very high degree of crystalline quality, and the impurity concentrations have decreased steadily as the crystal growth technique was being perfected. The original impurity concentration level started at the part per million level, but, as the crystal growth technique was developed, the impurity concentration has improved three orders of magnitude to the part per billion level. (Part per billion, and better, impurity concentrations are required in order for the material to be useful in

radiation detectors). Prototype detectors will be fabricated and tested, and effort will continue to develop the crystal growth technique in order to produce crystals with the characteristics required for radiation detectors.

Environmental Research

KP-02-01

ATMOSPHERIC AEROSOLS AND GLOBAL CLIMATE CHANGE (NOVAKOV)

Anthropogenic aerosol particles may influence global and regional climate directly by scattering and absorption of solar radiation or indirectly by modifying the radiative properties of clouds. The backscattering of solar radiation by anthropogenic aerosols tends to cool the atmosphere. In addition, increasing concentrations of anthropogenic aerosols may contribute significantly to cloud condensation nuclei (CCN) concentrations, which results in increasing albedos of clouds (i.e. increasing CCN concentrations will increase the cloud droplet concentrations, resulting in brighter clouds, which reflect a larger fraction of the incident solar radiation into space) and thus further contributing to atmospheric cooling. Modeling studies show that these aerosols may cause a globally-averaged climate effect comparable in magnitude but opposite in sign to that due to "greenhouse" gases, thereby masking, to some extent, the global warming induced by increasing concentrations of greenhouse gases.

Quantifying these aerosol induced climate effects is difficult, in part, because of the lack of sufficient empirical data on the regional and global distribution of major anthropogenic aerosol species and their optical and cloud nucleating properties. Namely, the anthropogenic aerosol is composed of varying amounts of chemical species, such as sulfates (from SO_2), organic particles (from gas-to-particle conversion of reactive hydrocarbon precursors, and primary organic aerosol from combustion processes), elemental or black carbon (from incomplete combustion), ammonium (from biological sources), and some nitrate . Out of these sulfate aerosol received most attention because of the relatively well documented global distribution, and the known optical and nucleation properties of this aerosol. In contrast, the data on the concentrations, sources and properties of other major components of anthropogenic aerosols are much scarcer. This is particularly true for the organic aerosol species because most systematic aerosol measurements have emphasized inorganic constituents. Obtaining such data is important because both optical and nucleating properties of anthropogenic aerosols depend on the size and chemical composition of all relevant species. To enable a more general assessment of anthropogenic aerosols in climate we have initiated research activities aimed at: (1) Determining the magnitude and the seasonal variability of organic and sulfate aerosol mass concentrations, and (2) Establishing the relative contributions of carbonaceous material and sulfate species to the total aerosol and CCN number concentrations. These activities are being pursued in parallel with the ongoing measurement and analyses of the relationships between sulfate aerosols and cloud droplet concentrations.

These objectives are being achieved by continuing measurements at our existing site on El Yunque Peak, Puerto Rico and at a newly established Pacific site at Point Reyes, California. Sampling at Point Reyes will allow the characterization of the aerosols advected to the western part of the U.S., and therefore aid in achieving the stated objective of the DOE Northeastern Pacific Air-Chemistry Study (NPACS). Sampling in the trade winds at the Caribbean site will characterize the aerosol that is carried to the Southeastern United States. Furthermore systematic measurements at these sites will provide the basis for establishing the differences in aerosol concentrations and properties between Equatorial North Atlantic, and Northeastern Pacific Oceans.

KP-02-03

RADON TRANSPORT THROUGH SOILS INTO HOUSES (SEXTRO, FISK)

Additional experimental and theoretical investigations of the factors controlling radon entry rates at our small basement structures will be conducted, with the goal of improving our understanding of radon entry into actual houses. This information will be useful in the development of methods to locate regions with high radon potential; it will also be important in designing radon-resistant building methods and codes. We plan to extend our

new understanding of scale-dependent soil permeabilities and examine other soils for these effects.

We have begun to experimentally examine the significance of transient flows of soil gas into houses caused by barometric pressure fluctuations. Experiments will also seek to investigate entry rates and source strengths for ^{220}Rn, a short-lived radon isotope that may, nevertheless, contribute 10 to 20 percent of the average radiation exposure due to radon. We will begin site assessment and planning activities for the location of a second experimental site in which the underlying geology, soils, and climate differ from those found at our present site in the Santa Cruz mountains.

THERMODYNAMIC PROPERTIES OF CHEMICAL SPECIES PRESENT IN MIXED ORGANIC-RADIONUCLIDE WASTES (AL MAHAMID)

The project experimentally determines the thermodynamic complexation constants, the speciation, the solubility and the reaction kinetics of complexes and compounds that can form between the metal ions of plutonium and organic constituents of mixed organic-radionuclide wastes present at DOE sites. The data are necessary as input to geochemical models predicting the most stable contaminant species and their possible release concentrations to soil and groundwater environments at contaminated sites. This experimental program provides a better understanding of the fundamental chemical phenomena that control contaminant mobility in the subsurface. It will provide understanding for designing possible pathways to manipulate the geochemistry in order to achieve remediation by either stabilizing or mobilizing contaminants in the subsurface.

Result thus far showed that the predominant plutonium species in the presence of NTA at low concentrations of plutonium and at pH = 7 are Pu(V) and Pu(IV).

FIELD HETEROGENEITY (MAJER)

This research is part of the Subsurface Science Program Heterogeneity Subprogram. It contributes to meeting the goals of the subprogram by improving means to relate subsurface physical properties to the microbial heterogeneity of natural systems. A large number of field-scale processes are affected by variations in the scale of heterogeneity, ranging from the pore scale to the bed scale and beyond. To relate the significant scales of variation of microbial and chemical behavior to the variation of physical properties, it is critical to know the scales at which various processes dominate. Without this knowledge, it would be impractical to attempt to relate a small-scale process (i.e., microbial behavior) to field-scale measurements.

The goal of the work is to use subsurface imaging to identify the fundamental scales of variation of physical parameters that control transport behavior relative to predicting subsurface microbial behavior. With this information, it may be possible to relate physical and chemical parameters (i.e., those parameters that geoscientists have experience measuring in situ) to the significant microbial properties and thus explain and predict their behavior in the subsurface. This approach uses controlled meter-scale field sites and supplementary laboratory and intermediate-scale information to characterize those physical properties that affect fluid flow and chemical transport and can be imaged with in situ methods. The work must be tightly integrated with chemical and microbial characterization and process definition efforts that will be undertaken in the subprogram. At DOE sites where information has been gathered on the microbial and chemical properties, field work will be carried out to define and characterize the natural subsurface physical heterogeneity.

Health Effects

Energy technologies provide many benefits to modern society. Each of the many energy technologies used to provide and distribute energy, however, has impacts on the environment and on humans and other biological organisms. In order to minimize such impacts, research is conducted in the biosciences and the environmental sciences to provide fundamental understanding of biological and environmental systems, from the molecular to the whole organism and environmental system level, and to understand the effects of specific technologies on life and environmental systems. In this research, the emphasis is on interdisciplinary, integrated studies that provide knowledge of basic processes and mechanisms. The knowledge gained from this research provides a basis for environmental and human health standards and for U.S. energy policies.

KP-03-02

AIR POLLUTANT EXPOSURES IN BUILDINGS (NERO, DAISEY)

Indoor environments are frequently the major determinants of adverse health risks from environmental pollutants and radiation. This is due to both the amount of time humans spend indoor, 90% on average, and to the substantially higher (2 to 10X typically) indoor than outdoor concentrations of many pollutants, including radon and volatile organic compounds. The fundamental goal of the Air Pollutant Exposures in Buildings project is to advance scientific understanding of human exposures to and health risks from indoor air pollutants. A specific focus is assessment of pollutant exposures and risks associated with reductions in building ventilation and related technologies to reduce energy usage in buildings. Two basic approaches are taken in this work: (i) existing data on various indoor pollutants are assembled, integrated and critically evaluated to assess indoor exposures to various pollutants (e.g., radon, combustion pollutants, and volatile organic compounds) and the risks associated with those exposures; and (ii) physico-chemical and statistical models are developed for the estimation of population exposures to indoor pollutants. Field measurements and laboratory experiments are made as required to assess exposures and develop and test exposure models.

LBNL has estimated that in perhaps 100,000 homes, concentrations of radon exceed 740 Bq m-3, implying occupant exposures at or above the occupational radiation limits. LBNL is now developing a statistically-based method to identify areas of the United States where the preponderance of such high levels occur. For this purpose, local information on physical factors such as soil types and local geology, meteorology, and housing characteristics is being correlated with available indoor radon concentration data from monitoring. This research involves application of advanced statistical methods to developing estimators of local radon concentrations, an approach that may prove important also for other environmental parameters. It also includes development of a survey methodology that can be used to efficiently provide the indoor monitoring data needed for normalizing the correlation results to actual occupant exposures. The outcome of the project is to be an integrated methodology, identifying data and statistical techniques that can be used to sweep across the nation, systematically and reliably identifying the high-radon areas.

Indoor exposures to volatile organic compounds (VOCs) and other complex mixtures are also being investigated. VOCs are emitted from many building materials and may cause adverse health effects in energy-efficient buildings with low ventilation rates. Efforts are underway to develop exposure metrics for mixtures of VOCs typically found in indoor settings that can be more readily related to health effects in exposed populations. The simple sums of concentrations of individual compounds which are currently used have been related to symptoms only at high concentrations, although the same symptoms are observed at low level exposures. More advanced exposure metrics will enable us to better understand environmental cause and effect and to link exposures to environmental agents to adverse health effects in energy-efficient buildings. Our current effort is focused on exposure metrics for mixtures of VOCs that can be related to irritancy and odor symptoms reported by the occupants of the 12 California office buildings of the California Healthy Buildings Study. The VOCs are suspected to contribute significantly to "Sick Building Syndrome," a complex of subchronic symptoms that occur during and generally decrease away from occupancy of the building in question. Prior attempts to link exposures to VOCs and symptom data have not considered the potencies, i.e., the level of response for a given dose, of these compounds, and have generally been unsuccessful in relating VOC exposures at low levels to SBS symptoms. We have also been collaborating with investigators at the California Department of Health Services in a study of exposures to environmental tobacco smoke (ETS) in office buildings. One of the objectives of this study is to develop and evaluate tracers in ETS that can be used to distinguish VOCs originating from ETS and those from other sources.

MOLECULAR MECHANISMS OF CELLULAR EFFECTS OF HEAVY IONS (BLAKELY)

The goal of this project is to obtain a quantitative understanding of the nature and kinetics of biological responses produced at the molecular and cellular level by accelerated atomic nuclei of varying energy density. In order to elucidate relevant mechanisms of action, we are measuring the dose-dependent yield of heavy-ion-induced DNA lesions that presumably originate as DNA strand breaks or DNA-DNA or DNA-protein crosslinks and then result in chromatin

breaks. We are examining how the chromatin breaks, which may be a complex lesion representing multiple strand breaks from DNA-base or -sugar damage, is modified by the cell, and how this type of damage affects the cell's ability to deal with additional insults. We characterize chromatin damage and restitution in individual chromosomes using the premature chromosome condensation (PCC) technique (combined with a fluorescence-in-situ-hybridization technique) to rapidly evaluate early yields of DNA damage. We follow the chromatin rejoining kinetics and quantitate the resulting aberration yields that are inherited by daughter clones.

We have shown that there is an LET-dependent variation in the efficiency for production of initial chromosome breakage per unit dose. We have also shown that the distribution of breaks becomes progressively overdispersed with an increase in the proportion of nonrejoining breaks as the LET increases. Increases in radiation quality up to linear energy transfer (LET) values of up to 100-200 keV/mm cause increases in the chemical and physical damage in DNA denoted by the general term DSB (double-strand break). These changes are accompanied by decreasing abilities of cells normally radioresistant to sparsely ionizing radiations to process DSBs in chromatin and to recover from radiation exposure, so they make significant contributions to the relative biological effectiveness (RBE) of a given radiation. This information is therefore relevant to evaluation of a diverse set of necessary estimates of risk from ionizing radiations, including radon in the home, diagnostic and therapeutic medical exposures, and the occupational exposures received by the radiation worker on each, and during extended interplanetary missions in space.

The damage associated with high-LET-induced DSBs continues to increase as the radiation quality changes with increasing LET. The efficiency of DSB induction and RBE values for DSB lethality decline at higher RBE values. The decline in RBE seems to mimic the overall decline in successful processing of DSBs. As a consequence, the quality factor (Q) for a given radiation cannot be based solely upon the pattern of energy deposition, a fact attested to also by the quite different RBE responses exhibited by repair-deficient mutant (or variant) cells in the high-LET region beyond 100 keV/mm. This biological diversity speaks to the need for further studies of coordinated investigations of structural and functional assessment of classes of DNA and chromatin radiation damage as a function of cell origin and cell cycle stage, and the ambient oxygen and other chemical environment within the cell. Experiments with a cell-cycle dependent DNA double-strand break repair-deficient mutant XR-1 cell will allow us to investigate genetic control of particle-induced DSb at different phases of the cell cycle in the same cell system, and to propose genetic mechanisms of action that are triggered by specific classes of DNA damage from ionizing radiation.

There are several recent reports in the literature attesting to chromosomal specificity of radiation-induced damage that persists in individual cells surviving a radiation dose. In addition, the evidence indicated that this chromosome-specific-damage is dependent on the LET of the radiation with for example, chromosome #2 rearrangements predominating in colonies surviving low-LET radiation, and chromosome #13 and #14 rearrangements persisting in high frequency after exposure to high-LET alpha particles. These data imply that individual chromosomes are not equally susceptible to ionizing radiations, and point to potential hot-spots of specific damage or repair-deficiencies that may be identified by our work.

DNA damage or genotoxic stress can be produced by many potentially damaging agents in the environment, as well as due to endogenous processes involving activated oxygen species as occurs for example during inflammation reactions in vivo. In mammalian cells a family of growth arrest/division delay (GADD) genes were originally isolated on the basis of rapid induction in hamster cells by UV radiation. It was later found that these genes were rapidly inducible by many other types of DNA-damaging agents especially those producing high levels of DNA base damage that results in growth arrest. DNA damage is known to activate transcription of many genes. Only one of the GADD genes (GADD 45) is inducible by ionizing radiation, and this response has recently been shown to involve both the p53 tumor suppressor and the ataxia telangiectasia gene product . The GADD 45 is inducible by ionizing radiation only in cells having wild-type p53. Two thirds of human tumor cell lines tested (including HeLa cells) have lost wild-type p53 and do not respond to ionizing radiation with an induction of GADD 45.

Radiosensitive cells from ataxia telangiectasia (AT) patients have a defective GADD 45 response. A wild-type p53 phenotype is required for the G1 checkpoint activated by x-rays. Both human and hamster GADD

45 genes contain a conserved sequence matching the recently described p53 binding site. The GADD 45 sequence strongly binds p53 protein. These results indicate that the radiation responsiveness of the GADD 45 gene includes a role for the tumor suppressor p53 in its regulation. It has been suggested that if the G_1 checkpoint is triggered by p53 acting as a transcription factor on other genes, GADD 45 may be one of these downstream genes that is defective in AT cells.

The Gadd 45 protein has recently been shown to complex with proliferating cell nuclear antigen (PCNA) which is a necessary component of the machinery that copies DNA so that cell division can take place, and is also known to be needed for the resynthesis of DNA after damaged portions are removed by the cell's nucleotide excision repair system after environmental insult. If Gadd 45 protein is removed, repair decreases and if it is added, repair increases. p53 protein stimulates Gadd 45 protein and is known to bind to a protein called ERCC3 which is one of several excision repair molecules that together recognizes and removes damaged segments from DNA. This implies that the p53 protein binding to ERCC3 affects repair and may be involved in damage recognition.

BIOLOGICAL EFFECTS OF MAGNETIC FIELDS (LIBURDY)

Electromagnetic fields can influence living systems and this is an important public health issue that is attracting growing scientific and public interest. Our research is directed at identifying biological response of cells to electromagnetic fields and the underlying biophysical basis of such interactions.
Cellular calcium ion concentrations or calcium dependent processes have been
shown in at least eleven laboratories to be influenced by electromagnetic fields

Research from our laboratory indicates that brief exposure of 30 minutes to 2450 MHz (CW) microwave fields promotes the release or shedding of protein from the erythrocyte and from the lymphocyte cell surface (0-60 mW/kg). This effect is mediated through structural changes in the cell membrane in two ways: 1) a temperature dependence implicates features of cell membrane structure at the structural phase transition; and 2) a calcium ion dependence implicates structural cationic bridges which form between calcium ions and protein/phospholipid moieties at the cell surface. This bioeffect is further mediated by antioxidants such as ascorbic acid and α-Tocopheral which implicate a role for free radicals and/or reactive oxygen species in the microwave interaction. In liposome studies 2450 MHz fields are reported to alter bilayer permeability, and we recently observed that antioxidants placed in the bilayer or in the interior of the liposome are effective in reducing microwave-induced membrane permeability. Here we present evidence that calcium ions in the extraliposomal media are important mediators of microwave-induced permeability changes. Calcium ions are known to bind to the polar,
anionic head groups of phospholipids in the liposome membrane. These calcium ion interactions stabilize the bilayer and shift the temperature of the main structural phase transition to higher temperatures. Large unilamellar liposome vesicles (DPPC/DPPG; 200nm diameter) loaded
with 3H-cytosine arabinofuranoside displayed a marked permeability increase at 39.5 C in 25mM Hepes buffer (pH 7.0) indicating a main phase transition. Experiments indicate that the presence of 10mM CaCl2 in the Hepes buffer shifts this main transition to 40.0 C consistent with a structural stabilization of the bilayer. Exposure of liposomes (no calcium present) to 2450 MHz microwave fields (0.6 mW/gm) for 5 minutes at temperatures between 36 - 43 C resulted in a shift of the main structural transition to a lower temperature of 38.0 C. This is consistent with previous reports from our laboratory [2 & ref. therein, 4]. When 10mM CaCl2 was present in the buffer microwave treatment was observed to shift the main transition to a lower temperature of 37 C and this is in the opposite direction of the stabilizing effect of CaCl2 by itself, mentioned above. Thus, the stabilizing effect of calcium ions which shifted the main transition from 39.5 C to a higher temperature of 40.0 C, was shifted by microwave fields to a lower transition near 37.0 C. The mechanism of interaction, we hypothesize, involves disruption of the stabilizing calcium cationic bridges with the negatively charged polar head groups of the DPPG phospholipids in the liposome bilayer. This is consistent with the role of calcium cationic bridges in stabilizing protein binding to the erythrocyte and lymphocyte, and the destabilization of these bridges by microwave fields which results in protein release from the cell surface [2 & ref. therein]. The above microwave exposures were carried out in a standard WR-430 waveguide operating in the dominant TE10 mode. A standing wave was set-up at the sample location with the sample exposed to an Emax (6.1 V/m) and a Bnull. Since the magnetic field component was nulled the above effects may be related to the E

field component of the microwave field. We are testing this hypothesis by conducting experiments at the equivalent SAR (0.6 mW/gm) but at a magnetic field maximum and an Enull.

THE RESOURCE FOR MOLECULAR CYTOGENETICS (GRAY)

The research projects in the Resource for Molecular Cytogenetics are: 1) Hybridization technology. In situ hybridization procedures will be improved to increase the speed, sensitivity and specificity of hybridization and to allow rapid identification and mapping of genetic aberrations. 2) Computer assisted microscopy. This technology will be developed for rapid, high resolution, multi-color imaging; analysis of comparative genomic hybridization (CGH); rapid metaphase finding and ranking; rare event detection; genotypic and combined genotype-phenotype analysis of interphase cells. 3) Probe development. P1 and PAC clones that contain genetically mapped polymorphic sequences or known genes will be selected at 5 Mb intervals over the human genome for use as probes for in situ hybridization.

The technology development supported by this project will facilitate application of molecular cytogenetics in several areas of interest to the Department of Energy including: 1) Assessment of clastogen induced genetic aberrations. FISH with whole chromosome probes (WCP) coupled with automation may facilitate monitoring of DOE workers for genetic damage as required by recent legislation; 2) Identification of genes that contribute to tumorigenesis or tumor progression. Identification of consistent aberrations may lead to improved understanding of the genetics of tumor progression and in improved therapeutic modalities (e.g. through attack on aberrant proteins/pathways). Comparison of radiation induced and spontaneous human tumors using CGH may reveal aberrations specifically caused by radiation; 3) Gene mapping. FISH is an essential component of physical map assembly allowing chromosomal localization of cloned probes and identification of chimeric clones. The Resource will facilitate this process through development of improved procedures for physical mapping and probe characterization; 4) Probe production. Molecular cytogenetic studies of genetic aberrations for diagnosis, prognosis and positional cloning throughout the scientific community are currently hampered by the lack of well mapped, easy to use probes. The Resource, in cooperation with the LBNL Genome Center, will provide probes for these studies.

Molecular Carcinogenesis

REGULATION OF TISSUE-SPECIFIC GENES IN NORMAL AND MALIGNANT TISSUES (BISSELL)

A fundamental problem in modern biology is the underlying molecular mechanisms in regulation of tissue-specific gene expression in normal and cancer cells. Over the last decade our laboratory has established that the microenvironment that surrounds the cells (including the extracellular matrix; ECM) plays a crucial role in maintenance of functional differentiation, in growth and in apoptosis (programmed cell death).

We have developed a unique model system to study regulation of normal function in the rodent breast whereby we can create an almost exact replica of the functional alveoli in a culture dish. Using this model we have shown that: 1) Cell-cell interaction is necessary for formation of basement membrane (BM), but if cells are cultured in an exogenous BM, even single cells can now function and express some milk genes in the absence of polarity and cell-cell interaction. Indeed we now have shown that the highly used viral promoters and enhancers such as MMTV and SV40, also respond dramatically to ECM. 2) Growth factors such as TGF-β and α are transcriptionally suppressed by ECM. 3) Interaction is mediated by one or more integrins, and laminin is an important ligand; most recently, we have shown that the E3 region of the laminin A chain is involved. 4) The BM is also essential *in vivo*. Studies with transgenic mice, in collaboration with the laboratory of Zena Werb at UCSF, where ECM-degrading enzymes are expressed inappropriately, and the use of elvax pellets embedded with inhibitors of ECM-degrading enzymes, indicate that there is a crucial balance between the ECM degrading and inhibiting enzymes. 5) Using a functional cell line developed in our laboratory, that can be transfected stably, we have shown that the ECM regulation is mainly transcriptional. 6) We have isolated the first ECM-response element from the 5′ region of the β-casein gene and we have begun a systematic study of its structure and regulation. Using site-specific mutagenesis, we find two regions that are important for activity. Band shifts and nuclear foot-printing indicate the importance of at least two transcription factors, C/E BP and STAT-5. 7) In collaboration with Judy Campisi's laboratory we have isolated pure cell populations from our cell strain; studies on growth

regulation of these functional epithelial cells indicate a crucial negative role for helix loop helix protein, ID-1. 8) We have uncovered novel regulation by ECM of another important milk protein, whey acidic protein. 9) Most recently and very excitingly, the ECM is now shown to also regulate apoptosis in the mammary cells via regulating the levels of interleukin converting enzyme (ICE; the "death" gene). 10) Finally and most surprisingly, the overexpression of ECM-degrading enzymes lead to tumor formation in the transgenic animals; these tumors appear to have gross chromosomal abnormalities . Thus, in addition to the classical agents that affect the DNA directly, those that perturb the normal milieu are shown also to be potential carcinogens. This observation has now afforded us an immensely useful model to assess the mechanism of microenvironmental control of cancer from normal to abnormal to premalignant and malignant. We will also continue our studies on the mechanism of signal transduction by ECM in both differentiation and apoptosis.

THE INTERACTION OF MICROENVIRONMENT AND GENOME IN GENESIS OF BREAST CANCER (BISSELL)

The mammary gland, both normal and cancerous, is composed of two discrete components, the epithelia and the stroma. The gland also contains an extensive ECM material between the two components and within the stroma itself. The classical models of epithelial function or dysfunction have often ignored the roles of both the connective tissue and the ECM. My colleagues and I hypothesized more than a decade ago that the ECM (which is made by the collective synthetic capability of various cell types in a tissue) not only plays a role in maintaining the cytostructure, but it also has information to actively direct and maintain tissue-specific genes. Work in many laboratories, including ours, has shown this to be the case in the rodent breast, as well as in many other tissues. It is our thesis that the mechanisms of human breast cancer induction and progression will not be understood unless there is an investigation of the roles of stroma and ECM, and of the nature of the dynamic interactions between them and the epithelial tissues.

Almost all normal and neoplastic human breast cells in a tissue culture plastic (2-D) have similar morphologies, grow at the same rate and share many common phenotypic characteristics. Embedded in a BM, however, they show profound differences in less than seven days. We have performed an in-depth analysis and review of the cell types in normal and cancerous human breast , and have identified the origins of the myofibroblasts in the *stromal reaction*. We also have used our assay to define a novel function for a putative -metastasis supressor gene, nm23-H1 gene. We believe the assay can be used to define the function of many other supressor genes. We have defined the pattern of integrin expression , and shown them not only to be useful markers for the onset of malignancy, but also instrumental in the cell's abilities to grow or to apoptose . In the coming year we intend to systematically define those genes that may be instrumental in the various steps of conversion of normal cells to a cancer cell. As such, a unique human cell line, developed by our collaborators in Denmark, HMT3522, will provide an exciting opportunity. These cells form a continuum from "normal" (as defined in our assay) to pre malignant to malignant (growth in the nude mice). We intend to use our 3-D model to distinguish these stages, and to make c-DNA libraries between normal and those sublines that begin to deviate from normal. We would like to detect the earliest changes where the cells lose their ability to respond to the ECM and the microenvironment in a controlled manner. We intend to identify differential genes, characterize, transfect and identify functions. The implications for both prognosis and therapy are evident. Finally, we would investigate the possibility that an aberration of apoptosis may precede the frank malignancy. Given our recent expertise in this area in the mouse model, we intend to also investigate the apoptotic response in the above model.

CELL CYCLE ACTIVATOR AND REPRESSOR GENES (CAMPISI)

Tumor cells generally contain the full complement of biomolecules necessary for normal survival, proliferation, and expression of many cell type-specific functions. However, failure to modulate these functions properly results in an altered phenotype and ultimately cancer.

Three cellular functions tend to be inappropriately regulated in a tumor. First, the normal constraints on cellular proliferation are relaxed. This is a necessary but often insufficient requirement for tumor formation. Second, differentiation can be distorted. The tumor cells may be blocked at a particular stage of differentiation, or they may differentiate into an inappropriate or abnormal cell type. Third, chromosomal organization may be destabilized in such a way that variant cells arise with high

frequency. Some variants may have an increased growth advantage; others may be resistant to killing by chemotherapeutic drugs or radiation. All three of these important areas are being studied at LBNL under the auspices of OHER.

Among the genetic lesions that are essential for tumorigenesis is a heritable loss of normal cellular growth control. Two types of genes are important for the control of cell proliferation: protooncogenes and tumor suppressor genes. In addition, cell-division control (cdc) genes have been identified which are critical components of protooncogene- or tumor suppressor gene-mediated pathways. There are many unanswered questions about how these genes interact, and it is clear that additional growth regulatory genes have yet to be identified.

Because growth control is under multigenic regulation, we are developing assays to detect growth regulatory genes with partial or incomplete activity. We are developing a quasi-genetic approach to identify these genes by using normal human cells, wild type or mutant forms of viral oncogenes and cellular genes of known and unknown function.

The first step in our approach is to introduce into the cells oncogenes that by themselves are unable to stimulate cell proliferation, but for which something is known about how they participate in oncogenesis. A prime example is the large T antigen of the SV40 tumor virus. T antigen stimulates quiescent or senescent human cells to enter the S phase of the cell cycle. Moreover, T antigen is known to bind and inactivate two cellular tumor suppressor genes: the retinoblastoma (Rb) and p53 proteins. It is also known that T antigen mutants that cannot bind Rb are somewhat less capable than the wild type of stimulating DNA synthesis in quiescent cells, and much less capable of doing so in senescent cells.

The second step in our approach is to identify cellular genes that can complement the defective stimulatory function of the introduced oncogene. In the case of the Rb-binding deficient T antigen, we found one human gene that can complement this defect. That gene is Id-1, and it encodes a nuclear factor that negatively regulates transcription factors of the bHLH family. Id-1 alone does not stimulate appreciable DNA synthesis, nor does the Rb-binding defective T antigen. But the two together stimulate DNA synthesis equivalent to a wild type T antigen. This finding was the first direct indication that Id-1 is a positive growth regulator, an activity that could only have been detected using our quasi-genetic approach. Because Id proteins are known to interact with bHLH proteins, this finding also suggests that the Rb-mediated pathway of growth inhibition and tumor suppression muse involve a bHLH suppressor protein.

We are preparing expression vectors of other viral oncogenes, and preparing expression cDNA libraries from human cells to identify other cell cycle activator genes and to identify the cell cycle repressor genes with which they interact.

In Vitro Transformation of Human Mammary Epithelial Cells (Stampfer, Yaswen)

The focus of our current studies has been the molecular dissection of factors which control expression of a specific differentiation marker, lactoferrin, which has previously been shown to be downregulated or absent in a large proportion of human breast tumor specimens. In initial experiments in collaboration with Mina Bissell's laboratory, we have found that expression of lactoferrin, normally synthesized in breast tissue during a broad range of functional states, is dependent upon cell interactions with basement membranes. We have continued to examine the mechanism mediating basement membrane regulation of lactoferrin expression in order to identify regulatory elements critical for coordination of growth and differentiation, and whose alteration may promote carcinogenesis. Our studies have shown that although receptors for extracellular matrix molecules are likely to be involved in signalling for lactoferrin expression, addition of exogenous basement membrane components is not necessary for induction of lactoferrin expression in cultured human or murine mammary epithelial cells. Simple suspension of these cells in non-adherent cultures is sufficient to cause cessation of growth and increase lactoferrin expression. In order to further distinguish between decreased adhesion and increased cohesion as driving forces for lactoferrin induction, we lowered extracellular calcium levels to minimize intracellular interactions. Under these conditions, lactoferrin was expressed by single cells. Thus, changes in cell shape/attachment are sufficient for modulation of this differentiation marker. We theorize that one mechanism through which basement membrane influences gene expression is through modulation of

cell shape. We are continuing to examine the intracellular circuitry which mediates this cell shape signal.

RISK ASSESSMENT—CARCINOGENIC POTENCY PROJECT (GOLD)

Rodent cancer tests were designed to maximize the chance of obtaining a positive result in a lifetime experiment with small numbers of animals; the maximum tolerated dose (MTD) and half MTD are used for that purpose. This experimental design, with a narrow range of doses tested, was never intended to provide the information to quantitatively assess the risk to humans from chemical exposures at low doses. In current regulatory policy, however, the results of these high-dose rodent bioassays are the main source of data used to assess human risk at exposure levels that may be hundreds of thousands of times lower than the MTD. Standard practice in risk assessment has been to estimate carcinogenic potency from bioassay data, and to obtain an upper bound on human risk simply by making a linear extrapolation to the human exposure level, i.e. risk = potency X human exposure.

The accuracy of this methodology is generally unverifiable, since data on humans are limited. Major goals of this project are to improve risk assessment methodologies and to develop strategies for setting research and regulatory priorities. The analyses are based on our Carcinogenic Potency Database, which we have been developing for 15 years, and which now includes results of 5000 animal cancer tests on 1200 chemicals.

About half of the chemicals tested, whether synthetic or natural, are carcinogenic to rodents at the high doses tested. A plausible explanation for the high frequency of positive results is that testing at the MTD frequently can cause chronic cell killing and consequent cell replacement, which is a risk factor for cancer that can be limited to high doses. Many of our analyses are consistent with this hypothesis, including positivity rates and shape of the dose-response for mutagens and non-mutagens.

The great bulk of chemicals ingested by humans is natural, by both weight and number. For example, 99.99% of the pesticides in the diet are naturally present in plants to ward off insects and other predators. Half of the natural pesticides tested (29/57) are rodent carcinogens. Reducing human exposures for the 0.01% of pesticides that are synthetic, either to individual chemicals or to mixtures, will not appreciably reduce cancer rates.

Humans also ingest large numbers of natural chemicals from cooking food. For example, more than a thousand chemicals have been identified in roasted coffee; more than half of those tested (19/26) are rodent carcinogens. There are more natural carcinogens by weight in a single cup of coffee than potentially carcinogenic synthetic pesticide residues in the average U. S. diet in a year, and there are still a thousand known chemicals in roasted coffee that have not been tested. This does not necessarily mean that coffee is dangerous, but that animal cancer tests and worst-case risk assessments, build in enormous safety factors and should not be considered true risks. The true risk may be zero, and assessment of risk requires research on mechanisms of carcinogenesis for each chemical.

The reason humans can eat the tremendous variety of natural "rodent carcinogens" in our food is that, like other animals, humans are extremely well protected by many general defense enzymes, most of which are inducible. Defense enzymes are effective against both natural and synthetic chemicals, such as potentially mutagenic reactive chemicals. One does not expect, nor does one find, a general difference between synthetic and natural chemicals in ability to cause cancer in high-dose rodent tests.

We have ranked possible carcinogenic hazards from known rodent carcinogens, using an index that relates human exposure to carcinogenic potency in rodents (HERP) . Our ranking does not estimate risks, which current science does not have the ability to do. Rather, possible hazards of synthetic chemicals are put into perspective against the background of naturally-occurring rodent carcinogens in typical portions of common foods. The residues of synthetic pesticides or environmental pollutants rank low in comparison to the background, despite the fact that such a comparison gives a minimal view of hypothetical background hazards because so few chemicals in the natural world have been tested for carcinogenicity in rodents. Linear extrapolation from the maximum tolerated dose in rodents to low-level exposure in humans for synthetic chemicals, while ignoring the enormous natural background, has led to exaggerated cancer risk estimates and an imbalance in the perception of hazard and the allocation of resources.

The validity of qualitative extrapolation between species is being examined by comparing results on positivity, target organ, and carcinogenic potency between near-replicate tests, between different routes of administration, and between species (rats vs. mice, nonhuman primates vs. rodents, humans vs. rats or mice for the known human carcinogens). We examine by simulation whether the observed concordance in carcinogenicity between rats and mice can be observed even if true concordance is much lower or much higher.

BONE MARROW STEM CELLS (NARLA)

In order to investigate *in vivo* erythroid stem proliferation we have engineered mice to model the human condition *Hydrops fetalis*. This common human condition results *in utero* lethality due to the absence of alpha globin chains in red cells. Using gene targeting in embryonic stem cells we have created mice with a 16 kilobase deletion including the two adult murine alpha globin genes. These animals faithfully mimic the human condition and die late in gestation. To demonstrate that the lethality of this condition is due exclusively to red cell globin abnormalities we have shown that the lethal phenotype can be corrected through the transfer of a human alpha globin transgene. These animals now serve as a substrate to investigate issues of early erythroid development and as a model of human *Hydrops fetalis*. A manuscript describing this studies is will appear in Nature Genetics.

KP-03-03

RADIOLOGICAL PHYSICS AND CHEMISTRY: THEORETICAL MODELLING IN RADIOBIOLOGY (CHATTERJEE)

There are several types of damages that can be inflicted on DNA by ionizing radiation. DNA contains the important genetic codes and hence its damage, if unrepaired, can have serious consequences. In this project the long-term goal is to correlate the physical and chemical changes that precede any specific type of damage to DNA with observable biological consequences to a cell. Ionizing radiation can cause strand breaks (single and double), base alterations, released bases, DNA-protein cross-links, DNA-DNA cross-links, etc. The short-term goal of this project is to correlate double strand breaks with mutation (specific types) and transformation frequencies as a function of dose for different qualities of ionizing radiation. The types of ionizing radiation being considered are X rays, Co^{60}-g rays, energetic protons, helium and other heavy charged particles. Each of these radiation qualities has a characteristic physical pattern of energy deposition (track structure) which is primarily responsible for chemical changes which can be sometimes qualitatively and quantitatively different. In addition the proximity of various types of damages (which is a function of radiation quality) on DNA may have a strong influence on the observable biological effects. In the last several years, we have developed a mechanistic theoretical model based on basic physical and chemical laws which can quantitatively estimate yields of strand breaks (single and double), base alterations by water radicals and base deletions for any quality of ionizing radiation. Now our approach is to use the yields of strand breaks as fundamental quantities in modeling mutation and transformation frequencies. These yields are computed over a variety of DNA structures, from a very simple linear DNA to 30 nm solenoidal fiber. Future studies will be extended to include even higher order structures. The immediate next step is to try to understand the mechanisms involved in some of the intermediate steps with special emphasis on enzymatic repair processes along the pathways to mutagenesis, cell transformation and eventually carcinogenesis. By understanding and mathematically modeling these steps we hope to be able to provide a systematic link between the various stages in the evolution of radiation damage beginning with the physical and physico-chemical processes. It is possible that some of the procedures involving the mathematical formulation of biological effects of radiation may be applicable to other carcinogens.

Theoretical modeling of chromatin in a 30 nm solenoidal form, along with an assessment of damage induced by various qualities of radiation, has yielded three very interesting results. A previously unknown phenomenon of production of small size fragments (80 bp - 2 kbp) with characteristic frequency distribution reflecting several structural features of the chromatin was predicted on the basis of the model. These predictions have been subsequently verified by experimental measurements in our laboratory. A second result was obtained which confirmed the formation of locally multiple damaged sites (LMDS) over a region of approximately 20 base pairs. The extent of multiplicity of damage was dependent on the quality of the radiation and the associated linear energy transfer (LET). A third result

was related to the formation of several LMDS over an extended region of 1 kbp (the total number of base pairs in one solenoidal turn). We have proposed that these types of damages be identified as regionally multiple damaged sites (RMDS). It is extremely important to examine the correlation between various classes of damage and the fidelity of repair. By correlating the available experimental data on chromosomal aberrations (deletion, translocations and dicentrics) with our calculated values of the yields of double strand breaks from the chromatin structure model, we have been able to obtain the fractions of unfaithful repair which lead to the formation of observed chromosomal aberrations. Following these studies, we are attempting to catalogue various degrees of LMDS (i.e., the number of damaged sites within 20 bp) for the same types of radiation quality for which unfaithful repair fractions are available from our theoretical analysis. This will allow us to classify damages from our catalogue, which are faithfully repaired and also those which are not faithfully repaired. Direct experimental measurements of fidelity of repair are underway in our laboratory. Instead of using the chromosomal aberration data, it is necessary that the data from these direct measurements correlate in the same manner with our damage classification criterion for the faithful damage repair fraction. Results for the chromosomal studies, as well as for the fidelity of repair studies, will provide new information. Based on the repair studies, a hypothesis on damage-repair correlation is being postulated, along with a possible experiment to test the validity of the hypothesis. According to the hypothesis, unusual damages i.e., damages which cells have never encountered during evolution, are the classes of damage which are difficult to repair and may have severe biological consequences. If this hypothesis proves to be true for radiation, then it may be tested for other carcinogens.

General Life Sciences

KP-04-01

STRUCTURAL BIOLOGY

LBNL's structural biology initiative takes a multidisciplinary approach to understanding the function of biological molecules and molecular complexes from the viewpoint of their respective three-dimensional structures. To help facilitate this objective, the Advanced Light Source (ALS) will offer new resource opportunities in the areas of x-ray microscopy, crystallography, and spectroscopy.

X-RAY ABSORPTION SPECTROSCOPY (CRAMER, RANDALL)

A cooperative effort is underway to develop new types of x-ray spectroscopy that will take advantage of the unique capabilities of the ALS. Three new techniques are under development: x-ray magnetic circular dichroism, site-specific x-ray absorption, and flow-pump-probe spectroscopy. Currently, these experiments are being conducted at the National Synchrotron Light Source (at Brookhaven) and the Stanford Synchrotron Radiation Laboratory (at SLAC).

X-ray magnetic circular dichroism (XMCD) measures the difference in absorption of left- and right-circularly polarized x-rays by a sample in a magnetic field. This year we recorded the first x-ray magnetic circular dichroism spectrum of a paramagnetic sample, the Fe(III) center in the protein rubredoxin. This technique allows determination of the magnetic orientation of specific metal in different oxidation states, and should have broad application to bioinorganic chemistry and magnetic materials science. Site-specific x-ray absorption is based on small shifts in fluorescence energies that occur with oxidation state changes. We have demonstrated fluorescence shifts for Mn(II), Mn(III), and Mn(IV), and are now building spectrographs to allow similar studies on Fe and Ni compounds. The technique of flow-pump-probe spectroscopy has in the past been used with a laser pump and millimeter scale hard x-ray probe beams. We are currently building an apparatus to use micron-sized soft x-ray probe beams from the ALS, along with a pulsed dye laser pump beam. Upon completion the smaller probe size will allow significantly shorter time scales to be examined.

SEMICONDUCTOR X-RAY DETECTORS FOR SYNCHROTRON APPLICATIONS (JAKLEVIC)

The program to develop semiconductor detectors for synchrotron applications focuses primarily on the detector needs of experimenters interested in biochemistry and structural biology applications of synchrotron radiation. However, the detector requirements for those experiments are very similar to

the requirements for a wide range of x-ray synchrotron experiments, and the detectors developed within this program will thus have a very broad use. A significant amount of research has focused on developing multi-element detectors and their associated electronics for spectroscopy applications requiring excellent energy resolution and high count rate capability. Towards this end, a second generation 200-element silicon detector, and a second generation low noise integrated circuit preamplifier chip, have been designed and fabricated. The detector and preamplifier achieve 350 eV FWHM energy resolution at room temperature, and <250 eV FWHM when cooled slightly, which is by far the best resolution achieved to date for multi-element detectors fabricated on high resistivity silicon with integrated pulse-processing electronics chips. The detector and preamplifier chip occupy only a few square centimeters of area, for all 200 detector elements. The detector and readout electronics will be further developed into a fully-functioning x-ray spectrometer, utilizing 64 of the available 200 channels. In order to take advantage of the full 200 channel capability, an additional IC electronics chip must be developed, which is out of the range of the current program funding. However, the equivalent electronics are being developed in a conventional manner, for 64-channels of data acquisition. (Utilizing conventional electronics technologies becomes prohibitively expensive for more than 64 channels. For >64 channel data acquisition, the electronics should be miniaturized onto a silicon chip). This 64-channel detector system will be demonstrated in two synchrotron applications: (1) in an EXAFS application studying actinide L-edges in organic compounds at the Stanford Synchrotron Radiation Laboratory and (2) in an x-ray microprobe experiment at the Advanced Light Source at Lawrence Berkeley National Laboratory. A parallel effort will begin a new project to integrate the preamplifier directly onto the detector for further improvements in energy resolution.

