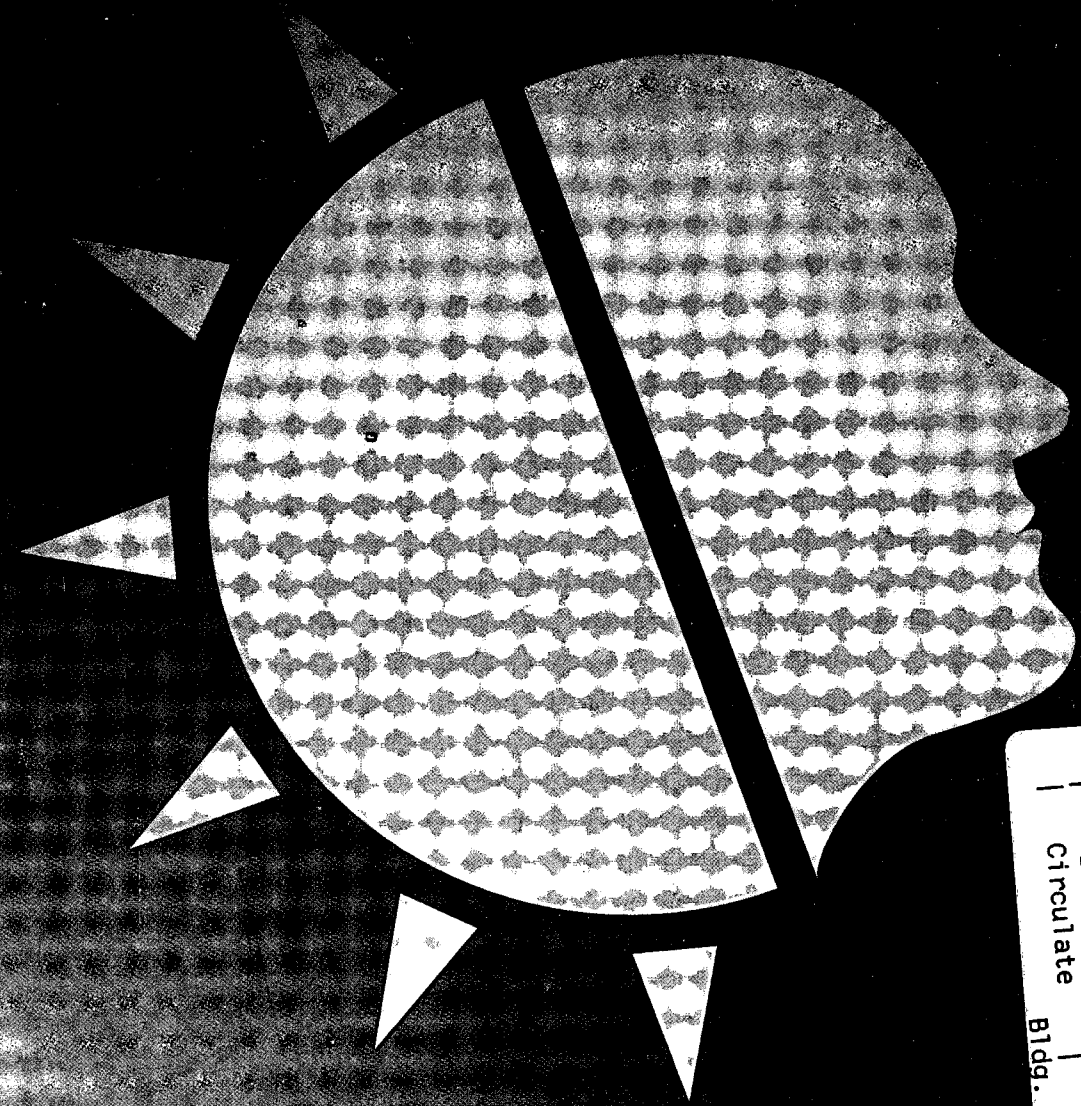
Lawrence Berkeley Laboratory

PROGRAM DIRECTOR'S REPORT FOR THE OFFICE OF

Health & Environmental Research



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

April 1994

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A. Program Director's Overview Statement

A. Program Director's Overview Statement

LBL performs basic and applied research and develops technologies in support of the 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; 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.

LBL 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 LBL plays in furthering the mission of OHER.

BIOSCIENCES AND BIOTECHNOLOGY

LBL's bioscience and biotechnology competency focuses on structural biology; genome research; bioinstrumentation; molecular cytogenetics; medical imaging; the biology of human diseases, and biomolecular design. LBL has pioneered the use of such techniques as x-ray and electron 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 LBL 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 microscopy, x-ray spectroscopy, and x-ray crystallography. The Donner 600-Crystal Positron Tomograph, provides better resolution for imaging biological functions in the body. The National Tritium Labeling Facility explores the labeling of compounds to high specific activity with tritium and provides a tritium-labeling service for investigators throughout the country. The Yeast Genetic Stock Center maintains a collection of approximately 1000 genetically defined strains of the yeast.

ENVIRONMENTAL SCIENCE AND REMEDIATION TECHNOLOGIES

LBL 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 LBL 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. LBL 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 biologically toxicity of mixtures of contaminants, and methods to eliminate the exposure pathways and monitor the effectiveness of remediation schemes. LBL 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). LBL 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. LBL is preeminent in conducting research on the nature, sources, transport, transformation, and deposition of indoor air pollutants including radon and combustion emissions. In addition, LBL'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 LBL 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 LBL'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 LBL competency on Chemical Dynamics, Catalysis, and Surface Science. LBL 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 LBL and DOE science programs. LBL 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. LBL 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 LBL 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 LBL 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

LBL maintains unique capabilities in the area of engineering design and fabrication technologies. The integrated capability at LBL 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

LBL has an underlying capability for designing, building and operating large shared research facilities for the benefit of the national scientific community. LBL 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 facility is available to visiting and in-house researchers in materials, interface and surface science, atomic and molecular physics, chemistry, the life sciences, earth science, and x-ray optics. It will also serve in the development of micro- and nanofabrication technologies and nanostructure characterization. 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, which produces compounds with high specific activities of tritium to serve as tracers in chemical and biomedical research, providing 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

LBL 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

LBL 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. In fusion energy, LBL leads the nation in the development of induction linear accelerators as drivers for inertial confinement fusion. LBL's advanced building technologies, such as more efficient window and lighting systems, offer cost-effective means of reducing energy use. Research on the thermal performance of buildings and indoor air quality addresses the objective of having energy-efficient buildings that do not compromise human health or productivity. Computational methods and simplified analytical tools facilitate the incorporation of energy-efficiency measures in the design, construction, retrofit, and operation of buildings. Electrochemical research provides a technology base for advanced batteries and fuel cells for transportation and stationary-power applications. Combustion and other fossil-fuel related research is directed towards increasing efficiency and reducing emissions for both energy supply (e.g., power plants) and end-uses (e.g., automobiles). Information on national and international energy use and on technical and economic assessments of energy options provide a basis for sound government and other decision-making. Electromagnetic and seismic imaging methods, numerical simulation, and methods to control and divert flow of subsurface fluids are critical to sustained supply of energy from the subsurface resources from which most of our energy is derived.

PARTNERSHIP INTEGRATION FOR TECHNOLOGY

LBL maintains an underlying capability for utilizing a multiprogram energy research laboratory as a national resource in partnership with industry, universities and other government agencies. In the last 2 years LBL has formed 30 partnerships with industry for the development and integration of complex technology through Cooperative Research and Development Agreements (CRADAs). LBL's California Institute for Energy Efficiency integrates new technology with the utility industry. The Center for Building Science, the Berkeley Electrochemical Research Center, and the Center for Advanced Materials are also examples of partnership arrangements. LBL is an active partner in AMTEX—a national laboratory partnership for advanced technology for the American Textile industry. LBL has developed the overall plan for many major detectors and now provides scientific leadership for partnerships of the most significant and complex experiments in high-energy physics (SDC, B-Factory), nuclear physics (STAR, Gammasphere), astrophysics (COBE), and is a major collaborator in other high-energy collider detectors (CDF, D0), high-energy heavy-ion studies (CERN), and in a neutrino experiment (SNO). The LBL capability in Engineering Design and Fabrication Technologies includes industrially important areas including custom integrated circuits, large-volume semiconductor-detector technology, and laboratory automation. In addition, the most productive form of technology partnerships, the direct exchange of personnel and ideas through the educational process, are strongly supported through LBL's fundamental capability of Education of Future Scientists and Engineers. LBL 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.

New and Expanded Programs in FY1994

While the programs in Analytical Technology (KP01) have remained static or have seen some tailoring back with respect to funding, Health Effects (KP03), General Life Sciences (KP04), and Medical Applications (KP06) programs at LBL are experiencing growth in areas reflecting the national research trends.

The OHER-funded subsurface science program includes high resolution seismic imaging, properties of material surfaces, and speciation of radionuclides. This basic research on the effects of natural physical and chemical heterogeneity on microbial populations in the subsurface will bear significantly on future applied remediation strategies.

The detector materials and detector instrumentation research areas have been integrated successfully in the fabrication of many instruments for diverse applications in high energy physics, astrophysics, x-ray fluorescence and diffraction, and gamma-ray spectrometry. Such a specialized expertise in these areas of radiation detection does not exist at this level outside of LBL. An area that would benefit considerably from this expertise would be in the Environmental Restoration and Waste Management program. The radiation detector technology we have advanced could be used to address the needs of environmental radiation measurement and monitoring.

Subsumed in the Health Effects category is LBL's premier national research effort on the indoor environment—studying the indoor air pollution and its relationship to the properties of buildings. In addition to this project, closely related efforts receive support from OHER, DOE/Conservation and Renewable Energy, EPA, NIH and other organizations. As we have honed our expertise in this area and developed unique facilities such as environmental chambers, the nation's attention has become attuned to the issues of indoor air pollution. Radon and so-called "sick building" syndrome are now part of the homeowner's lexicon. One of the most highly integrated programs at the Lab, our indoor environment effort would become more comprehensive with an expansion into the area of biologically based methods for risk assessment. The Air Pollutant Exposures in Buildings project, led by Joan Daisey, is expected to by funded at a level of $463,000 in FY1994, and additional funding is being sought for FY1995-96.

In FY1994, OHER has invested $171,000 into Judy Campisi's investigation of the Cell Cycle and Repressor Genes. It has been demonstrated that 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, Dr. Campisi is developing assays to detect growth regulatory genes with partial or incomplete activity and taking a quasi-genetic approach to identify these genes. Additional funding will be requested to expand this project for FY1995–96.

In the area of General Life Sciences, LBL's Human Genome Center, now under the direction of acting head Mohan Narla, will receive a $482,000 boost to the megabase sequencing effort. Manfred Zorn will receive $351,000 in FY1994 to launch a new project entitled Using Metadata to Generate Interfaces.

In the area of Medical Applications, there is an emerging national emphasis on application of medical imaging technology for the study of disease states at the molecular level. Radiopharmaceutical receptor imaging agents, developed at LBL, will be extremely valuable tools for determining physiologic, pathologic, and therapeutic effects on lipoprotein metabolism and cardiovascular disease risk. A new project led by Ronald Krauss, head of LBL's Molecular and Nuclear Medicine Department, is coming on line in FY1994 to address these opportunities. The Imaging of Apolipoprotein E-Binding Receptors project will 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.

Proactive Technology Transfer

Technology transfer opportunities abound at LBL and with technology partnerships firmly established as part of the Laboratory mission of LBL, the laboratory continues to expand, create and manage an organization and environment where technology transfer means business. LBL will effectively and aggressively translate its research and development projects into successful commercial products and services for the community and the nation. To accomplish effective technology deployment, LBL focuses on three critical elements: 1) innovative research and technical assistance; 2) outreach to strengthen industrial partnerships and; 3) commercialization of intellectual property.

To fulfill the long-term technology transfer objectives, our specific goals include:

- Strengthening research centers and management organizations that promote fundamental innovative science and technology development germane to industrial research needs;
- Developing specific innovative technology research projects and advanced technology demonstrations to the point where commercial interest is attained; and
- Developing research staff awareness and recognition of technology transfer opportunities.

LBL continues to emphasize technology transfer through the publication of research results, technical consulting, personnel exchanges, and conferences. An important technology transfer mechanism is training students who ultimately work for industry, universities, or government. More than 700 advanced students are supported at LBL. About 100 advanced degrees based on LBL research are granted annually, and approximately half of these students bring their technical talents to industry.

LBL has developed a "Technology and Invention Inventory." The inventory lists over 200 technologies (including patentable inventions, public domain technologies, and copyrightable developments) in database form. The database yields information regarding subject area, patent and licensing status, scientists, and activity comments.

LBL uses a multi-step approach to inform industry about LBL technologies. First, the attributes of the invention are summarized using attractive layouts and highlighting potential product applications. This package is sent to corporate executives in charge of company development; they are targeted through a high-technology corporate database that lists companies by product subject area. In addition, notices describing the inventions are distributed through technology newsletters that specialize in announcing new ideas to corporate executives. Articles are also sent to trade journals and are announced in general press releases.

FY1993 CRADA Highlights Related to Health and Environmental Research

Lawrence Berkeley Laboratory and Chiron Corp., a California biotechnology firm, have signed a CRADA to jointly study macrophage colony stimulating factor (M-CSF). M-CSF, one of approximately 18 signaling molecules that regulate growth and differentiation of both red and white blood cells, transmits its message into the cell interior only after binding to a receptor on the cell surface. Research under this CRADA will focus on gaining an understanding of the interaction between M-CSF and its receptor at the molecular level. This information may help in the design of drugs that, by binding the M-CSF receptor, can mimic or antagonize the behavior of natural M-CSF. M-CSF has shown promise when used in clinical trials to correct blood cell deficiencies and as an anti-fungal agent for infections that arise as a side effect of chemo- and radiation therapy.

Lawrence Berkeley Laboratory executed a CRADA with Glycomed Incorporated to study the development of potential therapeutics for the treatment of disease, including inflammation. Inflammatory diseases are caused when the body's immune system functions inappropriately. Carbohydrates are involved in this process at the most basic level because they are on most cell surfaces. They bind to various receptors which are adjacent to potential sites of inflammation in the body, and facilitate cells moving to these sites. By blocking these receptors with novel carbohydrates, the inflammation process may be halted. The project will include the identification, assembly, screening and testing of a vast number of peptides generated through solid phase synthesis. Using a random library generation approach with carbohydrate-based structures, this CRADA will allow Glycomed to greatly increase the number of compounds that it can assess for therapeutic efficacy.

Lawrence Berkeley Laboratory and E.I. du Pont de Nemours and Company have signed a three-year CRADA to jointly design, prepare and use organometallic catalysts for the "living" polymerization of isocyanates and carbodiimides. The new materials to be synthesized—polyisocyanates and polycarbodiimides—are unique organic polymers that display extended chain, helical conformation in both solution and the solid state. U.S. industry has sought a reliable route to the synthesis of these compounds, which have potential applications as high strength, light-weight structural materials, high impact plastics, thermoplastic elastomers, biocompatible materials, optically active polymers, and innovative optoelectronic (e.g., nonlinear optical) materials.

LBL is home to the California Institute of Energy Efficiency, a consortium of major California utility companies, universities, and national laboratories. This partnership, which operates under the terms of a $20 million CRADA, sponsors research aimed at saving energy, improving air quality, and avoiding the environmental costs associated with new power plant construction.

CIEE now funds and coordinates a large variety of research projects through the state. In FY1993, CIEE funded 27 CRADAs including:

- Indoor Ozone Concentrations: Experimental Quantification of Mechanisms of Outdoor Attenuation
- Analysis of Energy Efficiency and Air Quality
- Design of Phase-2 Study of Sick Building Syndrome
- Neighborhood Cooling by Trees
- Indoor Air Quality Study Employing Silicon-Based Sensors
- Interactive Information Kiosk: Providing Information about Energy Efficiency in Buildings

FY1993 Licensing Highlights

Advanced Photonics, Inc. licensed LBL's new application-specific integrated circuit for use with API's proprietary avalanche photodetectors (APD). As part of its work for the Superconducting Super Collider (SSC), LBL designed a chip that was readily customized to match API's avalanche devices. The circuit designed by LBL contains 16 ultra-low noise preamplifiers for use with API's new line of APD arrays, and will permit API customers and potential customers to more easily integrate and utilize API's advanced avalanche array products in their instrumentation.

ISM Technologies Inc. has licensed LBL's broad-beam direct current ion implantation device and related inventions for surface modification by ion implantation. Operating in continuous mode, with a wide beam, the beam efficiently modifies surfaces of materials. It can be used to harden parts such as medical devices. The U.S. economy will benefit from the added value of longer lasting parts, while ISM will be able to capture a larger share of the ion implantation machine market with more efficient products.

LBL has licensed to a Colorado small business the software for a new wellbore procedure, a high resolution instrument/software that characterizes groundwater contamination. It provides vital information for solving water quality and supply problems and for improved remediation of contaminated sites. Termed hydrophysical logging, the technique is based on a new concept for measuring fluid flow and allows characterization of growing groundwater problems more quickly, more cost effectively, and with higher resolution than current industry standards. By incorporating unique interpretation software with advanced downhole instrumentation (the hydrophysical logging tool), and through assessment of induced changes in the electrical conductivity of the wellbore fluids, the technique quantifies inflow locations, their associated flow rates, and basic water quality parameters of the associated formation waters. This product will aid the environment restoration industry.

LBL also entered into 10 options in FY1993, with:

- Motorola
- IBM
- Glycomed
- Intermagnetics General Corp.
- Chemagnetics
- Fusion Systems Corp.
- Bellcore
- Celtrix Pharmaceuticals Inc.
- Ion Microfabrication Systems, GmbH
- Chiron Corporation

Other FY1993 Technology Transfer Highlights

Researchers at LBL won an R&D 100 award for the invention of carbohydrate protein conjugates (CPC). These novel materials allow proteins to remain active in harsh industrial environments. They were developed through a joint effort by researchers from Lawrence Berkeley Laboratory (LBL), University of California at Berkeley, University of Ohio, and Cargill Corporation. CPC-enzymes and CPC-antibodies exhibit enhanced catalytic and binding activities at elevated temperatures and in organic solvents. Enzymes have the potential to revolutionize the synthetic chemistry of both current and new materials because they offer high catalytic activity and selectivity. This translates into decreased costs of some products and the availability of others not otherwise available. Enzyme catalysis also results in significant decrease in energy consumption since the enzymes operate at low temperature and pressure. In addition, enzyme catalysis significantly decreases (in many cases totally) the production of toxic or wasteful by-products. Use of this novel carbohydrate protein conjugate approach is applicable to a vast and diverse array of industrial reactions and offers a general solution for gaining protein stability at elevated tempera-

tures and in organic solvents. Potential applications include, but are not limited to, industrial uses of enzymes, diagnostic testing, drug delivery systems, medical devices, and protein synthesis.

FY1994 HIGHLIGHTS

LBL will have at least 7 ER-LTT Spinoff CRADAs in FY1994 including:

- LBL will work with SOMATIX to develop neurochemical imaging of gene therapies. The ultimate goal will be to develop a retroviral vector which will insert genes into host animal cells for the production of specific brain chemicals. Gene therapy, an emerging biomedical technology, offers the ability to treat human brain diseases such as Alzheimer's and Parkinson's disease.

- LBL will partner with General Dynamics to develop new microphotogrammetry instrumentation. They will produce a tool capable of making digital three dimensional surface maps of microscopic objects. The primary application will be semiconductor inspection, presently a $1 billion market. Next generation IC technology will require instruments capable of imaging in three dimensions. Present IC inspection units are only capable of scanning in two dimensions.

- LBL will work with a major manufacturer of eyecare products using novel polymers to create a new generation of biocompatible and bioactive soft contact lenses.

- Working with Teledyne Laars, LBL will develop low NO_x emission gas-fired furnaces and boilers. NO_x is a major source of air pollution. LBL's weak swirl burner technology has the advantage of reducing the actual amount of oxides of nitrogen produced, rather than mitigating NO_x already present in exhaust.

In FY1994, LBL will fund 10 technology maturation projects including:

- *Development of a colorimetric sensor for virus detection and treatment.* This project will refine LBL's patent-pending novel thin-film flu detection material. This film combines a molecular binding site and a molecular detection element within the same molecular assembly. In the presence of influenza virus, the film changes color, giving an immediate diagnosis. The film's capabilities will be extended to other viruses, and developed for drug screening.

- *Establishment of a borehole simulator user facility.* This facility will be used to perfect systems that characterize borehole fluids. Due to the complex nature of fluid dynamics and geological factors, it is difficult to predict how an instrument design will function in the field. This facility, using the leading-edge borehole simulation algorithms and expertise available at LBL, will allow manufacturers of borehole instruments to perfect their designs. Borehole measurement tools are used in oil reservoir management, and toxic site characterization. Improved downhole instruments will help to increase oil production and improve environmental restoration efforts.