Additional projects include the development of new detector contacts, which result in improvements in peak-to-background for enhanced detector sensitivity and improvements in low energy x-ray detection. Spin-offs from the synchrotron detector program include a spectrometer for detection of actinides in nuclear waste contaminated soil and water samples.

SOFT X-RAY MICROSCOPY (GLAESER, MORONNE)

Two principal objectives of the soft X-ray microscopy program include: i) the development of labeling and contrast enhancing methods uniquely suited to X-ray wavelengths, including radiation resistant fluorochromes, and ii) X-ray stereo and tomographic reconstruction techniques for three dimensional analysis of subcellular fine structure. In the past year, we obtained promising results that radiation resistant fluorescent labels suitable for soft X-ray microscopy can be made using lanthanide conjugated antibody and avidin probes. Potential applications include multiple label imaging of specific subcellular targets or compartments at significantly higher resolution than is possible by light microscopy. Using rigorous numerical simulations, we have also recently established that the radiation dose required for producing a high resolution tomographic three dimensional reconstruction is no greater than that required for a single high resolution projection image. This finding supports the feasibility of obtaining X-ray 3-D tomographic reconstructions of "thick" biological specimens with five times or better resolution than with light microscopy, and with samples that electron microscopy fails to show anything at all.

It is only in the last few years that the complimentary development of high resolution zone plate lenses and intense, tunable, monochromatic X-ray sources has made possible the construction of X-ray microscopes with high resolving power. In this context, both scanning and imaging microscopes have been produced that are capable of better than 50 nm resolution, with improvements expected to reach 20 nm or better. These values are 4-10 times better than the resolution of the best visible light microscopes. The Advanced Light Source (ALS) in Berkeley, which recently went into operation, is expected to generate soft X-ray fluxes suitable for X-ray microscopy that are at least 30 times brighter than any source available at present. These technological advances have created a situation where it is of great scientific importance to determine whether it is possible to make the X-ray microscope a significant tool for cell biologists.

High Resolution X-ray Excited Fluorescence Microscopy. With a scanning microscope configuration, the precise position of the exciting X-ray beam is well defined, even within a thick specimen. Visible light emitted from a fluorescent label can be collected and then used for digital image

reconstruction. Since the resolution of such a system depends on the spot size of the exciting radiation and not the wavelength of the emitted light, this approach offers the possibility of fluorescent labeling and detection with spatial resolutions five times or better than that available with visible light microscopy. Because of the significant potential increase in spatial resolution offered by X-ray excited fluorescence microscopy, we are vigorously pursuing the development of radiation hard fluorochromes. Conventional fluorescent labels are rapidly destroyed by X-ray excitation and are unsuitable for X-ray microscopy. As a consequence, we are working on new fluorescent formulations based on lanthanide elements and have produced compounds that continue to yield useful luminescence even after 10^{10} rad exposures of X-rays. Development of biologically useful probes based on lanthanide complexes linked to avidin and secondary antibodies are currently in progress. These compounds will ultimately form the basis of a useful new class of radiation resistant fluorochromes suitable for scanning X-ray microscopy.

Tomographic Reconstruction: Hegerl and Hoppe introduced a critical theorem stating, "A three-dimensional reconstruction requires the same integral dose as a conventional two-dimensional micrograph provided that the level of significance and the resolution are identical." To test this important theorem, we have implemented an extensive set of simulations of the 3-D reconstruction process using a realistic model created from an actual electron micrograph. In addition to verifying the basic conclusion of the Hegerl-Hoppe theorem, we have extended its validity to the non-ideal, but experimentally more realistic cases of high adsorption, varying contrast, and missing angular range. From these results we are now able to conclude that it is feasible to use tomography with soft X-ray microscopy to obtain 50 nm resolution of frozen hydrated specimens without causing extensive structural alterations. This should be possible in samples that are far too thick for any electron microscope and with five times or greater resolution than is available with the best visible light microscopes.

Study of Malaria Infections in Human Blood by X-ray Microscopy: A direct beneficiary of the 3-D and fluorescence techniques under development for X-ray microscopy include detailed studies of the life cycle of malaria parasites in human blood cells. Fluorescent labeling and tomographic studies will provide information about protein trafficking within infected cells, the expression of antigens on the outer surface of blood cells that prevent their scavenging by the bodies immune system, and the mechanisms that allow parasites to invade healthy cells in the first place. Information about these processes could ultimately help in the development of effective treatment strategies. Because of the small size of the parasite (~ 1μm) high resolution techniques such as those provided by X-ray microscopy are essential to obtain new information about one of humankind's oldest and most pervasive diseases.

BIOPHYSICAL CHEMISTRY (KLEIN, YACHANDRA)

Sulfur is an important element in biology and chemistry. In essentially all of its states it is spectroscopically silent and hence inaccessible for study or evaluation without degradation or destruction of the sample. X-ray Absorption Spectroscopy (XAS) can be performed readily at synchrotron radiation sources, especially the ALS. Our objectives are to determine the intracellular quantities of sulfur containing molecules in the several oxidation states, to measure the changes induced by drugs and to explore the idea that radiation damage to cells may be determined by the state of sulfur.

The iron-sulfur proteins, called ferredoxins, are ubiquitous as biological electron carriers. They occur as one, two, three, four and eight-iron clusters. Despite very similar structures they can exhibit a very wide range of redox potentials. We are interested in determining and understanding the subtle changes in electronic and metrical structure that accompany these redox changes.

All of these proteins have one or more paramagnetic forms that are easily studied by EPR spectroscopy. In fact, this entire class of proteins was discovered because they exhibit unusual electron paramagnetic resonance, EPR, parameters that had not been observed previously. More advanced forms of EPR, including electron nuclear double resonance, ENDOR, electron spin echo envelope modulation, ESEEM and now coherent Raman beat EPR, CRBs, permit us to observe very small magnetic couplings between the unpaired electron spin and that of paramagnetic nuclei that are close to the Fe-S cluster. We are collaborating with the group of Professor John Markley of the University of Wisconsin who has expressed genetically modified members of this class

of proteins in auxotrophic E. coli. These proteins contain specifically isotopically enriched amino acids. The magnetic couplings between these structurally known substituents and the Fe-S cluster will provide detailed geometrical and electron density information not otherwise available. To complement the information derived from the EPR measurements we will also make X-ray absorption measurements at the sulfur K-edge. These XANES data provide a direct measure of electron density on the sulfur moieties. Recent developments in XAS permit observation of spin selective measurements which should be particularly informative at the Fe edges. Of particular interest is the fact that Professor Markley's collaborators have single crystals of each of their proteins that will provide vectoral as well as scalar information. To supplement these studies on proteins we shall also make measurements on well characterized model compounds that will help to guide our interpretations of the biological materials.

MUTAGENESIS—FUNDAMENTAL CHEMISTRY (HEARST)

The ultimate objective of this project is a fundamental understanding of the mechanisms of mutagenesis. The essential features of our approach include the site-specific modification of nucleic acid substrates with psoralen. The complete stereochemical characterization of the adduct between the polynuclear photoreagent and the DNA allows for the separation of the various addition products on a chemically synthesized oligonucleotide. These modified DNA molecules have then been used for un vitro and in vivo assays of DNA excision repair and the mutagenesis associated with this process.

After eleven years, this project is entering a new structural phase for which a new infusion of money is being requested. In the past year this laboratory has perfected the large scale synthesis of psoralen modified DNA oligonucleotides. These products are being used to determine the three dimensional structure of the psoralen crosslink and the psoralen furan-side monoadduct using NMR in collaboration with David Wemmer. A crosslinked 8-mer duplex has been crystallized and the long term goal is the determination of the X-ray crystal structure of this same oligo in collaboration with Shing Ho.

Finally, the central feature of all of these studies has been the collaboration with the laboratory of Aziz Sancar of the University of North Carolina Medical School. The new emphasis has been the development of reliable assay systems, using our unusual substrates, for excision repair in human cells and cell free extracts. This is the first step toward the isolation of the excision repair enzymes of humans. Already, there is an indication that while the E-coli repair excises a damaged fragment of twelve nucleotides, the human enzyme system excises a different sized fragment, suggesting that human or mammalian excision repair incorporates an entirely new class of enzymes.

BIOMOLECULAR STRUCTURE ANAYSIS BY NMR (WEMMER)

Much of our recent work has been in trying to understand the interactions of genetic regulatory proteins with their targets. This has included a repressor, several transcription factors and an RNA binding protein. Multinuclear, multidimensional NMR is being used to determine the solution structures of the proteins, and examine their interactions with other molecules. Specific examples of the types of interactions we are trying to identify are: protein-DNA and protein-protein interactions in the DNA binding domain of a transcription factor; protein-protein interactions in activation domains of transcription factors; protein conformational changes upon phosphorylation in a regulatory domain of a transcription factor; metal ion interactions in a metal sensitive repressor; and protein-RNA interactions in a genetic switch protein. The emphasis of this work is to determine, and hence ultimately to be able to control, the machinery of the cell responsible for many different types of regulation.

We have also carried out structural studies on a self-cleaving hammerhead RNA to try to understand the structural basis for the autolytic reaction. Our recent studies have focused on labeled RNAs, applying both N15 and H3. These studies have indicated that for at least one of the constructs we are using there is conformational lability among the conserved bases. This suggests we need to alter the molecule before trying to extend the structural work. Studies of one such new construct have been initiated.

We have also initiated NMR studies on protein fragments using solid state NMR (to be able to tackle insoluble aggregates). Experiments involving selections and libraries of proteins have also been started to extend the number of protein sequences which can be recognized by particular protein motifs. Conformational studies of new biopolymers have also

been done. A variety of studies have also explored ways in which NMR methods can be applied to new systems, previously inaccessible.

CENTER FOR BIOMOLECULAR DESIGN (KIM, SCHULTZ)

The Center for Biomolecular Design bridges chemical science and biological science by combining the expertise in LBNL and the University of California, Berkeley. Our aim is to redesign biological molecules to create new classes of novel biomolecular structures with applications to major problems in the medical, biological and environmental sciences relevant to DOE missions. Examples of new technologies that are emerging from interdisciplinary studies at LBNL include designing and creating a new generation of biopolymers that have unique properties and redesigning natural proteins to create new properties. One example of the latter is catalytic antibodies tapping the tremendous diversity (>10^{10} molecules) and specificity of the immune system to create "custom made" catalysts. These molecules may lead to restriction enzymes for proteins and sugars for novel therapeutic agents for destroying cancer cells, viruses, or even atherosclerotic plaques. Chimeric receptors will be constructed that "reprogram" the transmittal of information to cells and may ultimately lead to novel cellular based sensory or memory devices. Powerful methods have been developed making it possible to generate proteins that contain synthetic amino acids with novel properties not limited to the natural 20 amino acids. These activities reflect the tremendous chemical potential inherent in the complex architecture of biomolecules.

The Initiative focuses on the development of new technologies for generating and screening large libraries of both biomolecules and synthetic molecules for new properties and function. These biotechnologies are being applied to key problems in the general areas of biocatalysis and biomolecular recognition with potential applications to human health and the environment. For example, structure-based libraries are being developed for discovering new inhibitors and antagonists for key signal transduction proteins involved in cancer. Libraries of biopolymers with improved pharmacological properties (relative to peptides) are also being developed for therapeutic applications. New *in vivo* cell based screens for assaying biological activity and function are being developed as well as new rapid in vitro screening methods. Libraries of large biological and synthetic molecules are being synthesized and screened in an effort to generate tailor made enzymes and antibodylike receptors. Active molecules and systems that came out of this work will be studied in detail by NMR and x-ray crystallography in order to make further improvements in their function.

CATALYTIC ANTIBODIES (SCHULTZ)

Our research program focuses on developing new methods for studying biomolecular recognition and catalysis in biological systems. The first area focuses on the use of new mutagenesis methodology, in which unnatural amino acids with novel structural and electronic properties can be site-specifically introduced into proteins, to study signal transduction and biological catalysis. Specifically we are introducing novel amino acids into loops 1, 2 and 4 of ras p21 protein in order to study the (i) mechanism of GTP hydrolysis, (ii) ras-raf interactions and (iii) 9-cation interactions in ligand binding. In addition, efforts are underway in collaboration with Sung-Hou Kim to crystallize the amino terminal SH2 domain of GAP. We are also studying the catalytic mechanisms of two novel biochemical reactions, the self-splicing reaction of DNA vent polymerase from Thermococcus litoralis and the hydrolytic mechanism of the novel dicobalt protease, methionine aminopeptidase. The second area focuses on the generation and characterization of catalytic antibodies. We are currently focusing on the development of antibodies that (i) catalyze epoxide hydrolysis (a glutathione-S-transferaselike activity) and (iii) catalyze chemiluminescence. At the same time efforts have begun to clone, crystallize and solve the three dimensional structure of an antibody with ferrochelatase activity in order to better understand the molecular basis for antibody catalysis.

STRUCTURAL BIOPHYSICS (KIM)

The eukaryotic cell division cycle involves a complex sequence of biochemical events. The timing and order of these biochemical events are tightly regulated, so that each event in the cycle only occurs after previous events have been completed. The timing and coordination of cell cycle events are governed by a class of protein kinases known as the cyclin-dependent kinases (CDKs). A detailed understanding of these kinases will not only provide a better understanding of normal cell cycle control but will also provide helpful clues about the abnormal cell

cycle mechanisms in the cancer cell transformed by radiation, chemical carcinogens, and environmental factors. These kinases undergo periodic activation at various cell cycle stages and are believed to directly trigger the major transitions of the cell cycle. Coordination of cell cycle events is thought to occur at the level of CDK activation: thus, a failure to complete one event (e.g. DNA replication) inhibits CDK activities required to initiate the next event (e.g.. mitosis). Similarly, cell cycle control by external growth regulatory influences, such as the ras mediated cell proliferation, is also thought to involve the regulation of CDK activity. Thus the regulation of CDK activity is of central importance in our understanding of cell division processes and how those processes are regulated by external growth controls. The overall objective is to determine the three-dimensional structures of several key proteins involved in the eukaryotic cell division cycle using x-ray crystallographic methods. Initially we will focus on two proteins, CDK2 kinase and cyclin A. We plan to determine the crystal structures of both proteins individually and as a complex.

THREE-DIMENSIONAL STRUCTURE OF CATALYTIC RNA (TINOCO, KIM, WEMMER)

The catalytic activity of RNA is perhaps one of the most unexpected discoveries in the field of molecular biology in recent years, offering profound implications for our understanding of catalysis. Since the discovery of the autocatalytic activity of some rRNA, the biological role of RNA has been found to be quite diverse and the occurrence of RNA self-processing much more widespread than expected. Biochemical evidence so far strongly indicates that the three-dimensional structures of the RNAs are essential for their autocatalytic activities. Although the crystal structure of one small catalytic RNA complexed with DNA inhibitor has been determined, the structure fails to show the mechanism of RNA catalysis due to DNA inhibitors. Our objective is to determine the first three-dimensional structure of a catalytic RNA with authentic RNA substrates by using a combination of chemical probes, NMR spectroscopy, and x-ray crystallographic methods.

MOLECULAR STRUCTURE OF MEMBRANE TRANSPORT SYSTEMS (JAP)

Our research has focused on the structural determination of proteins with major emphasis on membrane proteins, using electron and x-ray crystallographic methods. In the past year, we continued to make major progress on our structural studies of a number of membrane proteins, such as cytochrome reductase, water channel protein, maltose transporter and potassium channel. We are completing the structure determination of calmodulin-like protein and, in collaboration with the Max Planck Institute group, have obtained the molecular model of a proteasome at 3.4Å resolution. We have also established in our laboratory the vaccinia virus expression system for over-producing membrane proteins such as the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein for subsequent crystallization and structural determination.

We have greatly improved the purification process of cytochrome reductase. The purified protein shows no detectable contamination from cytochrome oxidase, which was found to be the major contaminant in our previous purifications. The use of the purified cytochrome reductase yields crystals that diffract to 3.2Å resolution. We have also been successful in freezing the crystals that yield diffraction to about 3.5Å resolution. This approach allows a full high-resolution data set to be collected from a single crystal. The successful freezing of the cytochrome reductase is probably the first case where membrane protein crystals have been frozen without significantly affecting their diffraction quality. A full native data set to 3.5Å resolution has been collected using the synchrotron source at the Brookhaven National Laboratory.

We have purified the water channel protein (CHIP28) from bovine red blood cell membranes and have successfully reconstituted the protein to form two-dimensional (2-D) crystalline patches suitable for high resolution structure determination by electron crystallography. The membrane patches diffract to a resolution of about 3.0Å. A preliminary high-resolution projection map at 3.5Å resolution has been obtained that shows several helices that were earlier predicted to be the components forming the channel. In the coming years, we will devote major effort toward obtaining the 3-D map, first at 6.0Å and followed by the molecular model at 3.5Å resolution.

We have developed a rather simple purification method for the maltose transporter, a member of the ABC superfamily, that gives several milligrams of purified protein in a fully active form. We have obtained 3-D crystals of the maltose transporter. The

crystals are fragile and sensitive to handling for x-ray crystallographic examination. Several reconstitution experiments have been performed to obtain 2-D crystalline patches but, as yet, have not resulted in any good crystals. More extensive crystallization trials are currently being performed.

We have successfully obtained the recombinant of the CFTR and the vaccinia virus. The cloning of the CFTR was done in two stages. The first involves the cloning of the CFTR to a transfer vector with a $(His)_6$ tag. The second was to perform the recombination of the transfer vector with the vaccinia virus. Although the recombination is an easy technique, it is time consuming. This work involves the selection of a properly combined vaccinia virus and CFTR clone that includes several rounds of infection and selection of potential clones. We have obtained the proper combination of CFTR clone and vaccinia virus and are currently purifying the recombinant vaccinia virus.

We have received research support from Pfizer Corporation to carry out structural studies of potassium channel kv1.3, which is believed to be involved in the regulation of the immune response. We have successfully obtained a few hundred micrograms of purified protein. Currently, we are scaling up the purification to obtain a few milligrams of protein for 2-D crystallization.

Significant progress has also been made on our structural studies of calmodulin-like protein. We have obtained new crystals of this protein that diffract to better than 1.7Å resolution and have determined a molecular replacement solution using calmodulin as the model. The structure model of calmodulin-like protein is currently being refined and the fully refined structural model will be completed this coming year.

KP-04-02

YEAST GENETICS (MORTIMER)

The research group led by Dr. Robert Mortimer has worked on the genetics and radiobiology of the yeast *Saccharomyces cerevisiae* for 40 years. Over most of this time work has focused on developing the genetic map of this organism. The current map describes the location of over 1150 genes on 16 metacentric chromosomes. Most yeast researchers accept it as part of their scientific responsibilities to map genes that they work on; as a result, new mapping data have been generated very rapidly. About 15 years ago, the group accepted the responsibility of collating and publishing the mapping results from the many laboratories worldwide working on yeast. Since then, the group has published four major compilations of mapping data and are currently working on the fifth such compilation. The group is relating this genetic map to the recently developed physical map of this organism and to the international efforts to sequence the yeast genome.

Radiobiological studies have targeted the induction of recessive and dominant lethal damage, examination of the relationship between ploidy and radioresistance, and studies of RBE-LET relations for induction of lethality and mutation. Recently, Dr. Mortimer's group has been concentrating on a set of genes, isolated mostly at LBNL, that are involved in recombinational-repair of DNA lesions. Using molecular and genetic techniques to study the nature, regulation and products of these genes.

The yeast *RAD54* gene product is involved both in DNA repair and recombination. Raising antibodies to this protein the group is purifying the protein to further characterize its cellular role. Two approaches to antibody production were selected. The first involves the use of a 20 amino acid synthetic peptide coupled to keyhole limpet hemocyanin as antigen source. The second approach involves the isolation of a Protein-A: *RAD54* fusion protein by affinity chromatography purification. Purified antibodies generated in rabbits from both antigen sources have been checked for cross-reactivity with total protein extracted from E. coli strains that overproduce the *RAD54* gene product. Both antibodies give a strong response to a protein of about the correct size in such bacterial strains. These antibodies are now used to detect the *RAD54* protein in extracts of yeast cells and to study changes in the levels of this protein in response to DNA damaging agents.

Earlier studies have shown that both *RAD54* and *RAD51* are inducible by DNA damaging agents. The studies on *RAD54* induction are fairly well advanced, yielding the identification of a maximum 29 base pair sequence (DamageResponse Sequence-DRS) responsible for induction. Interestingly, deletion of this sequence had no effect on the x-ray sensitivity of the strain carrying the deletion.

These results demonstrate that *RAD51* transcript levels vary during the cell and also are responsive to

DNA damage. However, unlike a number of other coordinately cell-cycle regulated genes with similar patterns of expression, *RAD51* has no clear role in DNA replication, but does have a role in DNA repair. We have recently isolated plasmids that contain the region which appears to be necessary for damage induction but which lack the *MluI* sites involved in cell cycle regulation. Plasmids have been made that lack the damage inducible region but which contain the *MluI* sites. Northern analysis will be used to determine whether or not DNA damage can be uncoupled from induction from cell-cycle regulation.

During the last year, Dr. Mortimer has embarked on a cooperative research project with a number of major wineries in the California wine industry. This work is focusing on the genetic characterization of yeast strains responsible for "natural" fermentation of wine. This project seeks to develop superior wine producing yeast strains through genetic manipulations.

KP-04-04

HUMAN GENOME CENER (NARLA, PALAZZOLO, MARTIN, RUBIN, JAKLEVIC, EECKMAN)

The LBNL Human Genome Center (HGC) supports the national Human Genome Program with support from both DOE, OHER and the NIH National Center for Human Genome Research. The major focus of the HGC is to develop and implement automated technology for the mass production of finished genomic DNA sequence.

LBNL has proved itself to be uniquely suited to the rapid development of the directed sequencing effort. The serious commitment to develop and support interdisciplinary research projects at LBNL has been a key ingredient in the ability to successfully pursue the directed sequencing approach. The directed sequencing approach places heavy demands upon custom automation and informatics. It would be difficult, if not impossible, to design and implement this approach in a more traditional biological research setting.

Another factor of key importance has been the focus on production sequencing as a primary goal. Continued success has been in large part due to the focused effort of the majority of the Center's resources to the goal of DNA sequencing technologies and production. The payoff for this significant investment of resources is the attainment of world class status in the field of genomic DNA sequencing and the opportunity for significant expansion of this effort in the future. As of the end of November, 1994, approximately 2 million bases of genomic DNA has been sequenced. This achievement, has been accomplished over two and a half years with a relatively small production sequencing group, which has only recently totaled as many as 13 people. The combination of submitted finished sequence coupled with the efficiency of production makes the LBNL directed sequencing effort one of the elite sequencing groups in the world. The total sequence submitted places LBNL second in the world and, in terms of efficiency ($/base), LBNL ranks at the top.

During the past year the LBNL HGC has been considerably strengthened with the addition of human biology component. There are a variety of different options being explored by the genome community in search of cost-effective options to annotate completed genomic sequencing. These include: the single-pass sequencing of expressed sequences, large-scale exon trapping, and parallel sequencing of syntenic human-mouse genomic regions. LBNL has taken a different gene finding approach in that it is actively coupling large-scale human genomic sequencing with genetic assays for phenotypes in mouse regions that are syntenic to the human genomic regions that are being sequenced at LBNL. The syntenic genetic searches coupled to computer gene discovery promise to be an attractive approach to finding the biological function of completed sequence information.

LBNL is in a particularly strong position in terms of developing an automated environment for sequencing. The Automation Group of the LBNL HGC has already put into practice modules that automate many of the basic operations of the sequencing production line and is planning to integrate these modules into interacting systems in the next couple of years. The Computation Group has developed software for the early stages of sequencing and has recently finished a detailed plan for an integrated package of data handling that is tailored specifically to meet the needs of the LBNL directed strategy.

The expectations within the community are that a number of Centers will emerge in the U.S., and other countries, which will collectively provide the capacity to sequence the human genome over the next 10-20 years. When one considers a 15 year effort to sequence the entire genome at $0.60 per base pair (in 1994

dollars), the effort will require a 200 megabase per year capacity and will require funding on the order of $120 million per year. With 10 Centers operating, each would average $12 million in production funding. However, the number of U.S. Centers that could put together the appropriate biological approach, strong management, and the automated environment may be less than 10. This challenges LBNL, and other sequencing leaders, to consider the possibility of expanding sequencing operations on an even larger level than currently anticipated.

Although the cost of sequencing the genome of man is quite high, the benefits to the nation's health and the biotechnology industry are also likely to be high. Amgen provides an illustrative example. This Fortune 500 company has only two products; together, they account for $1 billion in sales annually. The sequencing of the entire human genome will cost in the $1.5 to $4 billion range. However, it is hard to conceive of a result where the availability of this information would not accelerate the discovery of tens of such valuable products by several years. Thus, the value of the information of the human genome project is likely to more than pay for itself in both human health benefits and commercial returns.

DATA MANAGEMENT TOOLS FOR GENOMIC DATABASES (MARKOWITZ)

Genome applications generate large amounts of data that must be maintained and made available to scientists for analysis and manipulation. Due to their robustness and proven data management facilities, relational database management systems (DBMSs) such as Sybase and Oracle have been widely used for developing large genome databases such as the Genome Data Base (GDB) at Johns Hopkins School of Medicine, Baltimore, the Genome Sequence Data Base (GSDB) at the National Center for Genome Resources, Santa Fe, and the Protein Data Bank (PDB) at Brookhaven National Laboratory. Constructing, maintaining, and modifying genome database implemented with relational DBMSs, entail complex and time-consuming processes.

We are developing data management tools that provide facilities for efficiently constructing, maintaining, and modifying genome databases. These tools are based on the Object-Protocol Model (OPM). OPM provides constructs for modeling object and protocol structures specific to genome applications, and supports the specification of queries and views.The OPM data management tools allow specifying, querying, and browsing databases using object-oriented concepts on top of commercial DBMSs, and insulate users and applications from the underlying DBMS.

HUMAN GENOME COORDINATING COMMITTEE AND OPERATIONS (SPENGLER)

The Human Genome Coordinating Committee (HGCC) was formed in October 1988, as part of the management structure of the DOE Human Genome Program. As part of the Infrastructure of the Program, this office provides a variety of support activities, including meeting organization, report writing, and support for the Genome Subcommittee of HERAC. In addition, some new research and development activities have been organized through the office, permitting proof-of-principle research or other work. For example, a research subcontract to Allied Signal, Kansas City, supported modification of current technologies for the design and generation of models of biologically interesting molecules, such as the HIV protease. Additional effort went toward the generation of a major review article assessing the status of computational biology, particularly focused on structural biology. For FY95, the first effort was the DOE Genome Program Contractor-Grantee Workshop in Santa Fe, which had 400 people in attendance. This was the fourth workshop for the six year old DOE program. In addition, meetings of the HGCC are scheduled, as well as working groups in sequencing strategy.

LASER MICROSCOPY (SPENGLER)

In FY94, funds were received to support proof of principle of laser-feedback microscopy (LFM) for biological materials. The effort, a research and development subcontract to Dr. Alan Bearden, has lead to a system with data acquisition and real time data analysis. The current LFM is a scanning, confocal, laser-feedback interference microscope operating with a He-Ne laser as light source. The lateral (x,y) resolution of object details is on the order of 200 nm and the axial (z)resolution is 1 nm. Although STM and AFM have lower lateral resolutions, LFM furnishes images without the tip-forces inherent in probe techniques. This feature is especially important when imaging soft material in

fluids, e.g., biological cells. Although LFM can provide high signal-to-noise images from very weakly back scattering samples, more complete information about the structure of transparent samples, e.g., biological samples, is gained by placing the samples on a reflective substrate. LFM acquires and displays two digitized images: the optical path height image and the reflectivity image. Since LFM allows the determination of biological structure under physiological conditions, without using fixatives, stains or metal shadowing, it is particularly valuable for determining organelle size in developing cells and in measuring axonal growth in active neural processes. As an alternative to fluorescence-label confocal microscopy, LFM imaging of nanoscale gold-colloid labels seems promising. Of particular interest is the possibility of nanometer-scale resolution with LFM-incorporated reflectance *in situ* hybridization (RISH) applied to DNA localization and characterization.

MASS SPECTROMETRY DETECTORS (BENNER, JAKLEVIC)

Mass spectrometry offers the possibility that large molecules, such as DNA and proteins, can be mass analyzed rapidly. The development of this capability will eliminate a time consuming bottleneck associated with electrophoresis gel-based analytical methods. For mass spectrometry to become a substitute for gel-based methods, several developments need to occur and of these, one is the development of new ion detectors that respond to large ion impacts. Today's mass spectrometers function with ion multiplying detectors (microchannel plates) but this kind of detector will not respond efficiently to ions much larger than about 300,000 Da, or in terms of DNA, a fragment needs to be smaller than about 500 base pairs long. We are developing detectors that will register the heat pulse that occurs when an ion strikes a surface. During such an impact, the kinetic energy of an ion is converted to heat which subsequently warms the surface at the ion impact site. We are developing detectors that can respond to this small heat pulse but have chosen not to pursue the application of superconducting bolometric detectors because their low temperature operating requirements greatly diminish their attractiveness and potential applicability.

We are testing four detector principles. The first three are custom fabricated devices and the fourth is a commercially available avalanche photodiode. Metal-oxide-silicon (MOS) devices have been fabricated in our facilities and have been tested with surrogate particle and large ion impacts. This device experiences dielectric breakdown at impact sites and the breakdown current is used to detect particle impacts. A custom fabricated device has been assembled that utilizes heat sensitive phosphors. The phosphors can be stimulated to emit photons when warmed. Two phosphors have been tested and show sensitivity to small temperature pulses. The final detector is a piezoelectric film device has also been assembled in our labs and is undergoing similar tests. Feasibility studies have been completed with all the devices and we are now refining the ways these devices are fabricated and tested to determine if they are appropriate for commercialization as mass spectrometer detectors. Along with the practical implementation of these detector technologies, we are also examining the fundamental physical principles that control these detectors. In addition we are developing methods to mass analyze DNA.

Medical Applications

THE CENTER FOR FUNCTIONAL IMAGING

The mission of the Center for Functional Imaging is to perfect and use new biochemical and biophysical methods of noninvasive imaging for studies of the mechanism of human diseases. The emphasis is to advance nuclear medicine and nuclear magnetic resonance techniques toward the study of brain, heart, and metabolic disease in an integrated approach which combines tracer techniques, advanced detector instrumentation and mathematical methods for reconstruction and kinetic analysis. A strong theme of our approach is quantitation and statistical characterization.

The focal points of the Center include development, evaluation and dissemination of advanced medical imaging techniques in the study of mental illnesses, aging, atherosclerosis and cancer. The major emphasis has been the application of positron emission tomography together with advanced mathematical techniques of analysis, chemical synthesis of specific tracers and detection instrumentation.

The Center's program of basic and applied nuclear medicine research evolved from early studies at the laboratory on the use of radioisotopes to study human

diseases (e.g., the first use of radioiodine to evaluate thyroid function, 59Fe and 52Fe for hematopoesis studies, 11CO use in the first studies using a positron emitter for physiological measurements, the first 99mTc Anger camera studies, the original ^{201}Tl whole body dosimetry studies, the first quantitation SPECT and the first studies of brain, heart, and kidney using 82Rb). Instrumentation developments such as the well-counter, scintillation camera, quantitative whole body scanner, dynamic positron emission tomograph, and attenuation compensation emission tomography programs were also pioneered by this program.

The radiopharmaceutical arm of the Center made significant progress in generators but without a dedicated cyclotron the development program lacked a breadth in labeling activity. In the last four years we have built up a group of organic and radiopharmaceutical chemists through fellowship programs and the DOE experimental medicine chemistry project now led by Dr. VanBrocklin. This year a mini-cyclotron (11 MeV) will be installed on the LBNL site. With this installation we plan an expansion of the LBNL nuclear medicine radiopharmaceutical, instrumentation and applications program by setting up a resource culture to make available the highest resolution PET system, new reconstruction algorithms and innovative radiolabeling activities. We envision a scholar's program and a closer tie between our program and DOE-sponsored programs.

A major objective at the Center has been the elucidation of the characteristics of mental illnesses such as schizophrenia and dementias such as Alzheimer's disease and Parkinson's disease. Cardiac disease studies include a large study on the kinetics of rubidium in the human to establish the validity of dynamic PET in evaluating coronary artery disease. The significance of this work is that dynamic PET or SPECT might provide an inexpensive method of patient management wherein a decision is to be made regarding the appropriateness of coronary artery surgery vs. conservative medical management.

A significant portion of the PET applications at LBNL are the whole body PET studies in cancer. Breast cancer and prostate cancer patients are now being evaluated under protocols to ascertain the extent of disease and the response to specific therapies. This has been a very successful program with promise for major improvement in the diagnosis of patients at high risk for breast cancer and for the medical management of cancer particularly when a decision regarding the appropriateness of expensive therapy depends on reliable information on the presence or absence of metastases. The program will be extended to lung tumors in 1994 with two successful cases already having been studied by dynamic PET.

A major development in PET data analysis is the computer based description of volumes of interest which are physiologically realistic, objective and adequate from a statistical standpoint. The developments underway can become a significant tool for both SPECT and PET quantitative analysis packages in commercial and academic need.

The objective of the Positron Three-Dimensional Imaging project is the development of advanced detector concepts for the imaging of positron-labeled tracers in man and animals with substantial improvements in spatial resolution and data collection speed. This will allow improved regional measurements of blood flow and metabolism in the heart and brain, and improved studies of the relationship between the binding rate of specific neurotransmitters and brain function. Our tomograph designs encircle the patient with multiple rings of detectors having good detection efficiency, good spatial resolution, and low dead time.

To overcome the limitations in event rate that conventional tomographs have when imaging short-lived tracers in the heart, we are developing a detector module consisting of a group of small scintillation crystals coupled on one end to a rectangular phototube for timing information and coupled on the opposite end to an array of silicon photodiodes for position information. This design will provide good spatial resolution (3 mm) and improve maximum data rates by a large factor. For ultra-high resolution ($\leq$2 mm) imaging of tracer compounds in the brain, we are developing a version of the design that uses smaller crystals and can measure the depth of interaction in the crystal to correct for parallax error. To overcome the limitations of existing scintillators for PET, we are developing new scintillators by (i) systematically searching pure and doped heavy-atom compounds to find those exhibiting fast fluorescence, (ii) measuring the scintillation properties of optical crystals of promising compounds, and (iii) investigating scintillation mechanisms through the use of synchrotron radiation.

We are working with industry on the development of improved positron tomographs, silicon photodiodes, novel solid state photodetectors, and new, fast, high

efficiency scintillation crystals. Transfer of these techniques and devices to other research institutions and industry will permit the production of improved positron tomographs for the benefit of medical research throughout the world.

Experimental Medical Development of Radionuclides (Van Brocklin)

The experimental medicine chemistry program at LBNL was established to develop synthetic methods for the incorporation of radionuclides into biochemical substrates and to evaluate the potential efficacy of these radiolabeled compounds for the study of physiological processes and mechanisms in both normal and diseased states. The program has focused on the development of agents that will give information on how the neurochemistry of the brain is related to diseases of the brain, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, and other dementias as well as depression, hypertension, violent behavior, and brain aging.

We are in the process of developing three new tracers for brain studies. First, we have synthesized and evaluated 5-[123I]iodo-nitroquipazine, a potent and selective seretonin reuptake receptor ligand. This tracer demonstrated rapid penetration and high selectivity for seretonin receptor-rich regions of the brain in a series of SPECT studies in *macaca mulatta* monkeys. The organ dosimetry and toxicology of this compound is being studied in preparation for the first human studies using this non-invasive tracer.

The second tracer under development is an α_2-adrenergic receptor antagonist, 5-[^{18}F]fluoro-atipamezole, for in vivo receptor quantitation. The α_2-adrenergic receptor system, located in locus coeruleus, nucleus tractis lateralis, hippocampus, and frontal cortex, has been implicated in hypertension, depression, drug abuse and craving, and dementia. We are in the labeling chemistry development stages of the synthesis of this molecule with the intention of studying its biological potential once a suitable route of production has been achieved.

Thirdly, we are continuing to evaluate [F-18]fluorodihydrorotenone, a derivative of the natural plant product rotenone, as an imaging agent for mitochondrial function. The loss of Complex I of the electron transport chain, found in the mitochondrion, has been linked to Parkinson's and Huntington's diseases as well as the normal aging process. This tracer has demonstrated high uptake in the brain and the heart, organs containing higher concentrations of mitochondria. Validation studies for this tracer as well as other labeled analogs are progressing. These novel molecular medicine imaging agents for the evaluation of human brain oxidative metabolism also has applications to heart metabolism and cancer.

In addition to the new tracer studies, we provide labeled radiopharmaceuticals for on-going physiological studies. We routinely produce [F-18]fluorodeoxyglucose for the investigation of glucose metabolism in the brain, heart and tumors. Recently, we have synthesized [F-18]fluoro-m-tyrosine, a metabolic tracer, to image the dopaminergic pathway in the caudate and putamen. This tracer will be used to validate the treatment of Parkinson's disease through the implantation of genetically engineered cells. This work is part of a cooperative project (CRADA) between SOMATIX, a biotechnology company developing methods of gene therapy, and the Center for Functional Imaging.

Radiation Effects of Vascular and Blood Diseases (Ebbe, Taylor)

Work on the development of new radiotracers continues for the non invasive imaging of atherosclerosis via PET and SPECT. Our studies have centered on the use of porphyrins as imaging agents, specifically hematoporphyrin derivative and uroporphyrin. Procedures have been developed for the insertion of ^{68}Ga into these compounds, and they have been studied in vivo, including with positron emission tomography (PET), to study atheromata and organ uptake and blood clearance. Though neither compound has been found to have high enough aorta to blood levels to be useful as an imaging agent, the Ga-uroporphyrin exhibits some traits that make further study of this compound warranted. In addition, liposomes were examined as a possible carrier for enhanced uptake into lesions, but again cleared the blood too slowly to be of use with short-lived positron-emitting isotopes.

Work on the regulation of megakaryocytopoiesis and platelet production is on-going. By analyzing different species and genetic variants of mice, it is thought that insight will be gained into mechanisms that control megakaryocyte DNA replication and the release of platelets from megakaryocytes.

IMAGING OF APOLIPOPROTEIN E-BINDING RECEPTORS *IN VIVO*
(KRAUSS)

There are two overall goals of this project. The first is to develop and apply noninvasive imaging methodology for determining the distribution and activity of cell-surface receptors that are important in regulating plasma levels of atherogenic lipoproteins. A particular focus is the low-density lipoprotein (LDL) receptor, which has two major apoprotein (apo) ligands, apoE and apoB-100. Peptide analogs of apoE have been shown recently to compete effectively with LDL for specific binding to the apoB,E receptor in cell culture. These peptides, labeled to high specific activity with short-lived radioisotopes, will be used as probes for external detection of apoB and apoE-binding receptors *in vivo* by positron emission tomography (PET). In the initial phase of the work, candidate peptides have been chemically synthesized and are being characterized with respect to specificity and affinity of binding to the apoB,E receptor in cultured human skin fibroblasts. Future studies will focus on determining the optimum peptide configuration and mode of administration for *in vivo* studies, and examining the specificity of tissue uptake of radioisotopically-labeled peptides in normal and genetically receptor-deficient rabbits. Peptide derivatives labeled with the positron-emitting isotope [18-F] will then be used for PET scanning.

The second goal of the project is to characterize specific molecular forms of apoB-containing lipoproteins that are involved in the formation of atherosclerotic lesions, with the aim of defining features that can serve as a basis for the development of PET imaging of atherosclerosis. Of particular interest are two genetically influenced atherogenic lipoproteins: Lp(a) , and small, dense LDL.

In collaboration with memebers of LBNL's Functional Imaging Center, radiolabelled derivatives of these atherogenic molecules will be developed and evaluated for use as atherosclerosis imaging agents in experimental animal systems.

Table 6: Significant Science and Technology Achievements

Analytical Technology

KP-01-01
CHARACTERIZATION OF AIRBORNE RADON CONCENTRATIONS
(GADGIL, SEXTRO)

- Used experimental and theoretical methods to quantify the pressure field on the ground surface near the house resulting from the interaction of the house structure with wind. Using an experimentally verified model of radon transport and entry, quantified the significant impact of this wind-induced ground pressure field on radon entry rates into houses with basements.

- Made significant progress with an experimental effort to quantify the effectiveness of an approach to place an impermeable membrane under the sub-slab gravel as a means for passive or energy-efficiency radon mitigation technique. This effort was co-funded by the DOE office of building technologies.

KP-01-02
DETECTOR MATERIALS, INSTRUMENTATION (JAKLEVIC, HALLER)

- A new charge sensing technique have been developed. It provides an effective solution to the long-standing problem of poor hole collection in compound semiconductor detectors, enabling these room temperature detector to achieve performance levels competitive with cryogenic germanium detectors. A patent application has been filed.

- A CRADA was established between our group and eV Products (a division of II-VI Incorporated) to

develop CdZnTe materials and to commercialize detectors based on the use of our new charge sensing technique.

- We have successfully applied the amorphous-contact technology developed previously to a variety of high-performance germanium detectors. A monolithic detector array utilizing the amorphous contact has been developed and it provides a very substantial gain in high-rate performance compared to conventional detectors.

- Our work on defect gettering in p-type FZ silicon has resulted in our establishing a dialogue with a major silicon vendor on possible FZ growth technique improvements.

- Our gettering results have further suggested that the point defect diffusivities (vacancy and silicon interstitials) may be considerably higher than has been presently accepted. If correct, our results could have a major impact on diffusion modeling for sub-micron silicon devices.

- A carrier drift distance of 4 cm has been demonstrated in our p-type drift detectors. This is the first time that a p-type silicon drift detector has been realized and offers the possibility of substantially lower fabrication costs for these type of devices.

- Our work on p-type drift detectors also has resulted in our being awarded an SBIR with NOVA Research Inc., and an LDRD to develop p-type x-ray drift detector.

- High purity gallium arsenide epitaxial layers with impurity concentrations in the range of one part per billion have been produced. This approaches the level required for radiation detector applications.

Environmental Research

KP-02-01
AEROSOL & CLOUD CHEMISTRY (NOVAKOV)

- CCN concentrations cannot be explained by sulfate concentrations alone. To account for the "missing" CCN we have developed and validated a measurement and data analysis methodology that enables the quantification of contributions of sulfate, sea salt and organic aerosol mass concentrations to the CCN number concentrations.

- This is presently used at our measurement site The results obtained by this methodology at Point Reyes, California demonstrate that the contributions of organic and sulfate aerosols to CCN number concentrations are variable, ranging from ~20 to ~65% for sulfate and ~5 to ~80% for organic aerosol.