- *Development of precision manufacturing using deep x-ray lithography.* The next generation of nanotechnology will feature 3D microstructures with lateral dimensions in the micrometer range, vertical dimensions several hundred micrometers high, and sub-micron tolerances. Within a decade, these devices will be ubiquitous. Successful demonstration of this technology at LBL's Advanced Light Source will help the U.S. catch up in the race to develop this crucial technology.

- *High resolution direct quantum detection digital x-ray imaging techniques.* This project will develop the potential for silicon strip x-ray detectors. Such devices will have better resolution, sharper pictures, processed in real time digitally, at lower energies than conventional x-ray sources. They also obviate the need for film and associated chemicals, which pose an environmental hazard. They will find use in routine clinical radiology, mammography, CT scans, digital subtraction radiography, airport baggage inspection, industrial process control, and dental applications.

- *Ergonomic improvement in the Airvest design.* This project will make a production model of LBL's patented Airvest technology, a device to be worn by workers using exhaust hood vents, such as spray painters. The Airvest achieves a 50-fold reduction in breathing-zone pollutant concentration while reducing energy consumption of the exhaust hood. This invention will protect workers while saving energy.

- *Yellow phosphorus to bleach pulp.* LBL researchers will assess the practicality of adopting LBL's patented yellow phosphorus flue gas scrubbing system, PhosNOX to the bleaching of paper pulp. Yellow phosphorus uses one-fourth the energy of other systems designed to replace chlorine in the pulp bleaching process. The paper industry has been conducting an intensive search for a viable replacement for

chlorine treatment, the use of which results in the presence of dioxin in the paper mill waste stream.

- *Characterization and genetic improvements of wine yeast.* Using LBL's molecular biology expertise, and the unique resource of the Yeast Genetic Stock Center, the world's best source of yeast, located at LBL, researchers will determine which genetically-controlled traits are important in wine making. The ultimate goal is to breed yeast with optimum levels of these traits. These optimized strains will provide novel quality control measures to winemakers, and yield significant cost-savings to the wine industry.

LBL will also fund at least 5 mini-CRADAs in FY1994:

- LBL will team up with Intel Corp. and Chromex, Inc. to develop high-throughput semiconductor inspection instruments. Using LBL's unique Raman and photoluminescence capabilities, the partners will develop a microprobe that will give rapid and definitive chemical identification of the impurities that can lead to IC failures. Using such a device, the semiconductor industry will be able to better maintain the high production runs necessary to offset the huge capital investments required to build an IC factory. U.S. industry will gain a competitive edge in an extremely competitive field.

- LBL researchers, working with SynchroDesign and Orbit will perfect a recycling integrator IC chip. These chips, which combine analog and digital circuitry to greatly reduce the per-channel cost, are used in high-resolution ionization chambers and other scientific instruments. Commercial applications include radiation detectors, radiation source calibrators, and radiation therapy beam delivery monitors.

- LBL will collaborate with Thermatrix, Inc. to develop an optical detection and feedback system for Thermatrix's packed bed non-flame thermal reactor. This reactor is capable of destroying many hazardous wastes, including chlorinated hydrocarbons. The detection system, using LBL's Fourier Transform Infrared (FTIR) spectroscopy technology, will help optimize the performance of this reactor. Such a reactor has a wide range of application in environmental restoration.

- LBL will collaborate with two partners, Computer Technologies & Imaging and Advanced Photonics, Inc., to develop a positron emission tomograph (PET) with improved resolution and data rates. They will develop improved silicon photodiodes and very large scale integration (VSLI) chips, then incorporate them in a next generation PET, with resolutions in the 2–3mm range. PET scans have great value to the medical sciences, giving researchers high-resolution, real-time pictures of the metabolization of labeled molecules in the human body. Clinical applications include Alzheimer's disease, epilepsy, affective disorders, stroke, and coronary artery disease. This project will have added spinoff value in the silicon photodiodes, which are 2 to 3 orders of magnitude cheaper than other types of detectors, and will find large markets outside of PET applications.

- LBL will work with Wang NMR, Inc. to develop low-cost magnetic resonance imaging (MRI) for breast cancer diagnosis. MRI has the potential to improve on conventional x-ray mammographs, which often fail to detect small lesions, and cancer in younger patients. This project will work on solving several drawbacks of MRI, including discriminating between malignant and benign tumors, and reducing the bulk of the scanner so that doctors can perform rapid needle biopsy during and aided by the scan. The general public will benefit greatly from improved methods of detecting a major disease.

Other FY1994 Projections

- In FY1994, LBL expects to participate in 2–3 Technology Reinvestment Program CRADAs.

- LBL expects to execute between 15–20 licenses and options in FY1994.

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 75 students to the laboratory in FY 1993. Because the Lawrence Berkeley 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 1992, 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 LBL 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. 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 activities and direct research experiences. During the summer of 1993, forty-three teachers participated at the laboratory, over 20% of them working directly with Life Sciences Division scientific staff.

LBL is host to the Department of Energy's High School Honors Life Sciences Program. This two-week program brings 64 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 LBL 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. In FY 93, approximately one-fifth of the LBL volunteers were from the Life Sciences Division. Of the fifteen students who participated in the eight week summer high school student research program, two worked in the Life Sciences Division. The Life Sciences Division staff also assisted the Education Outreach in other ways: giving presentations on the Human Genome Project, the Dog Genome Project, and helping to edit biology curriculum guides for LBL. In the Summer of 1994, Life Sciences Division is planning to host eight high school students for internships in the Human Genome Project, The Dog Genome Project, Occupational Health Services, and Analytical Services.

B. Laboratory Facilities and Resources

B. Facilities and Resources

Laboratory Facilities

ADVANCED LIGHT SOURCE

The Advanced Light Source (ALS) will offer 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 will become resources for research that breaks altogether new ground. Life sciences research at the ALS will ultimately make use of three insertion devices, each the source of radiation for a different "resource center." An ongoing research effort in x-ray microscopy, using facilities at both Brookhaven National Laboratory and BESSY in Germany, is aimed at determining the most profitable path to pursue in capitalizing on the opportunities of this nascent field. Our current vision, however, includes two complementary x-ray microscopes, each offering its own advantages, illuminated by an undulator source of ultrabright soft x-rays. 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 OHER and the Office of Basic Energy Sciences. This circular polarization resource will consist of two beamlines, with the optics arranged so that either can intercept the central radiation fan. The facility will consist of a complete soft x-ray grating monochromator beamline and a hard x-ray crystal monochromator beamline, as well as several ultrahigh-vacuum chambers, detectors, and a 6-T magnet. For protein crystallography, the central technique of structural biology, a third resource is planned—one that would match or exceed

the performance of any facility currently in operation, and, at the same time, would exploit a user-friendly, multibranch design that would multiply the data-collection efficiency of a single linear wiggler by several-fold. This facility will consist of a 2.13-T wiggler and three end stations. Finally, since full utilization of the ALS for biological research requires the proximity of well-equipped biological laboratories, as well as adequate office space for visiting researchers, construction has been proposed for just such conventional facilities, as well as a full complement of office and laboratory equipment. The facilities will include 11,000 gross square feet on the second floor of the new ALS building and the second floor of the contiguous Building 80.

IMAGING FACILITIES

The Donner 600-Crystal Positron Tomograph was designed and built at LBL 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 LBL. 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, LBL has also developed the capability of imaging biological macromolecules by scanning tunneling microscopy. Several STMs are operational at LBL.

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.

RADIOPHARMACEUTICAL FACILITY

Nuclear and organic chemical synthesis instrumentation and laboratory facilities at LBL include the 88-Inch Cyclotron and radiation-containment devices for rapid synthesis of radiotracers. A mini-cyclotron facility is being acquired through support of OHER to provide positron-labeled isotopes for the PET nuclear medicine program.

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 LBL 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, LBL's Information and Computing Sciences Division maintains state-of-the-art information and computing resources. These resources include SEEDIS, the LBL 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 OER-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.

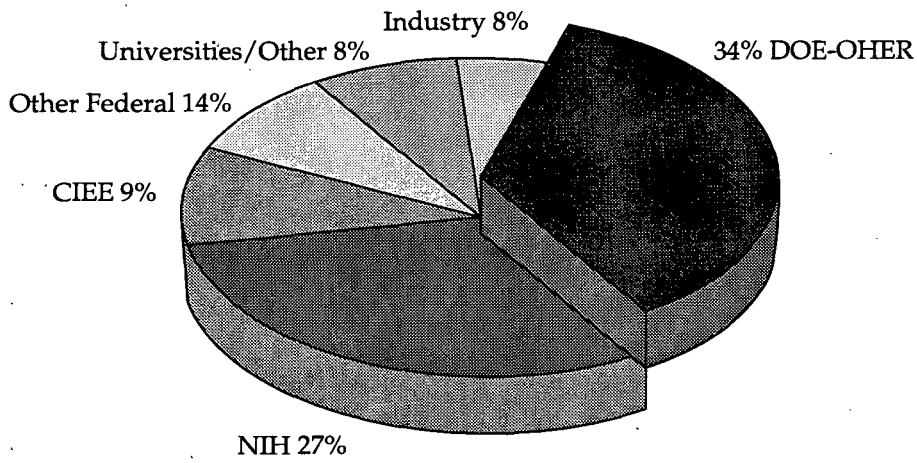
Laboratory Funding and Organization

On the following page are tables summarizing OHER funding and personnel levels, in the context of the Lawrence Berkeley 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 the new Life Sciences Division, Structural Biology Division and Energy and Enviroment Division, where the bulk of the OHER-sponsored research is conducted.

Laboratory Funding (millions of dollars)

Source	1992	1993	1994*
OHER			
Operating	16.8	17.9	19.0
Capital equipment	1.3	1.4	1.4
Construction	0.0	1.2	2.8
TOTAL	18.1	20.6	23.2
Other OER			
Operating	112.5	115.1	102.0
Capital equipment	12.7	15.0	20.4
Construction	22.5	24.8	22.6
TOTAL	147.7	154.9	145.0
Other DOE			
Operating	44.1	49.2	40.4
Capital equipment	1.0	0.5	0.0
Construction	0.5	1.2	6.8
TOTAL	45.6	50.9	47.2
WFO	34.5	35.4	58.0
Total Lab Funding	**245.8**	**266.5**	**273.4**
Laboratory Personnel (FTEs)			
OHER direct	110	115	110
Other DOE direct	1410	1415	1356
WFO	330	340	326
Indirect	750	780	748
Total Lab Personnel	**2600**	**2650**	**2540**

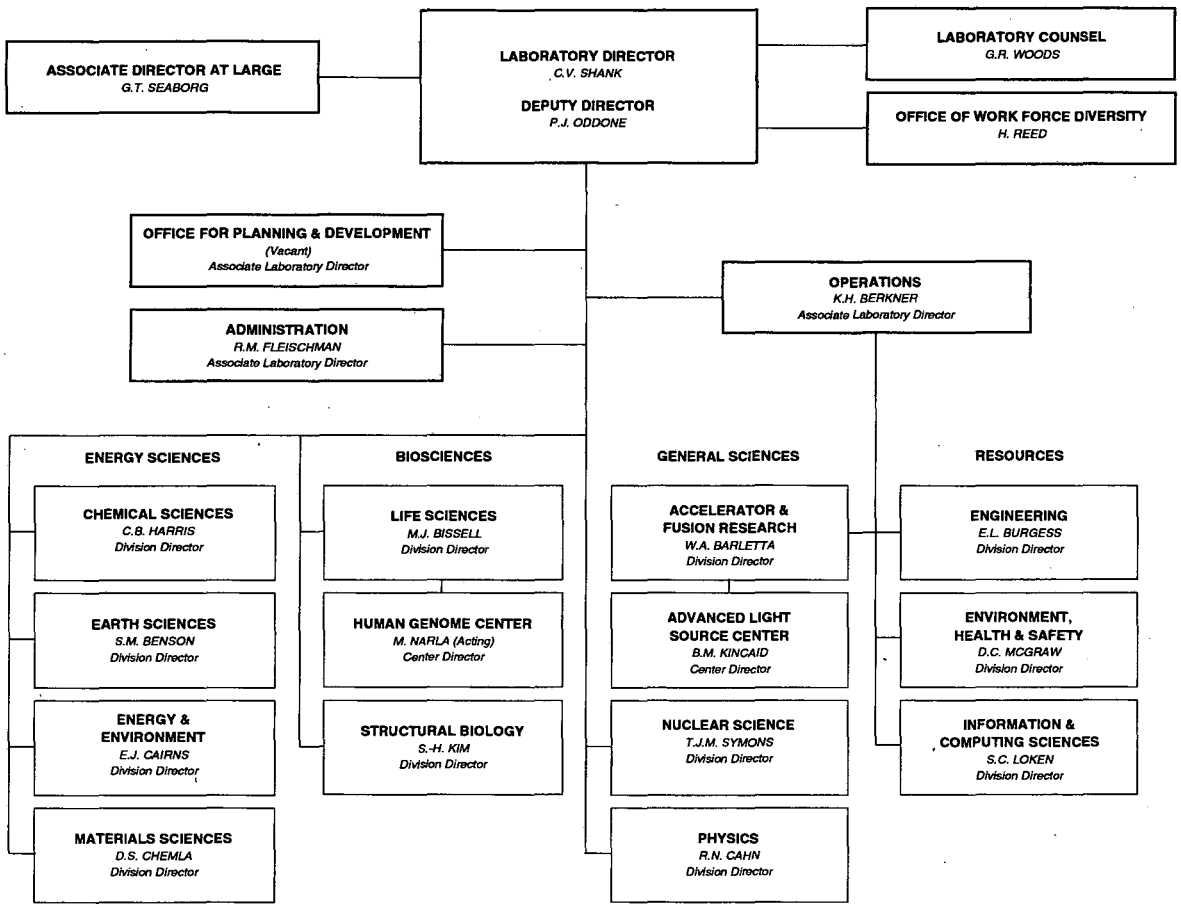
*Figures for 1993 are estimates.



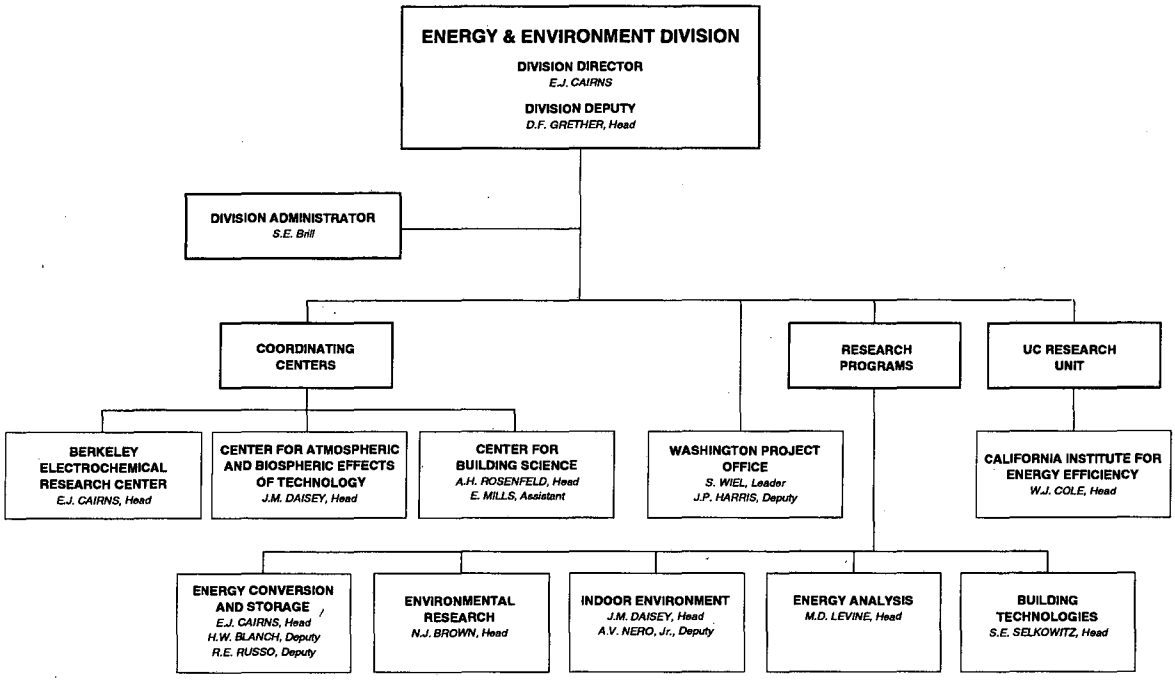
FY 1994 $56M

Sponsors of biosciences and environmental sciences research programs at Lawrence Berkeley 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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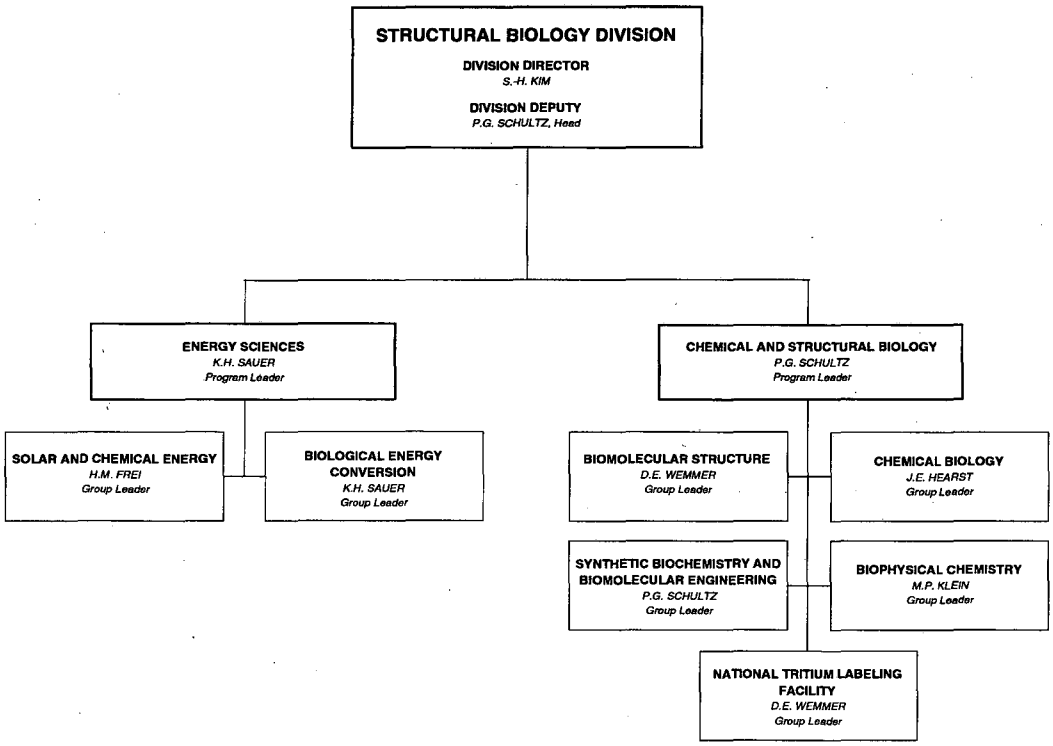