- These findings challenge the conventional view that CCN are composed mainly of sulfate species, and may significantly alter the results of model calculations of the aerosol effects on climate.

KP-02-03
RADON TRANSPORT THROUGH SOILS AND INTO HOUSES (SEXTRO, FISK)

- We have directly measured soil gas entry into one of our basement structures driven by atmospheric pressure variations. We found that the larger the rate of change in the atmospheric pressure the larger the soil gas flow rate. Although the overall flow into and out of the structure averages to zero, there is a net migration of radon into the structure because the radon concentrations in the soil gas are higher than that of the air inside the structure. This effect may help explain elevated indoor radon concentrations observed in some houses during periods when steady-state conditions driving advective transport are absent.

- We have made preliminary measurements of radon-220 concentrations in one of our basement structures (without the underlying gravel). With no induced pressure difference across the building shell, the radon-220 concentrations averaged ~0.4 pCi/L, yielding a diffusive entry rate for radon-220 of ~3 Bq/s, compared with 0.1 Bq/s for radon-222. Experiments to measure radon-220 entry rates under depressurization conditions are underway.

- Scale-dependent permeability measurements have been made at another location and the data suggest that permeability varies with length scale at this site as well. Additional measurements at this and other sites are planned when the portable apparatus for use in the field has been completed.

THERMODYNAMIC PROPERTIES OF CHEMICAL SPECIES PRESENT IN MIXED ORGANIC-RADIONUCLIDE WASTES (AL MAHAMID)

- Determination of the stability constant of Pu(V) with NTA at 0.1 M ionic strength by two independent

methods simultaneously : spectrophotometry and potentiometry

We studied the complexation behavior of Pu(V) with Nitrilotriacetic Acid (NTA) in the pH range 4 to 7. We determined the stability constant for the complex $[PuO_2NTA]^{2-}$ at 0.1 M $NaClO_4$ and at 25°C using two independent methods simultaneously: spectrophotometry and potentiometry.

- Study of the complexation behaviors of Pu(III), Pu(IV), Pu(V), and Pu(VI) with NTA at macroscopic concentrations of Pu (10^{-3} M) using spectrophotometry

  In a series of experiments, plutonium in four oxidation states, Pu(III), Pu(IV), Pu(V), and Pu(VI) was individually mixed with NTA, and the solutions were analyzed as a function of time for oxidation state changes. We observed that at ligand to metal ratios of about 6 to 1, at an initial plutonium concentration of about 10^{-3} M, and at pH ~ 5, the final stable species of plutonium, when mixed with NTA, is the Pu(IV)-NTA complex.

- Study of the complexation behaviors of Pu(III), Pu(IV), Pu(V), and Pu(VI) with NTA at environmental relevant concentrations of Pu using a solvent extraction technique.

  NTA solution was added to individual Pu(III), Pu(IV), Pu(V), and Pu(VI) solutions. Plutonium concentrations ranged from 3.8×10^{-7} to 7.0×10^{-6} M. The results showed that the presence of NTA in plutonium solutions inhibits the precipitation of plutonium and maintain it in the solution. The oxidation state distribution analysis showed that Pu(V) as well as Pu(IV) are the predominant oxidation state.

Health Effects

KP-03-02

AIR POLLUTANT EXPOSURES IN BUILDINGS (NERO, DAISEY)

- The High-Radon Project is developing a methodology for systematically identifying the areas of the United States containing most of the houses where occupants are receiving exposures to radon decay products comparable to or exceeding occupational dose limits.

- We have recently developed hierarchical statistical models of the correlation between physical factors and sparse indoor monitoring data, and also between different types of monitoring data, that yield estimates of mean indoor radon concentration by area, along with uncertainties in these estimates. This approach has been applied to Minnesota and Washington and appears to be capable of providing a topology of indoor radon exposures across the country.

- Although exposures to volatile organic compounds (VOCs) in indoor air are suspected to be one of the causative factors for "Sick Building Syndrome" (SBS) symptoms, efforts in field studies to link reported symptoms to VOC exposures, using the sum of the concentrations of all VOCs as the exposure metric, have been largely unsuccessful to date. As part of our effort to develop better metrics of exposure for VOC mixtures, we have assembled, analyzed and integrated existing animal and human data on the odor thresholds and irritancy of approximately 40 VOCs commonly found in indoor air.

- Using structure-activity relationships, we have estimated values for compounds for which we lack measured data. Metrics of exposure for odor and irritancy, based on weighting observed concentrations of VOCs by their relative potencies, have been developed and are being tested in a logit regression model for their ability to predict symptom reporting in office workers. Preliminary results indicate that the new metrics can be linked to certain SBS symptoms reported by workers in 12 California office buildings, i.e., eye irritation, nasal congestion. If confirmed, this will be the first scientific evidence from field studies which links VOC exposures and SBS symptoms.

MOLECULAR MECHANISMS OF CELLULAR EFFECTS OF HEAVY IONS (BLAKELY)

- GADD 45 response is dose- and LET-dependent—Examined the dependence of the GADD 45 gene induction response in normal human fibroblasts on the ionization density of the damaging radiation using x-rays (0.2 keV/μm), helium ions (7 keV/μm) and nitrogen ions (126 keV/μm). Confluent cultures of a normal human fibroblast (IMR-90 and AG-1522) and an AT fibroblast (AT-2SF) cell lines were irradiated in track segment experiments and assayed for GADD 45 mRNA immediately and up to 6 hours after exposure. At equivalent doses of cell killing, low-LET and high-LET radiations induce

approximately the same transcription levels of GADD 45, but with a delayed time course. These results therefore indicate an RBE effect since a lower dose of high LET radiation yields the same mRNA levels as a higher dose of low-LET radiation. In AT cells the GADD 45 mRNA induction after x-rays is diminished and delayed compared to the response of the normal human fibroblasts; induction after radiations with high-LET radiations are in progress. Our data indicate that differences in the nature of the lesions produced by radiations of different quality can lead to differences in the expression of the GADD 45 response. This mechanism is the first molecular handle that is available for investigating the cell's recognition of damage by radiations of different ionization density.

- Yield of translocations in human chromosome #2 after neon ions—Measured the yields of unrejoined breaks and exchange events per cell in human chromosome #2 in a normal human fibroblast (IMR-90) and in the UV24C2-3 human/hamster hybrid cell line. Using FISH combined with premature chromosome condensation (PCC) preparations that involve "painting" only the chromatin associated with the human #2 chromosome, we have measured 4.5 unrejoined breaks/cell immediately after a single dose of 10.6 Gy of accelerated neon ions at an LET of 183 keV/mm. Eight hours after irradiation of each cell line, the number of unrejoined breaks diminishes to 3 unrejoined breaks/cell, with a concomitant increase in the number of persistent exchange events over the same time period. These experiments indicate that the fate of the human #2 chromosome in the human/hamster hybrid is identical to that measured in the normal human fibroblast. Additional experiments are underway to examine the chromosome specificity and LET-dependence of persistent chromosomal lesions induced by ionizing radiations.

THE RESOURCE FOR MOLECULAR CYTOGENETICS (GRAY)

- Developed a high resolution technique to map clones probes along individual, linearized high molecular weight DNA molecules (e.g. YACs or P1 clones). This procedure allows localization of cloned probes along the linearized DNA with a precision of ~1kb. This technique will greatly improve the speed and accuracy with which physical maps can be assembled and will allow analysis of the extent of gaps in the map.

- The Resource operated a P1 clone selection and mapping facility with a throughput of ~30 loci per month. To date, 302 loci defined by STS have been screened with the P1 human genomic library. 400 P1 clones from 205 loci have been selected. 268 (68%) of the selected P1 clones mapped, by fluorescence in situ hybridization, to the expected target chromosome covering 155 loci (75.6%). Along with probes selected from other source, chromosome map location and FLpter (fractional location relative to the p-terminus) value have been determined for 257 probes covering 157 loci and 115 anonymous probes throughout the human genome. Probes are selected and mapped at a rate of ~40/month. The major focus of our probe selection is now on isolating P1 probes at chromosomal loci known or suspected to display copy number alteration or rearrangement in human disease. So far we have isolated P1's for tumor suppressor genes and oncogenes (c-MYC, GLI, SIS, SRC, MET, p53, Rb), translocation breakpoints of clinical significance (PML, RARA, TCRA, ETO, ALL), and regions involved in contiguous gene syndromes (Angelman, Prader-Willi, Cri du chat, Wolf-Hirschhorn and DiGeorge syndrome).

- The physical locations of 46 cosmid clones and 21 P1 clones were determined along the chromosome 20 axis relative to the p terminus (FLpter) using fluorescence in situ hybridization (FISH) and digital image microscopy. The cosmid clones were selected from the chromosomally enriched library LA20NC01. Nine P1 clones were selected from a pooled DuPont genomic library using PCR with primer pairs selected to amplify genetically mapped sequence tagged sites. This information was used to relate the physical map to the genetic map. Twelve P1 clones were selected from the same library using PCR primer pairs that amplified known genes. Two of these, E2F and BCLX, had not been mapped previously.

- We used fluorescence in situ hybridization (FISH) and digital image analysis to localize cosmids along human chromosome 17. Seventy one cosmids were selected at random from a chromosome 17 library constructed from a partial Sau3A1 digest of flow-sorted chromosomes from a mouse-human hybrid cell line. Sixty three of these (89%) gave a signal only on chromosome 17. The forty producing the most distinct hybridization signals in metaphase and interphase cells were precisely mapped using digital image analysis. An additional 20 cosmids, previously mapped by linkage analysis, were also mapped. The order of these probes determined by metaphase mapping was consistent with the order determined

by linkage analysis.

- The Resource collaborated on the development of a P1/YAC contig spanning a ~2Mb wide region on chromosome 20 that is amplified in ~25% of human breast cancer. This contig is now being used in efforts to identify genes from the region that may contribute to breast cancer progression. Gene identification will be accomplished by direct selection, exon trapping and direct sequencing (in collaboration with the LBNL Human Genome Center).

- We collaborated with Dr. Pieter De Jong to obtain a complete copy of his PAC library. This library will be pooled for screening in a manner similar to that used for the P1 library. Once completed, both the PAC and P1 libraries will be screened during molecular cytogenetic probe development.

- An Internet NCSA Mosaic browser has been developed for the Resource for Molecular Cytogenetics. This browser can be used to obtain information about probes, technologies and software developed by the Resource. In addition, the browser can be used to order probes. The Internet address is http://rmc-www.LBNL.gov/.

- Our program for comparative gnomic hybridization has been further improved. The new program is written in SCIL Image and is easily maintained. This program is now in routine use at LBNL, in the Division of Molecular Cytometry at UCSF and at research institutions in Finland, Japan, and Switzerland. An agreement has been made with Vysis, Inc. to distribute a version of this program commercially.

- Fluorescence in situ hybridization translocation analysis using chromosome-specific DNA libraries was used to determine occupational exposure of 50 x-ray technologists in U.S. hospitals. An automated metaphase finder developed by the Resource was used to analyze the large number of metaphase spreads required by the study (average=1150/ sample). The total number of metaphases analyzed during this study was 57,054.

- Molecular cytogenetics of prostate adenocarcinoma was advanced by the application of CGH to DNA samples from localized prostate cancer demonstrating excellent correlation between CGH and other molecular analyses (LOH) as well as detecting a region of DNA amplification (chromosome 8q) and five regions of DNA deletion (chromosomes 13q, 16, 17, 20q and Y) in localized prostate tumors. In recurrent and metastatic prostate cancer at least one genomic alteration was found in 89% of the tumors, with deletions occurring at twice the frequency of gains: average of 5.2 per tumor (range 1-11) for deletions versus 2.6 per tumor (range 0-12) for gains. Alterations ranged in size from whole chromosomes to less than 1/10 of a chromosome arm.

- An image acquisition tool, Xquips, featuring a Motif grafical user interface (GUI) was developed and is now in routine use with QUIPS imaging stations. In addition, a GUI interface, called sinapps, was added to the CGH analysis package. Both of these packages are "stand-alone", i.e. they run independent of any host image processing environment. This applications are part of our effort to develop stand-alone, single task applications for molecular cytogenetics research.

- A software package was developed to efficiently display 3D FISH images and to enable interactive enumeration of FISH signals in intact nuclei inside intact thick tissue sections. The package has been successfully tested using normal and cancer specimens.

- A procedure was developed correct images of autofluorescence. In this approach, autofluorescence is measured during excitation at a wavelength that does not excite probe fluorescence and a fraction of this is subtracted from images obtained when the illumination is adjusted to excite the probes. This procedure makes hybridization signals much more distinctive.

- The Resource was established with support from Vysis, Inc. Naperville, IL(formerly, Imagenetics). Vysis provides financial support to the Resource in return for the right to distribute probes and licence technologies developed by the Resource.

- The Resource and Vysis have been awarded a grant by the National Institute of Science and Technology to develop and microarray based procedure for comparative genomic hybridization. This grant will support development of technology to prepare arrays of probes on a solid substrate that will serve as a substrate for comparative genomic hybridization. In addition, the grant will support development of probes that will comprise the array.

- The Resource is collaborating with Vysis on

development of a commercial software package for comparative genomic hybridization. A CRADA application has been submitted to support this process.

MECHANISMS OF TUMOR PROMOTION (BISSELL)

- A well known metastatic suppressor gene, NM23, was shown to be a determinant of cellular structure. Its overexpression in metastatic human breast cells was shown to stop growth of these cells in three-dimensional cultures by allowing the cells to make a basement membrane.
- Integrins, extracellular matrix receptors, were shown to be involved in regulation of growth and apoptosis, but tumor cells were shown to lose both the regulation and the integrins
- We developed an exact replica of human breast tumors in three-dimensional cultures and for the first time conclusively described the cellular nature of stromal reaction, an early marker for breast cancer.
- A balance of extracellular-degrading enzymes (metalloproteinases) and their inhibitors are essential for functional differentiation and branching morphogenesis in vivo.
- Mammary glands from transgenic mice with stromelysin-1 (a metalloproteinase), under the control of a milk protein promoter, lose function and branch prematurely.
- Transgenic animals with a damaged basement membrane develop mammary carcinomas. Thus an abnormal microenvironment can cause genomic stability and epithelial cancer.
- Extracellular matrix (ECM) was shown to be crucial in preventing apoptic cell death. ECM does so by suppressing the Interleukin converting Enzyme (ICE, also called the Death Gene.)
- A change in cellular structure by ECM was shown to be crucial in regulating transcription of tissue-specific genes.
- We discovered a helix-loop-helix transcription factor (Id-1) that modulates growth and differentiation in breast epithelial cells.
- Determined that stromelysin transgenic animals have increased angiogenesis and developed "reactive stroma" in the breast. Using in situ hybridization, we showed that stromelysin-1 is expressed in the stroma, pointing to stromal-epithelial interactions in normal morphogenesis and function.
- Showed that the ECM-response enhancer in the promoter of ß-casein gene requires interactions with two transcription factors: CEBP-ß and STAT-5. Both are required, yet not sufficient. Stable integration into the genome is necessary for expression (i.e. transient transfections do not work). This finding and supportive evidence have major implications for transcriptional regulation in epithelial cells.

CELL CYCLE ACTIVATOR AND REPRESSOR GENES (CAMPISI)

- Constructed expression vectors for the following cDNAs: human Id-1; human Id-1'; human Id-2; T antigen defective in p53 binding; human mdm-2. These vectors were introduced into quiescent or senescent human fibroblasts with the following results.
- Identified that ability of the human Id-1 gene to complement an Rb-binding defect in T antigen was specific: the Id-related genes Id-1' and Id-2 showed only weak complementation. Thus, Id-1 is a specific cell cycle activator.
- Identified that a T antigen mutant defective in binding and inactivating the p53 tumor suppressor protein was less capable that the wild type of stimulating DNA synthesis. The mutant was much less active in senescent cells compared with quiescent cells. Thus, p53 is weakly growth inhibitory in quiescent cells, but strongly so in senescent cells.
- Identified that the human mdm-2 gene, which inhibits the ability of p53 to act as a transcription factor, complemented a p53 binding defect in T antigen.
- Constructed a human cDNA expression library for mammalian cells and yeast.
- Identified a novel ß-galactosidase activity an independent marker for cell senescence - unlinked to DNA synthesis—which we plan to use as an alternative screen for identifying genes important in

senescence and cell immortality.

IN VITRO TRANSFORMATION OF HUMAN MAMMARY EPITHELIAL CELLS (STAMPFER, YASWEN)

- Expression of lactoferrin, a differentiation marker which is often downregulated or absent in human breast tumors, has been shown to be critically dependent upon cell shape in human and mouse mammary epithelial cells.

- Cell shape has been shown to be modulated, in turn, by cell interactions with basement membrane components.

INTERSPECIES EXTRAPOLATION AND RISK ASSESSMENT (GOLD)

- Mutagenic heterocyclic amines formed in foods from heating amino acids or proteins show strong carcinogenicity in terms of positivity rates and multiplicity of target sites in rodents. However, concordance in target site between rats and mice is generally restricted to the liver. The levels of heterocyclic amines in typical cooked foods are relatively low, and their possible hazards rank quantitatively below those of many natural chemicals in foods.

- For several chemicals with reasonably reliable exposure data in the workplace, exposure levels are high when compared to rodent carcinogenic potency. The highest HERP (Human Exposure/Rodent Potency) values, all greater than 10%, are for ethylene dibromide (production workers, 1949-1975); styrene (fiberglass boat builders, 1983-1989); tetrachloroethylene (dry cleaners); and methylene chloride (triacetate fiber production workers, 1954-1977). By comparison, a risk of one in a million using standard regulatory methodology converts to a HERP of approximately 0.00001%.

- One measure of inter-species agreement in carcinogenicity is concordance: the percentage of chemicals that are classified in the same way for mice and rats, either both species negative or both species positive. Observed concordance in bioassays of the National Toxicology Program is about 75%, but we show by simulations that this 75% result can arise if the true concordance is 20%, 100%, or anything in between.

BONE MARROW STEM CELLS (NARLA)

- Engineered mice to model the human condition *Hydrops fetalis*. We have demonstrated that the lethality of this condition is due exclusively to red cell globin abnormalities and that the lethal phenotype can be corrected through the transfer of a human alpha globin transgene. A manuscript describing this studies is in press Nature Genetics.

KP-03-03
RADIOLOGICAL PHYSICS (CHATTERJEE)

- Interaction between energetic charged particles and chromatin provides important structural information.

- Ionizing radiation produces clusters of damage that are quite unique and may be difficult to repair.

General Life Sciences

KP-04-01
BIOPHYSICAL CHEMISTRY (KLEIN AND YACHANDRA)

- Performed the first Coherent Raman Beat EPR spectroscopy on transition metal complexes and proteins and established a significant improvement in sensitivity and spectral resolution compared with traditional electron spin echo experiments.

- Performed extensive set of EPR experiments on Anabena ferredoxins containing specific isotope substitutions in residues providing ligands to the iron-sulfur cluster. Compared Electron Spin Echo Envelope Modulation with Coherent Raman Beat measurements.

- Discovered that Coherent Raman Beats can be observed with only application of the observe microwave field. It was previously stated that a preparation sequence of hard or soft pulses is required.

MUTAGENESIS: FUNDAMENTAL CHEMISTRY (HEARST)

- Human Excision Repair
  Human excision repair of psoralen monoadducts has again been demonstrated in cell free extracts of

HeLa cells. The excision patch has been proven to be 29 bases. This is an initial step toward a detailed understanding of the mammalian excision repair mechanism.

- Solution structures of psoralen adducted DNA oligomers
  The large scale synthesis of DNA oligomers containing psoralen adducts which was developed in the past year has been used to synthesize enough of a specific adduct so that a two dimensional NMR analysis could be completed. The 3D structure of the monoadducted DNA oligomer and the crosslinked oligomer have been established and will be submitted for publication in the coming year. This work has been accomplished by Peter Spielmann in collaboration with David Wemmer of the Structural Biology Division. The structures are not highly kinked, suggesting that earlier conclusions from NMR data relating to the structures of psoralen adducted DNA may be based on a misinterpretation of the DNA. This question is crucial for the issue of how repair enzymes identify damaged sites in DNA.

X-RAY ABSORPTION SPECTROSCOPY (CRAMER, RANDALL)

- Taking advantage of the high brightness of the Advanced Light Source undulator beamlines, we were able to collect preliminary soft x-ray *L* emission spectra of Ni and Zn complexes.

- Collected high-resolution Kß x-ray fluorescence data on Mn, Fe and Ni complexes and proteins. Recorded site-selective EXAFS data of a Mn(II,III,III) trinuclear complex.

SOFT X-RAY MICROSCOPY (GLAESER, MORONNE)

- Determined that avidin and secondary antibody polychelate lanthanide conjugates yield useful luminescence with X-ray doses exceeding 10^{11} rads. Calculations using this data indicate that an Advanced Light Source based scanning X-ray microscope should be able to image fine structures such as microtubules with considerably better signal to noise ratios than by confocal light microscopy and with five times or better resolution.

- Succeeded in eliminating much of the background labeling associated with the first generation of lanthanide probes. Exhaustive modification of residual amino groups on the polychelates results in products with greatly improved molecular specificity. This was verified using tracer fluorescein and rhodamine labels and confocal light microscopy. Further, we have shown that biotinylated polychelates used in conjunction with standard avidin labeling provides an effective alternative labeling strategy. This makes it possible for biologists to use conventional labels requiring only a single additional incubation to attach the polychelate for X-ray visualization.

SEMICONDUCTOR X-RAY DETECTORS FOR SYNCHROTRON APPLICATIONS (JAKLEVIC, ROSSINGTON)

- Developed a second generation multi-element silicon detector, with integrated circuit readout electronics, for synchrotron x-ray fluorescence applications. The 200 element silicon detector, with a new low noise IC amplifier chip, achieves 350 eV FWHM at 6 keV at room temperature, and <250 eV FWHM when cooled slightly. The detector and IC electronics chip will be incorporated into an x-ray spectrometer for full data acquisition, utilizing 64 of the 200 channels, for use in synchrotron experiments.

MOLECULAR STRUCTURE OF MEMBRANE TRANSPORT SYSTEM (JAP)

- Successfully reconstituted the water-channel protein, CHIP28 (an integral membrane protein), forming two-dimensional crystalline patches suitable for high resolution structure determination by electron crystallography.

- Obtained preliminary high resolution projection map of CHIP28 at 3.5Å resolution, showing the architecture of the channel.

- In collaboration with Max Planck Institute in Germany, we have solved the structure of proteasome (the central enzyme of non-lysosomal protein degradation) to 3.4Å resolution.

- Greatly improved the quality of cytochrome reductase crystals so that the new crystals diffract to a resolution of about 3.4Å.

- Have successfully flash-frozen the cytochrome reductase crystal without significantly altering its diffraction quality. This procedure will allow a full high resolution data set to be collected from a single crystal.

- Obtained small three-dimensional crystals of maltose transporter.

- Obtained a tentative molecular replacement solution for calmodulin-like protein; it can be expected that the complete structure will be solved soon.

- Cloned the CFTR (cystic fibrosis transmembrane conductance regulator) into a vaccinia expression system.

BIOMOLECULAR STRUCTURE ANALYSIS BY NMR (WEMMER)

- NtrC is a bacterial transcription factor involved in regulation of nitrogen uptake. It has a receiver domain which becomes phosphorylated, and in then interacts with a neighboring domain of the same protein stimulating oligomerization and opening of the polymerase complex. We determined the solution structure of the receiver domain, and can interpret some mutants to identify the binding interface. We found conditions under which the protein can be maintained in the phospho form for long enough to collect NMR data. (Collaboration with Prof. S. Kustu, MCB)

- HSF, the heat shock transcription factor, is a critical component of the heat shock response in eukaryotic cells. We are studying the yeast version of this protein to understand its DNA binding and the nature of its activation domains. We have solved the solution structure of the DBD by NMR (and compared it with the crystal structure from the Nelson lab), which indicates that it is in the helix-turn-helix family but with an unusual distortion in one of the helicies. We have now made a dimer construct which has high binding affinity and similar binding characteristics to the native trimer. We have expressed an activation domain, which is the N-terminal segment of the protein bordering the DNA BD, linked to the DNA BD. (Collaboration with Prof. H. Nelson, MCB.)

- Sxl is a critical genetic switch which maintains the male/female character of an organism which is set very early in development. This regulation occurs through binding of Sxl to messenger RNAs, which affects splicing both of its own gene product and a number of others. Two central RNA recognition motif domains are critical for this. We have expressed these two domains, both linked together and each individually. The structure of the second domain is complete. The data show clearly that the two domains are individually structured. (Collaboration with Prof. D. Rio MCB.)

- FUR is a bacterial repressor which controls a gene cluster involved in iron uptake. The repressor binds iron, and in then binds DNA and represses transcription. We have separated the DNA binding and metal binding domains, and have much of the metal binding domain (a tetramer under NMR conditions) assigned. These data already indicate that a published model for the structure of this protein is incorrect, and should soon lead to a corrected version.

STRUCTURAL BIOPHYSICS (KIM)

- Two inhibitor drugs of CDK2, a human cell cycle controlling protein kinase, have been complexed with the CDK2, and the three-dimensional structures of the complexes have been determined. The structures revealed surprising ways the inhibitors bind to CDK2. These results provide a foundation for refined drug design to discover better inhibitors of CDK that may have anti-cancer effects.

ARTIFICIAL ENZYMES (SCHULTZ AND WEMMER)

- Synthesized a tertiary N-oxide hapten, an antigen to generate antibodies containing active site acidic and basic residues that might act in concert to catalyze the hydrolysis of the corresponding amide. Although catalytic antibodies were not obtained it was shown that a hapten containing only a secondary ammonium could induce antibodies that catalyzed the hydrolysis of the corresponding carbonate.

- Completed the synthesis of an azirdine hapten for generating antibodies with epoxide hydrolyase activity. Over thirty hybridomas have been generated and assay conditions are currently being developed. Beginning assays of the kinetics and stereochemistry of antibody-catalyzed epoxide hydrolysis. These reactions may be applied to both the detoxification of environmental pollutants and to stereoselective synthetic chemistry.

- Completed the synthesis of a phosphinate hapten for generating antibodies that selectively hydrolyze a prodrug to a cytotoxic agent. Specifically we hope to hydrolyze a trimethylbenzoate ester to a nitrogen mustard derivative, a commonly used anticancer

agent, to foster selective activation of prodrugs at cancerous cells. Hybridomas production has already generated over 20 cell lines have been generated.

THREE-DIMENSIONAL STRUCTURE OF CATALYTIC RNA (TINOCO, KIM, AND WEMMER)

- Obtained high resolution crystals of a hammer-heard catalytic RNA from a plant virusoid and x-ray crystallographic data collected to a resolution of 2.2Å.

SEMICONDUCTOR X-RAY DETECTORS (JAKLEVIC)

- Applied for a patent for a new, large area, low capacitance Si(Li) radiation detector for synchrotron applications in biomedical and environmental sciences. Spectrometers using this new detector are being utilized in synchrotron EXAFS and microprobe experiments, which improve the counting rate eight-fold over conventional spectrometers and improve the energy resolution two-fold.

KP0402
GENETIC STUDIES ON YEAST (MORTIMER)

- Found that the predicted amino acid sequence of the *RAD54* protein contains a potential zinc finger. This region shares considerable homology with the two zinc fingers of mammalian poly (ADP-ribose) polymerase, that have been extensively characterized and shown to be involved in the binding of this enzyme to both single-strand nicks and double-strand breaks.

- Published the sequences of *RAD51*, *RAD54* and *RAD57*. Both *RAD54* and *RAD51* are inducible by DNA damaging agents.

- Demonstrated that natural fermentations have as many as 16 different strains of *Saccharomyces cerevisiae* participating. This confirms and extends studies carried out in France and in Switzerland.

- Established that *Saccharomyces cerevisiae* exists on the grape surfaces and is introduced into the must at the time of crushing. This ends a long-standing controversy about the origin of these yeasts.

- Demonstrated that inoculated fermentations may have none of the inoculum at the end of fermentation; only natural yeast persist. This is an important finding given mat >90% of nearly 1000 California wineries use an inoculum.

- Established that the yeast entering a fermentation from given vineyards are not repeated from one year to the next. This may be one of the factors that determines "good" and "bad" years.

- Proposed a model for the rapid evolution of natural yeast strains that has gained wide acceptance.

- Testing a model that states that the natural strains of *Saccharomyces cerevisiae* reside in tree exudates and are transported to the grapes by insects during the few weeks prior to harvest.

KP0404
HUMAN GENOME CENTER (NARLA, PALAZZOLO, MARTIN, RUBIN, JAKLEVIC, EECKMAN)

Sequencing Production

- Developed a directed strategy for large-scale sequencing and implemented this strategy to complete more than 2 million base pairs of finished sequence.

- Successfully collaborated with the LBNL HGC Automation and Computation Groups to begin to develop an automated environment for sequencing.

Genome Informatics

- Developed a community database, 21Bdb, for the sharing of physical map information on human chromosome 21.

- Developed a database, Syndb, specifically designed to be integrated into the production sequencing factory at LBNL. Like 21Bdb, Syndb is built on the ACEDB framework, with special facilities for showing several different kinds of physical maps: cross species syntenic maps involving human chromosome 5 and mouse chromosomes; P1 STS content maps of any human chromosome; DOGtag maps of individual P1 clones; transposon maps of individual DOGtags.

- Created (with collaborators in Cambridge, England and UCB) a Macintosh version of ACEDB and our databases, so that the information contained in them can be widely disseminated by simply supplying a

compact disc, with the usual Macintosh look and feel.

- Collected application programs which provide image analysis (for STS-content maps and DOGtag maps), the display and analysis of transposon data acquired from the imaging station, a customized version of a widely used trace editor, the acquisition and incorporation of YAC physical map data from CEPH.

- Developed a database, Flydb, for the display of a detailed physical map of the organism *D. melanogaster*, as part of the collaboration with the Drosophila Genome Center at UC, Berkeley.

- Developed a system for data acquisition, display, quality control of markers and primer determination for STS-content mapping of clones appearing on the P1 physical map of *Drosophila.*

Human Biology

- Developed a clone based map of chromosome 21q22. This region encompasses much of the Down syndrome region at 21q22.2 and includes the markers CBR and PCP-4 between which mapping studies have indicated the weaver gene lies.

- Demonstrated YAC transgene expression at appropriate location. The STS CBR is contained within the gene for human carbonyl reductase which is considered to be a housekeeping gene. The STS resides within YAC 230E8 and a line of mice containing this YAC expresses the carbonyl reductase gene fairly uniformly in all tissues examined. The STS D21S267 is contained within a transcription unit which has been shown to be expressed predominantly within the human brain. This STS is found within the YACs 141G6 and 152F7 and two lines of mice each containing one of these YACs express the gene containing this STS and furthermore expression of the human gene is particularly strong in the mouse brain.

Sequencing Template Production/ P1 Clone Mapping

- Made progress on generating a P1 map in the interleukin gene cluster on chromosome 5q. This region is roughly defined by two genes, the distal IL3 and the proximal IL4, which are 700 Kb apart. Four YACs (A94G6, 811C11, 854G6 and 885G11) were identified by filter hybridization screening of a set of chromosome 5 YACs using IL3 and IL4 sequences.

- Closed the gap and a tiling path of 16 P1s covering approximately 1.2 Mb of contiguous DNA from the 5q31 has been provided to the production sequencing group.

- Carried out the screening of the P1 library using probes generated from the distal 14 Mb of the chromosome 21 and identified over 600 P1 clones.

DATA MANAGEMENT TOOLS FOR GENOMIC DATABASES (MARKOWITZ)

- The OPM data management tools have been extended in order to address specific requirements of developing genome databases such as GDB, GSDB, and PDB. The following OPM data management tools are currently available via World Wide Web at (http://gizmo.LBNL.gov/opm.html):

- The OPM Schema Editor for specifying OPM schemas; this editor provides facilities for specifying new OPM classes, modifying existing OPM classes, combining (merging) different OPM schemas, browsing OPM schemas, and outputting OPM schemas in Latex, PostScript, and HTML formats.

- The OPM Schema Translator automatically generates relational DBMS database definitions representing OPM schemas and DBMS procedures implementing the OPM (retrieval and update) methods; the OPM Schema Translator also generates data integrity procedures for maintaining the constraints (e.g., referential integrity and domain constraints) implied by the OPM constructs, and a metadata file containing the OPM mapping information;

- A querying, browsing and data entry tool for entering OPM data into OPM-based databases, specifying OPM queries, and browsing OPM data using a WWW interface.

- The OPM data management tools are currently used for developing several genome databases, such as version 6 of GDB and the new version of PDB, for constructing an object layer on top of GSDB, and for developing a large scale sequencing LIMS at Myriad Genetics, Inc.

HUMAN GENOME COORDINATING COMMITTEE AND OPERATIONS (SPENGLER)

- The HERAC Subcommittee on the Genome prepared and distributed its report assessing the status of the Genome Program.
- Made nine accurate models of biologically interesting macromolecules. These included: models of DNA (copies at OHER) for education projects, a model of the protein, subtilisin, now used by the Smithsonian in a permanent exhibit, models of the HIV protease monomer (at DOE headquarters), and the HIV protease dimer with inhibitor bound.
- Drafted a primer on statistics associated with the Genome Program. This is a companion to the Primer on Molecular Genetics for the Genome, DOE Report, ER-0544P.

LASER MICROSCOPY (SPENGLER)

- Decreased image acquisition time for LFM from 3 minutes to 20 seconds, permitting analysis of biological materials in a time-dependent manner and minimizing the setup time. Biological specimens imaged include types of yeast, chick embryo spinal ganglia, salamander photoreceptor cells, and the chloroplasts and mitochondria in green algae.

LABORATORY INFORMATION MANAGEMENT SYSTEMS (ZORN)

- Beta Release of SubmitData\GSDB for data submission to GSDB.
- Submission of over 1Mb of sequence data from LBNL to GSDB using SubmitData\GSDB.
- Announcement of a public web server for data produced at the Resource for Molecular Cytogenetics.
- Demonstration of sequence analysis on a workstation farm using BioPOET.

MASS SPECTROMETRY DETECTORS (BENNER, JAKLEVIC)

- Completed construction of a linear MALDI mass spectrometer and with it we obtained resolution as high as 1050 for 30-mers of ssDNA.
- Used the MALDI mass spectrometer to determine the molecular weight of 3 DNA-binding proteins.
- Used MALDI mass spectrometer as a charged particle/ion source for testing MOS and phosphor detectors.
- Upgraded an electrospray ion source for use with an orthogonal-type of time-of-flight mass spectrometer.
- Developed the capability to generate highly charged submicron diameter test particles from an electrospray source for use as surrogate large molecular weight ions
- Successfully tested a fiber optic module to decouple microchannel plate signals from a detector operated at high voltage; 5 ns wide pulses were transmitted and received without significant signal degradation.
- Fabricated several hundred MOS devices in the silicon labs at LBNL with a cost savings of more than $ 50,000 when compared to outside vendor fabrication estimates and these devices were tested as particle/ion detectors.
- Measured MOS detector bias voltage ramp rates, maximum operating voltages, and sensitivity to particle impact as a function of oxide thickness.
- Studied two heat stimulable phosphors to determine the areal density of light trapping centers and we estimate that about 1014 centers exist per cm2 of phosphor film. At this density, impacts should be registered because the dead area between light trapping centers is minimal.
- Measured thermoluminescent glow curves (a plot of photons released vs. temperature) for these phosphors.
- Tested a commercially available avalanche photodiode detector as a large ion detector. It was found to be incapable of detecting ions but registered the impact of electrons having energy greater than 15 keV.

Medical Applications

KP0601
VASCULAR AND BLOOD DISEASES (EBBE, TAYLOR)

- Working on the development of a porphyrin-based radiotracer for atherosclerosis. Work centered on two different porphyrin molecules, hematoporphyrin derivative and uroporphyrin, both of which exhibited significant and selective uptake as unlabeled compounds into lesions in hypercholesterolemic rabbits. Developed methods to radiolabel both compounds by complexing with ^{68}Ga, and in vivo tracer studies, including PET, were performed with both. The Ga-uroporphyrin exhibited the best stability and plasma clearance of the two, though the aorta to plasma ratios are still quite low.

- Developing methods for the ^{68}Ga-labeling of a similar set of compounds, the phthalocyanines. Phthalocyanines will accumulate in the extracellular matrix of the zone of smooth muscle cell proliferation that caps a fully developed atherosclerotic lesion in humans. Observed uptake of some non radioactive Ga-phthalocyanine tetrasulfonate into the diseased aorta following injection.

- Studied the potential of ^{68}Ga-loaded liposomes as imaging agents. ^{68}Ga-deferoxamine was loaded into phosopholipid/cholesterol liposomes for in vivo uptake studies. Blood clearance rates were too slow for these compounds to be utilized *as* PET agents.

- Determined megakaryocyte ploidy and size and blood platelet number and volume in genetically abnormal mice that have a defect in the ability of platelets to bind and release 5-hydroxytryptamine (5-HT). The platelets were normal, but the megakaryocytes were of larger than normal size for their nuclear DNA content. These findings suggest that 5-HT may play a role in the feed-back regulation of megakaryocytopoiesis. Canine megakaryocytes were found to be of higher ploidy than human megakaryocytes. In spite of this, platelet mass in the two species is similar. This supports the notion that megakaryocyte ploidy may not be an important determinant of platelet count and size.

EXPERIMENTAL MEDICAL, CLINICAL, AND DIAGNOSTIC ISOTOPES (BUDINGER)

- Accomplished quantitative whole body PET studies in breast, prostate and lung cancer in an on-going efficacy study to show the benefits of PET relative to X-ray CT and magnetic resonance methods.

- Assessed patient motion during PET heart studies.

- Studied kinetics of Rb and Tl in model and rabbit heart.

- Evaluated a mitochrondial avid radiopharmaceutical for PET.

- Image processing for 3-dimensional volumes of interest from 3-D datasets at arbitrary orientations.

- Developed new algorithms for reconstruction tomography applicable to functional imaging.

- Conducted an evaluation of the relative merits of hyperpolarized xenon ^{129}Xe MRI vs radioactive ^{127}Xe or ^{133}Xe.

- Developed a new prototype medical isotope cyclotron known as Deep Valley I cyclotron as a DOE SBIR program with CTI corporation. Through funding of this FTP, the first commercial model was completed and successfully brought to production performance. After a 1 year period of further development in Knoxville, Tennessee, this cyclotron is being moved to LBNL in June, 1995.

- Dynamic SPECT was shown theoretically to be able to measure quantitatively brain and heart blood flow.

- Conducted Parkinson's disease studies using PET to evaluate gene therapy in collaboration with Somatix Therapy Corporation. Imaging studies of monkey basal ganglia using high resolution PET and m-tyrosine labeled with $^{F}18$ were successful.

EXPERIMENTAL MEDICAL DEVELOPMENT OF RADIONUCLIDES (VAN BROCKLIN)

- Relocated, with the cooperation of CTI, Inc., our 11 MeV medical cyclotron, to the CTI facility in Berkeley. In addition to training on the machine, we participated in the production of [^{18}F]fluoride and [^{18}F]fluorine gas for radiopharmaceutical production and new tracer development.

- Produced 129 batches (~100mCi/ batch) of [^{18}F]fluorodeoxyglucose for neurologic, cardiac, and tumor imaging studies in non-human primates and clinical research patients.

- Produced [^{123}I]iodonitroquipazine for evaluation as a serotonin uptake site marker in non-human primates. Based on these studies, we anticipate that

this agent will be the first useful serotonergic agent for non-invasive studies in humans.

- Commenced construction of the Biomedical Isotope Facility (Bldg. 56) on September 15, 1994 with completion expected by June 1, 1995.

- Evaluated several ^{18}F and tritium (collaboration with the NTLF) labeled analogs of rotenone as mitochondrial imaging agents to study the loss of mitochondrial function in the normal aging process and the neurological diseases, Parkinson's and Huntington's. Studies in small animals and isolated rabbit hearts have shown this compound to be very specific for mitochondrial rich tissues. This represents a novel approach to evaluate brain energy metabolism and may also have applications to heart metabolism and cancer studies.

- Discovered an unexpected chemical rearrangement which occurred during the nitration of a precursor for fluorine labeling. This was reported in a note to the Journal of Organic Chemistry.

- Discovered a mechanism for the unexpected ^{18}F labeling of meta-substituted benzocyclanones. Understanding this mechanism will provide the potential of extending this methodology to label other important radiopharmaceuticals.

POSITRON EMISSION TOMOGRAPHY INSTRUMENTATION (DERENZO)

- We have developed a novel PET detector module using the following state-of-the art components (i) lutecium orthosilicate scintillators, (ii) low-noise solid state photodetectors, (iii) integrated circuit charge amplifier arrays. This design is a major advance in terms of 3D spatial resolution and data rates.

- We are working with one of the leading manufacturers of positron tomographs to construct a high resolution animal/brain tomograph at LBNL. Relative to the best previous commercial designs, this machine will improve the maximum data rate by a factor of ten and the spatial resolution by a factor of two.

- This project is credited with the discovery of the scintillators CeF3, PbWO4, and LuAlO3:Ce, all of which have been produced in the form of large, clear crystals and are being considered for large-scale production.

IMAGING OF APOLIPOPROTEIN E-BINDING RECEPTORS *IN VIVO* (KRAUSS)

- The effect of the EV trimer on LDL receptor binding was also examined in a direct binding assay using ^{125}I-LDL with addition of varying amounts of the trimer. With no added trimer LDL bound to a single site (the LDL receptor) with a Kd of 13μg/ml and a binding maximum of 19 ng. With addition of the EV trimer, the LDL bound to the same site, but the binding curve exhibited a second component, with approximately five-fold weaker binding, and a greater than three-fold greater binding capacity. Thus, the stimulation of LDL binding by the EV trimer may involve two binding sites—one, the LDL receptor, and a second, low affinity, high capacity site which may represent a second apoE-binding receptor.

- Results to date from five subjects reveal a substantial reduction of binding affinity of LDL-I fractions upon removal of apoE: Kd (μg/mg cell protein ± SD) was 11.5 ± 5.2 for the total fraction, vs. 18.0 ± 6.2 for the apoE-free fraction. (p=0.1). Thus, as was suggested by the EV peptide studies above, the addition of apoE to an LDL particle appears to confer substantial increase in LDL receptor binding affinity. This property may have important implications for the potential therapeutic value of agents that may confer this property *in vivo*.

TABLE 7: Major Examples of Collaborative Research

The Resource for Molecular Cytogenetics serves as a model of collaborative interaction. The resource staff collaborate with numerous groups throughout the the World. Active collaborations include:

UCSF/LBNL

Dr. Judith Campisi, Life Sciences Division, LBNL, Berkeley, CA. Mapping of genes involved in cellular senescence.