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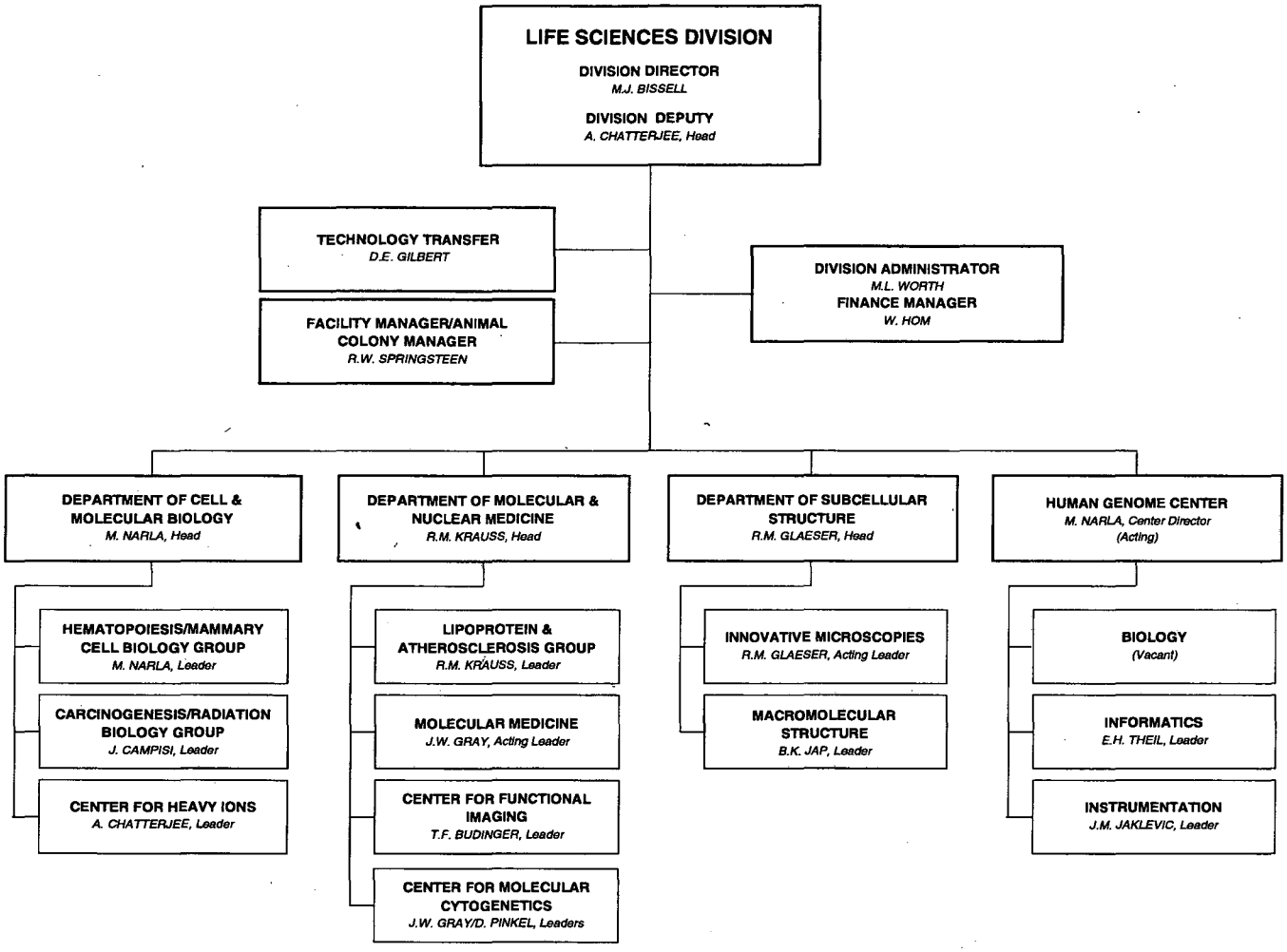
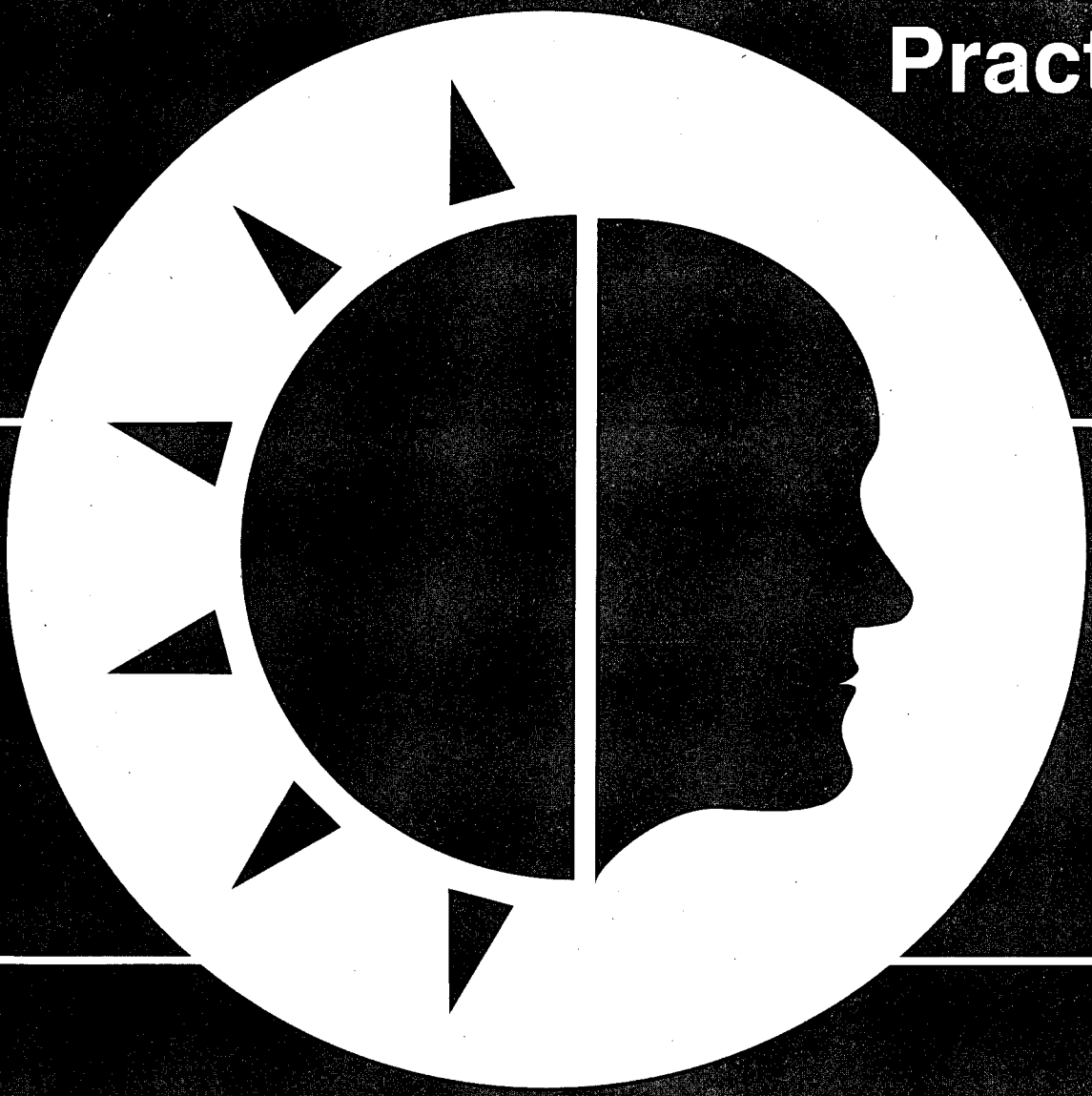


C. Research Management Practices



C. Research Management Practices

Scientific Review

The quality and direction of research in biosciences and environmental sciences at LBL 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 DIVISION

Department of Cell and Molecular Biology

Eleanor Adair
Fellow, Department of Physiology
John B. Pierce Laboratory

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

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

Errol C. Friedberg
Professor, Department of Pathology
University of Texas Southwestern School of Medicine

Yuet W. Kan
Howard Hughes Professor, Department of Laboratory Medicine
University of California San Francisco

David Larson
Associate Professor, Department of Radiation Oncology

University of California at San Francisco

J. Richard McIntosh
Professor, Department of Molecular
Cell and Developmental Biology
University of Colorado

Robert L. Ullrich
Professor, Department of Radiation Therapy
University of Texas Medical Branch

Department of Molecular and Nuclear Medicine

Richard L. Jackson
Vice President, Discovery Research
Wyeth-Ayerst

Kenneth A. Krohn
Professor, Department of Radiology
University of Washington

Gerald Matson
Department of Veterans Affairs Medical Center
San Francisco

Gustav Schonfeld (Chair)
Professor, Lipid Research Center
Washington University

Allan R. Tall
Department of Medicine
Columbia Collaege of Physicians and Surgeons

Michel Ter-Pogossian
Professor, Division of Radiation Sciences
Washington 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 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 LBL 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 Bassingthwaight, M.D., Ph.D.

Craig Malloy, M.D.

Albert Macovski, Ph.D.

Ronald Nutt, Ph.D.

J. Patrick Reilly, Ph.D.

William Strauss, M.D.

The Molecular Cytogenetic Resource

Resource directions are set during meetings of the Resource Project leaders. This process was initiated in December 1993 during a day-long Resource retreat attended by all project leaders. The retreat included a review a work originally proposed, a discussion of progress to date and a redefinition of short and long term objectives for probe development, in situ hybridization technology development, image cytometry and data base development. Progress and plans will be presented to an advisory committee during July–August, 1994. The advisory committee is being assembled now, based on names submitted by the Resource Project Leaders. Resource staff will present progress and plans to the Advisory committee in early September, 1994.

STRUCTURAL BIOLOGY DIVISION

Irwin D. Kuntz
Professor, Department of Pharmaceutical Chemistry
University of California at San Francisco

David Eisenberg
Molecular Biology Institute
University of California, Los Angeles

Mostafa El-Sayed
Department of Chemistry and Biochemistry
University of California, Los Angeles

William Parson
Department of Biochemistry
University of Washington

Peter Wright
Scripps Research Institute

Energy and Environment Division

Peter Blair
Office of Technology Assessment

Jack Chaddock
Emeritus Professor of Mechanical Engineering
Duke University

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

Paul J. Lioy
Environmental and Occupational
Health Sciences Institute

Adel Sarofim
Massachusetts Institute of Technology

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: LBL; 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

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

Thomas C. Reed
Quaker Hill Development

Sally Ride
Director, California Space Institute
University of California, San Diego

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, and Senior Vice President of Academic Affairs
University of California

John Nuckolls
Director
Lawrence Livermore National Laboratory

Charles V. Shank
Director
Lawrence Berkeley Laboratory

To advise the Division Directors on a more frequent basis, both with regard to scientific priorities and operational matters, each division collects and collates information from the research staff and from outside the division. Typically, 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, for most divisions of the Laboratory, a professional staff committee, a safety committee, a salary committee, 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.

FY 1994 OHER-Related Laboratory-Directed Research and Development:

EARTH SCIENCES

Doner:
Application of the ALS to the Study of Trace Element Adsorption and Distribution in Soil Carbonates—$18,000

DePaolo:
Characterization and Monitoring of Subsurface Biologic Activity Using Stable Isotope Soil Gas Analysis—$75,000

Holman:
Laboratory Studies of Microbial Transformation of Petroleum Hydrocarbons in Transient Subsurface Environment—$100,000

Tokunaga:
Synchrotron Light Source Applications to Transport of Contaminants in Soils and Rocks—$70,000

ENERGY AND ENVIRONMENT

Levine:
Development and Application of New Tools to Analyze the Impact of Clean Cars, Transport Policy, and Other Innovative Energy Programs on Air Quality in the South Coast Air Basin—$100,000

Rubin:
Defect Characterization of P-GaN and Fabrication of a Blue Laser—$90,000

ENGINEERING

Jaklevic:
Development of Microchemical Methods for Biological Assays—$95,000

Katz:
Development of Next-Generation Detectors for Use at the Advanced Light Source—$115,000

INFORMATION AND COMPUTING SCIENCES

Johnston:
Visual Servoing in a High Speed Network Environment—$150,000

LIFE SCIENCES

Head-Gordon:
A Comprehensive Approach to Protein Folding—$100,000

Jap:
New Initiatives of Electron Crystallography of Selected Membrane Proteins—$80,000

Pallavicini:
Genetically-Damaged Hematopoietic Stem Cells: Biological Dosimetry—$45,000

Roos:
Head Probe for Nuclear Magnetic Resonance Imaging and Spectroscopy at 10 T—$50,000

Rubin:
Creation of Transgenic Mice Containing a Library of P1 Clones Encompassing the Down's Syndrome Region from Chromosome 21—$190,000

Shyamala:
A Transgenic Model for Clinical Testing of Progestins and Analysis of Progesterone Receptor Function—$60,000

Tribble:
Variations in Susceptibility to Environmental Oxidants as Studied using Transgenic Mice Models—$125,000

STRUCTURAL BIOLOGY

Kim:
Genomes, Enzymology and Biology of Hyperthermophilic Microorganisms—$100,000

Shin:
A Molecular Description of Transmembrane Signaling—$70,000

MULTI-DIVISIONAL, ESD

Benson, Brown, Daisey, Gold:
An Interdisciplinary Environmental Research Center—$400,000

Animal Welfare and Human Use

The LBL 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 LBL 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 LBL 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 LBL activities involving human subjects, pursuant to Office of Science and Technology Policy (OSTP), UC Systemwide, and campus directives. An LBL advisory committee assists the CPHS in their review of LBL projects, and LBL research scientists serve as committee members. LBL protocols are submitted to the LBL Human Use Committee (HUC), which reviews all submissions and approves them before submission to the CPHS for review. The LBL HUC keeps LBL 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

LBL 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 LBL.

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 LBL 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, LBL 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.

LBL 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 LBL staff. Prototype testing of notebooks has begun, and implementation began in Spring, 1993. The safety committee network will provide assistance to responsible supervisors in meeting the requirements.

The Life Sciences Division ES&H program structure consists of the Division Director, the ES&H Team made up of four specialists with different expertise (a Senior Scientist Coordinator; an Environmental, Health & Safety Professional; a Facilities Manager; and a Database Custodian), and an ES&H Committee. In addition, Departments Heads, Group Leaders, Supervisors, Building Managers, Division Facility Supervisors, and Staff in the Division are responsible for ES&H compliance.

The Division's ES&H Team sets up special training sessions, reviews requirements, assists in the maintenance of ongoing programs (such as the chemical inventory), plans for implementation of new requirements or procedures, and escorts assessment teams from DOE, external agencies, or internal entities who visit the Division.

The Division's ES&H Committee network, with representatives from all buildings, and staff levels within the Division, serves as a resource to assist in the installation of recommended operational procedures required of management by specific DOE orders and federal and state laws. This Committee is chaired by an Environmental, Health & Safety (EH&S) professional. The Environment, Health and Safety (EH&S) Division and the Office of Assessment and Assurance (OAA) are prime resources in the implementation of DOE, state and other federal regulations. A member of LBL's Safety Review Committee (SRC) and five representatives from the Environment, Health & Safety (EH&S) Division sit on this committee to provide updated information and training to the committee network. These representatives also take input and concerns expressed by the membership about the needs and impact of procedures on the Division back to their respective organizations.

The ES&H Committee conducts self-assessment wall-to-wall inspections of all Division space. Topics reviewed during these inspections covers all aspects of Environmental, Health & Safety compliance. Findings are entered into the LBL LSAD file maintained by OAA to facilitate tracking, trending and budget planning. The Division's ES&H Team do follow-up inspections for verification, develop corrective action plans, assist the Facility Supervisors and Building Managers in resolving open findings, and alert the Division Director to specific problem or non-compliant areas.

D. Research in Progress

D. Research in Progress

Analytical Technology

AIRBORNE RADON CONCENTRATIONS

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 LBL 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. Other areas of focus include the effects of transport and entry of radon, and 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.

SEMICONDUCTOR RADIATION DETECTOR TECHNOLOGY

The program to develop detector materials and instrumentation for semiconductor radiation detector applications has focused on five main areas in the past year: (1) development of high purity GaAs epilayers for room temperature detector applications; (2) evaluation of other high Z materials, such as CdTe and CdZnTe, for room temperature detector applications; (3) completion of the study of amorphous germanium as a superior entrance window contact material for a wide variety of Ge detectors; (4) development of very large area (75 mm diameter) lithium-drifted silicon detectors using a recently developed procedure to getter defects in commercial grade float-zone silicon; and (5) fabrication of prototype large area, position-sensitive p-type Si drift detectors. Each of these programs is briefly outlined below.

High purity GaAs epilayers, grown on GaAs substrates, are now being routinely produced for future use in new detector technologies. GaAs material is being explored as an alternative to conventional Si and Ge in x-ray and gamma-ray spectroscopy applications, since the GaAs can be used at room temperature and can be fabricated into multi-element devices for high rate, field portable environmental survey applications. The GaAs epilayers are of a very high crystalline quality, but require further refinement in purity to reach the levels required for fully depleted detector devices. This program, to develop GaAs as an alternative to the standard Si and Ge, has now been expanded to include an evaluation of other high Z semiconductor materials for room temperature applications. We have recently initiated a program to evaluate new detector structures that will improve the carrier collection in such materials as HgI_2 and CdTe. The bandgap of these materials allows them to be used at room temperature, but the charge collection is poor and the energy resolution is nowhere near that offered by conventionally cooled Si(Li) and Ge. New detector designs could improve the charge collection and therefore improve the energy resolution of these II-VI materials.

The evaluation of amorphous Ge contacts on Ge detectors has resulted in the development of a material that has a number of useful characteristics. The amorphous Ge passivates the detector surface to reduce surface leakage current and improve detector resolution and reliability. The amorphous Ge has also been used to electrically isolate individual detector elements such that multi-element detector arrays can now be fabricated on large area Ge substrate material. (This particular attribute of the amorphous Ge has been exploited in the fabrication of a 140-element Ge detector now being used for medical imaging applications.) In addition, the amorphous Ge contact yields an entrance window with an extremely thin dead layer useful in the detection of low energy x-rays, and results in a detector with a superior peak-to-background ratio compared with conventional Si(Li) and Ge detectors.

The gettering of defects in float-zone silicon has become important for programs that require fabrication of large area (>40 cm^2) lithium-drifted Si detectors. Material for large area Si(Li) detectors has previously been unavailable, due to the high concentration of defects in the commercial grade silicon. A recently developed technique to getter defects in this material has now opened up the possibility of lithium-drifting a wide range of material from different vendors and fabricating very large area devices. To this end, 75 mm diameter Si(Li) detectors have been fabricated and successfully demonstrated in an accelerator environment. We are currently confirming the proposed gettering mechanism through systematic measurements of material and process characteristics.

The program to develop p-type Si drift detectors (SDD) has resulted in the fabrication of prototype p-type devices which demonstrate their potential use as detectors offering both position sensitivity and good energy resolution. P-type material is much more desirable as a starting material than the conventional n-type NTD material due to its wider availability, lower cost, simpler detector fabrication procedures and potentially higher resistance to radiation damage.

Environmental Research

RADON TRANSPORT THROUGH SOILS INTO HOUSES

The details of the interactions among environmental conditions, soil characteristics, building features and indoor radon concentrations are complex and poorly understood, and existing models of these are not verified. The two room-size basement structures we

have constructed at a site in the Santa Cruz (CA) mountains are designed to provide a well-controlled experimental facility to address some of these questions. These structures, designed to mimic the important details of actual basements, are identical except that in one structure, the floor rests directly upon the native soil, while in the other, a 10-cm-thick gravel layer was installed below the floor. The structures and site are extensively instrumented to permit continuous measurement of radon and other environmental parameters.

This project will help further elucidate these relationships so that indoor radon concentrations and the associated risks will be understood more fully and predictably. This understanding is critical to developing methods to identify regions containing homes with elevated concentrations and in developing techniques for limiting radon entry into new or existing buildings. The results from these studies also have implications for developing insights into potential exposures from other soil-gas-borne contaminants that may enter buildings from the surrounding soil, such as volatile organic compounds from nearby waste sites.

Atmospheric Aerosols and Global Climate Change

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.

Thermodynamic Properties of Chemical Species Present in Mixed Organic-Radionuclide Wastes

The goal of this research as it has been carried out over the past two years is to provide fundamental knowledge on the complexation behavior of nep-

tunium, plutonium and cobalt with synthetic organic-radionuclide wastes in the subsurface at DOE sites. A major part of this program is focused on collaborative research with other participants of the co-contaminant chemistry subprogram.

The task of our group, the Actinide Geochemistry Group, LBL, has been defined as the study of the complexation behaviors of Np, Pu, and Co with Nitrilotriacetic Acid (NTA), Ethylenediaminetetraacetic Acid (EDTA) and other synthetic organic materials. The stability of plutonium in the presence of organic materials is to be studied at macroscopic concentrations and at environmental concentration. The Pu-NTA system should be studied in the absence and presence of microbes and/or in the presence of other elements such as Ca, Al, Fe. Co-NTA and Co-EDTA systems should be studied in the absence and presence of Fe_2O_3-coated sand.

FIELD HETEROGENEITY

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.

RADIONUCLIDE CHEMISTRY

Fundamental knowledge of the chemical processes that occur between radionuclides and synthetic organic chemicals present in DOE waste sites will provide the ability to more accurately predict the processes that control contaminant mobilization and transport in the subsurface environment. We are conducting experiments to determine the thermodynamic and kinetic solution phenomena such as complexation constants, speciation, solubility of complexes and the nature of compounds that can form between constituents of mixed organic-radionuclide wastes. These experiments will provide the data necessary to predict and model the interaction and the mobility of co-contaminants and their maximum release concentrations in the subsurface, filling the gaps in the existing database used for transport modeling. Our efforts have also generated the basic information necessary to successfully integrate the multi-investigator research program involving collaborators from several National Laboratories and Universities. The collaborative research effort by the co-contaminant chemistry subprogram team will generate knowledge about the very complex behavior of organic-radionuclide mixtures in the subsurface much faster and far more comprehensively than any single-investigator effort. The collaborations will provide an increased understanding of intermediate- and macro-scale transport of cobalt with the complexing agents NTA, EDTA, citric acid, and oxalic acid with and without the presence of microbes and surfaces that can adsorb metal ions.

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 environ-

mental 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.

AIR POLLUTANT EXPOSURES IN BUILDINGS

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 10 times 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 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 and risk models.

LBL has initiated research project to develop a method to identify geographic areas of the United States where most of the houses with indoor concentrations above 750 Bq m^{-3} are located. For this purpose, local information on physical factors such as soil types and radium content, geology, meteorology, and housing characteristics are being used to develop simple indices that correlate with indoor radon concentration data from monitoring.