Dr. William Morgan, Dept. of Radiation Biology, UCSF, San Francisco, CA. Characterization of translocation breakpoints on human chromosomes following exposure to restriction enzymes.

Dr. David Young, Veterans Administration Medical Center, San Francisco. Vascularization of transplanted human skin.

Dr. Mike Skinner, University of California, San Francisco. Investigation of the distribution of bovine, murine and human tissues in multi-tissue ovarian tumor models developed in nude mice.

Dr. I. Craig Henderson, University of California, San Francisco. Investigation of the genetics of chemoresistance in human breast cancers.

Dr. Marco Zarbin, Eye Research Center, UCSF, San Francisco. Development of techniques to differentiate between graft and host cells in sex mismatched RPE-cells transplantations.

Dr. Charles Vidair, Dept. of Radiation Oncology, UCSF, San Francisco, CA. Heat shock induced aneuploidy in Chinese hamster cells.

Dr. Thea Tlsty, University of North Carolina. Investigation of genetic instability in human malignancies

Dr. Douglas Hanahan, University of California, San Francisco. Investigation of genetic progression in transgenic murine tumors.

Dr. Harold Varmus, University of California, San Francisco (and the NIH). Investigation of genetic aberrations in transgenic mammary tumors.

Dr. Devron Char, University of California, San Francisco. Investigation of genetic progression in Ocular Melanoma.

Dr. Fredric Waldman, University of California, San Francisco. Investigation of genetic progression in Breast and Bladder Cancer.

Dr. Burt Feuerstein, University of California, San Francisco. Investigation of genetic progression in human Brain Tumors.

Dr. Helene Smith. California Pacific Medical Center. Investigation of genetic progression in Breast Cancer.

Dr. Ronald Jensen, University of California, San Francisco. Investigation of genetic progression in human prostate cancer.

Dr. Maria Pallavicini, University of California, San Francisco. Investigation of genetic progression in human leukemias.

Dr. O. Clarke, Dept. of Endocrine Surgery, UCSF, San Francisco, CA. Cytogenetic aberrations in thyroid cancer.

North America

Dr. Teresa Yang-Feng, Yale University. Investigation of genetic progression in Ovarian Cancer.

Dr. Julie Korenberg, Cedars Sinai Medical Center, Los Angeles. Application of Comparative genomic hybridization in prenatal diagnosis.

Dr. Norman Doggett, Los Alamos National Laboratory. Investigation of genetic aberrations on chromosome 16 associated with breast and prostate cancer.

Dr. Peter K Rogan, Division of Genetics, College of Medicine, Penn State University. Application of P1 probes for clinical diagnosis of deletion syndrome.

Dr. Lillian Y F Hsu, Prenatal Diagnosis Laboratory of New York City and Dept. of Pediatrics, New York University, Medical School. Application of P1 probes for clinical diagnosis of deletion syndrome.

Dr. Peter Quesenberry, Cancer Center, University of Massachusetts, Worchester, MA. Application of mouse Y chromosome-specific probes to differentiate between graft and host cells in sex mismatched bone marrow transplantations in mice.

Dr. Santiago Munne, IVF-lab., Cornell University Medical Center, New York, NY. Cytogenetic studies in preimplantation embryos.

Prof. Andrei Dyban, Reproductive Genetics Institute, Chicago, Il. Cytogenetic analysis of germ cells in mice carrying balanced reciprocal translocations.

Dr. Marille Herrmann, Dept. of Surgery, Henry Ford Hospital, Chicago, Il. Cytogenetic aberrations in endocrine tumors.

Dr. Scott Cram, National Flow Resource, LANL, Los Alamos, NM. Generation of high complexity libraries for rat chromosomes by AP-PCR from flow sorted chromosomes.

Dr. Verne Chapman, Roswell Park Cancer Center, Buffalo, NY. Generation of high complexity DNA libraries for the mouse X chromosome.

Dr. Laurie Shepel, Cancer Center, University of Wisconsin, Madison, WI. Generation of high complexity libraries for rat chromosomes by AP-PCR from flow sorted chromosomes.

Dr. Andrew Wyrobek, Lawrence Livermore Natl. Laboratory, Livermore, CA. Determination of baseline frequencies of aneuploid sperm in normal human donors.

Dr. Ben Seon , Roswell Park Cancer Center, Buffalo, NY. Determination and control of cytogenetic markers during establishment of a mouse model for CLL.

Dr. Michael Andreeff, MD Anderson Cancer Center, Houston, TX. Detection of minimal residual disease in CLL by fluorescence activated cell sorting and fluorescence in situ hybridization.

Dr. James Allen, EPA, Research Triangle Park, NC. Specific probes for mouse and hamster chromosomes for detection of aneuploidy in various tissues after exposure to mutagenic or aneuploidogenic agents.

International

Prof. I.T. Young and Dr. Lucas van Vliet, Pattern Recognition Group, Delft University of Technology, The Netherlands. Characterization of CCD cameras.

Dr. Kohsuke Sasaki, Iwate Medical University, Morioka, Japan. Investigation of genetic progression in solid tumors.

Dr. Olli-Kallioniemi, Tampere University Hospital, Tampere, Finland. Investigation of genetic progression in human breast cancer.

Dr. James Piper, MRC Edinburgh, Scotland. Development of improved software for image cytometry.

Dr. Trond Stokke, Oslo Norway. Positional cloning of an amplified region of chromosome 20 in human breast cancer.

Dr. Masaru Sakamoto, Sasaki Institute of Medicine, Tokyo, Japan. Investigation of genetic progression in Ovarian cancer.

Dr. Lars Bolund, Aarhus University, Denmark. Investigation of the utility of comparative genomic hybridization in clinical genetics.

Prof. Manfred Bauchinger, gsf-Forschungszentrum, Neuherberg, FRG. Stable chromosomal changes following low level ionizing irradiation in humans.

Dr. Beate Miller, Hoffmann-La Roche, Inc., Basel, Switzerland. FISH applications in the micronucleus assays for assessment of aneuploidogenic and clastogenic effects of various agents.

Drs. J.J. Cassiman and I. Wlodarska, Center for Human Genetics, University Leuven, Leuven, Belgium. Characterization of marker chromosomes in haematological tumors by WCP-FISH.

Dr. Sandor Szuecs, University Munich, Munich, FRG. Cytogenetic abnormalities in renal cell carcinomas (RCC's).

Dr. K. Harry Scherthan, University Kaiserslautern, FRG. Direct visualization of homologous regions on metaphase chromosomes from different mammals by chromosome painting.

TABLE 8: Patents, Licenses, & CRADAs in FY 1994 & FY 1995

BIOTECHNOLOGY & HEALTH CRADAS

Amgen/Schwartz, Rick
Tendon Repair Factor
Develop a drug for improving tendon and ligament repair.

Glycomed/Nagy, Jon
Synthesis of New Carbohydrate-Based Materials for use in the Construction of Biocompatible Structures and Devices. Vehicles for controlled delivery of pharmaceutical agents and bioactive materials.

Chiron/Kim, Sung-Hou
Structure Determination of M-CSF and its Variants.
Determine 3-dimensional structure of M-CSF and several site-specific mutant. Understand the complex mechanism of immune regulation and development.

Somatix Therapy/Jagust, William
Neurochemical Imaging of Gene Therapy.
Apply two technologies, neurochemical imaging and gene therapy, which will permit the insertion of genes into cells to develop therapeutics for Parkinson's disease.

General Dynamics/Bastacky, Jacob
Microphotogrammetry
Combine photogrammetry and scanning electron microscopy to develop a new field, microphotogrammetry, which will apply the methods of photogrammetry to images obtained with the scanning electron microscope.

Rhône Poulenc-Rorer/Rubin, Edward
Cloning Genes for Diabetes, Obesity and Atherosclerosis. Clone mouse genes responsible for diabetes and obesity in the mouse and isolate therapeutic agents for the treatment of these diseases in humans.

Vysis/Pinkel, Daniel (NIST)
Development And Commercial Application Of Genosensor Based Comparative Genomic Hybridization (CGH). Develop and evaluate techniques and DNA reagents for performing CGH to arrays of DNA on solid supports.

General Atomics/Chu, William
Medical Accelerator Technology
Adapt and transfer LBNL-developed technologies relevant to the use of accelerated beams (e.g. heavy charged-particle radiotherapy) for the treatment of human cancer.

Kaiser Foundation Hospital/Johnston, William
National Information Infrastructure (NII) Prototype for Distributed Health Care Imaging
Develop enabling technologies that will provide extremely high speed distributed computing and health care imaging data such as coronary angiograms.

Amgen, Inc./Earnest, Thomas
Structure of the Erythropoietin Receptor
Determine the 3-dimensional structure of the erythropoietin receptor (EPO-R is the primary hormone involved in the production of red blood cells.) by the use of electron crystallographic techniques.

SMALL BIOTECHNOLOGY & HEALTH CRADAS

Computer Tech. & Imaging (CTI Positron)/ Derenzo, Stephen
Positron Tomograph with Improved Resolution and Data Rates.

Wang NMR/Roos, Mark
Low Cost Magnetic Resonance Imager for Breast Cancer Diagnosis.

BIOTECHNOLOGY TECHNOLOGY MATURATIONS

Select California Wineries/Mortimer, Robert
Characterization and Genetic Improvement of Wine Yeast

Gatan/Bastacky, Jacob
A Specimen Holder for Low Temperature Scanning Electron Microscopy.

BIOTECHNOLOGY PERSONNEL EXCHANGES

BioGenex Laboratories/Bissell, Mina
ECM Markers as Prognostic Indicators of Breast Cancer Using LBNL's Reconstituted Basement Membrane Assay.

OTHER BIOTECHNOLOGY PARTNERSHIPS

Cotton Biotechnology Project
AMTEX - Cotton Incorporated/John McCarthy TReC
Textile Resource Conservation Project
Fish, Richard H. /Mehlhorn, Rolf J.
Implement appropriate genome technology to increase cotton fiber performance and reduce the cost of the product for the U.S. textile industry.

ENERGY & ENVIRONMENT CRADAS

Enzymol International/Glazer, Alexander
Enzymatic Remediation of Waste Streams
Develop the enzyme soybean peroxidase as a commercial product for the degradation of toxic waste chemicals such as polychlorobiphenols (PCBs), nitroaromatics and chlorophenols.

DuPont Company/Somorjai, Gabor
Catalytic Conversion of Chloro-Fluorocarbons over Palladium-Carbon Catalysts
Develop alternate refrigerant chemicals to replace chloro-fluorocarbons because of their adverse effects on the earth's protective ozone layers.

ENGINEERING SMALL CRADAS

eV Products Inc./Luke, Paul
Development of Room Temperature Semi- Conductor Radiation Detectors

DIGIRAD/Millaud; Nygren
Instrumentation of Gallium Arsenide Pixel Detectors

Personnel Exchanges

Fisons/Kevex/Rossington, Carolyn S.
Technology Transfer of Fabrication for New Si(Li) X-Ray Detector

ENGINEERING TECHNOLOGY MATURATION

Applied Electron/Walton, Jack
X-Ray P-type Silicon Drift Detector (x p-SiDD)

PATENTS FILED

Inactivation of the E-Cadherin Gene in Breast Cancer Through Mutation and Deletion
J. Gray; H. Nakamura; C. Collins

Co-Planar Grid Electrodes for Single Polarity Charge Carrier Sensing in Ionization Detectors
P. Luke

High-Speed Thermal Cycler for Biochemistry
A. Hansen; J. Jaklevic

In Vivo Biomarker for Cell Senescence
G. Dimri; J. Campisi; M. Peacock
(Licensed to Geron Corporation)

Transgenic Mice Expressing Genomic Apolipoprotein
E. Rubin

Low-Force Pipet Tip Ejector
D. Uber; W. Searles

Direct Colorimetric Detection by Polymeric Thin Films
D. Charych; R. Stevens; A. Reichart

Direct Colorimetric Detection by Polymeric Thin Film Liposomes
D. Charych; R. Stevens; A. Reichert

The Combinatorial Synthesis of Novel Materials
P. Schultz; X. Xiang

Polymer Pendent Sulfonated and Linear Catechol Amids Ligands for Selective Removal and Recovery of Metals from Aqueous Environmental Waste Systems
R. Fish

Polyacrylamide Gel Loader
M.W. West; E. Polonsky

OTHER LICENSES

Eli Lilly and Co.
Apo B 100 Transgenic Mice
E. Rubin

Work for Others

The success of DOE life and environmental sciences programs at LBNL has depended not only on DOE support but also on complementary work, sponsored by others, that is closely coupled to the DOE programs and benefits directly form LBNL facilities and expertise. Examples include the National Dairy Promotion and Research Board's support of atherosclerosis work, the National Heart, Lung and Blood Institute's support of nuclear imaging methodologies, and the National Institute of Health's support of programs at the National Center for Electron Microscopy and the National Tritium Labeling Facility. Some projects supported by outside agencies have not been strictly dependent on large facilities or unique instrumentation but have made contributions that could only have been made in a national-laboratory type environment. Examples—in fields ranging from cell and molecular biology to environmental research to structural biology—abound. Non-OHER support expands the base of investigative activity and makes possible a wide diversity of staff expertise, thus greatly enriching the accomplishments of OHER programs themselves.

DOE Office of Basic Energy Sciences (BES)

The DOE Office of Basic Energy Sciences (BES) sponsors a continuing effort directed toward using knowledge of green plant photosynthesis to design artificial systems for the utilization and storage of solar energy. In addition, we focus effort on chemical reactions that suggest new concepts for photo-associated synthesis of high-valued chemicals from abundant chemicals, photocatalysis, temporary chemical storage of near infrared photons, and conversion of photon energy into electricity.

Also under the sponsorship of BES, we are studying the synthesis of chlorophyll in a purple, photosynthetic bacterium, *Rhodobacter capsulatus*. We have recently

completed the sequence of all of the genes required for pigment synthesis in *R. capsulatus*. As many as twenty gene products may be involved in bacteriochlorophyll (Bchl) production, and many of the enzymatic steps require the combined activity of two or more gene products. *Erwinia herbicola*, a non-photosynthetic bacterium, has a 12.7kb gene cluster of its carotenoid biosynthesis genes. This region has been completely sequenced and functions of the six genes determined. The sequencing of these regions has facilitated the understanding of the interrelationships and regulation of some of these genes. Our efforts are now concentrated on understanding how these enzymes work and how their gene expression is regulated by environmental factors. We are also studying photosynthetic oxygen evolution in higher plants and cyanobacteria.

Another BES-funded project concerns the absorption of visible light photons, followed in less than a nanosecond by excitation transfer and trapping in reaction centers of photosynthetic membranes. We are investigating the detailed kinetics and energetics of this process using wavelength-resolved transient absorption change and fluorescence decay measurements applied to well-defined preparations of antenna pigment proteins or reaction center complexes. Recent x-ray crystallography studies in several laboratories have provided detailed structural information for several of these proteins. This has enabled us to carry out excitation transfer calculations using exciton theory and/or Forster inductive resonance transfer applied to pigment arrays of known geometry.

DOE Office of Energy Efficiency and Renewable Energy (EERE)

Approximately 13 Quads of energy are used annually to condition and move the outside air supplied to residential and commercial U.S. buildings. This energy is used to maintain acceptable indoor air quality and occupant health, comfort, and productivity. A major goal of DOE, specified in the National Energy Strategy, is to increase the energy efficiency and ventilation efficiency of buildings, while maintaining or improving indoor air quality. The DOE Office of Conservation and Renewable Energy (CRE) supports an LBNL project that is developing and disseminating the information and technology needed to achieve this goal. Research covers the relationships among building energy usage, ventilation and infiltration, indoor air quality, and human factors.

We employ a combination of modeling, laboratory experiments, and field studies. In addition to developing methods for reducing energy use by heating, ventilating, and air conditioning systems, we are investigating indoor air pollutant concentrations and source strengths and their dependence on ventilation and building characteristics. Factors that cause the occupants of large buildings to have building-related health symptoms are also being investigated. Much of the research conducted for EERE depends upon the OHER-supported research to develop a sound scientific understanding of exposures and health risks from indoor air pollutants.

National Institutes of Health (NIH)

Activities sponsored by the National Institutes of Health (NIH) complement OHER-supported work in the following theme areas: hematopoiesis, carcinogenesis, atherosclerosis, aging, mental disorders, and physical health effects. In these areas NIH-supported work is strongly related to OHER missions through exploitation of unique instruments, methods and scientific personnel at LBNL.

An example of NIH's interest in improved instrumentation is support for the development of positron emission tomography (PET) technology. Topics range from scintillation mechanisms for PET to algorithms and processing architectures for tomography. NIH also sponsors several studies that use LBNL's unique PET instrumentation to characterize diseases of the brain.

A new emphasis in PET and nuclear magnetic resonance (NMR) is the practical interpretation of the human genome project. As the OHER program in medical applications seeks to modernize nuclear medicine activities and couple them with research in molecular genetics and gene expression, LBNL has emphasized those areas of clinical medical science that have potential for correlation to genome mapping activities.

Another area of NIH's interest is atherosclerosis research based on unique methodology for lipoprotein analysis developed at LBNL over the past 45 years. These tools are being applied in NIH-supported studies directed at understanding genetic, cellular, and metabolic mechanisms of heart disease. In addition, the NIH-supported research on PET and NMR enhances our understanding of the pathophysiology of atherosclerosis. Transgenic mouse studies and other atherosclerosis research programs supported by NIH call on unique LBNL facilities such as PET instrumentation, as well as the preliminary work for a very high field NMR spectrometer that will allow precise metabolic studies.

Research support from NIH also encompasses broadly all areas of current LBNL research activities in cell and molecular biology. One program project provides support for determining the high-resolution structure of various membrane proteins, while another program project provides support for delineating the detailed molecular structure of the red blood-cell membrane. In addition, a large number of research grants to individual scientists support research in various aspects of cell and molecular biology. Topics include growth regulation in normal and transformed cells, the molecular mechanisms of cell senescence, molecular analysis of differentiation of human mammary cells, the physical structure of viruses, molecular mechanisms involved in DNA damage and repair, mechanisms of mutagenesis in human cells, and the molecular and cellular basis for sickle cell anemia. These projects enhance OHER-sponsored studies in cell differentiation, carcinogenesis, and DNA damage and repair.

The National Tritium Labeling Facility (NTLF), which receives funding from NIH as well as DOE, serves as one of the few facilities in the nation equipped to label compounds to very high specific activities of 3H. It thus serves as a laboratory where researchers from across the U.S. can carry out labeling and radiopurification procedures that would be impossible at their home institutions. The mandated functions of the NTLF are to engage in research and development of advanced labeling techniques and to disseminate the results, to promote collaborative research using labeled molecules, to provide labeling services to the nation's scientists, and to train researchers in labeling methodologies. One of the NTLF's most important activities is to supply labeled biomolecules for tritium-NMR spectroscopy, a key technique in our OHER-funded structural biology research.

Research supported by NIH complements OHER-supported research to characterize air pollutant exposures in buildings and to assess the risks associated with human exposure by providing information on the gas-particle distributions and particle-size distributions of carcinogens in environmental tobacco smoke. This information is needed to understand the risks from exposures to this complex mixture.

The National Institute for Environmental Health Sciences supports research that directly benefits work we are doing for OHER by providing information on a source and mechanism of indoor exposures, i.e., soil-gases. This research will also be of value to DOE in its environmental restoration efforts since it will provide information to assess the potential for human exposures at contaminated DOE-sites.

National Aeronautics and Space Administration (NASA)

The National Aeronautics and Space Administration (NASA) utilizes the unique capabilities of accelerators, detectors, and scientists at LBNL to assess the health risks of space exploration. The nation's need for LBNL facilities for the Space Exploration Initiative is readily understood by the following example: The major radiation on extended manned missions to the moon and planets is galactic cosmic particles consisting of atomic nuclei from hydrogen to iron and even uranium. These particles penetrate the space craft and tissues at high velocity and cause biologic damage varying from cell death to cancer cell induction. Though the total conventional radiation dose is only a few rads to the whole body, the true situation is that each cell could be hit by one high-energy projectile on a two-year mission. Thus, to learn the consequences of this form of radiation, NASA needs the DOE facilities and personnel at LBNL to perform cell and animal studies. NASA sponsors a number of research projects aimed at assessing radiation-induced DNA damage and the mutagenic and tumorigenic potential of radiation.

In 1992 NASA awarded LBNL its Specialized Center for Research and Training (NSCORT) in Radiation Health. This is a five year grant. The major objective of the NSCORT is to conduct basic and applied radiobiological research with HZE (high atomic number and energy) particles that is directly applicable to the assessment of the radiation risk associated with extended manned space missions. Proper knowledge of these risks will allow NASA to determine the measures needed to protect human beings against the effects of ionizing radiations in space. The NSCORT also represents a Consortium with the Department of Radiological Health Sciences at Colorado State University and the Lawrence Berkeley National Laboratory. The Consortium has as a second major objective to further the nation's scholarship, skills, and exploitation of opportunities in space life sciences and related technology areas toward a goal of enhancing the pool of trained research scientists to meet the forthcoming challenges of the nation's commitment to prepare for future human space exploration missions. Several individual research projects are supported by the NSCORT.

OHER-sponsored detector studies in the Engineering Division are supplemented by more applied research supported by other offices in DOE and by NASA. These

projects benefit from the basic knowledge gained in the OHER projects and at the same time provide an excellent background of knowledge of real-world problems, which helps focus our fundamental OHER work.

Environmental Protection Agency (EPA)

The EPA supports research on numerical modeling of radon entry into houses, as well as analysis of animal cancer tests and studies of chemically induced damage to human mammary epithelial cells.

California Air Resources Board

The California Air Resources Board is sponsoring research to measure emission factors of selected toxic volatile organic compounds (VOCs) and respirable particulate matter in environmental tobacco smoke (ETS) using the LBNL room-size environmental chamber. Environmental tobacco smoke is suspected to be a major source of exposure for many of the toxic VOCs of concern to both the state of California and to EPA. Emission factors are being determined for selected N-nitrosamines, nicotine, aldehydes and other VOCs, including 1,3-butadiene, and for respirable suspended particles for a subset of cigarette brands which have the largest market shares. Emission factors for freshly generated sidestream smoke are also being measured and compared to those measured for ETS, which is more diluted and aged. This research will provide data needed to model indoor exposures to toxic VOCs for the California population.

University of California Tobacco-Related Disease Research Program

Environmental tobacco smoke is a major indoor air pollutant. LBNL has support from the UC Tobacco-Related Disease Research Program to investigate factors affecting the size distribution and concentration of environmental tobacco smoke particles to provide a more accurate estimate of the lung dose of particles and radon progeny attached to these particles. This research, thus, extends our OHER-supported research efforts to characterize indoor air pollutant exposures and assess their risks. It also complements and extends our OHER research to characterize indoor radon and radon decay products, their attachment to airborne particles, and their removal through deposition to indoor surfaces.

LBNL also has funding from the UC Tobacco-Related Disease Research Program to conduct a more basic study aimed at establishing, at the molecular level in human epithelial cells, the mechanisms of the inter-related phenomena of procarcinogen activation, cocarcinogenesis, and oxidative DNA damage involved in tobacco-related cancer. A detailed understanding of these inter-related processes could lead to effective preventive treatment for individuals previously exposed to cigarette smoke.

Table 9: Summary of Work for Others

The budgets shown on the following pages do not include Laboratory overhead costs.

Analytical Technology

E. Haller	NASA, Far-Infrared Semiconductor Detectors and Materials	$127,446
E. Haller	NASA Evaluation of Pyroelectric Materials	$100,456
E. Haller	NASA, Far-Infrared Advanced Photoconductors	$138,753
A. Hodgson	CPSC Volatile Organic Chemical Emissions from Carpet Cushions	$11,968
J. Jaklevic	SBIR NOVA R&D Inc., Riverside, CA A Feasibility Study of P-type Silicon Drift Detectors for High Precision Radiation Measurements	$26,000
J. Walton	NASA Various Special Silicon Detectors	$331,197
Jaklevic	WFO—AMGEN Development of an automated environment for construction of cDNA libraries	$1,200,000
J. Jaklevic	WFO—CRIS Dectectors II Various Special Silicon Detectors	$170,000
J. Jaklevic	WFO—12000 Channel Digital Angiography Electronics System	$60,000
J. Jaklevic	NASA–Ames Si(Li) Detectors	$35,000
R. Sextro	EPA Radon Entry into Florida Homes	$51,735
J. Walton	NASA Various Special Silicon Detectors	$331,197

Health Effects

E. Alpen	NASA Tumorigenic Potential of HZE Radiations	$123,182

M.H. Barcellos-Hoff	NIH grant Stromal Influence on Expression of Preneoplasia	$44,154
M. Bissell	UCSF NIH subcontract Role of Metalloproteinases in Mammary Gland Remodeling	$76,299
M. Bissell	Research Gift—Monsanto	$75,000
M. Bissell	DOD Mechanisms of Abnormal Cell Extracellular Matrix Interactions in Human Breast Cancer	$7,895
J. Campisi	NIH grant Cellular Senescence and Control of Gene Expression	$106,236
J. Campisi	NIH grant Growth Regulation in Normal and Transformed Cells	$93,958
J. Campisi	NIH grant Senescence- and Longevity-Modulating Genes	$80,214
A. Chatterjee	NASA Specialized Center of Research and Training	$654,525
J. Conboy	NIH grant Red Cell Band 4.1: Dev. Changes in RNA Splicing	$150,747
P. Cooper	NIH grant Mechanisms for Repair of Radiation Damage in Human Cells	$13,637
S. Curtis	NASA Risks to Normal Organ Function from Solar Particle Events	$15,622
J. Daisey	DOE (EERE) Infiltration, Ventilation and Indoor Air Quality	$1,000,000
J. Daisey	NIH grant Environmental Tobacco Smoke: Physico-Chemical Properties	$247,346
J. Daisey	UCB/NIH Subcontract Soil Gas Transport: A Mechanism of Indoor Exposures to VOC	$252,482
J. Daisey	CARB Toxic Volatile Organic Compounds in Environmental Tobacco Smoke	$70,286

J. Daisey, R. Sextro	UC Tobacco-Related Disease Research Program, Characterization of Particulate-Phase ETS in Differing Environments	$61,651
P. Durbin	NIH grant Biological Evaluation of New Actinide-Chelating Agents	$87,200
R. Goth-Goldstein	EPRI grant New Strategy for Coal Tar Remediation	$38,462
R. Goth-Goldstein	NASA grant Carcinogenic & Mutagenic Effects of Protons & Heavy Charged Particles	$14,193
A. Kronenberg	NIH grant Heavy-Ion Mutagenesis	$38,481
A. Kronenberg	NASA grant Mutagenesis in Human Cells with Accelerated H & Fe Ions	$71,978
A. Kronenberg	NIH Radiation Delayed Mutation and Instability in Human Cells	$7,335
R. Liburdy	NIH grant High Field NMR Bioeffects: Lymphocyte CA^{+2} Metabolism	$74,727
R. Liburdy	DOD Human Breast Cancer Proliferation and Modulation by Melantonin and Environmental Magnetic Fields	$9,613
J. Miller	NASA Experimental Study of Nuclear Interactions Relevant to High Energy HI Transport	$233,866
M. Narla	NIH grant Red Cell Deformability *In Vitro* and Survival *In Vivo*	$132,177
M. Narla	NIH grant Red Cell Membrane Studies	$1,003,890
M. Narla	Univ. British Columbia Subcontract Rheological and Adherence Properties of Sickle Cells	$215,430
M. Narla	UCSF/NIH grant Cellular Consequence of Hemoglobin Membrane Interaction	$75,539

G. Shyamala	NIH grant Normal and Neoplastic Growth: Role of Estrogens and HSP90	$124,454
G. Shyamala	NIH grant Progesterone Receptors in Mammary Development Neoplasia	$20,716
B. Singer	NIH grant Alkylation of Polynucleotides *In Vitro* and *In Vivo*	$175,748
B. Singer	NIH grant Biochemical Mechanisms of Vinyl Chloride Carcinogenesis	$124,784
M. Stampfer	NIH Merit Award Characterization of Human Mammary Epithelial Cells	$228,498
M. Stampfer	DOD Human Mammary Cell Resource	$7,746
M. Stampfer	Clonetics Corp. Grant Differentiation of Human Mammary Cells (Post-Doc Fellowship)	$5,000
P. Yaswen	NIH grant Calmodulin - Related Gene and Epithelial Transformation	$140,290

General Life Sciences

J. Bastacky	UC Tobacco-Related Disease Research Program. Effects of Tobacco on Rat Lung Alveolar Lining Liquid	$119,481
J. Bastacky	NIH grant Surface Tension Gradient Along the Airway in Lung	23,643
J. Bastacky	MIT/NIH Subcontract	$8,729
K. Downing	NIH grant Tubulin Structures by Electron Crystallography	$136,121
R. Glaeser	NIH grant	$362,179
R. Glaeser	NIH grant High Resolution Electron Crystallography of Proteins	$151,316

E. Golder	AHA Fellowship Maturation of HDL by LCAT in Transgenic Mice	$13,721
T. Head-Gordon	USAF grant Neural Networks as a Predictive Tool for Constrained Optimization Protein Folding	$14,578
B. Jap	WFO—Pfizer Crystallographic Studies of Potassium Channels	$38,868
M. Maestre	NIH grant Physical Structure of Viruses	$110,462
R. Mortimer	NIH grant Molecular Analysis of X-Ray Damage and Repair in Yeast	$106,781
M. Palazzolo	UCB subcontract (NIH) Drosophila Genome Center	$1,164,972
M. Palazzolo	NIH grant Drosophila Genome, Phase III, Sequencing Technology	129,406
M. Palazzolo	WFO—AMGEN Development of an automated environment for construction of cDNA libraries	$485,000
E. Rubin	AHA Established Investigator Analysis of HDL Atherosclerosis in Transgenic Mice	$56,751
E. Rubin	UCSF/NIH subcontract Construction of a Transgenic Mouse Model for Sickle Cell Anemia	$262,942
D. Wemmer	NIH grant National Tritium Labeling Facility	$481,525

Medical Applications

J. Alonso	NIH grant Design Study for UCD Proton Facility	$227,922
J. Bielicki	AHA Fellowship Extracellular HDL Assembly with Apolipoprotein AI Variants	$21,169
T. Budinger	NIH grant Cardiovascular Flow and Metabolism	$658,739

T. Budinger	NIH grant Cerebral Blood Flow Patterns in Alzheimer's Disease	$169,959
T. Budinger	Univ. Of Illinois/NSF subcontract New Technology for High Field NMR Imaging and Spectroscopy	$20,939
T. Budinger	NIH training grant Quantitative Cardiovascular Research	$47,367
M. Callow	AHA Fellowship Atherogenesis in Mice Expressing Lp(a)	$21,600
J. Castro	NIH grant Treatment of Cancer with Heavy Charged Particles	$398,952
P. Coxson	NIH grant Statistical and Modeling Uncertainties in Dynamic PET	$60,835
S. Derenzo	NIH grant Search for Ultrafast Heavy Atom Scintillators	$284,997
J. Enas	NIH grant Radiolabeled Alpha-2 Adrenergic Receptor Ligands	$22,669
T. Forte	NIH training grant Lipoprotein Methodology, Structure and Function	$44,164
T. Forte	UC Tobacco-Related Disease Research Program, Effect of Cigarette Smoke on High Risk Lipoprotein Profiles	$83,754
B. Ishida	NIH grant Pre-Beta Migrating High Density Lipoproteins	$58,431
W. Jagust	NIH grant Alcohol and Memory: A PET Study	$136,573
W. Jagust	NIH grant Longitudinal SPECT and PET Studies in Dementia	$78,800
R. Krauss	NIH grant Lipoprotein Subclasses: Structure, Origin, and Metabolism	$1,290,482

R. Krauss	Children's Hospital/National Dairy Promotion Board Subcontract (Calendar year) Genetic Influences on Lipoprotein and Atherogenic Responses to Dietary Fat	$494,234
R. Krauss	Sequoia Hospital Contract Metabolic Atherosclerosis Management	$346,789
R. Levy	LOMA LINDA Proton Radiosurgery for Arteriovenous Malfunctions	$125,614
R. Marshall	NIH grant Fluorodeoxyglucose Kinetics in Myocardium	$116,875
W. Moses	NIH grant High Resolution PET Detectors Using Solid State Photonics	$194,545
A. Nichols	NIH grant Apolipoprotein-Specific HDL and Cholesterol Transport	$132,169
T. Nordahl	NIH grant Hippocampal Metabolism in Schizophrenia	$68,410
M. Roos	UC Berkeley NIH subcontract Development of NMR Assisted Cryosurgery	$34,126
T. Sargent	NIH grant Methyl Carbon Pathway in Psychosis	$52,076
L. Stoltzfus	NIH grant In Vivo Effects of Apolipoproteins AI Variance on HDL	$21,004
D. Tribble	UC Tobacco-related Disease Research Cigarette Smoke Exposure and Antioxidant in Blood	$38,666
P. Williams	NIH grant Effects of CHD Prevention on Lipoprotein Subclasses	$15,758
P. Williams	NIH RCDA Effects of Exercise, Diet and Fat Lost on Lipoproteins	$32,465
P. Williams	NIH grant Weight Set Point and HDL Concentration in Runners	$282,330

5.0 Program Orientation and Future Directions for FY 1995–1997

Analytical Technology

AIRBORNE RADON CONCENTRATIONS (GADGIL, SEXTRO)

During the next year, the Indoor Atmosphere Program studies on radon progeny behavior will concentrate on completing experimental and theoretical research on two fronts: the deposition of radon progeny on to indoor surfaces, and wind-house interaction producing ground pressures as a significant factor influencing the entry of radon-bearing soil gas into buildings. In the research on deposition, we aim to resolve the large (about a factor of four) discrepancy between model predictions and experimental estimates for rates of removal of radon progeny by deposition on surfaces. In the research on wind-induced ground pressures and their effects on radon entry, we aim to investigate both the steady-state and the non-steady state effects through theoretical and experimental work. A significant non-steady state effect appears to be the entry driven by "pumping" action of wind pressure fluctuations.

In FY 1996, we also plan to initiate a strategic analysis of the influence of the indoor environment on occupant health, and costs to the economy including effects of the indoor environment (thermal comfort, indoor pollutants, indoor propagation of airborne diseases) on office worker productivity.

SEMICONDUCTOR RADIATION DETECTOR TECHNOLOGY (JAKLEVIC, HALLER)

The funding provided under this existing contract has in the past years allowed us to carry out a broad-based research program to develop a wide array of semiconductor detectors, materials, characterization techniques, electronic and system designs. Technologies developed in these areas have benefited many programs related to DOE's missions, as well as other federal and civilian research projects. Many of the technologies have been, and is still being, transferred to the private sector. With, in effect, the termination of this program this coming year, our effort will necessarily become more narrowly focused and be directed towards specific projects. At this point, pending funding from a new proposal with OHER, we are expecting to focus on the development of field portable detector systems for environmental monitoring employing room temperature CdZnTe and silicon drift detector designs.

Environmental Research

ATMOSPHERIC AEROSOLS AND GLOBAL CLIMATE CHANGE (NOVAKOV)

To obtain data most useful for modeling of direct and indirect climate forcing by multi-component aerosols. These include:

1) Measurements of aerosol composition and physical properties at a variety of remote locations;

2) Establishing the sources of carbonaceous aerosols (natural vs. anthropogenic);

3) Development of global emissions and concentrations inventories of carbonaceous aerosols.

RADON TRANSPORT THROUGH SOILS INTO HOUSES (SEXTRO, FISK)

Additional experimental and theoretical investigations of the factors controlling radon entry rates at our small basement structures will be conducted, with the goal of improving our understanding of radon entry into actual houses. We will extend our new understanding of scale-dependent soil permeabilities and examine other soils for these effects. Based on current model predictions that barometric pressure fluctuations may enhance radon transport into basements, we will undertake experiments to assess the significance of these transient flows. Experiments will also seek to investigate entry rates and source strengths for ^{220}Rn, a short-lived radon isotope that may, nevertheless, contribute 10 to 20 percent of the average radiation exposure due to radon. We will begin site assessment and planning activities for the location of a second experimental site in which the underlying geology, soils, and climate differ from those found at our present site in the Santa Cruz mountains.

THERMODYNAMIC PROPERTIES OF CHEMICAL SPECIES PRESENT IN MIXED ORGANIC-RADIONUCLIDE WASTES (AL MAHAMID)

Stability of Pu-NTA System in the Presence of Microbes

We will focus on studying the behavior of the Pu-NTA system in the presence of microbes. The ability of microorganisms in the environment to metabolize NTA when complexed with plutonium, may be a major determinant of the migration of plutonium species in the environment.

Sorption Studies of Plutonium-NTA on Geological Materials

To predict mixed contaminant migrations in the subsurface, it is necessary to carry out studies on the adsorption, dissolution, and surface catalyzed reactions on natural mineral surfaces. We will initiate several series of experiments to study the behavior of Pu-NTA in the presence of natural mineral surfaces such as iron coated-sand, goethite, and gibbsite. Speciation analysis for the solutions and the mineral surfaces will be carried out to determine the predominant oxidation states and the sorption of Pu on the natural mineral surfaces. Our data will be used to predict a model for the transport of plutonium.

Equipment Needs :

An inert glove box that comply with the regulations of EH&S ($ 40K) is needed to perform the sorption experiments and the preparation of plutonium stock solutions. It is very hard for a small project like this ($ 225K) to obtain capital equipment funding of $ 40K.

Health Effects

AIR POLLUTANT EXPOSURES IN BUILDINGS (NERO, DAISEY)

LBNL's research program on air pollutant exposures in buildings seeks to advance scientific understanding of exposures and risks from key indoor pollutants and to provide a sound basis for the more applied research on buildings in DOEís Office of Energy Efficiency and Renewable Energy. The emphasis is on pollutants associated with energy usage in buildings and on those whose indoor concentrations are influenced by reductions in building ventilation to save energy. These include radon, combustion pollutants and volatile and semi-volatile organic pollutants. Our current effort to identify high radon areas of the country has three major components to be completed. First, the statistical modeling approach developed at LBNL will be applied consistently to three states, Minnesota, Washington, and New York, which are being used as a basis for methodological development. This will provide an assessment of the power of this approach in three different situations with a range of indoor radon concentrations and geological conditions. Second, we will complete a new survey being conducted in Minnesota to acquire long-term indoor monitoring data in homes sampled across the state and use these more complete data to further develop our analytical approach, with the objective of yielding concentration estimators for areas as small as census tracts (about 4000 people). Finally, we will formulate the methodology so that it can be used systematically across the United States, thereby providing a reliable identification of high-radon areas, so that monitoring and control efforts can be focused more effectively and so that the homes causing "occupational" exposures can be given the highest priority.

In the next two years, we will complete the development of logit regression models to relate irritancy and odor symptoms in 880 office workers in 12 California Office buildings to several newly developed metrics of exposure for volatile organic compounds (VOCs). Since indoor exposures to these compounds are strongly influenced by building ventilation, it is important to determine whether such exposures have any adverse health impacts at levels found in typical office settings . We will also complete analyses of the field data on tracers of exposures to toxic volatile organic compounds from environmental tobacco smoke (ETS) in office buildings with different types of ventilation and other pollutant control measures.

When these efforts are completed, we will initiate research efforts to develop methodologies for estimating population exposures to other key indoor pollutants for which reductions in ventilation and related technologies to reduce energy usage in buildings are known to be important, e.g., toxic VOCs from building materials and other indoor sources. Since building characteristics vary across regions, we will develop and test the methodology for selected regions in the U.S. using data from the population-based U.S. radon survey, emissions factors that we and others have developed measured for these species, and the infiltration model developed here at LBNL. Finally, we will formulate the methodology so that it can be used systematically across the United States, as we have done for radon, so that DOE weatherization programs to save energy in residential buildings can be better designed to minimize risks of indoor air quality problems and their associated health risks due to reduced ventilation.

THE RESOURCE FOR MOLECULAR CYTOGENETICS (GRAY)

Trends: The Resource for Molecular Cytogenetics was established in August 1993 and has as its main goal, development of molecular cytogenetic techniques, instruments and reagents and to make these broadly available. The Resource is now routinely screening >30 loci/month to select molecular cytogenetic probes that contain genetically mapped polymorphic sequences or known genes. Our goals for the next two years are to meet the Resource objectives outlined in the peer reviewed proposal. We anticiapte that these will be essentially complete in 1997.

New directions: Beginning in 1997, we plan to begin application of techniques and reagents developed by the Resource to identification and cloning of "susceptibility" genes that, when mutated in somatic cells, lead to cancer and/or, when mutated in germinal cells, lead to increased risk of developing cancer. Specific projects may include genomic localization of genes (e.g. by complementation analysis, genetic linkage or mapping in human and model tumors), assembly of detailed physical maps in suspect regions, isolation and sequencing of genes from these regions, analysis of differential gene expression, and generation of transgenic mice carrying candidate oncogenes.

Anticipated achievements: The Resource will within the next two years: 1) Develop and distribute >500 mapped P1 clones distributed at ~5Mb intervals over the human genome. Most of these will contain genetically mapped polymorphisms or known genes. 2) Develop and distribute software for physical mapping according to fractional location along metaphase chromosomes, comparative genomic hybridization, high speed scanning and genotype-phenotype analysis in three dimensions. 3) Develop procedures for comparative genomic hybridization to arrays of well mapped clones in order to allow detection of relative gene dosage abnormality with <5Mb resolution. 4) Maintain a Internet database of molecular cytogenetic probes.

MECHANISMS OF TUMOR PROMOTION (BISSELL)

We now know that extracellular matrix is a central regulator of not only differentiation, but also growth, apoptosis and cancer. The refined techniques and cumulative knowledge acquired in the rodent model will be invaluable in studying human breast cancer. In this model, we have succeeded in demonstrating the significance of the three-dimensional environment and the role of extracellular matrix molecules in regulation of supressor genes. We will now concentrate on molecular mechanism of stromal-epithelial interaction and function of supressor genes in the human breast project. In addition, the mechanism of loss of apoptotic activity and the progression to malignancy will be studied in a novel human breast cancer model. We are confident that our unique approach to the problem of tissue-specificity will bear additional fruit in the human system, where for so long the mechanism of breast cancer induction has remained elusive.