Indoor exposures to volatile organic compounds in office buildings are being investigated in a collaborative research project with investigators at the California Department of Health Services. Efforts are being made to develop metrics of exposure for complex environmental mixtures such as the mixture of hundreds of volatile organic compounds (VOCs) to which humans are exposed in indoor environments. The goal is to develop metrics based on biological mechanisms of toxicity that can be more readily related to health effects in exposed populations than the simple sums of concentrations which are currently used. The current effort is directed toward developing a metric of exposure for complex mixtures of VOCs that can be related to irritancy and odor. More advanced cancer risk assessment methods based on fundamental biological mechanisms are also being developed for selected indoor pollutants.

MOLECULAR MECHANISMS OF CELLULAR EFFECTS OF HEAVY IONS

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 break, 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 using the premature chromosome condensation (PCC) 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 the 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 earth, 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 repair 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. This is the continued focus of this proposal, planned for continuation at LBL's 88" cyclotron and at the Advanced Light Source (ALS).

Biological Effects of Magnetic Fields

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. In addition, we have a strong program component directed at developing new life sciences technologies based on our discoveries of biological responses to electromagnetic fields. We are currently investigating ELF (extremely low frequency) fields associated with electricity distribution systems and appliances, microwave fields associated with developing telecommunication systems, and high intensity NMR/MRI fields used in clinical diagnostic procedures. Spin-off studies have evolved into patented technology in the area of drug delivery using microwaves fields and liposomes.

An important focus of our recent work has been the development of unique exposure systems for performing cellular studies during exposure to very well-defined electromagnetic fields. Such exposure systems do not presently exist and our design efforts have established a standard for long-term exposure of cultured cells *in vitro* using specialized mu-metal shielding and Merritt 4-square coils to optimize exposure area while shielding the cells from exogenous fields generated by cell-culture incubators, which are significant. In addition, in companion single-cell investigations our laboratory has a lead in the development of specialized non-metallic perfusion chambers and exposure coils for microscope-based studies that can maintain cells while exposures to electromagnetic fields are conducted.

Using these new, well-characterized exposure systems we have carried out studies to determine if human breast cancer cell growth is influenced by environmental level magnetic fields. These studies were motivated by several recent epidemiology reports that human risk for breast cancer is elevated in individuals living or working near magnetic fields, and by the fact that magnetic fields are reported to suppress the secretion of melatonin which is elevated at night; melatonin is a natural oncostatic agent for breast cancer.

In cell culture we observed that environmental-level 60 Hz magnetic fields completely block melatonin's natural oncostatic growth inhibition of human breast cancer cells. This represents the first report linking human breast cancer, 60 Hz magnetic fields, and melatonin, and it suggests a very plausible working hypothesis for understanding how environmental-level magnetic fields influence breast cancer by blocking melatonin function. Currently, we are investigating whether the estrogen receptor is involved in this interaction, and we are planning to expand these studies to include Video Display Terminal (VDT) frequencies associated with the operation of computer monitors.

A second focus of our studies involves assessing changes in immune system function at the cellular level. We have investigated signal transduction during lymphocyte activation and specifically the early changes that occur in intracellular calcium and in the early response genes c-MYC and c-FOS. To do this we have used fluorescence to monitor in real-time changes in intracellular calcium during exposure to electromagnetic fields. We are planning to develop a fluorescent reporter enzyme assay to follow activation of these genes in real-time in living cells during lymphocyte activation. To date we have observed that several mitogens, PHA, Concanavalin A, and anti-CD3 monoclonal antibody, activate lymphocytes and can elevate both intracellular calcium and c-MYC

mRNA, but that magnetic fields profoundly activate cells in the presence of suboptimal mitogen. Thus magnetic fields can act as a co-factor during signal transduction and can drive the cell into mitogenesis despite suboptimal activation by mitogen. We have recently undertaken single-cell studies to observe changes in intracellular calcium in real-time in living cells during field exposure. These studies will reveal changes in the spatial and temporal development of calcium oscillations and changes in cell morphology that occur during signal transduction. Collaboration with the Imaging Technology Group at LBL (Dr. W. Johnston) will enable digital imaging to be performed rapidly at state-of-the-art speeds by distributed computing over fiber optic lines. Our digital imaging microscope will be one of the first at LBL to use fiber optics for visual servoing on living cells.

Studies with microwave fields use liposome vesicles as a model for the cell membrane. We have observed that brief exposure causes the release of chemical compounds from the liposomes and this phenomenon is mediated by antioxidants, implicating free radicals. Recently we have observed that calcium ion bridges are disrupted by microwaves—these bridges are noncovalent and stabilize the outer phospholipid bilayer through calcium ion interactions with the anionic polar head groups of phospholipids. These studies provide insights into how microwaves influence the structure of membranes and form the basis of understanding permeability changes in the bilayer during field exposure. Spin-off technology using microwaves and liposomes holds promise for drug delivery applications involving pulsatile, on-demand drug release.

Our expertise in fluorescence has lead to the development of the first prototype fluorescence assay for intracellular calcium in living cells that is performed using an automated fluorescence plate reader. This work was performed in collaboration with Millipore Corporation.

The Molecular Cytogenetic Resource

The clinical and biomedical utility of molecular cytogenetic analysis has increased dramatically in recent years as improved procedures and reagents have been developed. Important advances include: (a) Fluorescence in situ hybridization (FISH) for visualization of one or several gene sequences in individual cells; (b) PRimed IN Situ labeling (PRINS) for rapid hybridization and the potential for near single base detection sensitivity; (c) Comparative Genomic Hybridization (CGH) for rapid detection and mapping of regions of altered gene copy number anywhere in the genome; (d) Procedures and reagents for non-radioactive probe labeling with reporter molecules; (e) Digital imaging microscopy for sensitive multi-color detection, display and semiautomatic detection and mapping of normal and aberrant genes; (f) Informative probes for detection of several disease-linked genetic aberrations. As a result of these and other developments, molecular cytogenetics now plays a prominent role in probe mapping for genomics research, detection of genetic damage after exposure to toxic agents, prenatal and neonatal screening for genetic abnormalities, and genetic analysis of leukemia and solid tumors for diagnosis, prognosis, monitoring the progress of therapy, and searching for cancer genes.

The purpose of the Molecular Cytogenetic Resource is to stimulate progress in molecular cytogenetics through: (a) development of improved hybridization technology; (b) design and development of computer assisted fluorescence microscopy; (c) production of probes optimized for molecular cytogenetic studies; (d) support of the molecular cytogenetic community through collaboration, distribution of probes and analysis technology and education and; (e) by transfer of useful reagents, processes and instruments to the private sector for commercialization. This will be accomplished by integrating the technical capabilities of the Human Genome Center and Engineering Departments of the Lawrence Berkeley Laboratory (LBL), the chromosome microdissection and cancer genetics expertise from the National Institutes of Health (through collaboration with Drs. Trent and Meltzer), and the myriad of clinical and genetic studies being undertaken by cooperating investigators at the University of California, San Francisco (UCSF), the Lawrence Berkeley 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. Specific activities include:

Probe mapping: The development of simple methods for blocking hybridization of interspersed repetitive sequences in cloned genomic DNA has made it possible to map most cloned segments greater than 1 kb in extent. While substantial effort is still required for small probes, the process is simple and rapid for probes larger than 10 kb. Thus, FISH has made

mapping sufficiently simple that mapping of tens or hundreds of probes can be accomplished by a single group of researchers. In the Molecular Cytogenetics Resource, mapping has been almost fully automated and mapping speed is limited only by the rate at which high quality metaphase preparations can be found. Automation and multiplex strategies will be developed as part of the Resource to speed the process even further.

Toxic exposure assessment: Physical and chemical agents including ionizing radiation induce such genetic damage as single base mutations, aneuploidy and structural chromosome rearrangements. The frequency of these aberrations can be measured using molecular cytogenetics techniques as an indication of the extent and type of genetic damage. This may be especially important for population monitoring. FISH with centromeric probes is used to detect aneuploidy while FISH with whole chromosome probes allows detection of structural aberrations. Structural aberration analysis is especially important since the aberration frequency seems to persist years or decades after exposure. This allows assessment of genetic damage years after exposure. Aberration analysis is now limited by the rate at which aberrations can be detected and scored. The Resource will automate this process.

Analysis of human malignancies: Cancers develop and progress through the accumulation of genetic abnormalities at critical loci. These may involve alterations of one or a few bases of DNA, deletions ranging from sub-microscopic to whole chromosomes, duplications or higher level amplifications of chromosomal regions, or rearrangements producing abnormal juxtapositions of DNA sequences. FISH is useful for detection and characterization of genetic abnormalities such as aneusomy (abnormal chromosome number), translocation, gene amplification, and deletion. These can be detected in individual cells, thereby enabling assessment of the tumor genotype, analysis of genetic heterogeneity, and detection of rare malignant cells. This information on the number and type of aberrations present in a tumor may allow prediction of the future course of the tumor including response to therapy. In addition, FISH with probes distributed along the genome and application of Comparative Genomic Hybridization (CGH) appear useful for discovering and refining regions of the genome that contain cancer genes. These studies currently are limited by lack of informative probes with high hybridization efficiency so they can be used for reliable analysis of clinical samples and by the labor intensive nature of the cell analysis process (e.g., especially hybridization signal enumeration). Development of improved probes and improved multicolor computer assisted fluorescence microscopy to facilitate molecular cytogenetic studies of human cancers are major goals of the Resource .

Prenatal and neonatal diagnosis: The most frequent genetic abnormalities found in prenatal diagnosis involve trisomies of chromosomes 13, 18, 21, and aneusomies involving the sex chromosomes. Such abnormalities can be detected using FISH to interphase amniocytes or chorionic villus cells with probes to these chromosomes. In fact, this assay is now being offered commercially as a method of obtaining rapid preliminary information on high risk patients while waiting for the results of conventional banding analysis. In addition, substantial effort is being expended on development of methods to permit identification and molecular cytogenetic analysis of fetal cells in the maternal circulation. Neonatal diagnosis of physical malformation or mental retardation frequently involves the detection of the deletion or duplication of chromosomal segments. In some cases, the deletions are so small that they are undetectable by conventional cytogenetics. FISH with probes for the specific region is often the best way to detect these abnormalities. These studies are now limited principally by the lack of high quality probes and by the labor intensive nature of microscopic scoring. Development of probes and microscopic technologies to facilitate these studies will be pursued by the Resource.

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; rapid metaphase finding and ranking; rare event detection; genotypic and combined genotype-phenotype analysis of interphase cells. 3) *Probe development.* A collection of P1 clones that contain genetically mapped polymorphic sequences will be selected at 5 Mb intervals over the human genome will be selected for use as probes for in situ hybridization. The set will include probes to specific regions of the genome known to be involved in genetic disease. In addition, probes for whole chromosomes, chromosomal regions, and specific repetitive sequences will be developed.