IN VITRO TRANSFORMATION OF HUMAN MAMMARY EPITHELIAL CELLS (STAMPFER, YASWEN)

The central theme of this project is to determine how normal mechanisms which coordinate growth and differentiation of human mammary epithelial cells (HMECs) are altered as a result of immortal and malignant transformation. This work is performed primarily utilizing the well-characterized HMEC culture system developed here at LBNL. As the fidelity of our culture system improves, our ability to define characteristics which distinguish normal and tumorigenically transformed HMEC will also improve. We plan to employ several approaches for analysis of key regulatory pathways which are altered in HMEC in vivo and in vitro as a result of carcinogenic transformation. One such approach will be to continue to study the regulation of specific differentiation markers, such as lactoferrin, whose presence or absence is associated with transformation.

Another approach, for which we have recently received LBNL LDRD funds to develop, is a molecular biological methodology for the functional isolation and identification of gene products whose downregulation is directly involved in cell transformation. This promising method relies on the ability of segments of genes, when overexpressed, to interfere in a dominant fashion with functions of the wild-type genes from which they are derived. Such gene segments, termed genetic suppressor elements (GSEs), act by encoding inhibitory antisense RNAs or truncated proteins. Because this technique can potentially be adapted to any system for which a biological selection regime is available, widespread utility is anticipated. We will use the GSE technique initially in conjunction with assays for three properties associated with cell transformation: a) resistance to TGFb mediated growth inhibition, b) anchorage independent growth, and c) cellular immortalization.

CELL CYCLE ACTIVATOR AND REPRESSOR GENES (CAMPISI)

During the year of the study of transcriptional control, the program will seek to:

- Use T antigen, the T antigen mutants, candidate cellular genes and cDNA expression libraries to identify additional cellular genes active in either the Rb or p53-mediated pathways of growth inhibition and tumor suppression.
- Elucidate the mechanisms by which Id-1 and mdm-2 stimulate DNA synthesis in cooperation with the T antigen mutants to stimulate DNA synthesis.
- Set up assays that will enable us to identify genes important for progression through the G2 phase of the cell cycle. Apply the approach and techniques successful for identifying genes important for G1 progression, described above.
- Develop the β-galactosidase senescence marker as a screen to clone novel suppressor genes.

Anticipated Milestones: We expect to be able to understand how the activities of Rb and p53 interact with known cellular genes such as E2F and SDI-1 to regulate cell proliferation. We also expect to identify novel cellular genes that modulate Rb and/or p53 activity, or that constitute novel growth inhibitory/tumor suppression pathways.

INTERSPECIES EXTRAPOLATION AND RISK ASSESSMENT (GOLD)

The broad areas of future work are the broadened perspective on possible cancer hazards for prioritization purposes, the development of risk assessment methodologies, and the use of our large database of animal cancer test results to address issues of dose-response, interspecies extrapolation, and bioassay design. We will continue to rank and compare possible carcinogenic hazards (HERP). Our focus is on rodent carcinogens in the diet, in indoor air and workplace air, and from exposures due to hazardous waste sites. In the coming year we will address exposures to rodent carcinogens in indoor air from tobacco smoke and other sources.

An area of current emphasis is the evaluation of possible hazards due to exposures at hazardous waste sites. We are comparing hypothetical cancer risks from groundwater contamination to hypothetical substitution risks from chlorination by-products that result if people are removed from contaminated water and put onto chlorinated surface water.

The validity of qualitative extrapolation of carcinogenicity between species will be evaluated by comparing results in monkeys to those in rats and mice. Where possible, results in humans will be compared to those in monkeys.

In all years, we will continue to address theoretical questions of mechanism and how information about mechanism can be incorporated into risk assessment methodology in order to improve the extrapolation from animal bioassays conducted at the MTD to human exposures at low doses. In the coming year, in our collaborative LDRD project SELECT (see below), we will investigate the pharmacokinetics and uncertainties in risk estimation for trichloroethylene.

In the area of risk assessment methodology, based on our earlier findings that potency estimates are bounded by the doses administered in a bioassay (MTD), we are evaluating the use of the MTD for a preliminary estimate of a virtually safe dose, in the absence of any cancer tests on a given chemical. Since potency in bioassays is constrained, and the default regulatory assumption is linear extrapolation, a reasonable estimate of carcinogenic risk should be possible without running a 2-year bioassay. The uncertainty in such an estimate will be compared to variation in potency estimation in near-replicate tests when the same chemical is tested more than once in the same species, sex, and strain by the same route of administration.

Other work on risk assessment methodology will focus next on (a) differences in positivity and potency for chemicals that have been tested by both oral and inhalation routes in rodent bioassays. (b) In current risk assessment methodology, positive results for chemicals tested in rodent bioassays are summarized qualitatively by EPA by ranking as either A, B, or C. We intend to improve the use of rodent bioassay data by providing a profile on the strength of evidence of carcinogenicity for a chemical, e.g. lethality of tumors, number of target organs, proportion of experiments that are positive. These measures will be combined in a more detailed categorization which can then be used in risk assessment.

General Life Sciences

SEMICONDUCTOR X-RAY DETECTORS FOR SYNCHROTRON APPLICATIONS (JAKLEVIC)

A very strong emphasis will be placed on the continued development of one- and two-dimensional detectors with integrated readout electronics for high count rate synchrotron biochemical spectroscopy and cystallography applications. These detectors will offer hundreds of individual elements in a few square centimeters of silicon, capable of excellent energy resolution, high count rate throughput, and position information. The development of integrated circuit pulse-processing electronics allows for parallel pulse processing of hundreds of channels of data, again at the cost of a few square millimeters of silicon chip. Parallel efforts will focus on detector materials issues, such as the development of electrical contact technologies which yield detectors with improved low energy x-ray detection and increased peak-to-background levels for improved sensitivity. Further developments in the IC electronics technologies will include a new program to fabricate the pulse-

processing electronics right onto the detector wafer, which allows for an additional flexibility in detector geometries and the possibility of further improvements in energy resolution.

As this program becomes more multi-disciplinary, as is crucial for the success of developing fully functioning spectrometers for use in synchrotron experiments, it becomes more and more important to draw on the expertise of a variety of engineering and scientific disciplines. The current funding level for this program supports only one full-time engineer, and there is very little additional money to pay for support from other engineers and technicians. This program will have fewer and fewer successes, if the funding levels remain constant (or decrease, as they have been) while the internal laboratory costs of supporting engineers escalates.

MOLECULAR STRUCTURE OF MEMBRANE TRANSPORT SYSTEMS (JAP)

With the research support from LDRD, we have successfully cloned the CFTR gene into a vaccinia expression system. We are establishing the capability to obtain high expression of a wide range of proteins using this expression system. Such a capability will allow us to carry out structural studies of clinically important proteins starting from their cDNA. We hope to receive substantial support from DOE to further develop our capability in expressing membrane proteins. Our goal is to be able to quickly respond to the late breaking results from Human Genome Center research and to immediately begin the structural studies of gene products associated with diseases.

We have received research support from Pfizer to carry out structural studies of the potassium channel kv1.3, which is believed to be involved in the regulation of the immune system. We have successfully obtained a few hundred micrograms of purified protein. Currently, we are scaling up the purification protocol to obtain a few milligrams of protein for two-dimensional crystallization.

MUTAGENESIS: FUNDAMENTAL CHEMISTRY (HEARST)

This program focus can be divided into three projects: Project 1—Solution structures of psoralen-modified DNA oligonucleotides using nuclear magnetic resonance; Project 2—X-ray crystallography of psoralen-modified DNA oligonucleotides and; Project 3—The characterization of covalent linkages between DNA psoralen furan-side monoadducts and uvrB.

In Project 1, the E-coli excision repair pathway and very likely the mammalian pathway as well recognize a large variety of covalent adducts to DNA including thymine dimers, psoralen monoadducts and crosslinks, cis-platin, aflatoxin, and benzo-pyrene. The versatility of this repair pathway suggests that the recognition signal for the repair enzymes is not the local damage done to the DNA duplex, which is different for each chemical mutagen and carcinogen, but a common modification of duplex structure. We have suggested in our earlier work that the cause for repair is likely to be a kink in the DNA duplex. The new structural work on psoralen adduct structure by NMR and by others using electrophoretic gel retardation, suggests this may not be the correct answer. We are now convinced that these issues will not be resolved until atomic resolution structural information is available. This is therefore the new emphasis of this project using both NMR and X-ray crystallography.

A procedure for the large scale preparation of pyrone-side monoadduct has also been developed, and a 8mer pyrone-side monoadduct substrate will be the next substrate whose structure will be determined. As a continuing effort, new substrates of these three classes will be made and their structures determined by NMR which are longer and which vary in sequence from the first substrates analyzed in order to establish the variety of structural motifs which emerge.

In project 2, the X-ray crystallographic structures of the adducted oligomer molecules are being determined in collaboration with Professor Pui Shing Ho and Blaine Mooers of the Department of Biochemistry and Biophysics at Oregon State University, Corvallis. While we have had preliminary success in crystallizing an 8mer-cross link oligonucleotide, the crystals are not large enough or stable enough to gather high resolution diffraction data from. Dr. Hearst is also receiving essential training for his role as a user of the scientific biological research program in X-ray microscopy. Ultimately, the ALS is viewed to be an important resource for the long term success of many X-ray experiments.

In Project 3, the various steps in the E-coli excision repair pathway are complex. One of the many very

important achievements of the collaboration between the Hearst laboratory and the Sancar laboratory has been the elucidation of the steps involved. First uvrA dimer binds tightly to the damage site, a step requiring ATP and very likely forcing the structure of the DNA into a new metastable state (perhaps single stranded near the damaged region). The positioned uvrA dimer directs the binding of uvrB to the DNA at the damaged site where the uvrB binds tightly (probably irreversibly), and the uvrA dimer leaves this site. The amazing thing about this mechanism is that uvrB has no ability to bind to DNA on its own, but once the site has been prepared by uvrA dimer and ATP, the binding of uvrB alone becomes irreversible. The positioning of uvrB at the damage site then directs uvrC to that site and the excision or endolytic cleavage of the DNA occurs.

Ultimately we need to establish the structures of each of these complexes with DNA and are pursuing any biochemical advantages which will help us achieve these goals. We have recently discovered that when a furan-side monoadducted oligonucleotide is bound to uvrB using the above steps, and then irradiated with 360 nm ultraviolet light, a covalent bond is formed between the psoralen adduct and the uvrB protein. Since this is a direct route to mapping the active site of this enzyme, we are characterizing all aspects of this photochemistry. We have determined which tryptic fragment of uvrB binds to the psoralen. This project is now in the hands of Professor Shrinivas Sastry at the Rockefeller University in New York City. While we are in constant contact with these activities, no further experiments are planned for Berkeley.

MASS SPECTROMETRY DETECTORS (BENNER, JAKLEVIC)

Future directions in the mass spectrometry detector project include:

- Detector sensitivity will be determined as a function of ion or particle energy and ion/particle mass.
- Detector efficiency will be determined as a fraction of ions/particles that strike a detector vs. those that register a signal.
- Mass spectrometry of DNA will be developed more extensively. Limits of delectability will be determined as a function of the size of a DNA fragment
- The high resolution we attain will open the door for the application of mass spectrometry to genomic science.

HUMAN GENOME CENTER (NARLA, PALAZZOLO, MARTIN, RUBIN, JAKLEVIC, EECKMAN)

Sequencing Production Goals

There are currently two teams in the sequencing production group. One of these teams is funded by the NIH and is focused on sequencing the *Drosophila* genome. The second team is funded by the DOE and is working on templates from human chromosome 5. The teams are currently capable of sequencing about 700 kb per year per team. The goals is to continue these sequencing efforts but to try and increase the effectiveness of each group by the incorporation of novel automation and computational tools. The sequencing rate of each team is expected to increase to about 1 megabase per year per team.

The sequencing production group has submitted a grant to the NIH to increase the number of teams sequencing *Drosophila* templates. The grant was submitted in October of 1994 and after a site visit in January 1995, new teams are slated to be added to the sequencing effort beginning in August 1995. The grant requests funds for an additional 6.5 member team every quarter until a total of 12 teams are working on the fly genome. If completely funded the production output is predicted to finish 72 megabases of genomic sequence during the five years of the grant. A two to three year extension on the grant would allow complete sequencing of the 120 megabases of the euchromatic portion of the *Drosophila* genome by the year 2003.

Research and Development Goals

Working towards improved sequencing efficiency is a long term commitment and is an interdisciplinary project at LBNL. The collaboration with the Automation group is focused on several novel instruments developed at LBNL that have completed their final testing phase. We are currently attempting to incorporate the oligosynthesizer, the water-based thermocycler, the ORCA pooling robot, and the colony picker into everyday production use. It is expected during the course of next year that the turbo-PCR will finish its test phases and become a candidate for entry into the sequencing assembly line.

The collaboration with the Computation group during the past year has focused on a detailed formulation of plans for automating assembly, editing, analysis, and submission of completed sequence. These tools will be introduced into the production arena in a timely fashion.

The group is also attempting to develop more robust protocols wherever necessary for the individual biological steps of the sequencing procedures. A major area of interest is the DOGtag system. This stage of the high resolution physical map has a relatively high false positive rate in its current implementation. A variety of alternatives are being assessed to improve the efficacy of this operation.

Expanding Sequencing Production

The LBNL Human Genome Center would like to position itself to play a competitive and substantive role in the elucidation of the sequence of the human genome. A major limitation in this area is a detailed plan for acquiring sufficient funding to scale up the effort on human templates. The sequencing production group is interested in pursuing every avenue to search for funding to increase this effort.

Genomic Applications

A major challenge in genome research is to develop effective mechanisms to use the information and tools generated by the genome project to do hypothesis driven research in new ways. It seems logical to think that large benefits will be derived simply by the presentation of the sequence data to the worldwide research community for effective use in biomedical research. However, it is equally likely that those doing genome research, because of their intimate knowledge of the emerging reagents and tools, may have the opportunity to develop additional programs that lead the way to new approaches to biology.

Some examples of this type of research are already underway in our laboratory. The group has completed the sequence of the Bithorax complex. Analysis of the region has led to some insights about the structure and function of the region. However, the regions is largely composed of regulatory sequences which are probably best understood by doing sequence comparisons of homologous regions in evolutionarily related organisms. *Drosophila virilis* is such a related species. Undergraduates working in the lab are attempting to clone regions of the virilis genome that are homologous to the sequenced portions of the melanogaster bithorax complex. Such regions can be sequenced and sequence comparisons done to identify conserved regions that might be key to the regulatory control of this complicated system of related genes. The same group of undergraduates is also working on the putative downstream genes that might be regulated by the homeobox containing DNA binding proteins of the bithorax complex.

Automation and Instrumentation

Over the next five years, the instrumentation group activities will gradually move through several stages. In the immediate two to three year period, we will continue to focus on automation in support of the scale-up of directed sequencing to a production level. At the same time, we need to be active in the area of new technology development. Increased interactions with the commercial sector will likely lead to technology transfer. On the longer term, we need to be working closely with the biologists to develop new program areas where we can continue to exploit our unique synergism in combining modern instrumentation with innovative biological research.

The present emphasis on the development of instrumentation and robotics applications for automation of specific procedures will soon shift to issues concerned with development of integrated functional modules. The trend will be toward automated materials and reagent tracking coupled with reduced operator involvement in data acquisition and interpretation where possible. At the same time, the scale-up of the *Drosophila* project coupled with increasing interest in our instruments from entities outside the laboratory will require an increased effort in production and technology transfer.

Advanced Instrumentation for Mapping and Sequencing

LBNL's in-house advanced genome instrumentation programs are currently focused in two areas: fluorescence sequencing and mass spectrometry. In the sequencing project, we have developed a unique excitation and detection geometry which employs a linear array of cylindrical lenses coupled to an optical fiber array which efficiently gathers the fluorescence light and transforms it into an image which can be captured with a cooled-CCD camera. The detection system has been coupled to both a conventional polyacrylamide gel and a thin-gel system. In the former application, we have demonstrated the

successful detection of sequencing ladders at a spacing which corresponds to 100 lanes in a 25 cm gel. In the latter application, the detector is a significant improvement over direct CCD imaging of the gels over a limited size range.

The mass spectrometry project is focused on instrumentation development, particularly innovative ion detection methods. However, we have also been able to demonstrate the sizing of DNA molecules at a level which is adequate for the detection of point mutations in short fragments in a manner which could be adapted for genetic screening. In the area of large fragment detection and measurement, we are at a technology level comparable to other groups in the field and are experiencing similar difficulties in extending the range of applications to molecular sizes adequate for sequencing.

Informatics

The Genome Informatics Group at the LBNL Human Genome Center has three primary goals: (1) to support the production of human and model organism DNA sequence information at LBNL by the development of a highly integrated, customized computer system for the construction of high resolution maps leading to sequencing, and the automatic assembly, analysis and dissemination of the resulting data; (2) to contribute to the broader genome community, by the introduction and distribution of shareable, intuitive visual interfaces to genomic data on several different hardware platforms; and (3) to engage in a long range program of research and development in the areas of database development, high throughput sequence analysis and automatic annotation and measures of quality for primary data.

To achieve the first goal, we have been introducing a variety of software modules during the past three years, ranging from comprehensive databases to specific analysis programs. All of these have been designed in conjunction with the Center's biologists, and all address either specific bottlenecks or are solutions to data flow problems.

To achieve the second goal, we have deliberately chosen to align ourselves with other centers of software who are also interested in producing shareable programs and modules. One outcome of this strategy has been the availability of 21Bdb (see below), which provides graphic and textual information about physical maps of human chromosome 21. Another result is the recent introduction of MacAce, the Macintosh version of ACEDB (as well as our variations on ACEDB), which is freely available, and will be distributed by compact disk.

To achieve the third goal, we are proposing (and in some cases have already started) a series of automatic quality checks for the sequence data produced at LBNL, as well as a detailed program of research in improvements to basecalling and associated figures of merit. We also need to develop methods for automatic annotation of sequences and, given the large amount of genomic contiguous sequence that will be generated at the Center, we want to develop analytical ways to study long range motifs and structure.

Human Biology

The human biology component of the LBNL Genome Center, plays a key role in putting forth the rationale for deciding what regions of the human genome will be sequenced. Once the regions are defined the group is responsible for developing clone based maps and DNA templates to be sequenced by the production sequencing group and also developing technologies to decode this raw sequence data into the identification of genes and their function in collaboration with the informatics group.

Gene Discovery/Technology Development
Full utilization of the sequence information resulting from the Human Genome Project will require the deciphering of genes and their functions. The mouse will clearly play a fundamental role in both the discovery of important human genes and aid in the subsequent analysis of these genes and their functions in development and disease. Approaches for the investigation of regions of the human genome targeted by the LBNL program include: the creation of *in vivo* libraries of regions of the human genome and creating targeted mutations in mice of syntenic regions of the mouse genome. New technologies will need to be developed to make these gene discovery approaches feasible. The focus, thus for the next five years with regard to gene discovery will be to utilize available tools in the analysis of data generated by the production sequencing group as well as the development of new tools to accelerate this process.

Creation of *in vivo* libraries of the human genome. Evidence from mice and humans indicates that over-expression of distinct regions of the genome are associated with distinct phenotypes. It is also known

that many, if not most, regions of the mouse genome are syntenic with human chromosomes. The development of the YAC system in sequencing followed by our ability to produce YAC transgenics provide a technically feasible approach for creating in vivo libraries of defined segments of the genome. In vivo libraries will provide both the ability to study novel phenotypes in the animal as well as enable the complementation of existing mouse mutations. The latter approach will allow us to recover previously undetermined human homologues for existing mouse mutations.

Targeted Deletions

Targeted deletions of regions of the genome provides a powerful method for screening for genes and deciphering their function. Presently, the experience at creating large deletions in targeted regions of the genome is minimal. We are proposing three general strategies to create deletions of 100-500 Kb regions of the genome:

- Insertion of two targeting vectors containing lox P sequences at distinct sites in a region of DNA. Following insertion of these two lox P sites, expression of Cre recombinase in either a ubiquitous or tissue-specific manner will result in the targeted deletion of large specific DNA regions. This approach utilizes known technologies.

- Creation of targeting vectors in P1 or YAC clones. This would involve isolation of P1 or YAC clones from the region of the mouse genome to be targeted. The availability of a 129 P1 library makes isolation of the clones feasible. Lox P sites could be inserted at both ends of the human P1 insert. We have recently developed methods for manipulating 90 Kb P1s [M. Callow and E. Rubin *Site Specific Mutagenesis of a 90 kb P1*. Nucleic Acid Research in press].

- Extending the limits of homologous recombination through building targeting vectors with large regions of 5 and 3 prime homology. The maximal degree of deletion with standard targeting vectors is the 17 Kb alpha one and alpha two region of the globin region recently achieved in our laboratory. Other laboratories have been unable to achieve larger deletions, perhaps because they have been utilizing standard targeting vectors. It is possible that targeting vectors with large regions of 5' and 3' prime homology might enable the deletions of large regions of DNA. Using our ability to manipulate P1s using the rare recombinase system, we propose to build targeting vectors with extensive stretches of homology. The failure of the community to create such large deletions using the targeting vectors may be due in part to the inability to manipulate large DNA.

The preceding strategies are means to efficiently produce large deletions in embryonic stem cells with the plan to create mice containing these deletions.

Expansion of the P1 map in the distal portion of chromosome 5q

We expect to begin an extensive P1 screening and mapping effort before FY 96. The priority target will be the ~3 Mb region between the IL3 and IL9 genes. A P1 map in this region will provide templates for generating a continuous 4 Mb stretch of human sequences. We propose to begin this effort in six months when a set of non-chimeric YACs are mapped, and complete the P1 map in 12 months providing sufficient budget for two full time employee.

The next target will be the ~5 Mb region between the IL9 and EGR1 genes. This region contains about 10 framework markers and two disease loci (corneal dystrophies and myeloidysplastic syndrome). A P1 map will be completed in one year.

The third target will be the ~4 Mb region between the EGR and GRL genes. This region contains about 6 framework markers and two disease loci (limb-girdle muscular dystrophy and post-lingual deafness).

After the third year, the high resolution mapping effort will continue in the growth factor receptor gene cluster region at the distal end of chromosome 5 q with an slightly increased rate of 6-8 Mb per year. The determination factors for an increased mapping rate include the improvements made by others in high resolution clone library constructions, and the improvements in generating a non-chimeric YAC map with better genome coverage. In this stage, some portions of the procedures (e.g. the YAC selection) can be avoid and more efforts can be put into P1 map construction.

P1 mapping of the syntenic region of human 5q in mouse

We propose to map the syntenic region of human 5q in mouse. The first target would be the region containing the interleukin gene cluster on mouse chromosome 11. The sequences of mouse IL3 and GM-

CSF genes are available, and some physical mapping information is also documented in this gene cluster. These early studies provide us starting materials for the isolation of this gene cluster. A set of YAC will be screened out by PCR using the DNA pools generated from a mouse YAC library. These YACs will serve as DNA sources for generating a high resolution map in this region. The clones mapped to this region would likely be subjected to sequencing. The sequence information will be compared with the human sequence to assist in the identification of evolutionarily conserved elements outside the protein coding regions for functional studies.

Expansion of the P1 mapping to other biological important regions
New biological projects will be established in LBNL Human Genome Center. These projects will make use of the transgenic mouse technology and mapping and sequencing information developed by the LBNL Genome Center to study regions of the human genome under investigation by the LBNL Center. One of the candidate genes currently under investigation is the gene responsible for the *Weaver* phenotype in mice. This gene is localized genetically within a 3 Mb region on mouse chromosome 16, and the syntenic region in humans is responsible for the major phenotypic features seen in Down's syndrome. It is likely that the *Weaver* gene will be localized by studies in transgenic mice of a single YAC, and subsequently narrowed down to a single P1, provided a complete P1 contig in that region is available.

Estimates of needed resources
To carry out the technology development at the LBNL Transgenic Resource described above would require one full time postdoctoral fellow working on development of vectors and a full time technician who would participate in work with the embryonic stem cells. Tissue culture supplies for the embryonic stem cells would cost $25K per year. Mouse purchases would be $35K per year. Mouse housing charges would be $30K per year.

DATA MANAGEMENT TOOLS FOR GENOMIC DATABASES (MARKOWITZ)

OPM and the OPM data management tools will be extended with additional facilities needed for the development of GDB, GSDB, and PDB, such as facilities supporting inter-database references, database replication, object versioning, and object views. Furthermore, we will develop OPM data exchange tools, tools for retrofitting OPM schemas on top of existing genome databases, physical database design tools, and database reorganization tools. The OPM tools will be ported to additional commercial DBMSs. The proposed work will be conducted in close collaboration with GDB, GSDB, and PDB staff.

LABORATORY INFORMATION MANAGEMENT (ZORN)

The data submission tools will be expanded to cover submissions to multiple databases at the same time without requiring the user to repeat information. We plan to interface SubmitData with sequence analysis tools to map sequence annotations directly from the analysis programs into the submission formats. Direct connectivity to the public databases will provide immediate response for large scale submitter.

Parallel processing provides an easy mechanism to increase performance on time consuming tasks, like sequence similarity searches. Within the framework of POET, which acts as a mediator between the application and the parallel processing environment, we have developed BioPOET as a special example of a "Bag of Task" problem, i.e., one sequence is compared with one database sequence for all the sequences in the database. Multiple processors, e.g., workstations, can work independently on the tasks, and thus achieve a performance increase linear in the number of processors. This project has been funded internally by LBNL. Increased interest warrants to pursue this project with a dedicated effort and staffing of at least 1.5 FTE.

Medical Applications

VASCULAR AND BLOOD DISEASES (EBBE, TAYLOR)

Work will continue on the synthesis and selection of radiotracers for non invasive imaging of atherosclerosis. The breadth of our project will expand to include the imaging of carotid arteries, in light of recent reports that strongly conclude that early surgical intervention is the proper course of action to prevent strokes in patients with partially occluded carotid arteries. The ability to determine the extent of blockage using a non invasive modality will be of great diagnostic importance. Consideration will be given to the following classes of agents: 1) porphyrins, specifically ^{68}Ga- or ^{18}F-labeled

benzoporphyrin derivative-monoacid (BPD-MA), which has been shown to accumulate rapidly and specifically in atheromata (and tumors), and is being tested elsewhere in clinical trials in its unlabeled form as an anti-cancer agent utilizing photodynamic therapy. This work will involve the development of methods for the fluorination of porphyrins, resulting in radiofluorinated compounds that are better suited for PET than their ^{68}Ga-analogs, due to a longer half-life and lower energy positrons; and 2) development of radiolabeled aptamers to proteins associated with atherosclerosis. Aptamers are single stranded DNA oligonucleotides that specifically bind to selected peptides. The first work will be done in collaboration with Dr. M.H. Barcellos-Hoff to develop an aptamer against TGF-ß, which we have shown to accumulate in atherosclerotic lesions of our rabbit models. We are currently using a thrombin aptamer as a model compound to study the most efficient means of attaching radiotracers to the DNA oligomer.

A limitation on the current research is the identification of an animal model for atherosclerosis that closely mimics conditions found in humans. The current rabbit model that utilizes dietary fat to induce atheromata is limited in that it does not develop the cap of smooth muscle cells covering the lesion as in found in human atherosclerosis, thus the target tissue for ligand selection is limited to the fatty portion of the lesions. Further development of other models, including a combination of high fat diet and deendothelialization, use of endothelial and smooth muscle cell cultures, and possible usage of transgenic or knock-out mice for preliminary ligand selection, will be considered in order to obtain the best selection protocols.

Additional studies of comparative megakaryocytopoiesis and platelet production are necessary in different species and in genetic variants in mice to test our initial findings and thereby to define the roles of 5-HT and megakaryocyte ploidy in regulating the production of blood platelets. Some of these experiments will be done in collaboration with a veterinary hematologist. It is conceivable that analysis of some of the unique genetically engineered mice, produced at LBNL, will be informative, and very preliminary observations have been made in collaboration with Dr. Maria Pallavicini.

THE CENTER FOR FUNCTIONAL IMAGING (BUDINGER)

New Initiatives — Long Term Goals

Biodistribution Service to the Boron Neutron Capture Therapy

The fact that the boron compounds of the porphyrin class usually require 24 hours to reach a tumor to blood optimum ratio has been seen as a limitation to the use of radioisotope imaging methods which generally employ short-lived radionuclides. The longer lived radionuclides appropriate for imaging such as gallium-67 for single photon studies and iodine-124 for positron emission tomography have some limitation of high dose for the required statistics or limited resolution for acceptable quantitation (e.g. gallium-67). Over the past 10 years we have labeled a wide variety of porphyrin compounds with gallium-68 on a DOE project to investigate imaging atherosclerosis (Ebbe/Taylor). Other radionuclide substitutions of metalloporphyrins have been investigated, and although most of the porphyrins have been found to localize in the atheroma in animal studies, blood clearance has been too low for human imaging studies within a few half-lives of the tracer. The candidate boron compounds could be fluorinated or labeled with Cu-64 and used as tracers for biodistribution studies.

Within this background and that of our long-standing kinetic analysis projects we have derived a strategy for determining the equilibrium distribution of candidate boroporphyrin or other boron compounds using quantitative PET studies over a few hours after injection of traces of the labeled compound. The procedure is to caculate using measured kinetic parameters the tissue to blood concentration expected near equilibrium or sooner for compounds which interchange between blood and tissue when the tracer has a half life shorter than the time needed to reach equilibrium. These kinetic constants are readily determined by dynamic PET or SPECT imaging.

Serotonin

In the next project year, we anticipate performing new studies to investigate the serotonergic system. Initial studies will utilize a new ligand, developed for use as a potential SPECT tracer, with the ultimate development of a PET tracer as well. This tracer, [^{123}I]5-iodo-6-nitroquipazine ([^{123}I]5-I-6-NQP) is a marker of presynaptic serotonergic terminals, which shows favorable *in vitro* and *in vivo* characteristics indicating its potential utility as a SPECT tracer. Initial studies with and without pharmacologic

blockade in sub- human primates will be performed using the tracer with an upgraded multidetector SPECT system (the Strichman 910) capable of 6-8 mm resolution in plane. These studies anticipate future high resolution PET studies using ^{18}F analog studies of the raphe nucleus and other small mid-brain structures requiring the high resolution approach unique to LBNL. With the arrival of our cyclotron, we have the opportunities for studies with ^{11}C ligands for the serotonergic system and this approach might have priority particularly with the ready availability to study the raphe nucleus in humans using the 600 crystal high resolution PET.

m-Tyrosine Studies in Gene Therapy

Evaluation of the effectiveness of gene therapy of Parkinson's disease Imaging studies in monkeys with Parkinson's disease induced by lesioning will be used to study the effectiveness of implanted transfected cells. Fluorinated m-tyrosine and high resolution PET studies of normal and Parkinson's lesioned monkeys Preliminary imaging studies of animals with genetically modified fibroblasts have started. and studies are done two-times each week with sub human primates from U.C. Davis and Dr. Chris Bankiewicz of Somatix.

High Spatial Resolution Proton Spectroscopy in Neurodegeneration and N-Acetylaspartate Evaluation as a Marker of Neuronal Mass

We hypothesized that NAA content would be a measure of neuronal mass; and proton spectroscopy would allow us to normalize PET glucose metabolism to neuronal mass. At the time of submission we observed a nearly equal content of NAA in white and gray matter. This observation and the well documented postmortem ratio of 4:1 in NAA concentration between gray and white matter lead us to hypothesize:1)The rate of NAA enzymatic digestion is a few times faster in white matter than gray matter after death ;and2)NAA content can not be used as a measure of Gray:white matter. The progress this year in proving these hypotheses

Preliminary studies with New Zealand white rabbit brains, obtained from animals used for ex-vivo red blood cell perfused heart experiments were done with NMR spectroscopy over 2.5 hr periods. The NAA concentration changes in postmortem brain at 36° C showed NAA degrades more quickly in white matter brain than in gray matter brain. If we extrapolate our data using the same speed of degradation to the moment of rabbit death we find the same relative amount of NAA in both white and gray matter at the beginning of the experiment.

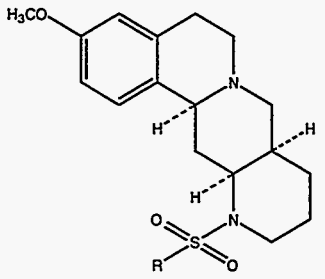
We will confirm these results in the live animal by studies before and after sacrifice using very high spatial resolution spectroscopy. We also have commenced human studies with 0.2cc volume resolution on our AD patients at UCSF. Contemporary results with volumes so large that all the volume elements are contaminated by mixtures of white matter, gray matter and CSF.

α2 - Adrenergic Receptor System Studies

The goal of this work is to non-invasively quantitate α_2-adrenergic receptor sites using positron emission tomography (PET). It is anticipated that the development of these radiopharmaceuticals will aid in the study of the brain-cardiovascular system, behavioral disorders and Alzheimer's disease. Of particular interest to us is imaging the α_2-adrenergic system of the human cortex, hippocampus, locus coeruleus, and nucleus tractis lateralis. This research has been underway for the last 5 years with the development of a suitable ligand and PET instrumentation and MRI alignment work needed to study subcortical regions and nuclei in the periaquaductal gray matter. We can use the 2.6 mm PET scanner with our MRI based localization technique to place the single section through the locus ceruleus. We have shown it is possible to bring the frontal cortex of the human brain near the center of the tomography to achieve the resolution of 3 mm. This project awaits the high affinity α_2 ligand.

Due to the problems encountered with the originally proposed synthesis of 5-[^{18}F]Fluoroatipamezole, an alternate route is now being pursued pursued by Dr. Joel Enas of the chemistry group.

In an effort to find other radiopharmaceuticals with equal or better potency and selectivity towards the α_2-receptor as atipamezole ($\alpha_2/\alpha_1 = 8000$), we are currently collaborating with Syntex Pharmaceuticals of Palo Alto to investigate the potential of compounds of the type shown below as PET ligands. This class of yohimbine analogs, the "isoquinonaphthyridines", exhibit α_2/α_1 selectivities in the range of 2000-15000 with pK_i values in the range of 8.6-9.4 making them among the most selective and potent α_2 ligands known.



R = fluoroalkyl

Fully 3D Reconstruction for Septaless PET Systems in New Geometrical Configurations

We intend to investigate and evaluate fully three dimensional reconstruction algorithms for PET systems. Of special interest are small special purpose devices for imaging human breasts and lymph nodes. These systems would not have some of the spatial symmetries which are exploited to reduce the computational burden of image reconstruction and data analysis.

A particular design we have been considering has a rectangular aperture instead of a circular one. The arrangement of the detectors consists of four planar arrays configured as two pairs of opposing faces of a rectangular parallelepiped. Transverse sections cut through the detector array to form rectangles instead of circles which is the norm. Sensitivity is increased by placing the detectors as close as possible to the patient and by eliminating scatter reducing septa. Electronics for such a system would place detection of an annihilation photon from any of the four faces in coincidence with detection of an annihilation photon from any other face. In addition to two dimensional position information of each photon detection in its respective plane, we will also estimate the depth of interaction of each photon in its respective detector.

Although such a special purpose device is physically small, the attainable resolution and the increased sensitivity (without scatter reducing septa) lead to very high data rates and very large data set sizes. The lack of symmetry increases the difficulty of using computational tricks to decrease the complexity of the problem.

We intend to pursue several avenues to solve the image reconstruction and data analysis problems associated with such devices.

EXPERIMENTAL MEDICAL DEVELOPMENT OF RADIONUCLIDES (VAN BROCKLIN)

With the completion of the Biomedical Isotope Facility in June 1995 and the commissioning of the medical cyclotron by September 1995, we will have the wherewithal to expand our efforts on the development of labeling strategies for the incorporation of short-lived isotopes into medicinally useful compounds. We will embark on new studies of the metabolic pathways peculiar to cancer and metabolic pathways associated with human genome patterns including obesity, hypoglycemia, diabetes and neurodegeneration. One example will be our program to develop novel molecular medicine tumor imaging agents based on the phenomenon of protein binding to oligonucleotides (DNA, RNA). Classical approaches using monoclonal antibodies specific for intracellular proteins, tumor antigens, or membrane receptors have shortcomings which compromise image quality. A novel approach to tumor imaging involves the labeling of short chain single stranded DNA also known as aptamers, designed specifically to bind tumor proteins or"membrane receptors. These aptamers will be an attractive alternative to monoclonal antibody imaging.

IMAGING OF APOLIPOPROTEIN E-BINDING RECEPTORS *IN VIVO* (KRAUSS)

Lipoprotein receptor imaging, near-term objectives: Optimization studies of PET imaging of the receptor probes in rabbits will be used to determine appropriate doses and PET scanning conditions for our human studies. Once these conditions are established, results from normal subjects will be compared with those from patients with heterozygous familial hypercholesterolemia, who express approximately half-normal apoB,E receptor activity. These studies should allow evaluation of PET imaging as a means of quantifying apoB,E receptor activity, particularly in the liver, the organ responsible for the major portion of receptor-mediated LDL clearance.

Atherogenic lipoprotein subtypes for atherosclerosis imaging.
Among the various human Lp(a) and small dense LDL species to be examined, those exhibiting the greatest uptake and localization in sites of atherosclerosis in cholesterol fed rabbits will be characterized in greater detail so as to identify

potential determinants of atherogenicity. Characterization will include studies of physical properties (size, charge), chemical composition (lipids, apoporteins, carbohydrates), and importantly, functional characteristics including binding to endothelial cell and smooth muscle cell receptors, binding to extracellular matrix components, such as chondroitin sulfate, and susceptibility to oxidative modification.

Long-term objectives:
The receptor imaging agent(s) to be developed in this project will give an overall assessment of tissue uptake capability for LDL as well as potentially atherogenic apoE-containing lipoprotein particles. This methodology would have considerable investigative and diagnostic utility in studying function and regulation of apoB and apoE receptors under physiologic conditions, with diet and pharmacologic therapy of hyperlipidemia, and in genetic disorders characterized by defective clearance of LDL and other atherogenic lipoproteins.

Ongoing and projected programs in the Department of Molecular and Nuclear Medicine at Lawrence Berkeley National Laboratory include a number of specific research projects which would enhance the potential utility of the lipoprotein receptor imaging methodology described here:

1. Refinement of methodology for whole body PET imaging in humans.

2. Studies of LDL and apoE receptor variants in human disease states.

3. Studies of dietary and drug influences on human lipoprotein metabolism.

4. Investigation of genetic influences on lipoprotein metabolism in humans and in transgenic animal models.

It is expected that identification of determinants of localization of atherogenic lipoprotein subtypes to atherosclerosis lesions will lead to the development of novel atherosclerosis imaging agents based on these properties. These agents will be tested in experimental animal models, and ultimately, in patients with known atherosclerotic disease.

Table 10: Facility Construction and Modifications

LBNL Human Genome Laboratory

- Funds totaling $24,700,000 for the construction of the new Human Genome Laboratory at Lawrence Berkeley National Laboratory were approved by Congress in December 1993.
- 44,400 gross square feet.
- 24,200 assignable square feet.
- A planned total of 92 staff would be accommodated, including senior scientists, postdoctoral associates, graduate students, technicians and support personnel in light laboratories, equipment rooms and office space in support of the DOE Biological and Environmental Research Program.
- The Environmental Impact Report (EIR) and was approved by the Regents of the University of California on October 26, 1994.
- An Environmental Assessment was prepared in accordance with the National Environmental Protection Act (NEPA) and a Finding of No Significant Impact was provided by DOE on March 30, 1995.
- Groundbreaking is scheduled for mid-August 1995.
- Building to be completed and occupied by 1998.

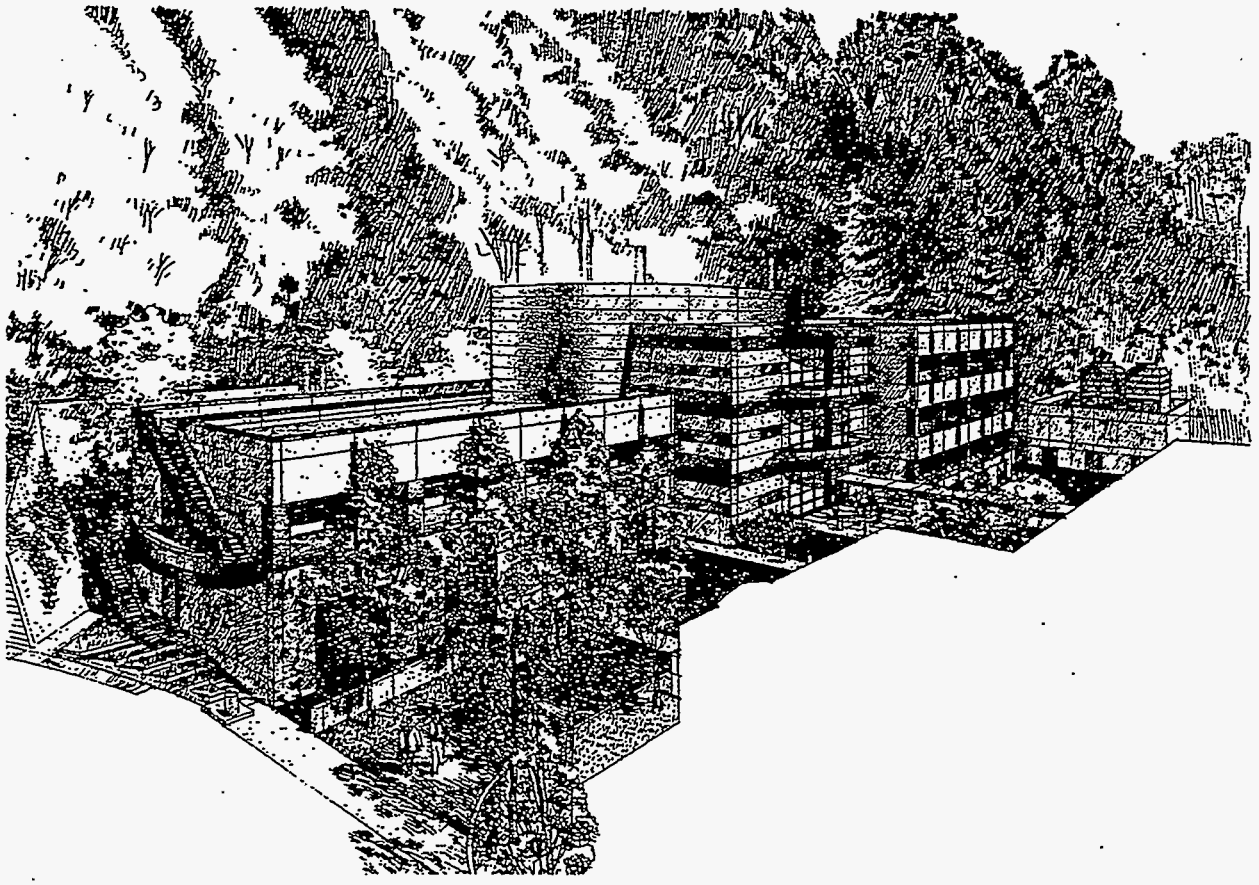


Table 11: Major Equipment Requirements for FY 1995-FY 1997

PRIORITY LIST OF KP EQUIPMENT REQUESTS, LIFE SCIENCES DIVISION

- Water purification system needed for new laboratories in Building 70A—$11k
- Indigo Extreme Graphics Workstation needed for quantitative analysis of PET and other images in the Center for Functional Imaging—$30k
- FPLC protein purification setup needed for preparation of clean fractions of lanthanide-calabeled antibodies and avidin, for the soft x-ray microscopy program—$20k.
- Robotic slide handling system needed for further development of automated molecular cytogenetics—$42k.
- ß-scintillation counter, to replace non-functional 20-year old unit in Donner Laboratory—$28k
- Laminar flow hood, two double-door $C0_2$ incubators, refrigerated centrifuge and tissue culture centrifuge, needed for new investigator—$43k
- Helium leak detector. Needed to support the operation of the mini-medical cyclotron—$11k
- Hamamatsu CCD camera. Required to record images from straightened DNA molecules up to 420 µm long, in the Resource for Molecular Cytogenetics—$34k
- Vacuum evaporator used for routine electron microscopy in Donner Lab. Needed to replace non-functional, 20-year-old instrument—$20k
- Environmental shaker for bacterial cultures, to replace old machine, worn out and is no longer cost-effective to repair—$7k
- Ultracentrifuge. Needed for lipoprotein separations, to replace a 20-year-old instrument—$35k

Total $281,000

In addition, there are a "big ticket" items, any of which would consume the entire Life Sciences equipment allocation of $109,000 when overhead is added.

- Phosphoimager. For rapid, sensitive readout of auotradiograms—$80K.
- Automated DNA extraction instrument is needed for comparative genomic hybridization (CGH) in the Resource for Molecular Cytogenetics—$106K.
- Image Plate detector system is needed for protein crystallography on the rotating anode x-ray set in Donner—$200 K.
- Pulsed x-ray facility within the Center for Functional Imaging—$100 K.

See the "Contemporary Issues" section for further discussion of major capital expenditures.

6.0 Contemporary Issues

"The laboratories' research role is a part of an essential, fundamental cornerstone for continuing leadership by the United States. We know that the studies and discoveries of science unravel the elements of nature and shower benefits on mankind. We know by intelligent estimates that there is much more to be learned and shared. We know that these scientific revelations will unfold from many sources: a brilliant insight by an individual, a research team at a university, a corporate or government laboratory — by accident, or on purpose. We know progress is hastened where diversity of personnel and institutions is encouraged.

We note that many of the least exploited investigative paths involve the need for extraordinarily sophisticated multidisciplinary teams using sophisticated instruments and tools. It is that role for which the national laboratories are uniquely qualified. It is the case for—the justification of—the existence of the DOE laboratories.

We are inclined to typecast these institutions simplistically by a few prominent contributions such as yesterday's bomb or the discovery of an element on the periodic table (both grand achievements), but overlook the multitude of other continuing achievements. We must reach out to know enough of this vast spectrum of accomplishments to justify our deserved support of these institutions that have contributed, are contributing, and will in the future contribute vital knowledge while continuing to revitalize themselves—just as science always renews itself. We must be in quest of that which we do not know in the field of science in every relevant way. Each revelation will enrich us manyfold."

—Robert Galvin—Chairman
Task Force on Alternative Futures for the Department of Energy National Laboratories—1995

"Vision 2000 is our strategic blueprint for the future. It focuses on basic research as our primary endeavor, but also establishes a commitment to reach out and forge new partnerships with industry, academia, and other national laboratories, to develop our resources and our people to create value for the economy, to enhance education, and to contribute to the community."

—Charles V. Shank, LBNL Director—1993

The core of the "Galvin Report" and the mission set forth in LBNL's "Vision 2000," have much in common. We, the investigators in LBNL's OHER-funded programs, mean to build on this fertile common ground.

Based on its basic research strengths, LBNL can make major contributions to the mission of the Office of Health and Environmental Research. In particular, LBNL's bioscience research holds promise for the understanding and prevention of both hereditary and environmentally caused disease, as well as for establishing health and environmental protection standards. LBNL's Human Genome Center (HGC) is a national resource of expertise for automated techniques for sequencing human chromosomes and selected microorganisms. In addition, the HGC is advancing the use of transgenics and knockouts to understand the function of human genes, and data analysis and interpretation methodologies to speed the access to the information being generated. The Advanced Light Source is strengthening DOE's international position in structural biology by providing biologists access to the world's brightest source of soft x-rays and new beam lines for x-ray crystallography. LBNL's Center for Functional Imaging is developing the highest-resolution medical diagnostic systems in the world, while the molecular medicine program is providing insights into the development and prevention of atherosclerosis. LBNL's cell and molecular biology research is pioneering the understanding of many aspects of gene expression, aging, and radiation-sensitive cancers in selected areas complementing the expertise on the UC Berkeley campus. Environmental research capabilities in pollution sensing, transport, effects, and cleanup promise to reduce costs for environmental remediation problems.

ANALYTICAL TECHNOLOGY

The current level of funding has allowed us to carry out a broad-based research program to develop a wide array of semiconductor detectors, materials, characterization techniques, electronic and system designs. Technologies developed in these areas have benefited many programs related to DOE's missions, as well as other federal and civilian research projects. Many of the technologies have been, and are still being, transferred to the rest of society. With, in effect, the termination of this program this coming year, our effort will necessarily become more narrowly focused and be directed towards specific projects. At this point, pending funding from a new proposal with OHER, we are expecting to focus on the development of field portable detector systems for environmental monitoring employing room temperature CdZnTe and silicon drift detector designs.

ENVIRONMENTAL RESEARCH

The "Galvin Report" discusses the benefits of ensuring that individual laboratories have a critical mass of expertise and technical capability in specific program areas rather than having these resources dispersed at sub-threshold levels across the entire system of laboratories. The Report suggests the creation of "Centers of Excellence" within the laboratory system as one approach for institutionalizing the necessary areas of specialization.

The Center for Environmental Biotechnology

One such area developing a critical mass at LBNL and deserving of OHER funding consideration is the Center for Environmental Biotechnology. This new initiative was born out of a workshop last year where LBNL was identified as having the capabilities of assuming a central role in uniting players in the field of environmental reseaerch from University of California and the national laboratories. The Center for Environmental Biotechnology will leverage the capabilities from such resources as those being cultivated in the Advanced Risk-Based Environmental Technologies and Remediation Initiative. This initiative provides integrated, interdisciplinary technologies to support the development of risk-based environmental policy on problems of national importance, such as remediation of subsurface contamination, urban air pollution, and stratospheric ozone depletion. This is a partnership between LBNL and the Department of Defense (DOD)/ McClellan Air Force Base. The initial focus of the collaboration is the development of user-friendly software, called SELECT, for evaluating and comparing environmental remediation technologies with respect to their risk reduction and cost effectiveness. The software builds upon existing codes for such sub-components of methodology as transport and transformation of multimedia contaminants, background and site-related exposures, and relative ranking by carcinogenic hazard for chemicals of concern.

Under the auspices of the Center, programs are being established in key focus areas of pollution prevention, bioremediation, waste site characterization and monitoring, hazardous waste treatment, human exposure and comparative risk assessment, and ecological risk assessment and restoration. In this way, the Center is bringing together not only the capabilities at LBNL, but also link those established within academic

institutions and government research institutions. The Center's goal is not to be redundant but rather to build bridges that link different capabilities within the DOE laboratories and associated universities and to focus on key environmental clean-up problems by enhancing synergy rather than competition between the various institutions.

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Basic Research on Indoor Air Quality

Over the past decade, OHER's collaboration with the Office of Energy Efficiency and Renewable Energy (EERE) on indoor air quality and buildings ventilation research has contributed substantially to DOE's efforts to reduce the energy used in buildings. OHER's support of the more basic research on indoor air quality, and on radon in particular, has provided a strong scientific basis for the more applied research and technology transfer that EERE has supported. This partnership has yielded some important results. For example, the fundamental research on indoor radon sources, entry mechanisms, and indoor concentrations supported by OHER indicated that properly-designed building energy efficiency programs (both weatherization programs for existing homes and efficiency measures in new homes) could proceed without causing significant increases in indoor radon concentrations. The basic research on radon sources and transport into buildings also led directly to the development of highly effective radon mitigation methods that save energy compared to simply increasing building ventilation rates. In addition, OHER-sponsored basic research has provided the necessary knowledge for ongoing EERE research on new radon mitigation technologies which have the potential to further reduce energy use by more than 85% and eventually save $200 million annually in energy costs.

Additional basic research is needed to support EERE's goal of further reductions in buildings' energy usage through reduced ventilation, advanced ventilation systems and advanced building materials for energy-efficient building shells. This research is also needed to support DOE's efforts to promote a more productive and competitive economy. Although much progress has been made in understanding the indoor behavior of radon and reducing uncertainties in the risks of radon, substantial scientific uncertainties remain with respect to a number of other equally important indoor pollutants that result from, or are affected by building materials, building systems (e.g., heating, ventilating, and air conditioning systems), and building operation and maintenance practices. These pollutants include volatile organic compounds (VOCs), bioaerosols, particles, and indoor combustion source gaseous pollutants (CO, CO_2, NOx).

In order to address these uncertainties, basic indoor air quality research that will support the applied research and technology transfer in EERE need to be focussed in two major areas:

Indoor Atmospheric Science. In contrast to our understanding of the troposphere and stratosphere, the physical and chemical behavior of pollutants other than radon in indoor atmospheres is not well understood. Since most of the population spends 90% of its' time indoors, indoor pollutant exposures often are the major determinant of health risks associated with airborne pollutants. A strong scientific understanding of the indoor atmosphere will provide the basis for the predictive capabilities required to select energy-efficient ventilation technologies and building materials which optimize human health and productivity.

Building-Related Health Effects. There is substantial and convincing evidence that several adverse health effects are related to chemical and microbiological pollutants that are emitted by building materials or building equipment, including heating, ventilating, and air conditioning equipment. In addition, building ventilation systems and naturally-occurring indoor air motion affect the transport of these pollutants from sources to occupants. Indoor micro-biological and chemical pollutants are known to trigger symptoms of asthma. In 1985, asthma was responsible for approximately 1.8 million emergency room visits. Health care costs for asthma were estimated to be $6.2 billion in 1990. Sick building syndrome (SBS), which appears to affect about 20% of new office buildings and a smaller but significant fraction of older buildings, is another serious problem related to the quality of indoor environments. SBS is a source of costly litigation as well as lost work days and productivity. Building ventilation systems and their mode of operation also affect the rates of transmission of important infectious diseases such as influenza (approximately 20 million cases occur annually), tuberculosis, and common colds. The cause and effect relationships between these health effects and specific building factors are not well understood.

If DOE is to be successful in meeting its energy efficiency goals, which will require advanced and energy-efficient technologies for buildings, a better understanding of methods to reduce or eliminate there building-related adverse health effects will be required. Consistent with

the DOE mission, there is also a potential for substantial gains in human health (and reduced health care costs), improved office worker productivity, and increased economic competitiveness of the U.S. through improvements in indoor environments that are compatible with energy-efficiency. The value of these benefits, in fact, is likely to be substantially greater than the value of all of the energy used in the buildings sector.

HEALTH EFFECTS/GENOMICS/GENERAL LIFE SCIENCES

Genome

Ground-breaking for LBNL's new Human Genome Laboratory is scheduled for August 1995 with completion of the facility set for early 1998. Within the next five years, LBNL will seek a dramatic increase of the current level of funding in Life Sciences. We anticipate an increased emphasis on our unique multi-disciplinary capabilities—large-scale sequencing with advanced instrumentation and computational support systems. Genome management has redirected the LBNL megabase sequencing effort to include chromosomes 5 and 20, and with support from the National Institututes of Health, the sequencing of *Drosophila melanogaster.*

LBNL has recently taken the initiative to form a consortium with the other DOE/UC Genome Centers at Los Alamos and Livermore, to engage in a collaborative effort in sequencing nearly one-third of the human genome. Fruitful discussions have already taken place with DOE Headquarters in this regard. It is expected that LBNL will continue to play an important role in this effort.

The directed sequencing strategy is less than three years old. As such, it is in the first of what are likely to be several rounds of technology development involving novel automation, informatics, and biological approaches. Each round encompasses the assessment of current bottlenecks, the design of new modules of automation, informatics, and biology, followed by the assessment of the behavior of the new system. The directed sequencing strategy is in competition with and attempting to replace the current shotgun sequencing strategy, which is over 15 years old and has a much more mature technology base in place. With this new technology, 2.5 megabases of genomic sequence has been generated in the last two years.

One of the most critical elements to the success of the megabase seqencing effort at LBNL is the automation in support of the directed sequencing strategy. The ability to work with the automation group and develop novel instrumentation has been a key to our ability to develop the directed sequencing process to its current level of success. It is essential that this significant effort continues for the foreseeable future. First, this is needed so that successive rounds of LBNL designed custom automation can be developed and brought into production use. Second, it is important to have the ability to identify and utilize those new technologies that may be developed outside of LBNL and be able to move them from advanced research stages (where they typically are left by non-production R&D groups) to a level of development where they can be used in sequence production operations. The government is currently funding a wide array of sequencing technologies at numerous sites. The current high level of commitment to custom automation needs to be continued for the foreseeable future in order to ensure the competitiveness of the directed strategy.

A second requirement is in the area of software development. A major challenge is the development of software that captures and utilizes the information content of the directed sequencing strategy. It is this information that gives the strategy much of its advantage over the current shotgun strategy, especially when applied to mammalian templates. Custom software tailored to support the strategy in use at LBNL is another component critical to the program's success. The large scale of operations and the uniqueness of the informatics requirements of the directed sequencing strategy will require a significant software development effort. The current software modules are stopgaps, with the development of future modules essential for increases in productivity and the ability to track and manage an expanding directed sequencing effort. The uniqueness of the LBNL strategy and the long term dominance of the informationally simpler shotgun strategy makes the majority of the currently available software tools for sequencing poorly suited for our use. The current use of available software modules has a significant cost in terms of the efficiency of our operations. New software developments should emphasize the integration of tools and databases into a common tool set. Too many individual programs perform well on a single task but cannot be integrated or interfaced with other programs making it necessary to write nuisance routines to reformat data, etc. Separating the analysis tools from the database access modules and graphical user interfaces is a first step in this direction.

Transgenics

The various centers providing transgenic and targeted

mice for DOE researchers must devise a standard protocol regarding the structure and exchange of materials with regard to the creation of the transgenic and targeted mice. User facilities are available at multiple institutions to provide models for this. With regard to the question of which center provides animals to which investigators, the directors of the various resource centers might divide up the requests. An advisory board consisting of members from all DOE labs would be available to oversee this process. Quality control in the process would come from the fact that the different resource centers will by necessity function at similar levels of efficiency; resource centers must exchange technology such that DOE investigators can receive transgenic and targeted mice in a rapid and cost effective manner.

As each resource center has the capability to make both targeted and transgenic animals, it seems unnecessary to divide the work into targeted versus transgenic centers. None of the potential resource centers have unique capabilities with regard to providing embryonic stem cells, targeting strategies, or transgenic mice. Division of the work load should result from other factors such as turnaround time (in order to prevent a backlog situation), location of the investigator, and project interests of the investigator.

LBNL has already committed a large portion of its General Plant Projects(GPP) funds to renovate and provide modern equipment for the Transgenic Facility. We have requested funds for science projects related to the Facility including deciphering the function of unknown human gene sequences.

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Environmentally-induced malignancies represent a major health concern following accidental and/or cumulative exposure to genotoxic agents such as radiation and carcinogens. Biomonitoring is an important federally mandated effort to address public concerns about increased risk of developing malignancies. Biological dosimetry and risk assessment is often based upon analysis of genetically-damaged reporter populations present in the circulation which are believed to derive from genetically-damaged stem cells.

LBNL has embarked on a new project in genetic susceptibility using a mouse model system that would eventually be applied to humans. Translocation frequency analysis suggests that radiation dose estimation can be accomplished at extended intervals post radiation exposure. Recent developments using fluorescence *in situ* hybridization for translocation analysis allow monitoring of translocation frequency in reporter populations in acute and chronically-exposed individuals. However, underlying assumptions in using reporter subpopulations for risk assessment remain untested and the biological basis and significance of these measurements remains poorly understood. A proposal in this area has been submitted to OHER. In FY 1996, we intend to pursue this topic with additional projects focusing on the concept of genomic instability.

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Center for Applied Genomics

LBNL is forging a new initiative to take full advantage of the capabilities available in the Resource for Molecular Cytogenetics, the Human Genome Center, as well as the expertise in cell and molecular biology, human cell cultures, and transgenic and knock-out technologies. A single integrated unit will emerge as the technological touchstone to be known as the Center for Applied Genomics.

Motivation

Assessment of the health effects of radiation and other clastogenic agents is a long term goal of the DOE. This has been attempted for several decades by measuring frequencies of chromosome aberrations in peripheral blood lymphocytes and/or frequencies of mutant peripheral blood cells (e.g. red blood cells carrying mutant glycophorin A). These measures seem to perform reasonably well for populations but not for individuals. Individual risk assessment is more difficult since it requires an understanding of individual susceptibility that most certainly will vary from person to person. Thus, understanding the basis for individual variation in susceptibility is essential to the goals of the DOE Health Effects Research Program. In fact, pursuit of this goal was a major motivating factor in the establishment of the DOE

Human Genome Initiative.

It is exciting that the technologies being developed by the human genome program are now maturing to the point that they can be applied to understanding individual risk to health from exposure to radiation and other by-products of energy production and use. Thus, the time is appropriate to establish a Center of Applied Genomics (CAG) that will develop the basic knowledge, applications and technologies for use by researchers at LBNL and elsewhere. The center will be the nucleus of a comprehensive program to elucidate the genetic bases of susceptibility to radiation (or other energy related clastogen)-induced human disease. Cancer is one of the most prominent radiation induced diseases and it is the main focus of this proposal. Several biological pathways/processes have been identified that may contribute to cancer induction and/or progression when altered by mutation. These include transmembrane signaling, cell cycle regulation, apoptosis, senescence, telomere structure, and cell adhesion. However, other diseases (e.g., atherosclerosis) whose development and/or progression is accelerated by radiation also may be studied.

Strategy

Several approaches will be taken to identify genes associated with cancer (and other relevant diseases as the program develops). For cancer, one approach is to begin by identifying regions that are frequently abnormal in advanced tumors identified using techniques such as comparative genomic hybridization (CGH) and analysis of loss of heterozygosity (LOH), to progressively narrow the regions of abnormality and eventually to identify and characterize the genes in these regions that effect the abnormal (neoplastic) phenotype. Another approach is to start from analysis of specific biological processes (e.g. cell-extracellular matrix disruption, escape from replicative senescence, loss of telomere function or DNA repair capacity, etc.). The aberrations affecting target genes may result in either gain or loss of function. Depending on the starting point, any one project may draw upon different components of the center. Possible components/functions of the center are described below.

I. Definition of regions of consistent abnormality. Numerous tumors (especially human cancers of the breast, ovary, brain, prostate and colon and murine model tumors) have now been assessed using CGH with the result that numerous regions of consistent abnormality have been identified and localized with the ~10 Mb resolution allowed by CGH. This information, and in most cases the tumor samples, are available at LBNL from UCSF and collaborating institutions to support positional cloning efforts.

II. High resolution aberration definition. Positional cloning of genes associated with regions of common deletion or amplification begins with localization of the region to within 2-3Mb through analysis of the physical location of the aberrations in many tumor samples. This approach is based on the assumption that tumor suppressor genes or oncogenes that contribute to tumor initiation or progression when inactivated/activated will be located in a small region of reduced/increased copy number that is common in many tumors (even though the region of deletion/amplification may be large in any one tumor). With current technology, tumors are analyzed every few Mb using FISH with mapped probes. The Resource for Molecular Cytogenetics is developing a set of closely spaced and well mapped probes for this purpose. Unfortunately, locus by locus analysis is time consuming and labor intensive. CGH to arrays of mapped probes (CGHa) should overcome these problems. However, CGHa is not yet developed and resources for its development are not adequate. LDRD funds are requested to support 2 FTE to assist in development of CGHa (one with a molecular biological background for technique development/application and one with an engineering background for array production) and then to apply this technique to high resolution tumor characterization.

III. Contig construction. The next step in positional cloning is to further narrow the region of common abnormality. This will be accomplished by high resolution analysis using CGHa to distribute probes contiguously over the 2-3 Mb region already defined. Except in rare instances, these contiguous probes must be developed. The LBNL's Resource for Molecular Cytogenetics and Human Genome Center already have developed procedures for efficient probe selection and physical map assembly (e.g. Probe selection by PCR screening and filter hybridization, STS content mapping and DNA fiber mapping).

IV. Gene identification. Once the regions of interest are narrowed as much as possible by high resolution CGHa, candidate genes already mapped to the region can be assessed for involvement in tumor progression. Unfortunately, this approach is not very powerful at present because of the limited number of mapped cDNAs. Thus, it is likely that the assembled contigs will have to be screened for additional genes. This may be accomplished using exon trapping, cDNA selection and/

or by genomic sequencing but genomic sequencing seems the most powerful approach for the future. The LBNL Human Genome Center is one of the world leaders in this technology.

V. The identified genes that appear as likely candidates based on gene structure that may be involved in tumor progression or initiation will be explored further (e.g. homology to genes known in other organisms to be involved in processes such as cell cycle regulation, membrane signaling, apoptosis, senescence, adhesion, etc.). The approach taken will depend, to some extent, on whether the gene is expected to be a tumor suppressor gene or an oncogene. In either case, functional analyses will require cell culture systems appropriate to the postulated function. For many gene functions (e.g., those involved in some aspects of cell cycle progression, replicative senescence, telomere function, DNA repair) fibroblasts, which are easy to culture, will be appropriate. For other genes, however, more specialized cell cultures (e.g., breast epithelial cells, keratinocytes, B or T cells) will be established.

(a) Tumor suppressor gene identification. Tumor suppressor genes located in regions of LOH/reduced DNA sequence copy number are likely to be present in only one allele in these regions and to carry significant mutations. Screening for mutations in human tumors may be accomplished by DNA sequence analysis of genomic DNA or cDNAs isolated from human tumors. Candidate genes can be further analyzed for reversal of one or more aspect of the tumor phenotype in cultured tumor cells or cells transformed in culture (e.g. in breast epithelial cells grown on matrigel in culture) . Gene knockout experiments in cultured cells or transgenic mice will provide further proof that the gene is involved in tumorigenesis. This approach is complicated by the fact that knock-out mice often are uninformative (e.g. because the knockout is a lethal event). This limitation can be partially overcome by knocking out genes in a tissue- or adult-specific manner in mice (e.g. by replacing an endogenous gene with an unmutated transgene that is flanked by sequences recognized by excision enzymes). Mice with the substituted gene are crossed with mice expressing the excision enzyme under control of a late-expressing tissue-specific or inducible promoter so that the knockout occurs only in the tissues expressing the enzyme.

(b) Oncogene identification. Increased expression or function relative to normal is a hallmark of oncogenes. CGH to arrays of cDNAs isolated from the regions of common abnormality with mRNA from normal and tumor tissues will be developed to allow efficient analysis of the relative expression of the many genes expected from the region of common abnormality. Mutations that might alter function could be screened by sequence analysis, single strand confirmation polymorphism (SSCP), and denaturing gradient gel electropheresis (DGGE). Candidate genes can also be tested for oncogenic potential in model cell culture systems and ultimately in transgenic animals.

VI. Structural analysis. Cell and/or tissue localization and protein structure analysis are essential to a detailed analyses of gene product function. Digital imaging microscopy (e.g. confocal microscopy, energy transfer studies of inter-protein distances, and 3D localization) will be used for analysis of protein function at the cellular level.

Enabling technologies:

Creation of this Center for Applied Genomics should yield several enabling technologies that are not now available at LBNL:

- CGH to arrays for genomic analysis.
- A Contig Assembly Production Unit for efficientcontig assembly.
- A genomic sequencing facility that is *available* for applied research.
- CGH with mRNA on small genomic clones and later cDNAs for quantitative mRNA expression analysis.
- Re-sequencing and SSCP from tumor material. High through put PCR and sequencing/ analysis to identify mutations.
- A imaging facility for cellular analysis of protein function.
- Cell/animal models for gene function.

Involved groups:

- Cell and Molecular Biologists.
- Genomics researchers (from the Human Genome Center and the Resource for Molecular

Cytogenetics).

- Engineering
- Computation biology.
- Structural Biology.

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Structural Biology

New applications of advanced imaging, diffraction, and spectroscopy techniques will greatly strengthen DOE's emerging national program in structural biology. A structural biology research program is beginning to take shape at the Advance Light Source. On May 15, 1995 ground was symbolically broken for the ALS Structural Biology facility. LBNL intends to build on this emerging Structural Biology program at the ALS.

One particularly critical component of this program is the soft x-ray microscopy project. Although this project is still in the formative stages (see pages 42-43, "Research in Progress," "Significant Achievements," page 61, and section 7.6, "Selected Research Highlights," *Getting Closer to the Malaria Vector with Soft-X-rays*) we expect that by next fiscal year a decision to be made on whether to proceed on the construction of a facility for biological x-ray microscopy. LBNL has already launched an effort, partially funded by LDRD, to bring in additional users to the existing x-ray microscopy resources, in order to generate additional scientific data in support of new resources.

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The most significant near term issue facing many of the structural biology projects is the slow erosion of programmatic support, coupled with increased overhead and increased load of paperwork which negatively impact our ability to accomplish high quality scientific work. This clearly propagates to the long term as well. Another long term issue is how to develop support for instrumentation and computing resources which will keep us at the leading edge of science being done in this country. There is also a continuing need for more facilities dedicated to biological x-ray spectroscopy. In many instances, the community is hampered by the detectors rather than the storage rings. Funding needs to be secured for improving detectors that can take advantage of the brightness and flux of the new synchrotron radiation facilities.

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It has been difficult to maintain the current level of productivity in the Mass Spectrometry Detectors project into a second three-year budget renewal cycle because of extensive capital equipment requirements that a project such as this one requires. We have finally acquired the necessary test equipment, much of which had to be built in-house. Commercial mass spectrometers could have used as test stands in which we could have exposed our new detectors to ion bombardment, but they cost more than $200K. We are now progressing according to expectations, having built the testing equipment that we need to evaluate the new ion detectors. This detector development progress is demonstrated at the end of a three-year budget cycle, a time during which we spent most of our effort designing and building the prerequisite test equipment and only recently have we been able to test detectors.

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It is becoming clear that a new generation NMR instrument (at a cost of approximately $2 million) will be required in the moderately near term for the LBNL Structural Biology program to remain competitive in the area of structure determination by this methodology.

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While the funding levels for the Semiconductor X-Ray Detectors for Synchrotron Applications program are decreasing (in real dollars as well as in inflation-adjusted dollars), the internal laboratory costs of supporting staff are increasing. For example, in 1989 when this program was first funded at a level of $275K/year, the primary staff scientist working on this program cost ~$100K/yr. Today, with a reduced funding level of ~$250K/year, this same staff scientist costs $200K/yr. It does not need to be explained that it becomes nearly impossible to run a successful research program with only one staff scientist, with very little money to buy equipment and supplies or pay for additional technical staff. OHER's choices are to increase the funding for this program, or officially acknowledge that they can expect only a fraction of the technical progress in this program today, compared with just five years ago.

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A coordinated program in computational biology is needed to link the advancements of biological sciences with computer and information science at LBNL. Such an endeavor crosses all biological and biomedical research activities sponsored by OHER and will encompass databases of human, model organism and bacterial genomes, macromolecular structure databases, and medical databases. A computational biology initiative will also provide solutions to common problems of data representation, search strategy, pattern recognition, data visualization, integration of experimental data, new computational simulations and drug design. LBNL will be seeking funding from OHER to launch a computational biology initiative in FY 1996.

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In the field of Radiobiology, theoretical modeling and experimental measurements have revealed that the basic structure of a chromatin fiber inside a cell is like a three dimensional zig-zag model rather than the widely accepted solenoidal model. The zig-zag model needs to be examined in cells from different systems such as *Drosophila*, yeast, humans, etc. In addition to structural analysis of DNA, novel experimental techniques have been devised to study the induction of double strand breaks and the fidelity of rejoining in defined genomic locations. Furthermore, collaborators at LBNL and from another laboratory have demonstrated that damage in transcribed strands of active genes is preferentially repaired for all qualities of ionizing radiation. This group is at the cutting edge of the discipline and needs to be supported.

MEDICAL APPLICATIONS

Medical imaging technology for the study of disease states at the molecular level resides at the top of the national agenda for medical applications research. Center for Functional Imaging researchers are vigorously pursuing the development of high resolution, high sensitivity, and high speed positron emission tomography systems. An emerging program in the Department of Molecular Medicine is developing and applying innovative radiochemical probes for receptors and other molecular species involved in cancer, cardiovascular disease, and brain disorders. A new dedicated minicyclotron (11 MeV) will be installed on the LBNL site this summer. With this installation we plan an expansion of the LBNL nuclear medicine radiopharmaceutical, instrumentation and applications program by setting up a resource culture to make available the highest resolution PET system, new reconstruction algorithms and innovative radiolabeling activities.

New Opportunities— Doing More With Less

Despite the apparent endorsement made by the Galvin Taskforce of the key role the national laboratories play in long-term economic security and advancement of basic science, national laboratory initiatives continue to be constrained by limited budget resources. Constrained funding is the reality of a restructuring DOE and integral factor in health and environmental research strategic planning at LBNL. The costs of state-of-the-art facilities and added operational requirements limit the opportunities for setting new research objectives. Under these conditions, it is essential that initiatives be well coordinated and managed and involve effective scientific review. Setting priorities and improving cost effectiveness will be essential to initiating new programs that are vital to the national interest.

Strategic Partnerships

Establishing partnerships with other national laboratories, government agencies, and state governments will contribute to the success of LBNL's strategic planning effort. LBNL's research in structural biology, molecular genetics, and research medicine will greatly strengthen national programs supported by both the Office of Health and Environmental Research and the National Institutes of Health. LBNL's alliances with other national laboratories, including the LLNL, ORNL, PNL, and SLAC, offer promise to DOE for efficiently and effectively utilizing the expertise of the national laboratory system. The Laboratory is revising its internal organization to utilize its resources most effectively in meeting these challenges.

Historically, the research and development missions of the DOE national laboratories have supported energy research, defense, and the physical and life sciences. As we examine our mission in today's world, we must expand our efforts to realize our R&D capability with an eye to addressing the economic security of the nation. These changes in LBNL's role have been facilitated by DOE management policies that enable the national laboratories to work more effectively with

other government agencies, such as the National Institutes of Health, the Environmental Protection Agency, the National Aeronautics and Space Administration, and the Advanced Research Projects Agency and industry. LBNL has identified the following issues that affect our ability to fulfill the laboratory's mission:

- Implement OHER's mission for biotechnology research. DOE programs need to more fully support research that is broadly responsive to the long-term economic vitality of the nation. LBNL is working with OHER and other national laboratories to develop programs that make full use of current technical capabilities to benefit the nation.

- Enable industrial R&D partnerships. The Laboratory is working with DOE to develop work-for-others conditions and Cooperative Research and Development Agreement documents that reduce obstacles to the development of R&D partnerships and technology transfer.

- Establish alliances and agreements with NIH, NASA, EPA, and other agencies. The Laboratory's research relationships with NASA, NIH, and EPA have strengthened national research programs in space research, health, and environment. Agreements need to be forged to reinforce these mutually constructive relationships and reduce administrative barriers.

- Fully utilize laboratory and university capabilities. LBNL is unique among the multiprogram national laboratories in being located next to a large university. The Laboratory and DOE must continue working constructively to strengthen relationships with the University of California campuses as well as with other universities, and to devise an administrative framework that minimizes barriers to university and other partners seeking access to facilities. The Laboratory's unique research facilities must also be effectively utilized. We are delighted with the ALS's Structural Biology facility support, but effective utilization of the Advanced Light Source will require research support for users, to gain the full benefit of the investment in these unique national facilities.

Constraints on New Initiatives

LBNL's initiatives are constrained by limited budget resources, despite their key role in long-term economic security. Constrained funding is a recognized factor in OHER strategic planning. The costs of state-of-the-art facilities and added operational requirements limit the opportunities for setting new research objectives. Under these conditions, it is essential that initiatives be well coordinated and managed and involve effective scientific review. Setting priorities will be essential to initiating new programs that are vital to the national interest.

LBNL is undertaking management performance improvements in many sectors of our research and support operations to meet best management practices. These activities call for improved environmental, health, and safety programs; strengthened financial, acquisition, and personnel management; and improvement in the quality of services and delivery of information at all levels. A laboratory must have sufficient research funding to support these raised performance expectations. Additional program growth will permit enhancement of LBNL's support services while maintaining its high level of research capabilities and performance.

Limited DOE resources set constraints on the range and scope of LBNL's initiatives. In this environment, scientific excellence alone is no guarantee of success; the Laboratory must also demonstrate that its initiatives contribute to national needs. The Laboratory is working with DOE, the scientific community, state, and industry participants to establish priorities and to support initiatives that can best serve the nation under financial constraints.

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Developing Alternative Funding Strategies

The major critical constraint to the further development of several OHER projects at LBNL, and our ability to institute new initiatives is the DOE rule that prevents us from applying for small grants that do not provide full cost recovery. The scientific value of such grants cannot be underestimated. They provide essential support for developing new ideas in specific areas that are likely to be of interest to the DOE, but have not yet matured. Funding agencies that can provide seed or supplementary support to projects of interest to the DOE include the American Cancer Society, the Leukemia Society, the March of Dimes and the American Heart Association.

The DOE's insistence on full cost recovery places immediate economic gain above scientific considerations and long-term economic gain. With DOE's stated

intention to provide the nation with the highest quality science, the public would expect more of the national laboratories, and obtaining resources should not be so limiting.

Quality Science Requires Quality Equipment

During the last few years, the severe shortage of unrestricted equipment funds for basic health effects and medical applications has perilously eroded our ability to do quality science. This disintegration of support has also led to a retrenchment mentality which will also decimate the ability of LBNL and the other national laboratories to maintain their unique competitive edge in contributing to the advancement of science in the United States.

For example, one particular area where equipment needs are endangering our international preeminence is in electron crystallography. In order to maintain our extraordinary productivity (see *Water Channel Protein* and *Taxol Binding Site* in section 7.6, "Selected Research Highlights"), we will need to acquire a replacement for our currently disabled electron microscope. A comparable 300 KeV electron microscope with Field Emission Gun will entail resources on the order of $2 million, plus allocation and renovation of space.

We propose that restriction of the use of equipment funds be lifted and the laboratory be allowed to make internal decisions as to how to use the available funds. While the percentage of equipment funds to operating funds remains below 5% it will be difficult to remain productive. Thus, it is imperative that this trend be reversed.

7.0 Appendices

7.2 Staffing by Program Area

7.3 Information Transfer

7.4 Conferences, etc.

The following information is tabulated by division; a summary table is given at the end. The totals (T) given in the tabulations of personnel data reflect DOE support of the entire division, except for the Engineering, Information and Computing Sciences, and Earth Sciences Divisions,and Structural Biology, where the totals reflect only effort directly related to OHER-sponsored research.

Life Sciences

Personnel (FTE)	FY 1993a		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[b]	31.5	93.0	32.1	91.1	31.7	91.0
Post-doctorates	4.2	15.0	3.3	30.7	3.3	31.0
Technicians	20.4	64.0	31.2	91.3	31.5	91.7
Animal care	0.9	3.2	0.4	2.7	0.4	3.2
Total direct personnel	57.0	175.2	67.0	215.8	66.9	216.9
Professional (adm.)	5.4	15.0	6.0	17.0	6.2	17.5
Clerical, adm. support	6.1	17.0	6.7	19.1	7.4	19.8
Maintenance	—	—	—	—	—	—
Total indirect personnel	11.5	32.0	12.7	36.1	13.6	37.3
Visiting scientists[c]	10.0	17.0	14.0	40.0	13.0	39.0
Graduate students[c]	9.0	17.0	7.0	21.0	6.0	20.0
Undergraduates[c]	20.0	58.0	22.0	65.0	31.0	86.0

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	279	117	144
Chapters, reviews	7	22	26
Books, proceedings (edited)	—	22	24
Reports/documents	8	30	15
Presentations at technical meetings	202	105	161
Meetings organized	10	8	12

a Figures reflect the reunification of the Cell & Molecular Biology and the Research Medicine & Radiation Biophysics Divisions

[b]Number of OHER-sponsored scientific professionals by highest degree:

12 M.D.

53 Ph.D

45 MS/BS

1 D.V.M.

[c]Data given as head count (number of participating individuals).

Structural Biology

Personnel (FTE)	FY 1993		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	5.0	25.0	5.0	26.0	5.0	29.0
Post-doctorates	1.0	26.0	1.0	26.0	3.0	34.0
Technicians	0.5	2.0	3.0	6.0	2.0	7.0
Animal care	—	—	—	—	—	—
Total direct personnel	6.5	53.0	9.0	58.0	10.0	70.0
Professional (adm.)	0.5	0.5	0.5	0.5	0.5	1.5
Clerical, adm. support	0.5	5.5	0.0	5.5	0.5	4.0
Maintenance	0.2	1.4	1.4	1.4	—	1.0
Total indirect personnel	1.2	7.4	1.9	7.4	1.0	6.5
Visiting scientists	0.0	2.0	—	2.0	0.0	6.0
Graduate students	5.5	47.0	10.0	32.0	13.0	36.0
Undergraduates	5.0	15.0	9.0	17.0	1.0	31.0

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	65	37	31
Chapters, reviews	—	—	—
Books, proceedings (edited)	—	—	—
Reports/documents	10	1	—
Presentations at technical meetings	32	25	15
Meetings organized	—	—	—

[a]Number of OHER-sponsored scientific professionals by highest degree:

12 Ph.D

2 MS/BS

Earth Sciences

Personnel (FTE)	FY 1993		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	3.7	3.7	3.7	3.7	4.5	4.5
Post-doctorates	0.8	0.8	0.8	0.8	1.0	1.0
Technicians	0.5	0.5	0.5	0.5	1.0	1.0
Animal Care	—	—	—	—	—	—
Total direct personnel	5.0	5.0	5.0	5.0	6.5	6.5
Professional (adm.)	—	—	—	—	—	—
Clerical, adm. support	0.3	0.3	0.3	0.3	0.3	0.3
Maintenance	—	—	—	—	—	—
Total indirect personnel	0.3	0.3	0.3	0.3	0.3	0.3
Visiting scientists	—	—	0.2	0.2	0.3	0.3
Graduate students	0.7	0.7	1.25	1.25	3.0	3.0
Undergraduates	0.2	0.2	0.2	0.2	0.5	0.5

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	6	3	3
Chapters, reviews	0	0	0
Books, proceedings (edited)	0	0	0
Reports/documents	3	3	5
Presentations at technical meetings	3	4	5
Meetings organized	1	2	4

[a]Number of OHER-sponsored scientific professionals by highest degree:

6 Ph.D.

4 MS/BS

Energy and Environment

Personnel (FTE)	FY 1993		FY 1994		FY1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	4.3	94.0	5.2	118.0	4.79	99.0
Post-doctorates	2.1	9.0	1.1	11.0	1.0	12.0
Technicians	1.5	77.0	1.3	71.0	1.73	91.0
Animal care	—	—	—	—	—	—
Total direct personnel	7.9	180.0	7.6	200.0	7.52	202.0
Professional (adm.)	0.2	11.0	0.2	11.0	0.26	11.0
Clerical, adm. support	1.4	19.0	1.3	20.0	1.26	20.0
Maintenance	—	—	—	—	—	—
Total indirect personnel	1.6	30.0	1.5	31.0	1.52	31.0
Visiting scientists	0.3	85.0	0.3	85.0	0.25	90.0
Graduate students	2.2	22.0	2.5	21.0	2.72	24.0
Undergraduates	0.3	10.0	—	—	—	—

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	19	20	34
Chapters, reviews	1	2	2
Books, proceedings (edited)	—	18	2
Reports/documents	26	6	12
Presentations at technical meetings	10	29	7
Meetings organized	—	—	1

[a]Number of OHER-sponsored scientific professionals by highest degree:

8 Ph.D

10 MS/BS

Engineering

Personnel (FTE)	FY 1993		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	5.0	9.0	5.0	9.0	5.0	9.0
Post-doctorates	—	—	—	—	1.0	1.0
Technicians	2.0	4.0	2.0	4.0	2.0	4.0
Animal care	—	—	—	—	—	—
Total direct personnel	7.0	13.0	7.0	13.0	8.0	14.0
Professional (adm.)	—	—	—	—	—	—
Clerical, adm. support	1.0	1.0	1.0	1.0	1.0	1.0
Maintenance	0.5	0.5	0.5	0.5	0.5	0.5
Total indirect personnel	1.5	1.5	1.5	1.5	1.5	1.5
Visiting scientists	—	—	—	—	—	—
Graduate students	1.0	1.0	1.0	1.0	—	—
Undergraduates	1.0	1.0	1.0	1.0	2.0	2.0

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	11	15	12
Chapters, reviews	1	0	0
Books, proceedings (edited)	0	1	1
Reports/documents	10	20	19
Presentations at technical meetings	8	15	10
Meetings organized	0	1	1

[a]Number of OHER-sponsored scientific professionals by highest degree:

5 Ph.D.

2 MS/BS

Information and Computing Sciences

Personnel (FTE)	FY 1993		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific, Ph.D.)[a]	2.0	2.0	6.8	6.8	4.7	4.7
Post-doctorates	—	—	—	—	—	—
Technicians	1.5	1.5	—	—	—	—
Animal care	—	—	—	—	—	—
Total direct personnel	3.5	3.5	6.8	6.8	4.7	4.7
Professional (adm.)	—	—	—	—	—	—
Clerical, adm. support	—	—	—	—	—	—
Maintenance	—	—	—	—	—	—
Total indirect personnel	—	—	—	—	—	—
Visiting scientists	—	—	—	—	—	—
Graduate students	—	—	—	—	—	—
Undergraduates	—	—	0.6	0.6	0.3	0.3

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	—	—	—
Chapters, reviews	—	—	2
Books, proceedings (edited)	—	—	2
Reports/documents	—	—	7
Presentations at technical meetings	—	—	9
Meetings organized	—	—	1

[a]Number of OHER-sponsored scientific professionals by highest degree:

1 Ph.D.

2 MS/BS

Summary Total

Personnel (FTE)	FY 1993		FY 1994		FY 1995	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	59.6	214.7	57.8	254.6	55.69	237.2
Post-doctorates	15.0	35.8	6.2	68.5	9.3	79.0
Technicians	26.2	89.9	38.0	172.8	38.23	194.7
Animal care	8.2	8.2	0.4	2.7	0.4	3.2
Total direct personnel	109.0	348.6	102.4	498.6	103.62	514.1
Professional (adm.)	13.0	27.6	6.7	28.5	6.96	30.0
Clerical, adm. support	21.0	42.0	9.3	45.9	10.46	45.1
Maintenance	1.9	1.9	1.9	1.9	0.5	1.5
Total indirect personnel	35.9	71.5	17.9	76.3	17.92	76.6
Visiting scientists	10.5	104.0	14.5	127.2	13.55	135.3
Graduate students	22.4	56.7	21.75	76.25	24.72	83.0
Undergraduates	21.5	69.2	32.8	83.8	34.8	119.8

Information Transfer (OHER-Sponsored Research)

	FY 1992	FY 1993	FY 1994
Journal articles (peer reviewed)	380	192	182
Chapters, reviews	9	24	20
Books, proceedings (edited)	—	41	15
Reports/documents	57	60	48
Presentations at technical meetings	255	178	147
Meetings organized	1	3	8

[a]Number of OHER-sponsored scientific professionals by highest degree:

12 M.D.

85 Ph.D

1 D.V.M.

65 MS/BS

7.4 Extramural Activities

Selected 1994 Awards, Honors and Editorships

Henry Benner

- LBNL Outstanding Performance Award

Mina J. Bissell

- AAAS Fellow
- HERAC; Chair HERAC Subcommittee
- Member, Chicago University Advisory Board
- Member, Scientific Advisory Board, Canji (San Diego)
- Chair, Program Committee of the 6th International Congress on Cell Biology; Chair, 1996 Keystone Symposium on Breast & Prostate Cancer.
- ASCB Women in Cell Biology Career Recognition Award
- John Simon Guggenheim Fellowship
- Editorial Board: *Cancer Research*
- Associate Editor: *Molecular Carcinogenesis*
- Associate Editor, *In Vitro Cellular and Developmental Biology*
- Member, Board of Directors, International Society of Differentiation
- Member, Pathology B Study Section, National Institutes of Health

- Governing Board, Gordon Research Conferences

Thomas F. Budinger

- Board of Trustees, Society of Nuclear Medicine
- Elected Fellow of the American Institute of Biomedical Engineering;
- Scientific Advisory Committee, Whitaker Foundation.

Judith Campisi

- Post-Member, Biological and Clinical Aging Review Committee, NIH
- Member, Editorial Board, *Experimental Cell Research*
- Member, Editorial Board, *Journal of Gerontology*
- Member, Scientific Advisory Committee, Tobacco-Related Disease Research Program
- Member, Board of Scientific Councilors, National Institute on Aging

Aloke Chatterjee

- Councilor (Physics), Radiation Research Society
- Member, Program Committee, Radiation Research Society

Joan M. Daisey

- Member, Editorial Advisory Board, Environmental Science and Technology
- Chair, Science Advisory Board Committee on Indoor Air Quality and Total Human Exposure, U.S. Environmental Protection Agency
- Member, Executive Committee, Science Advisory Board, U.S. Environmental Protection Agency
- Chairman-Elect, International Society of Exposure Analysis
- National Forum on Environment and Natural Resources Research & Development, sponsored by the White House Office of Science & Technology Policy and Agencies of the Committee on Environment and Natural Resources - participant in forum to develop long-term research strategy for U.S., March, 1994
- Member, Advisory Committee of the Health Effects Component of the University of California Toxic Substances Research & Teaching Program
- Member, DOE Laboratory Directors Environmental and Occupational/Public Health Standards Steering Group

William Fisk

- Member, Advisory Council of the Bay Area Air Quality Management District, Chair of Technical Committee
- Member, Editorial Advisory Board, *Indoor Air*
- Member, Steering Committee, ASHRAE IAQ '96 Conference

Robert M. Glaeser

- Elizabeth Roberts Cole Award, Biophysical Society
- Associate Editor, *Journal of Structural Biology*

Lois Gold

- Member, Society of Toxicology
- Board Member, Harvard Center for Risk Analysis, Harvard Risk Management Reform Group

Joe Gray

- President-elect: International Society for Analytical Cytology

John E. Hearst

- American Society for Photobiology Research Award
- Sigma Xi National Lecturer

William Jagust

- Editorial Board, Journal of Neuroimaging

Bing Jap

- Member, NIH Biophysical Study Section

Sung-Hou Kim

- HoAm Prize, The Samsung Foundation, Seoul, Korea
- Fellow, The American Academy of Arts and Sciences
- Member, The National Academy of Sciences

- Member, Editorial Board, *Nucleic Acids Research*
- Steering Committee, Structural Biology Synchrotron Users Organization
- Scientific Advisory Committee of the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation
- Member , Advisory Committee, National Science Foundation of Korea
- Board of Scientific Councilors, National Center for Biotechnology Information, National Institutes of Health
- Advisory Board, *Molecules and Cells* , Seoul, Korea (1993-)

Melvin P. Klein

- Chair, Advisory Committee to the Pittsburgh NMR Center for Biomedical Research, Carnegie-Mellon University and University of Pittsburgh
- Charter Council Member, International EPR Society
- Member, Advisory Committee, Biotechnology Research Resource, SSRL
- Member, Executive Committee, Users Organization, Advanced Light Source, LBNL
- Member, Advisory Committee, BioCAT, Advanced Photon Source, Argonne National Laboratory

Ronald M. Krauss

- Chair, Nominating Committee, Council on Arteriosclerosis, American Heart Association
- Member, Executive Committee, Council on Arteriosclerosis, American Heart Association
- Member, Nutrition Committee, American Heart Association
- Member, Data and Safety Monitoring Committee, Postmenopausal Estrogen-Progestin Intervention Study, NHLBI, NIH

Steven Lockett

- Presidential Research Award from the International Society for Analytical Cytology

Narla Mohandas

- Member, Editorial Board, *Blood*
- Member, Editorial Board, *Blood Cells*
- Member, Hematology Study Section, Division of Research Grants, NIH
- Member, Subcommittee on Red Cell and Hemoglobin, American Society of Hematology

Anthony V. Nero

- Chair, Forum on Physics and Society, American Physical Society
- Member, OHER Radon Principal Scientist Advisory Committee
- Member, International Academy of Indoor Air Sciences

Tihomir Novakov

- Co-chair 5th International Conference on Carbonaceous Particles in the Atmosphere (August 22-26, Berkeley)
- Guest editor special issue Journal of Geophysical; Research (Proceedings of above conference)

Dan Pinkel

- Editorial boards: Genes, Chromosomes and Cancer

Henry Rapoport

- Member, NIDA, Biochemistry Review Section
- Member, The Protein Society
- Member, International Society for History, Philosophy, and Social Studies of Biology

Peter G. Schultz

- Member, Editorial Advisory Board, *Biocatalysis*
- Member, Editorial Advisory Board, *Bioconjugates*
- Member, Honorary Advisory Board, *Synlett*
- Member, Editorial Advisory Board, *Accounts of Chemical Research*, American Chemical Society

- Board of Consulting Editors, *Bioorganic and Medicinal Chemistry*
- Editorial Board, *Perspectives in Drug Discovery and Design*
- Editorial Board, *Chemistry and Biology*
- Editorial Advisory Board, *Protein Science*
- Editorial Advisory Board, *Molecular Medicine*
- Chairman, Scientific Advisory Board, Affymax Research Institute
- Scientific Advisory Board, CV Therapeutics
- Advisory Board, Table Ronde Roussel UCLAF
- Advisory Board, Searle Scholars Program

Richard Sextro

- Member, OHER Radon Principal Scientist Advisory Committee
- Member, Radiation Advisory Committee, Science Advisory Board, U.S. Environmental Protection Agency

Martha Stampfer

- Editorial Board, *Cancer Research* (publication of the American Association for Cancer Research)

Ignacio Tinoco

- Member, California Council on Science and Technology
- Member, Health and Environmental Research Advisory Committee, U.S. Department of Energy
- Member, Editorial Board, *Nucleic Acids Research*
- Member, Editorial Board, *Bipolymers*
- Member, Editorial Board, *Biochemistry*
- Member, Editorial Board, *Biophysical Chemistry*
- Member, Editorial Board, *Biochimica et Biophysica Acta*
- Member, Editorial Board, *Cell Biophysics*
- Fellow, American Physical Society

Henry VanBrocklin

- Ninth Annual Award for the Most Outstanding Scientific Poster 19th Western Regional Meeting of The Society of Nuclear Medicine

David Wemmer

- Associate Editor, *Biopolymers*
- Member, Editorial Board, *Concepts in Magnetic Resonance*
- Member, Editorial Board, *Macromolecular Structures, Current Biology*
- Member, Editorial Board, *Journal of Biomolecular NMR*
- Member, Editorial Board, *Journal of Magnetic Resonance*
- Member, Editorial Board, *Journal of Structural Biology*
- Member, Editorial Board, Annual Reviews: *Biophysics and Biomolecular Structure*

Harold Wollenberg

- Member, U.S. DOE/BES advisory committees on Deep Observation and Sampling of the Earth's Continental Crust; Continental Scientific Drilling Forum.

7.5 OHER-Supported Publications

ANALYTICAL TECHNOLOGY

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Appendix 7.6
Selected Research Highlights

The following brief narratives describe significant research highlights of OHER-sponsored research at LBNL during FY 1994-1995. This selection also reflects the diversity of research interests at the Laboratory and represents efforts in the following divisions: Life Sciences, Structural Biology, Energy and Environment, Engineering, and Information and Computing Sciences. The titles of the narratives follow.

- Atmospheric Pressure Fluctuations as a Source of Soil-gas Transport into Buildings.
- High Resolution Electron Crystallography of the Water Channel Protein (CHIP 28) at 3.5A.
- First 3-D Images of the Taxol Binding Site.
- Toward Design Of Drugs That Fight Retroviruses.
- Soft X-ray Fluorescence Spectroscopy.
- Site-Selective EXAFS Spectroscopy.
- Getting Closer to the Malaria Vector with Soft-X-rays.
- Ranking of Possible Carcinogenic Hazards: Rodent Carcinogens in the American Diet.
- Construction of a P1 Map in the Region of Chromosome 5q31-q35.
- Automation of Directed DNA Sequencing.
- "Matrix is the Message" The Cell's Microenvironment Effects Gene Expression.
- A New Assay for Cell Senescence.
- Metadata-driven User Interfaces.
- Data Management for the Molecular Cytogenetic Resource.
- DNA Fiber Mapping.

Atmospheric Pressure Fluctuations as a Source of Soil-gas Transport into Buildings

Richard Sextro (510-486-6295)

Advective flow of soil gas is the primary transport mechanism of radon into houses. Advective soil-gas entry has usually been studied as a steady-state process in which a slight underpressure of the interior of a house, typically on the order of a couple of pascals (Pa), draws radon-laden soil gas through cracks in a building's substructure. This small depressurization is caused by indoor/outdoor temperature differences, wind blowing over the building shell, or operation of an unbalanced building ventilation system. However, several field studies have observed elevated indoor radon concentrations in the absence of any steady driving mechanism.

Fluctuations in atmospheric pressure several orders of magnitude larger than these steady driving pressures commonly occur. Pressure changes of the order of a few Pa occur over periods of minutes, and fluctuations of a few hundred Pa occur diurnally. A weather front can produce large, rapid changes in atmospheric pressure of over 1000 pascals. These atmospheric pressure changes cause soil-gas flow between the soil surface and the soil interior due to the time constant of the soil to pressure changes. Similarly, gas will flow between the house interior and the underlying soil.

We have directly measured the transient flow of soil gas into an experimental basement structure. This primarily below-grade structure was designed and constructed to study the effect of structural and environmental factors on radon and soil-gas entry into houses. The floor slab of the structure rests on a 0.1-m-thick gravel layer. Gas flow between the structure interior and the gravel layer occurs through a 3.8-cm-diameter hole in the center of the floor of the structure. As illustrated in the figure, the larger the rate of change of atmospheric pressure, the greater the pressure difference between the soil and the interior of the structure and the larger the soil-gas flow rate. The soil-gas-flow profile, shown in part (c), is almost identical in shape to the dP/dt spectrum in part (b).

Although the overall average flow rate of soil gas into the structure, driven by fluctuations in atmospheric pressure, is zero, this flow causes a net flux of radon into the structure because the radon concentration of the soil gas is several orders of magnitude larger than air inside of the house. The effective net radon entry rate is equivalent to that produced by a steady structure depressurization of ~0.5 Pa; for this structure the equivalent radon entry rate is 0.6 Bq /s, which is approximately six times that observed for radon diffusion. Thus soil-gas entry driven by atmospheric pressure fluctuations may explain the elevated indoor radon concentrations observed in some houses during periods when steady driving conditions are absent.

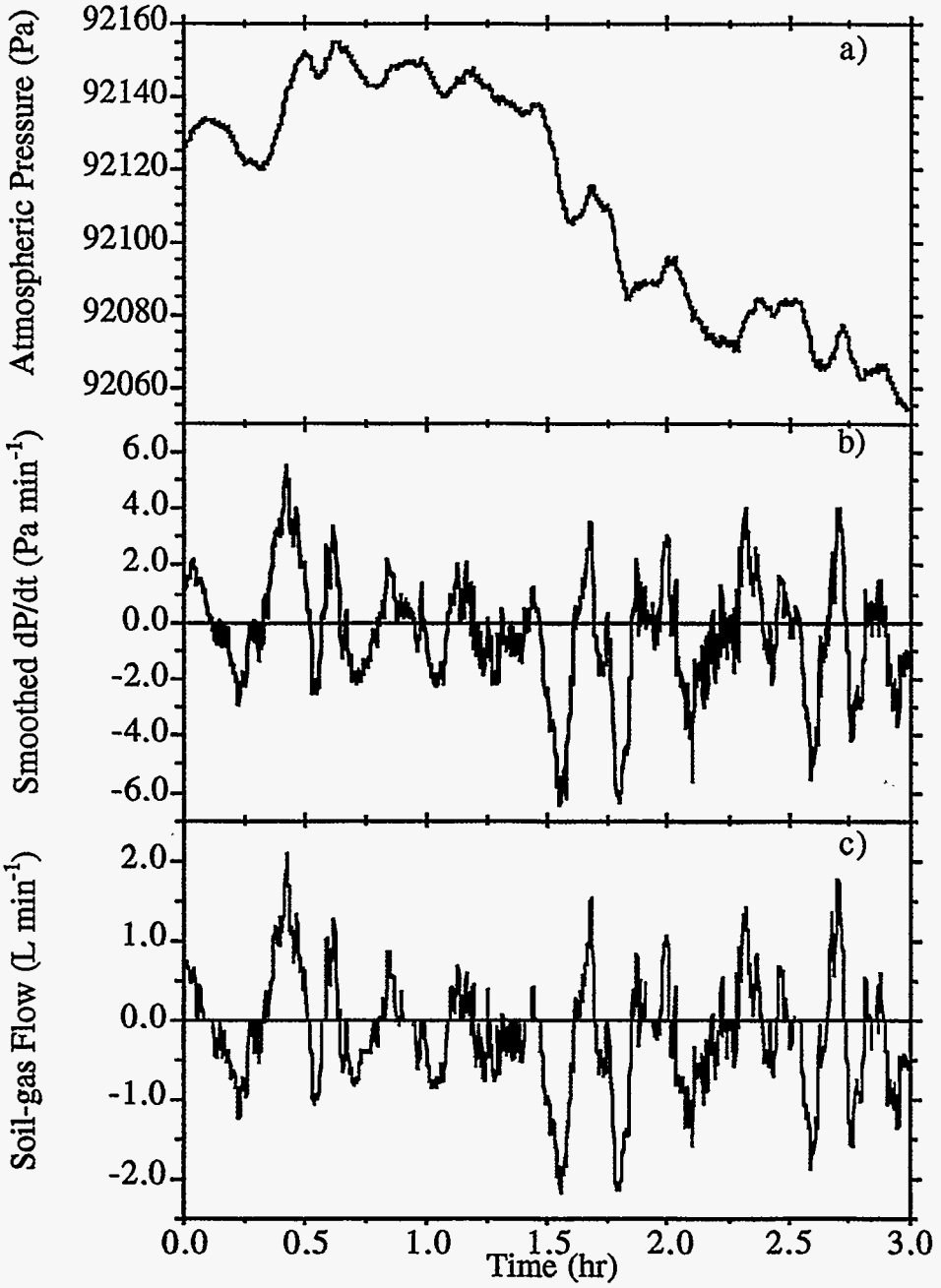


Figure Caption:

Part (a): Variations in atmospheric pressure as a function of time.

Part (b) Time-rate of change in atmospheric pressure; data smoothed with an exponential weighting factor.

Part (c) Soil-gas flow rates driven by fluctuations in atmospheric pressure; negative values indicate flow of soil gas into the structure while positive values indicate gas flow out of the structure into the soil.

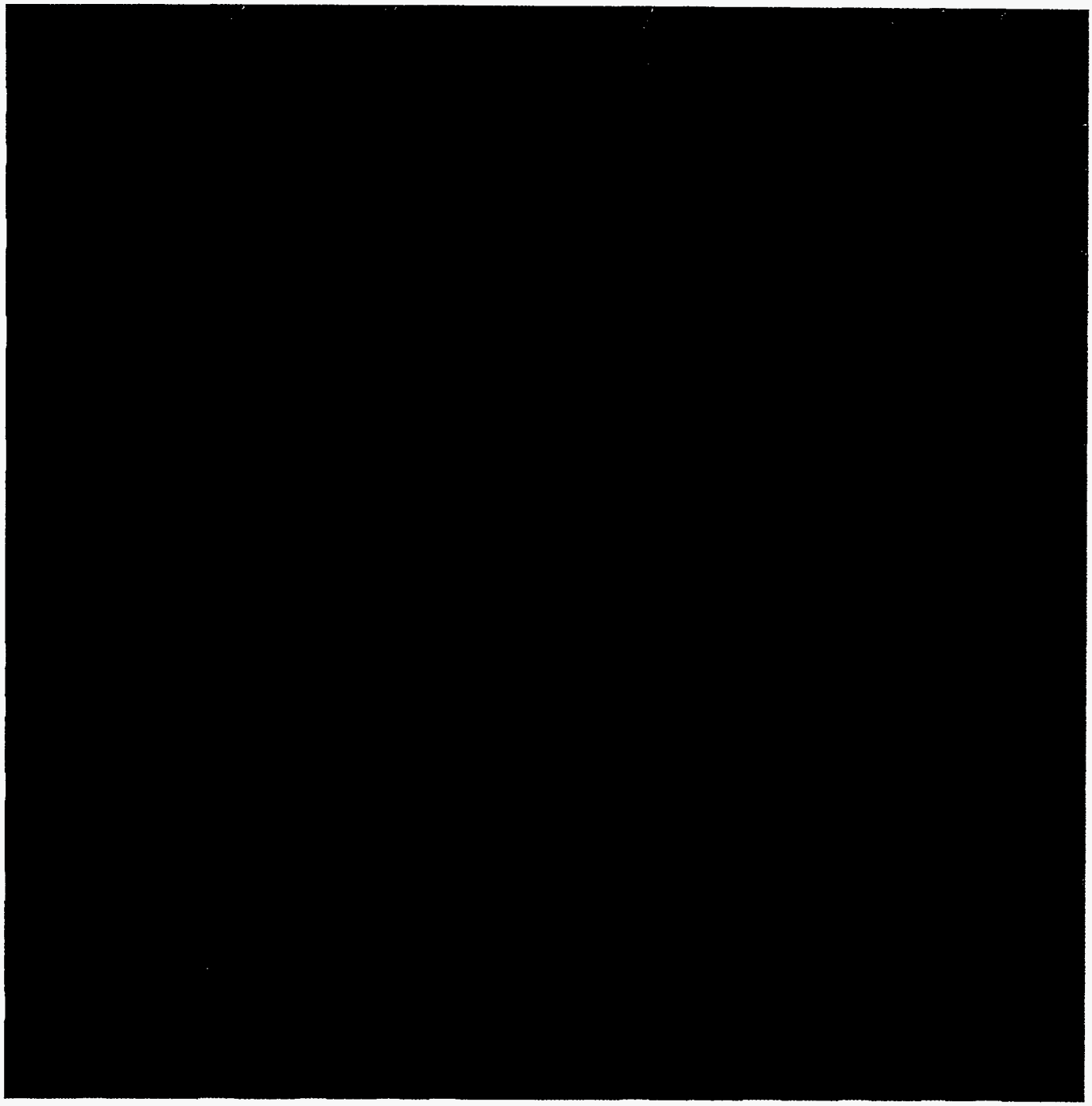
High Resolution Electron Crystallography of the Water Channel Protein (CHIP 28) at 3.5A

Bing Jap (510-486-7104)

Water is the major component of all living cells as well as the surrounding extracellular spaces. Transport of water into and out of cells occurs during digestion, respiration, circulation, regulation of body temperature, elimination of toxins, neural homeostasis, and during many other essential body functions. Water crosses cellular plasma membranes by two fundamentally distinct pathways: simple diffusion through the lipid bilayer; and channel-mediated water transport.

CHIP28, a 28-kDa channel-forming integral membrane protein, is the archetype member of the aquaporins, a growing family of membrane water channels from diverse plant and animal species. CHIP28 is a major protein of the red cell membrane, allowing rapid swelling or shrinkage to occur in response to small changes in extracellular osmolality. CHIP comprise 4% of the total brush border membrane protein in renal proximal tubules where it promotes reabsorption of water from the glomerular filtrate. CHIP is also expressed in several other mammalian tissues including choroid plexus, ocular ciliary epithelium, hepatobiliary ductules and capillary endothelium, where the protein participates in various secretory or reabsorptive processes.

Elucidation of this protein structure will yield a better understanding of certain kidney diseases. Concentration of urine in mammals is regulated by the antidiuretic hormone vasopressin. Binding of vasopressin to its V2 receptor leads to the insertion of water channels in apical membranes of principal cells in collecting ducts. In nephrogenic diabetes insipidus (NDI), the kidney fails to concentrate urine in response to vasopressin. A male patient with an autosomal recessive form of NDI was found to be a compound heterozygote for two mutations in the gene encoding aquaporin-2, a water channel. Functional expression studies in Xenopus oocytes reveled that each mutation resulted in nonfunctional water channel proteins. Thus, aquaporin-2 is essential for vasopressin-dependent concentrations of urine.



This high-resolution image of CHIP 28 was obtained by electron crystallography at a resolution of 3.5Å. Each cube pictured here in red depicts a tetrameric molecule of CHIP 28. Each monomer consists of at least six transmembrane α-helices. The channel itself is believed to be located in the region enclosed by these helices.

First 3-D Images of the Taxol Binding Site

Ken Downing (510-486-5941)
Eva Nogales (510-486-6437)

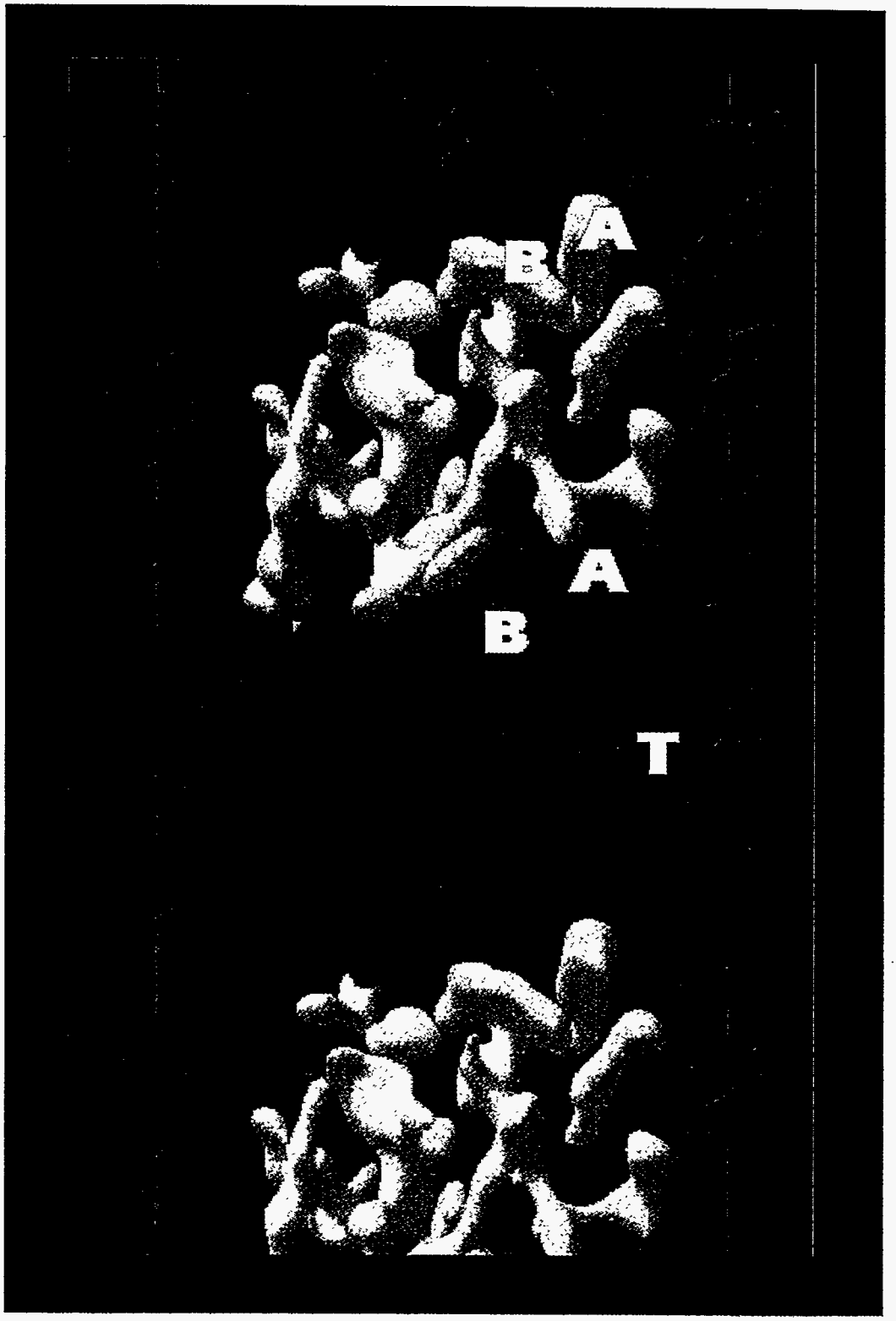
LBNL scientists have produced the first high-resolution three-dimensional image of the site where the anti-cancer drug taxol interacts with vital cell proteins. This could be a major step towards the commercial production of a synthetic version of taxol that would be safer and even more effective than the scarce commodity nature has provided.

Taxol is a natural substance found only in the bark of the Pacific yew tree (the name "taxol" has been trademarked by Bristol-Myers-Squibb). Clinical tests have shown it to be an effective treatment for a number of cancers including ovarian, breast, and lung. However, the bark from several yew trees is required to treat a single patient and stripping the bark kills the trees. Furthermore, taxol can also destroy healthy as well as cancerous cells. An improved version is needed, but the synthesizing processes used to date are too elaborate to be commercially viable. Experts agree that rational drug design and efficient synthesis will require structural knowledge of the taxol molecule and its interactions at the atomic level.

The LBNL researchers have been using electron crystallography to produce three-dimensional images of a protein called tubulin. At an atomic-scale resolution tubulin is the major constituent of microtubules, a mesh of hollow cylinders, like tiny drinking straws, that serve as a skeletal system for cells and are crucial to a number of vital functions including mitosis (cell division). The LBNL images have revealed the site where taxol binds to tubulin and prevents the protein from performing its necessary tasks.

The research team was led by Kenneth Downing and Eva Nogales, chemists with LBNL's Life Sciences Division, and included Sharon Wolf, of LBNL, and Israr Kahn and Richard Luduena, from the University of Texas in San Antonio. The team has reported their research in the journal Nature.

by Lynn Yarris



Tubulin in a zinc-induced sheet embedded in tannic acid. In the central protofilament the α-subunits are yellow and the β-subunits are red. Adjacent protofilaments are blue. Features tentatively identified as α-helix and β-sheet are indicated by the letters "A and "B," respectively. "T" marks the approximate position of the taxol binding site.

Toward Design Of Drugs That Fight Retroviruses

Ignacio Tinoco, Jr. (510-642-3038)

LBNL chemists in the Structural Biology Division have produced the first 3-D image of an RNA structure that plays a vital role in enabling retroviruses to replicate within cells.

The structure, a double-looped strand of RNA that forms what is called a "pseudoknot," was revealed to contain a bend in its shape that may serve as the site where key host proteins interact. It may be possible, the researchers say, to design drugs that could fight retroviruses—the most notorious of which is HIV—by binding to the pseudoknot at this site and blocking these interactions.

The research was reported in the April 14 issue of the Journal of Molecular Biology, in a paper co-authored by Ignacio Tinoco, Jr., who is also a professor of chemistry at UCB, and his student, Ling X. Shen.

A retrovirus is a protein-coated packet of RNA (ribonucleic acid) that requires the chemicals of a host cell to make viral DNA and proteins from its RNA genome. When a retrovirus invades a cell it synthesizes three enzymes: integrase, protease, and reverse transcriptase. These enzymes enable it to transform the host into a virus replication factory. The mechanism by which this enzyme synthesis is carried out is called "ribosomal frameshifting" and involves a shift in the order in which the virus's RNA genetic code is read. Retroviruses use a "minus-one" frameshift, which means the reading of the code starts one nucleotide from where it should.

"The efficiency of frame-shifting is modulated by messenger RNA structures such as a pseudoknot downstream of the frameshift sight," says Tinoco. "The minus-one frameshifting translational mechanism allows controlled synthesis of viral enzymes and structural proteins."

To understand how pseudoknots promote frame-shifting, scientists need detailed structural information. Tinoco and Shen, working in collaboration with the UC San Francisco group of Harold Varmus (head of the National Institutes of Health), used nuclear magnetic resonance (NMR) spectroscopy to produce a 3-D, high-resolution image of a 34-nucleotide pseudoknot that is known to cause high-efficiency frame-shifting in the mouse mammary tumor virus.

In NMR spectroscopy, atomic nuclei are identified and spatially located by their characteristic absorbence of radiowaves in a magnetic field. Tinoco is one of the few researchers to use NMR to study RNA, which is the workhorse of the genetic world. It transcribes the coded instructions of DNA and assembles amino acids into proteins. In 1992, Tinoco led a research team that produced the first 3-D image of a stem-loop "hairpin," a common and highly stable RNA structural element with critical folding and protein-recognition properties.

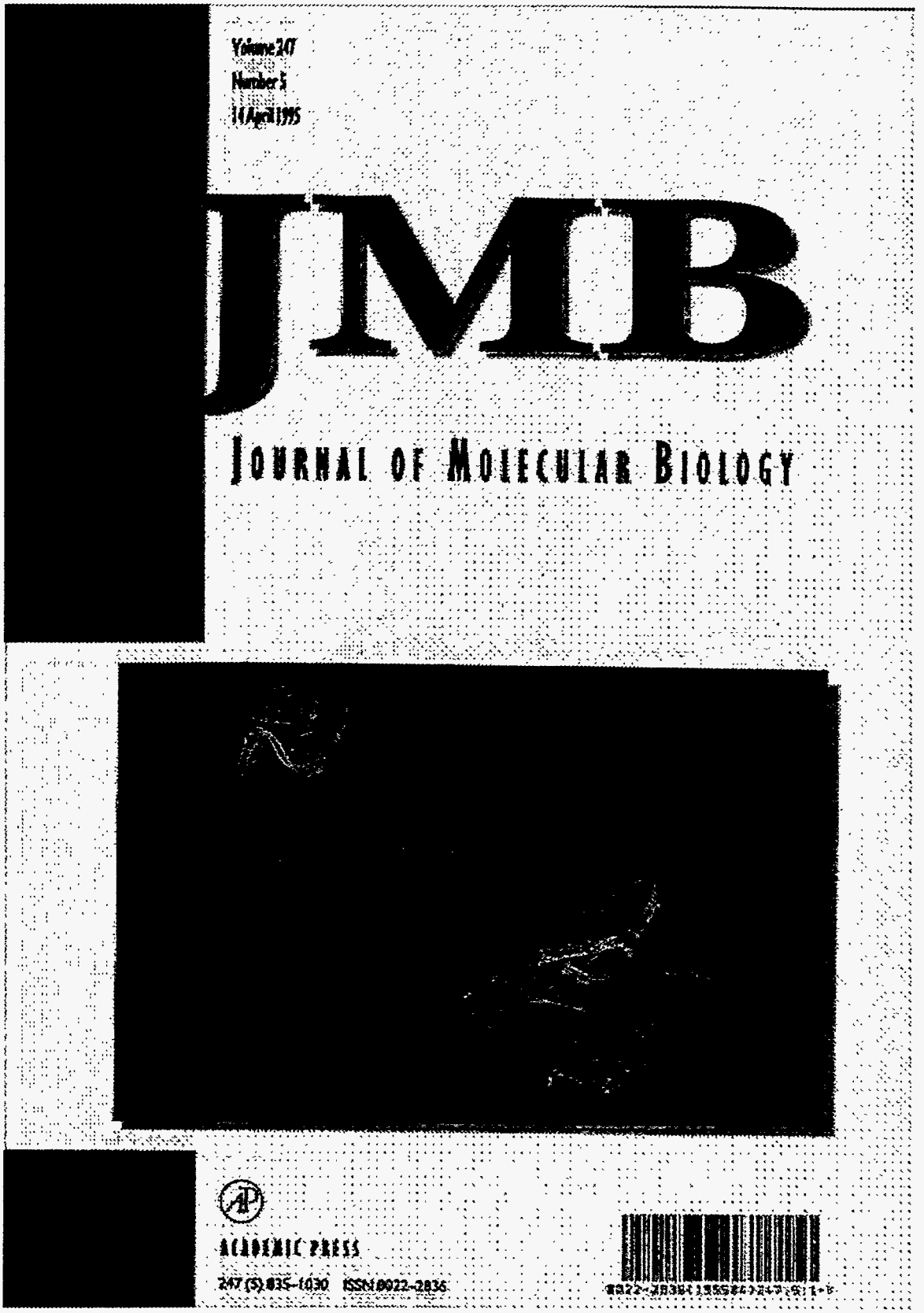
"When the loop of a stem-loop hairpin pairs with a complementary sequence outside the loop to form a second stem, the resulting structure becomes a pseudoknot," Tinoco says. The structure is only partially twisted, otherwise it would form a knot.

From their NMR images, Tinoco and Shen discovered that the presence of the nucleotide adenine at the junction of the two stems of the pseudoknot derived from the mouse mammary tumor virus creates a bend in the shape of the pseudoknot. Subsequently, the Tinoco and Varmus research groups experimented with modifying the pseudo-knot's nucleotide sequences. The idea was to find out which sequences resulted in frame-shifting and which did not.

They found that with the bend in its shape, the pseudoknot promotes high-efficiency (up to 20 percent) frameshifting. If the intervening adenine nucleotide is removed, a pseudoknot is formed without a bend and no frameshifting occurs. The next step will be to find which ribosomal proteins recognize this bend and interact with it in order to frameshift.

"Our NMR data indicate that there are internal dynamics associated with the pseudoknot," says Tinoco. "The unique, compact structure and conformational flexibility of the pseudoknot may be required for recognition and favorable interaction with the translating ribosome, or with the translation factors associated with the ribosome."

by Lynn Yarris



A three-dimensional image of the mouse mammary tumor virus frameshifter RNA "pseudoknot," generated from NMR spectroscopic data by Shen and Tinoco. J. Mol. Biol. 247, 963-978.

Soft X-ray Fluorescence Spectroscopy

Clayton Randall, (510-486-5583);
Stephen Cramer, (510-486-4720)

A better survey of the chemical sensitivity of Mn, Fe, Ni, Cu and Zn *L* fluorescence is our first goal. Although spectra have been recorded previously for pure metals and a few oxides and halides, there is no literature at all for bioinorganic model systems. Just as *L* absorption has shown great promise, *L* emission spectroscopy could also be very informative. The simplest comparison is that *L* absorption spectroscopy probes the empty metal 3*d* levels, while *L* fluorescence probes the filled *d* states. Complexes with filled d^{10}, nearly-filled d^9, or low-spin d^8 valence shells will have *L*-edges with no "white lines," or single, featureless peaks in their absorption spectra. In contrast, the emission from such complexes could be quite rich, since it will reflect *d*-orbital splittings.

Taking advantage of the high brightness of the Advanced Light Source undulator beamlines, we were able to collect preliminary soft x-ray *L* emission spectra of first-row metals. We obtained nickel emission data (Figure 1) on ALS beamline 7.0 in collaboration with Prof. J. Nordgren (U. Uppsala).

Our *L* fluorescence spectra are exciting because of both the large chemical shifts and the sharp lines that are observed. Note the approximately 2 eV difference between the lower energy (~850 eV) peaks in the spectra of low-spin and high-spin Ni(II) in Figure 1. A similar difference is seen between high-spin Ni(II) and Ni(III). The preliminary data indicates that *L* emission shows greater percentage chemical shifts than does *K* emission.

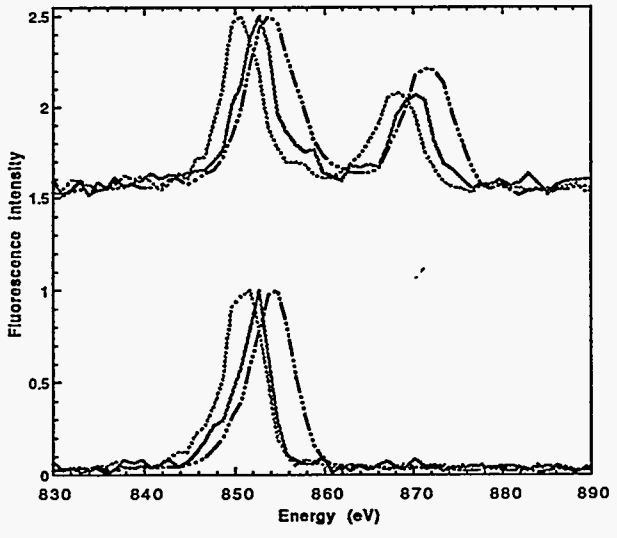


Figure 1. Preliminary nickel L *emission spectra from the ALS. Low-spin Ni(II), (—); high-spin Ni(II), (–); Ni(III), (-...-). Top, excitation at ~ 873 eV. Bottom, excitation at ~ 853 eV.*

Site-Selective EXAFS Spectroscopy

Melissa Grush, (916-752-1156);
Andrew Froeschner, (916-752-2812);
Clayton Randall, (510-486-5583);
Stephen Cramer, (510-486-4720)

We recently obtained high-resolution Kß fluorescence spectra of a $Mn(II,III_2)$ trinuclear complex, $Mn_3O(O_2CC_6H_5)_6(py)_2(H_2O)$ (Figure 2, left). For comparison, the emission spectra of MnF_2 and $Mn(OAc)_3{\cdot}2H_2O$ are shown. They explain our rationale in selecting the energies to monitor in recording EXAFS spectra. Preliminary analysis shows marked differences between the EXAFS spectra (Figure 2, right) obtained with monitoring the Kß emission at 6492.6 eV (Mn^{2+} side) and at 6488.9 eV (Mn^{3+} side).

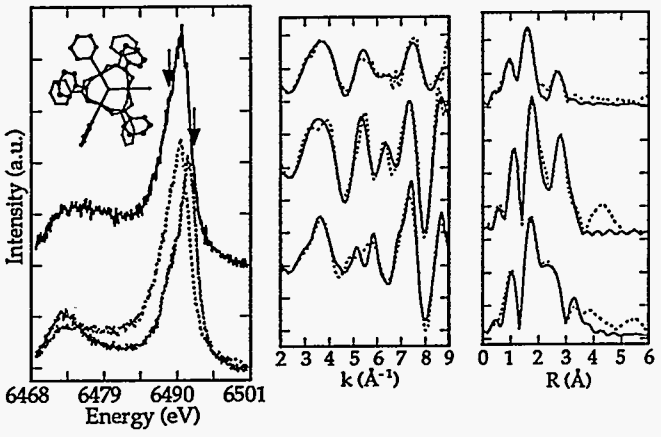


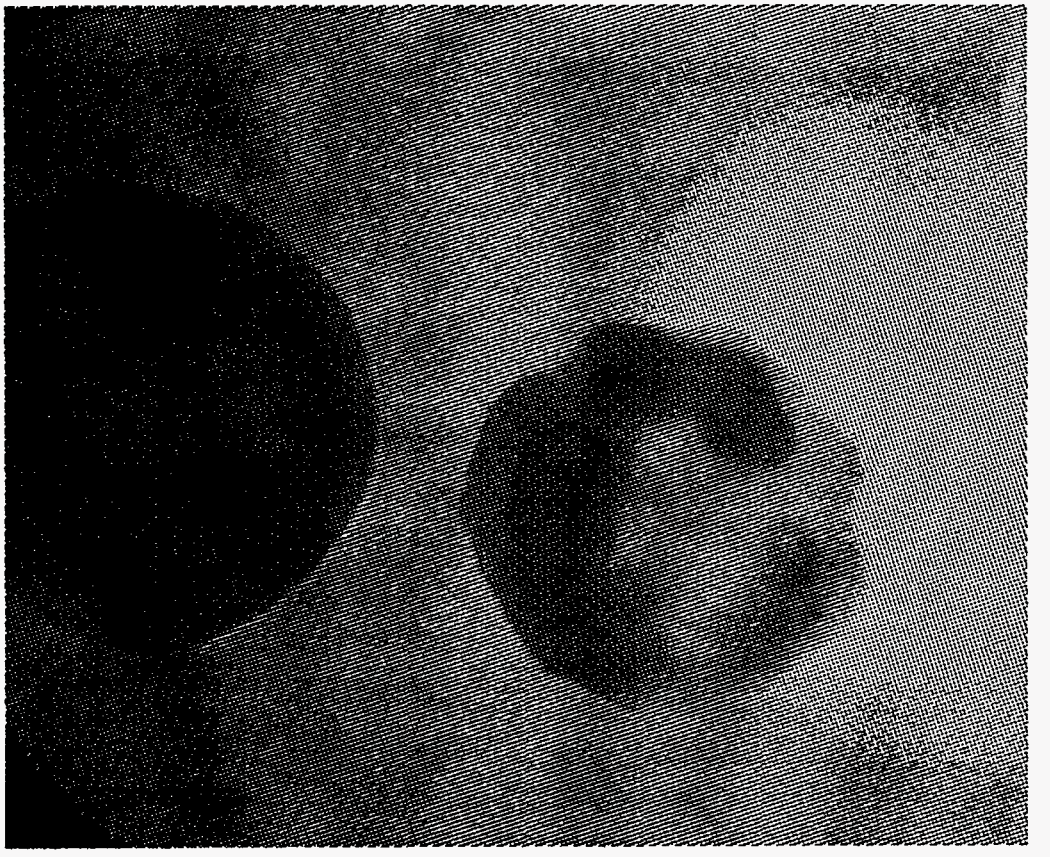
Figure 2. Left: Kß emission spectra of $Mn_3O(O_2CC_6H_5)_6(py)_2(H_2O)$ (top, structure in inset), $Mn(OAc)_3{\cdot}2H_2O$ (bottom, —) and MnF_2 (bottom, –). Middle: Preliminary EXAFS spectra of $Mn_3O(O_2CC_6H_5)_6(py)_2(H_2O)$: transmission (top), site-selective Kß fluorescence spectrum recorded with monitoring at 6492.6 eV (middle) and at 6488.9 eV (bottom). Right: Fourier transforms of EXAFS spectra in middle figure.

Getting Closer to a Disease Vector with Soft-X-rays

Mario Moronne (510-486-4236)
Cathie Magowan, (510-486-6439)
Werner Meyer-Ilse (510-486-6892)

According to the World Health Organization, each year between 300 to 500 million people living in tropical or subtropical regions of the world will become infected and suffer the burning fever and severe pain that the disease inflicts. Nearly three million malaria victims will die.

Malaria is caused by infection of red blood cells with a parasite called "plasmodium" which is transmitted through the bite of a female anopheles mosquito. Drug therapies and insecticides have been brought to bear against this ancient scourge, but resistant strains of both the parasite and the mosquito continue to emerge. At LBNL, researchers have been studying the deadliest strain of the parasite, seeking to better understand its life cycle in the human blood stream and, in particular, exactly how it defeats the body's natural defenses. With this knowledge, medical researchers will be better equipped both to prevent and to heal infections. This past year saw the start-up of an x-ray imaging microscope at LBNL's Advanced Light Source. Working with laser-like beams of x-rays that can be used to look deep inside living cells, the researchers were able to produce one of the clearest, most detailed images ever obtained of a red cell infected with the malaria parasite. With the accumulation of more of these images, the researchers expect to acquire a complete picture of the pathology behind malaria.



An intact red blood cell infected by the malaria parasite, imaged by the x-ray microscope at LBNL's Advanced Light Source. The microscope is dedicated to high resolution microscopy for biology and materials sciences.

Ranking of Possible Carcinogenic Hazards: Rodent Carcinogens in the American Diet

Lois Gold (510-486-7080)

(heterocyclic amines in italics)

Possible hazard: HERP (%)	Daily human exposure	Human dose of rodent carcinogen	TD_{50} (mg/kg) Rats	Mice
4.7	Wine (250 ml)	Ethyl alcohol, 30 ml	9110	(–)
0.3	Lettuce, 1/8 head (125 g)	Caffeic acid, 66.3 mg	284	(4970)
0.1	1 Mushroom (15 g)	Mix of hydrazines, etc.	(?)	20,300
0.1	Basil (1 g of dried leaf)	Estragole, 3.8 mg	(?)	52
0.07	Mango, 1 whole (245 g; pitted)	D-Limonene, 9.8 mg	204	(–)
0.07	Brown mustard (5 g)	Allyl isothiocyanate, 4.6 mg	96	(–)
0.06	Diet cola (12 oz; 354 ml)	Saccharin, 95 mg	2143	(–)
0.06	Parsnip, 1/4 (40 g)	8-Methoxypsoralen, 1.28 mg	32	(?)
0.03	Safrole: US avg from spices	Safrole, 1.2 mg	(436)	56.2
0.03	Peanut butter (32 g; 1 sandwich)	Aflatoxin, 64 ng	0.003	(+)
0.03	Comfrey herb tea (1.5 g)	Symphytine, 38 μg	1.91	(?)
0.006	Bacon, pan fried (85 g)	Diethylnitrosamine, 85 ng	0.02	(+)
0.005	Coffee, 1 cup (from 4 g)	Furfural, 630 μg	(679)	197
0.003	1 Mushroom (15 g)	Glutamyl *p*-hydrazino-benzoate, 630 μg	(?)	277
0.003	Bacon, pan fried (85 g)	*N*-nitrosopyrrolidine, 1.45 μg	(1.05)	0.679
0.002	Apple juice (6 oz; 177 ml)	UDMH, 5.89μg (from Alar, 1988)	(–)	3.94
0.002	Bacon, pan fried (85 g)	Dimethylnitrosamine, 255 ng	(0.2)	0.2
0.002	Coffee, 1 cup (from 4 g)	Hydroquinone, 100 μg	82.8	(225)
0.002	Coffee, 1 cup (from 4 g)	Catechol, 400 μg	336	(–)
0.001	Tap water, 1 liter	Chloroform, 83 μg (US avg)	(262)	90
0.001	Heated sesame oil (15 g)	Sesamol, 1.13 mg	1540	(4490)
0.0005	1 Mushroom (15 g)	*p*-Hydrazinobenzoate, 165 μg	(?)	454[a]
0.0003	Carbaryl: daily dietary avg	Carbaryl, 2.6 μg (1990)*	14.1	(–)
0.0002	Toxaphene: daily dietary avg	Toxaphene, 595 ng (1990)*	(–)	5.57
0.0001	*Salmon steak, baked (3 oz; 85 g)*	*PhIP, 306 ng*	4.29[a]	(28.6)[a]
0.00008	*Salmon steak, baked (3 oz; 85 g)*	*MeIQx, 111 ng*	1.99	(24.3)
0.00008	DDE/DDT: daily dietary avg	DDE, 659 ng (1990)*	(–)	12.5
0.00006	*Hamburger, pan fried (3 oz; 85 g)*	*PhIP, 176 ng*	4.29[a]	(28.6)[a]
0.00003	Whole wheat toast, 2 slices (45 g)	Urethane, 540 ng	(41.3)	22.1
0.00003	*Hamburger, pan fried (3 oz; 85 g)*	*MeIQx, 38.1 ng*	1.99	(24.3)
0.00002	Dicofol: daily dietary avg	Dicofol, 544 ng (1990)*	(–)	32.9
0.00002	Cocoa (4 g)	α-Methylbenzyl alcohol, 5.2 μg	458	(–)
0.000005	*Hamburger, pan fried (3 oz; 85 g)*	*IQ, 6.38 ng*	1.89[a]	(19.6)
0.000001	Lindane: daily dietary avg	Lindane, 32 ng (1990)*	(–)	30.7
0.0000004	PCNB: daily dietary avg	PCNB (Quintozene), 19.2 ng (1990)*	(?)	71.1
0.0000001	Chlorobenzilate: daily dietary avgan	Chlorobenzilate, 6.4 ng (1989)*	(–)	93.9
<0.00000001	Chlorothalonil: daily dietary avg	Chlorothalonil, <6.4 ng (1990)*	828	(–)
0.000000008	Folpet: daily dietary avg	Folpet, 12.8 ng (1990)*	(?)	2280
0.000000006	Captan: daily dietary avg	Captan, 11.5 ng (1990)*	2690	(2730)

Daily human exposure: reasonable daily intakes are used to facilitate comparisons; references are reported in [15]. *Possible hazard:* the human dose of rodent carcinogen is divided by 70 kg to give a mg/kg of human exposure, and this dose is given as the percentage of the TD_{50} in the rodent (mg/kg) to calculate the Human Exposure/Rodent Potency index (HERP). TD_{50} values used in the HERP calculation are averages calculated by taking the harmonic mean of the TD_{50} values of the positive tests in that species from the Carcinogenic Potency Database. Average TD_{50} values have been calculated separately for rats and mice and the more sensitive species is used for calculating possible hazard. A number in parentheses indicates a TD_{50} value not used in HERP calculation because it is the less sensitive species; (–), negative in cancer test. (+), positive in cancer test(s) not suitable for calculating a TD_{50}. (?), not adequately tested for carcinogenicity.

[a]The CPDB includes experiments on the hydrochloride salt. The TD_{50} value reported is expressed as the free base.

*Estimate is based on average daily dietary intake for 60–65 year old females, the only adult group reported for 1990. Because of the agricultural usage of these chemicals and the prominence of fruits and vegetables in the diet of older Americans, the residues are generally slightly higher than for other adult age groups.

Construction of a P1 Map in the Region of Chromosome 5q31-q35

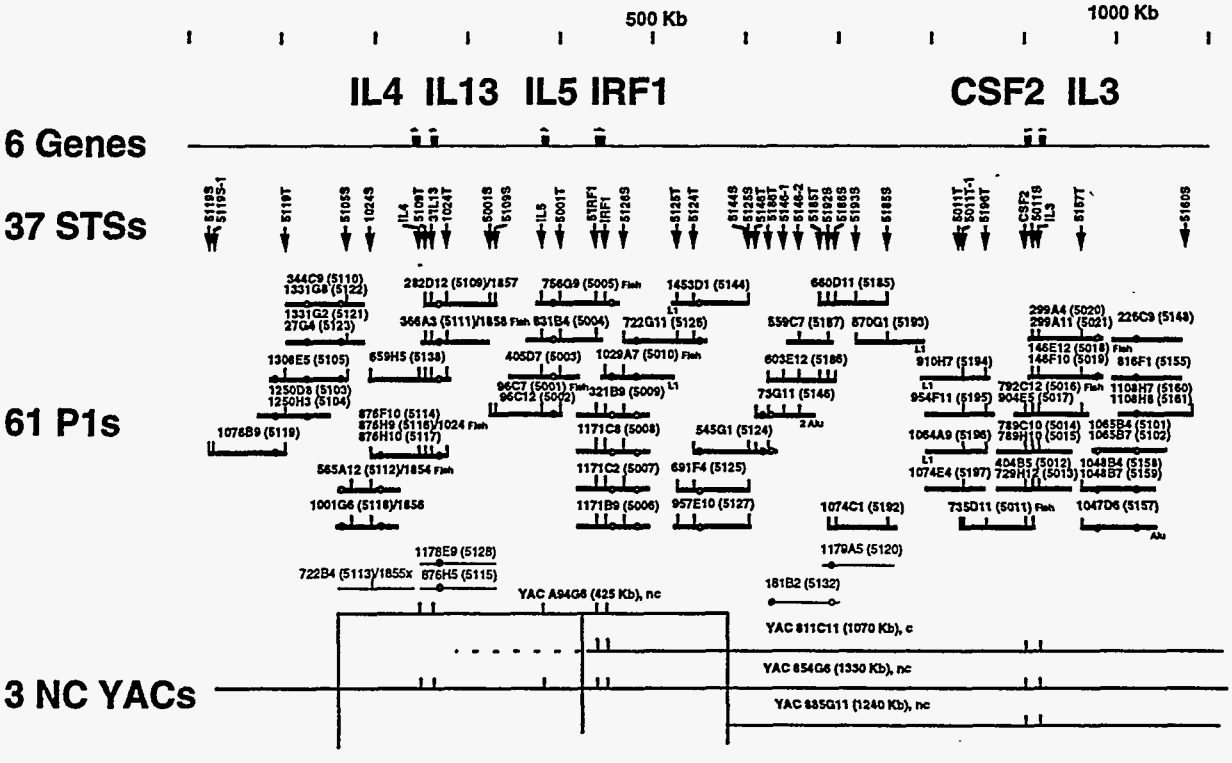
Jan-Fang Cheng (510-486-6575); Steve Lowry; Yiwen Zhu; Duncan Scott; Eddy Rubin (510-486-5072)

The mapping project at the LBNL Human Genome Center has focused on generating a set of P1 clones providing a complete coverage of the target region so that cloned genomic fragments with minimal overlaps can be determined and selected as templates for production sequencing. The q31-q35 region of chromosome 5 was chosen as the primary target for sequencing because this region contains a cluster of growth factor or receptor genes and it is likely to yield new and functionally related genes through long range sequence analysis. We have selected the 1.1 Mb interleukin gene cluster region in 5q31 as a starting point because: (1) that it contains a number of genes with related biological function, therefore, it exists the high likelihood that other genes resulting from gene duplications will be identified through sequence analysis; (2) that the genes localized to this region are of considerable health and medical importance. The known genes regulate hematopoietic cell proliferation; (3) the fact that multiple, previously sequenced genes (IL3, IL4, IL5, IL13, IRF1 and CSF2) are dispersed throughout this region would serve as a quality control for production sequencing; (4) the fact that sequence and functional information about the genes already characterized from this region may well assist in the difficult task of assigning importance and function to genes derived from the production sequencing program.

There are three key experimental steps in our mapping strategy, and they were designed to generate a clone map for specific regions of a chromosome in a time- and cost-effective way. The first step is to use inter-Alu fragments generated from YACs to isolate regionally specific P1s covering the target region. The second step is to establish the order and overlaps of the isolated P1s in a clone-limited approach. The third step is to close gaps and verify the integrity of the cloned fragments.

Three non-chimeric YACs were identified to spin the 1.1 Mb interleukin gene region, and 61 P1s have been isolated using probes derived from these YACs. Informative STSs were developed from 6 known genes and 19 ends of key P1s to resolve a minimal tiling path of 17 P1 clones (marked in red on the map). These 17 clones are templates for the large scale sequencing project implemented at LBNL. All STSs are now being used to generate restriction maps from both genomic DNA and cloned DNA in this region. The restriction map comparison should allow us to identify gross structure rearrangement, if any, in the cloned P1 DNA.

A P1 Map in the Interleukin Gene Cluster on Chromosome 5q31



Automation of Directed DNA Sequencing

HUMAN GENOME CENTER INSTRUMENTATION GROUP

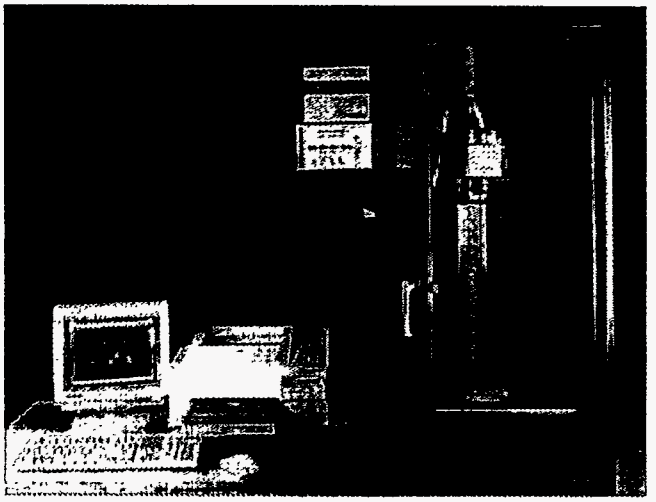
Joseph Jaklevic 510-486-5647/Martin Pollard 510-486-4561

The Goal of the Human Genome Project is to sequence the 3 billion basepairs of the human genome as well as the genomes of selected organisms such as Escherichia coli, Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila Melanogaster. It was recognized from the inception of the Human Genome Project that robotics and automation are an essential component for the ultimate success of the project given the shear enormity of the task. The Lawrence Berkeley National Laboratory Human Genome Center (HGC) is currently developing a large-scale directed sequencing project, for both human and Drosophila DNA, with a targeted multi-megabase per year capability. The directed portion of the sequencing protocol relies on STS (sequence-tagged site) content mapping of P1 inserts followed by high-resolution mapping of 3 kb fragments and transposon assisted sequencing. Automation of this directed approach presents many unique challenges and opportunities in the area of laboratory automation and systems design.

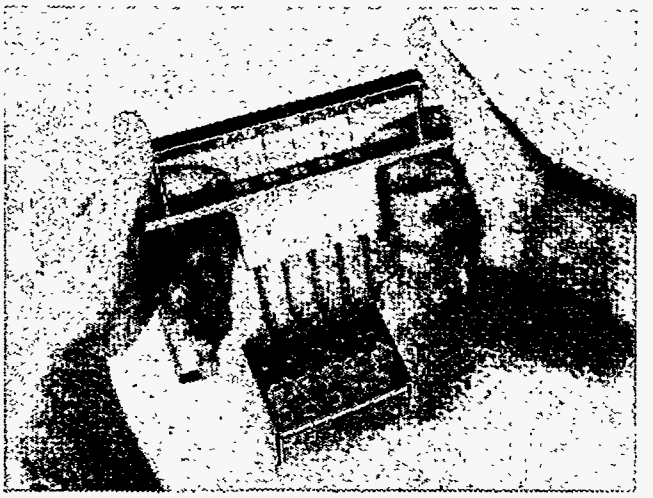
LBNL's approach to automation has been to implement a bottom-up strategy of addressing the most important bottlenecks in the overall sequencing protocol as they arise while maintaining the view that we are developing an integrated system of instrumentation modules. This bottom-up strategy allows us to address critical problems first in the shortest time frame. The modular approach builds flexibility into the final system to incorporate new technology as it becomes available. Our engineering accomplishments range from simple mechanical mechanisms such as the sequencing gel loader, to unique software and hardware applications of commercial instrumentation (ORCA robot), to custom built instrumentation and robotics (Colony Picking Machine, Agarose Imaging Station, and 12 Channel DNA Synthesizer). We have also implemented a wide variety of space efficient and ergonomic laboratory shelving systems and instrument carts, unique software/hardware Beckman Biomek Robot applications, and currently have a number of projects in the development stage for high throughput PCR setups, sequencing reaction setups, agarose gel load, and DNA plasmid preps.

The Colony Picking Machine was one of the earliest automated instruments developed at the HGC. Colonies are spread on 10x10 cm colony plates. A digital camera locates the positions of the colonies and selects the colonies to be picked. The position information is used to direct the XY motorized tables that place the colony source plate and the microtiter destination plate underneath a carousel of picking needles. Picking, place, and needle cleaning occur simultaneously leading to a pick rate of 1200 colonies/hour.

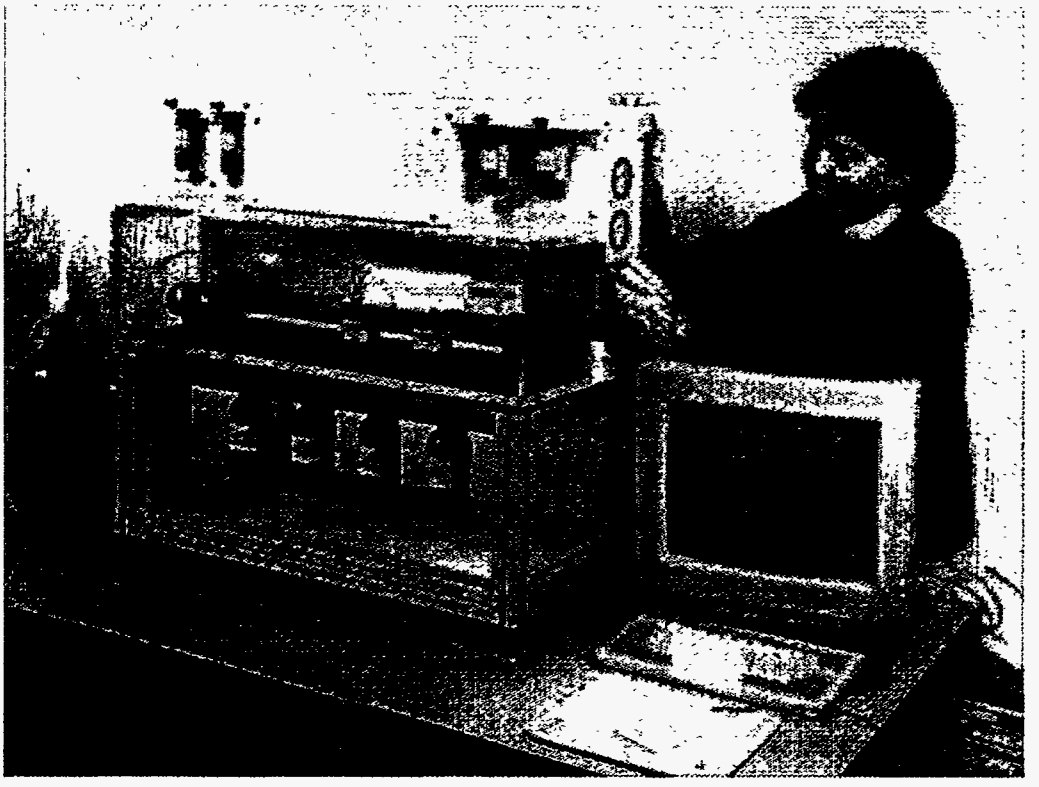
The Hewlett ORCA robot is an articulated robot arm mounted on a 2 meter linear rail. The robot hand is capable of manipulating microtiter plates and a custom built 12 channel pipettor. A wide variety of hardware components have been developed to implement various applications required in the HGC. This type of robot can easily be reconfigured for applications such as colony library replication, plate filling, and library pooling protocols.



Imaging Station—Images of samples run on agarose gels are captured using a cooled CCD camera. A complete gel casting system has been devised to accurately and reliably cast the sample lanes in known locations in the gel. This greatly simplifies the imaging requirements. This instrument has improve the throughput of agarose gel imaging and analysis 10 fold.



This 5-syringe gel loader has been designed to efficiently load the data lanes of a polyacrylimide gel in an ABI 373A DNA Sequencer.



The HGC's most recent instrument is a 12-channel DNA synthesizer. This instrument can make 12 different 21 mers in 2.5 hours. The instrument dispenses reagents very efficiently so that the cost per sample is a fraction of that produced on commercial machines.

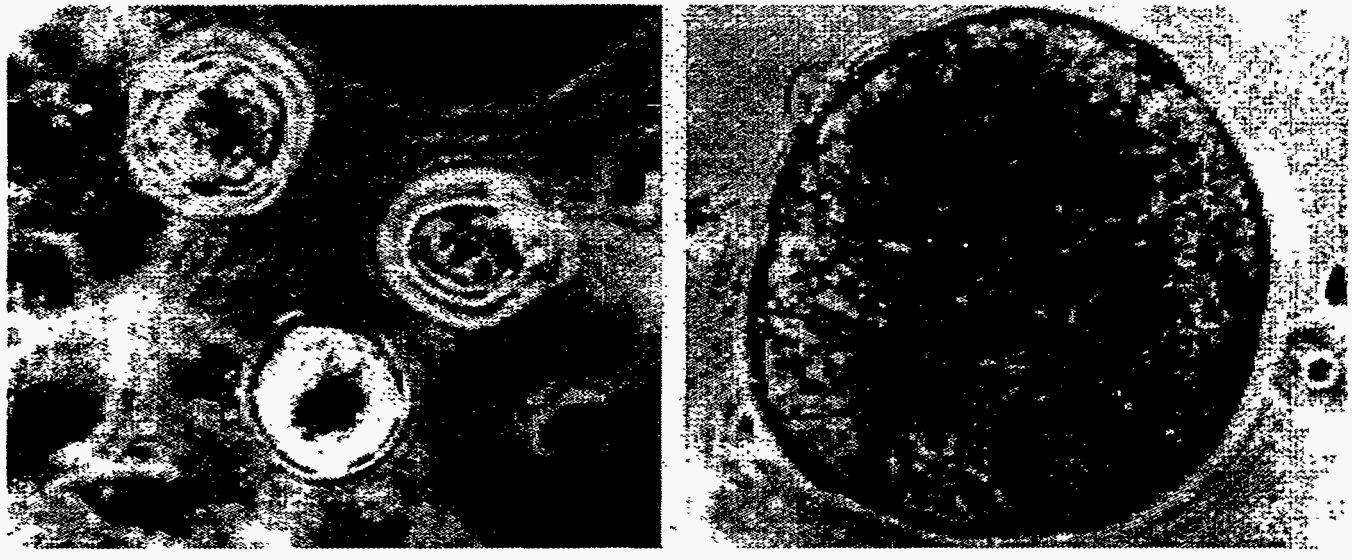
"Matrix is the Message"

The Cell's Microenvironment Directs Gene Expression

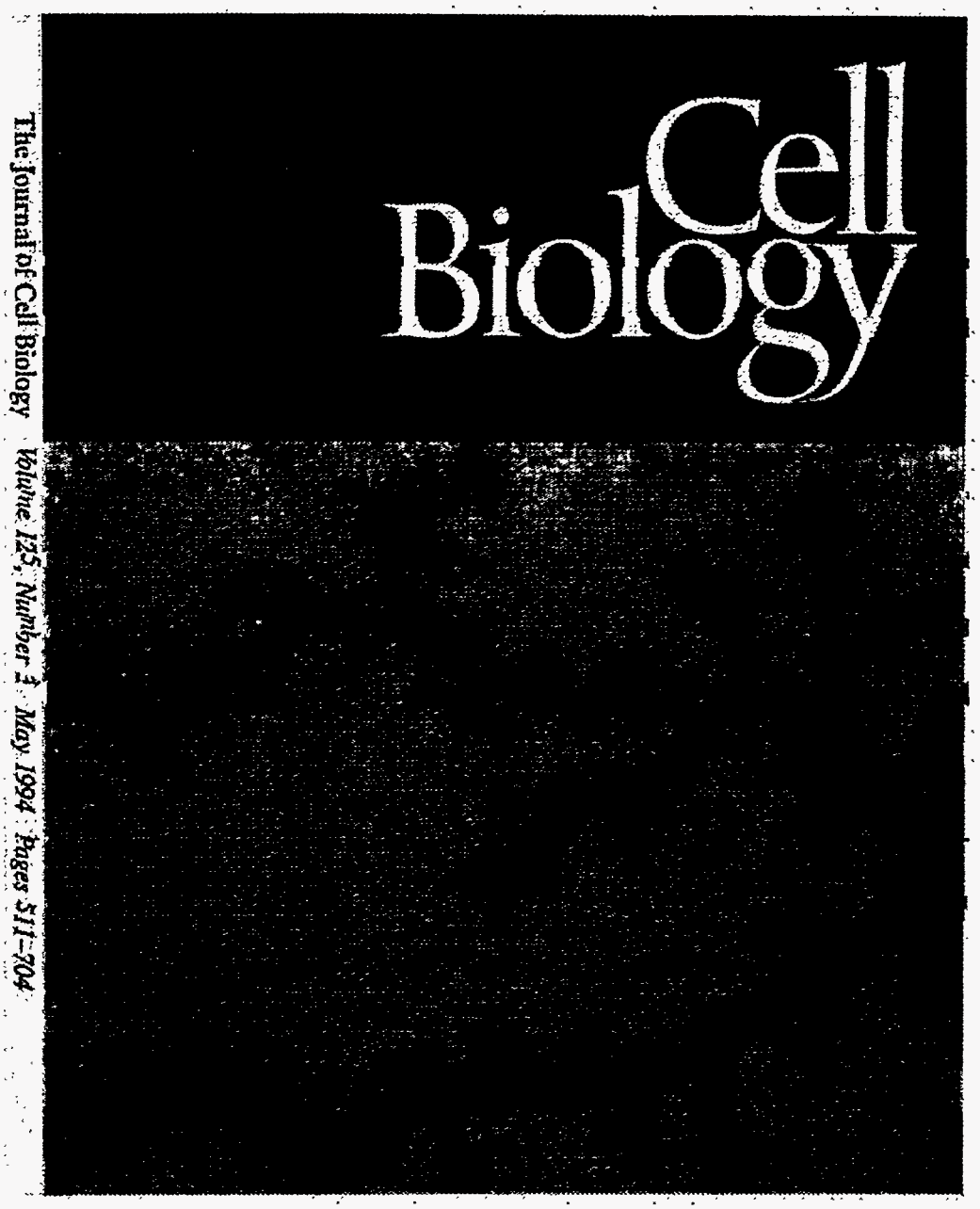
Mina Bissell, (510-486-6890)

One in eleven of all U.S. women will develop breast cancer. Approximately one-third of these women, when treated with current therapy, will survive for about 20 years after diagnosis. At LBNL, researchers have been studying the processes by which cells carrying identical genetic programs are able to differentiate at some point during their development to become a specific type of tissue or organ. They have then been applying their findings to understanding how this specificity is lost during malignancy.

A major discovery was the demonstration that the microenvironment of the cell, including a mass of fibrous and globular proteins called the "extracellular cellular matrix" (ECM) which surrounds a breast cell, plays a vital role in cell growth and development and, consequently, cancer. The biology and biochemistry of a normal cell and a cancerous cell have many common features. The difference is that cell division in the cancerous cell runs rampant. In addition, the cancer cell apparently is unable to sense the microenvironmental cues. Starting with the ECM discovery, LBNL scientists have built up a picture of the interaction between breast cells and ECM, which was presented this past year at a special symposium sponsored by the American Association for the Advancement of Science. A highlight of the presentation was the announcement of the first three-dimensional rapid assay for distinguishing normal from malignant human breast cells in tissue culture. This simple but informative assay should serve as a powerful tool in breast cancer research. Already it has been used at LBNL to provide evidence that a "tumor suppressor gene" indeed is capable of arresting the growth of metastatic tumor cells in the 3-D assay.



In collaboration with researchers at the Universtiy of Copenhagen, the Bissell lab has developed a rapid culture assay to distinguish normal human epithelial breast cells from their malignant counterparts. This is achieved by using basement membrane , a delicate noncellular matrix with which epithelial cells interact. The figure shows the differential response of normal breast epithelial cells (left) and carcinoma cells (right) to basement membrane.



Carmine staining of mammary glands in transgenic mice showed that breast cancer can occur from the breakdown of the "extracellular matrix" a mass of proteins that give strength and texture to cells.

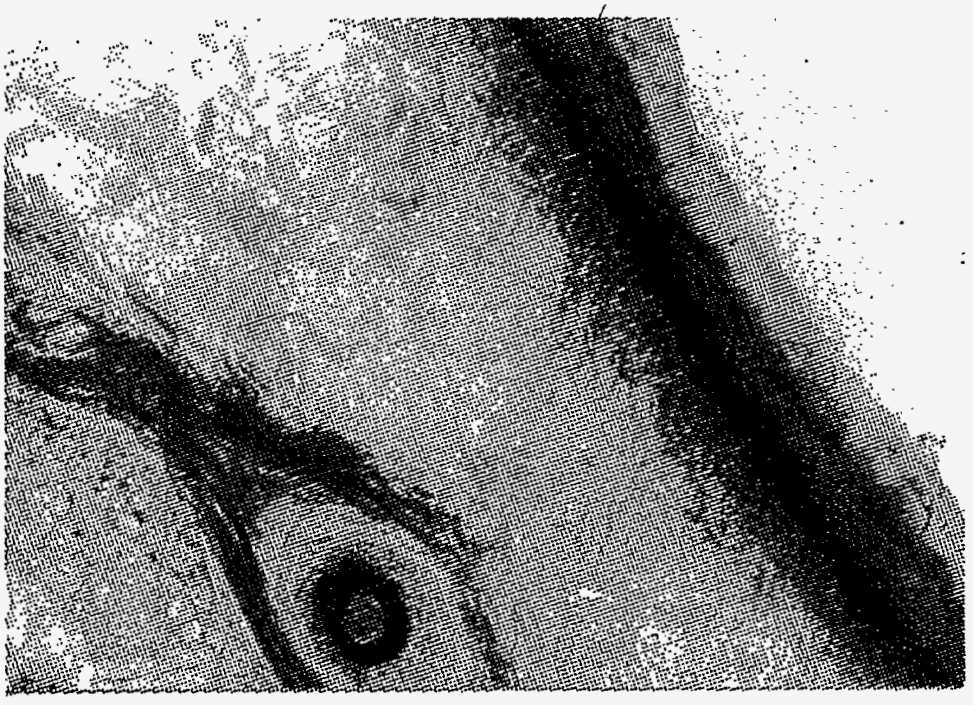
A collaboration between the Bissell laboratory at LBNL and the laboratory of Zena Werb at the University of California, San Francisco produced this paper in the May edition of The Journal of Cell Biology. The paper, authored by Carolyn J. Sympson of LBNL, Talhouk, Alexander, Chin, Clift, Bissell and Werb is entitled "Targeted Expression of Stromelysin-1 in Mammary Gland Provides Evidence for a Role of Proteinases in Branching Morphogenesis and the Requirement of an Intact Basement Membrane for Tissue-specific Gene Expression."

A New Assay for Cell Senescence

Judith Campisi, (510-486-4416)

Scientists know senescence is an irreversible stage in the life of the cell but want to learn more about it because—as the reverse of cancer—its secrets might help prevent the uncontrolled growth of a malignancy. This past year, LBNL scientists were instrumental in developing the first simple, rapid, and inexpensive test for identifying senescent cells that can be used on living organisms. Until now, senescence research has been limited to the study of cells grown in culture.

The new test was developed by Cancer Biology Department Head Judith Campisi who discovered that senescent cells produce an enzyme called beta-galactosidase that is virtually absent in normal cells. Based on this finding, a simply assay was developed that uses a stain to detect the presence of the enzyme. Old cells—those that express beta-galactosidase—turn blue. The ability to identify senescent cells on living creatures will allow researchers to take a much closer look at the role of these cells in tumor suppression. Now scientists can screen compounds for senescence-inducing or senescence-delaying activities that could have anti-tumor or anti-aging properties. The test can also be used to identify genes that might be able to stop cancerous cells from replicating, and genes that trigger premature aging. The assay has been licensed to a biotechnology company interested in new developing drugs.



This assay show the facial skin cells of a 73-year-old man. The blue stain indicates where the tissue has become senescent.

Metadata-driven User Interfaces

Shirdi R. Prem and Manfred D. Zorn

Metadata describe a particular set of data, i.e., specify data format, types, etc. We have developed software to interpret metadata and generate graphical user interfaces. In one example, database definitions are transformed into a configuration file that in turn is interpreted by a standard user interface program to create a forms user interface for a particular database. Based on the configuration file, buttons, labels, and fields are arranged in the window; functions are created that map data values from database representation into one of the user interface; and retrieval and update procedures are generated that access a Sybase relational database. Thus a working user interface is created directly from the metadata. At present, we are working on a graphical editor for the configuration file that will facilitate the metadata transformation.

In a second example, the protocol for submitting data to public databases is used to generate simple data submission tools. The metadata are used to define a set of forms for the user to fill out to format laboratory data into the required format for submission to a public database. The tool checks for mandatory fields, valid field values according to types, ranges, or controlled vocabularies. It even allows the user to specify templates that may include variables that will be replaced by values from data files. SubmitData\GSDB is being used by a few laboratories. The dependence on metadata allows to adapt the tool rapidly for other databases. At present, a submission tool for GDB is being developed.

Data Management for the Molecular Cytogenetic Resource

Jenny E. Marstaller (510-486-7695);
Manfred D. Zorn (510-486-5041)

The LBNL/UCSF Resource for Molecular Cytogenetics has been created to facilitate the application of molecular cytogenetics in clinical and biological studies. Work is being pursued in three areas: Development and application of improved hybridization technology, selection of probes optimized for use in fluorescence in situ hybridization (FISH) and development of digital imaging microscopy. All of these areas entail creation and manipulation of large images and other laboratory data. Our group is focused to provide data management support for all the activities in the Resource. To facilitate the free data exchange between researchers at UCSF and LBNL which are a few miles apart, we developed a Mosaic interface to access and modify information using the World Wide Web. The data are located on a central database. The Mosaic client allows the user to formulate retrieval and edit operations that are sent to the database. Results are filtered through a Perl script which generates HTML documents with Hypertext links that are sent back to the Mosaic client. Data from the Resource are made available using a similar mechanism that is open to outside access (URL: http://rmc-www.LBNL.gov). In order to handle large amounts of images we are developing an image annotation database. The images themselves are automatically transferred to the LBNL Mass Storage System. The annotation will be reformatted and loaded into a relational database to allow efficient query processing.

DNA Fiber Mapping

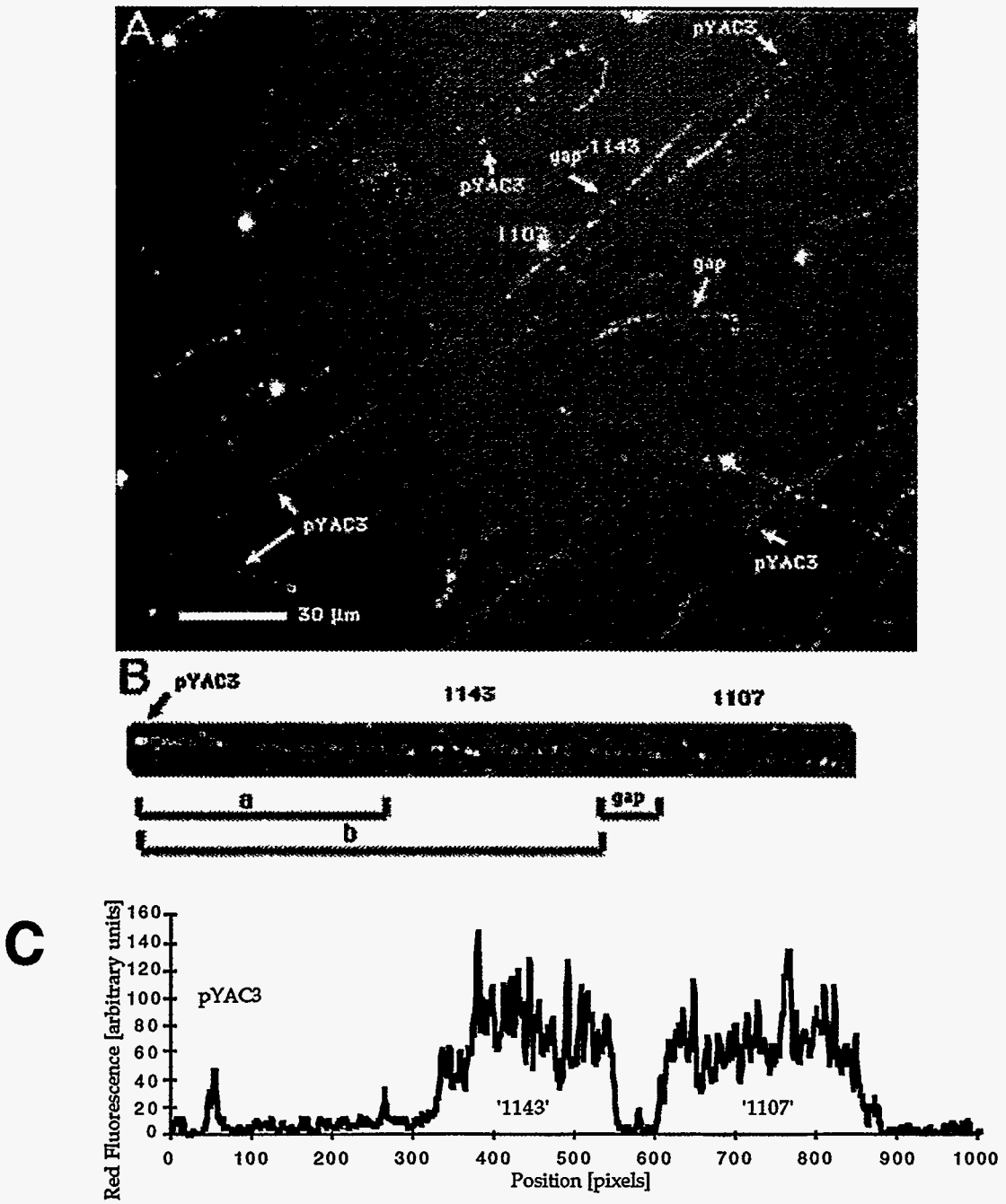
Heinz-Ulrich Weier (510-486-5327)

The construction of high resolution physical maps of the human genome and model organisms in a form suitable for DNA sequencing continues to be one of the major goals of the human genome project. In general, such maps are comprised of overlapping cosmids, P1s, BACs or DNA fragments cloned into other vectors that are amenable to sequencing. Assembly of these maps requires identification of cloned DNA sequences that contain overlapping regions of the genome. This has been accomplished by various forms of clone fingerprinting, by hybridization to clone arrays on filters and by identification of overlapping sequence tagged sites. These techniques, while effectively used by the genome community, are limited because they do not readily yield information about contig orientation, the extent of overlap of contig elements or the extent of gaps in the maps.

Fluorescence in situ hybridization provides additional important information for physical map assembly. For example, FISH to metaphase chromosomes allows localization and ordering of cloned DNA fragments with few-megabase resolution, FISH to interphase nuclei allows probes to ordered with 50-100kb resolution and FISH to preparations of decondensed nuclear or isolated cloned DNA allows visualization of probe overlap and provides some information about the existence and size of gaps in the map. However, none of these techniques provides quantitative information about the extent of clone overlap or about the separation between elements in the map because the chromatin onto which clones are mapped is condensed to varying degrees from site to site in these preparations.

We have reduced or eliminated these problems by mapping cloned DNA onto linear, fully extended DNA molecules using FISH. In our approach, DNA onto which probes are mapped is made linear using molecular combing following the recent work of Bensimon et al., In molecular combing, a solution of large DNA molecules is placed on a flat glass surface prepared so that the DNA molecules randomly attach at one or both ends. The solution is then covered with a coverslip and allowed to dry. The DNA molecules are straightened and more or less uniformly stretched during drying by hydrodynamic action of the receding meniscus between the slide and the coverslip. DNA molecules prepared in this way can be as long as 1 Mb in length. We have shown that FISH to such linearized molecules allows localization of cloned DNA sequences along the linearized molecules with near kilobase resolution.

This technique should greatly facilitate physical map assembly, especially in regions of biological interest; and should contribute to the human genome program by allowing detection and characterization of gaps in existing physical maps—critical to map closure.



Physical mapping of P1 clones hybridized to YAC DNA molecules and determination of gap sizes.

(A) Straightened molecules from YAC '141G6' (~490 kb, green) were hybridized with digoxigenin-labeled probes from P1 clones '1143' and '1107' (red) and the plasmid pYAC3 containing the vector arms (red). The figure shows a full length YAC molecule and numerous fragments. The hybridized P1 clones and pYAC3 binding sites as well as the gap between '1143' and '1107' are indicated. The resolution of the recorded image was reduced 3-fold to produce a figure of this size.
(B) A typical YAC DNA molecule (fragment) shown at full resolution carrying the hybridization signals of plasmid pYAC3 and the two P1 probes '1143' and '1107'. The distances a and b are determined for physical mapping of '1143' on YAC '141G6'. The interval 'gap' represents the size of the gap region between the two P1 clones.
(C) The average of 10 red fluorescence profiles along individual YAC molecules. Analysis of this average profile allowed determination of the gap size between P1 clones '1143 and '1107'. The hybridized P1 clones, the proximal pYAC3 binding site and the approximate locations of 50% values of the red fluorescence (dotted vertical lines) are indicated.