Carcinogenesis

CELL CYCLE ACTIVATOR AND REPRESSOR GENES

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 LBL 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 EPITHELIAL CELLS

Studies on NB-1: Utilizing the well-characterized human mammary epithelial cell (HMEC) culture system pioneered at LBL, we are elucidating the factors which might influence the capacity of HMEC to transform in culture. In particular, we are investigating how the differentiated state, including cell-cell and cell-matrix interactions, (i) changes as a result of immortal or malignant transformation; (ii) may effect the ability of the cell to undergo transformation.

Because of the critical role that calcium regulation has been shown to play in the growth and differentiation of epithelial cells, proteins involved in calcium mediated pathways are potential targets of carcinogenic changes. A comparison of transcripts expressed in normal vs transformed human mammary epithelial cells (HMEC) has led to the identification of a calmodulin-like protein (CLP), encoded by the intronless NB-1 gene, which is often downregulated or absent in tumorigenically transformed epithelial

cells with respect to levels in normal HMEC. Unlike the ubiquitous distribution of calmodulin, CLP expression is normally restricted to certain epithelial cells, and appears to be modulated during differentiation. Since a large number of calcium regulated cellular activities are thought to be mediated by the calcium sensitive binding of calmodulin to regulatory sites on responsive enzymes, our current studies have been performed in order to compare the biochemical activities of CLP and calmodulin, and to identify potential cellular targets of CLP. We have found that CLP activates the multifunctional Ca^{+2}/calmodulin-dependent protein kinase II with kinetics which are identical to those of calmodulin itself. In contrast, other calmodulin dependent enzymes, cGMP phosphodiesterase and myosin light chain kinase, display much stronger activation by calmodulin. In fact, in the case of myosin light chain kinase, CLP competitively inhibits calmodulin activation of this enzyme. These studies illustrate the variability in calmodulin interactions with target enzymes, and suggest that CLP may have evolved as a specific agonist which can activate certain calmodulin dependent enzymes, and as a specific antagonist which can inhibit other calmodulin dependent enzymes.

Proteins which bind CLP *in situ* were isolated from HMEC extracts by co-immunoprecipitation with CLP. In soluble extracts of [^{35}S]-methionine labelled cells, a 110 kD protein was identified which specifically co-immunoprecipitated with CLP in the presence of high salt and calcium. The protein could not be immunoprecipitated from extracts of cells lacking CLP, or in the presence of EGTA, indicating a specific calcium dependent interaction with CLP.

Molecular Carcinogenesis

Rapid assay for distinguishing normal and malignant cells: Having established the conditions for development of functional differentiation in the mouse system, we feel that we now have the tools to finally address these problems in the human cells. It is well known that human breast cells are very difficult to culture and to maintain, but what is most disturbing is that there are almost no markers for normal breast cells in culture. Indeed, normal cells grow as well if not better than malignant cells making it difficult to tell the two apart. In rare occasions, a culture of transformed cells may become "immortal," potentially giving rise to a tumor in the animal. But this process is extremely infrequent and unpredictable as to be almost an impossible marker.

A new rapid assay to distinguish normal from malignant cells has emerged from our systematic analysis of normal human breast cells obtained from reduction mammaplasties. Using the same rationale developed for the mouse model—growth within the basement membrane matrix—we succeeded in recreating normal ascini with complete homology to these structures *in vivo*. By comparison, cells from primary tumors or tumor cell lines, continued to grow within the three-dimensional matrix and furthermore were not polarized. In addition, the normal cells were capable of making an endogenous basement membrane, while tumor cells appear to have lost this capability. All these differences could be observed within six to seven days in culture, making this system the fastest assay for distinguishing normal and malignant cells. We are now studying the possible underlying mechanism of this fundamental difference, which we believe could be viewed as a function of a tumor suppressor gene.

Differentiation: The hallmark of malignant transformation is the loss of morphological and functional differentiation as well as loss of growth control. We have argued in the past that to understand the mechanisms that are involved in cancer induction, one has to understand the factors that allow the cells and tissues to retain their normal phenotype. Given that all cells have the same genetic information and almost all retain the ability to grow, what tells the cells in tissue to stop growing? What allows them to retain form and function? What makes a liver cell a "liver" and a breast cell a "breast"?

For more than a decade, Dr. Bissell has laid the foundation for designing, in tissue culture dishes, an environment that recreates a functional tissue—in her case—the breast. What we have accomplished has broad application to other tissues and cells enabling others to study the physiological basis of many biological processes in culture. What has emerged is that the same cells, with the same genetic material, behave very differently when confronted with different microenvironments.

In a three-dimensional matrix, created by giving cells a basement membrane-like material, breast cells that had lost both their ability to make milk proteins, regain form and function and produce copious amounts of milk. We now know that milk protein genes contain novel tissue-specific enhancers that respond to extracellular matrix components and allow the cells to express milk genes in the presence of lactogenic hormones. We also know that these

enhancers can drive the expression of foreign genes once the environment is appropriate, thus providing a technology that could be harnessed for the production of commercially important proteins. Such "matrix-response elements" in the promoter regions of other tissue-specific genes are being revealed both in the breast and in other tissues such as liver. This discovery has opened a completely new dimension in the study of transcriptional regulation. We are now in the process of determining the nature of the regulatory proteins that bind to these regions and by using site-specific mutagenesis, we have identified at least two regions that are crucial for the enhancer's activity; this should allow us to identify and clone the binding regulatory proteins. We have now shown that promoters and enhancers of well know viral vectors are also responsive to ECM. We have further elucidated aspects of the signal transduction pathway of the ECM.

RADIOLOGICAL PHYSICS AND CHEMISTRY

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}-γ 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.

RISK ASSESSMENT—CARCINOGENIC POTENCY PROJECT

In several analyses we have tried to broaden the perspective on possible carcinogenic hazards to humans by ranking rodent carcinogens on an index—HERP (Human Exposure/Rodent Potency) that compares human exposure (mg/kg/day) to the dose that induces tumors in rodents. This year, possible carcinogenic hazards in the workplace have been assessed for rodent carcinogens in our Carcinogenic Potency Database (CPDB) of 4800 experiments on 1200 chemicals. For rodent carcinogens that have a Permitted Exposure Level (PEL) set by the U.S. Occupational Safety and Health Administration (OSHA), we have ranked hazards using an index, PERP (Permitted Exposure/Rodent Potency), which expresses the worker permitted lifetime exposure (mg/kg/day) as a percentage of the lifetime dose that induces tumors in 50% of rodents (TD50 in mg/kg/day). PERP values for 75 rodent carcinogens differ by more than 100,000-fold from each other, indicating that rodent cancer test data are not the basis for the OSHA standards. A similar ranking would be expected on the basis of standard risk assessment methodology. For nine chemicals workers are permitted to receive more than 10% of the tumorigenic dose rate in rodents; among these are 1,3-butadiene, tetranitromethane, and glycidol. We have also identified 120 chemicals that require further regulatory attention because they have no OSHA PEL, workers are exposed to them, and they are rodent carcinogens.

Two aspects of current risk assessment methodology have recently been investigated: the potential impact of a reduced protocol for carcinogenesis bioassays and the use of a surface area correction when extrapolating carcinogenic potency from rodents to humans. Subsequent to our reporting that about 90% of rodent

carcinogens which are identified by the current standard test protocol of four sex-species groups could be identified by a reduced protocol of one test in each species; the Environmental Protection Agency (EPA) and the National Toxicology Program have been pursuing the possibility of reducing the standard four sex-species protocol to two sex-species. Our recent analyses indicate that chemicals which are carcinogenic in more than one species or which induce tumors at more than one target site on the basis of results from four sex-species groups are also identified as two-species or multiple-site carcinogens on the basis of tests in one sex of each species. Carcinogenic potency values for the most potent target site are similar when results are based on two compared to four sex-species groups. Thus, further consideration of reducing the standard protocol is warranted. Consideration should be given to using some of the cost savings to add studies on mechanisms of carcinogenesis to the standard protocol.

Body surface area has been used by EPA to extrapolate risk estimates from rodents to humans. We examined the literature on body surface area measurement and found that all measurements have relied solely on gross features of the body surface and, furthermore, have not taken into account more recent ideas of fractal geometry. It remains unclear whether the skin is a fractal dimension; however, measurements of body surface area have ignored microscopic features that contribute substantially to surface area; some of these features, such as pores, differ in their distribution between humans and rodents. Since the accuracy of surface area measurement is dubious, risk extrapolation from rodents to humans would be improved by use of other measures, such as species differences in metabolism.

Interspecies extrapolation of carcinogenesis has been further evaluated by comparing results in monkeys and rodents for 15 chemicals with positive rodent results in the CPDB and for which long (20-year) bioassays in monkeys were recently completed by the National Cancer Institute (NCI). Six of the 15 rodent carcinogens (40%) were not considered carcinogenic in monkeys by NCI. Among the nine chemicals positive in monkeys, a positive result was obtained in both cynomolgous and rhesus monkeys when tested in both. These nine monkey carcinogens have also tested positive in rats, and there is good target site concordance between monkeys and rats; the liver is the most common target site in monkeys and in rats.

We showed earlier that given the current bioassay design potency estimates based on the one-hit model are constrained to a narrow range surrounding the high dose tested, the maximum tolerated dose (MTD) (unless all dosed animals develop tumors, which rarely occurs). Over larger numbers of chemicals, the MTDs for rats and mice are highly correlated and span many orders of magnitude. Hence the potency correlation between rats and mice follows statistically. We recently examined how much of the observed correlation in potencies between species is artifactual and found that over 80% of the inter-species correlation in carcinogenic potencies for chemicals positive in both rats and mice can be explained by the interspecies correlation in toxicity (MTD) and the correlation between log potency and log MTD. Thus, while there may be some basis for extrapolation from rodents to humans, the interspecies correlation of potencies does not say much about the validity of that extrapolation.

Bone Marrow Stem Cells

Our hematopoiesis program focuses on characterizing the biology of long-term repopulation of stem cells. Stem cells receive particular attention because they are essential for sustained hematopoiesis over the entire lifetime an organism. Characterization of this blood component entails the use of monoclonal antibody surface markers to monitor stem cell populations from various sources such as bone marrow and fetal liver tissue. Our objective is to identify and solve the cell's long-term ability to repopulate and in so doing establish stem cell therapy to mitigate the effects of chronic, inherited hematopoietic disorders such as thalessemia. By using fetal liver stem cells to reconstitute erythroid elements in the mouse model system we are making appreciable progress toward this objective.

General Life Sciences

Structural Biology

LBL'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

Circularly polarized x-rays from synchrotron radiation sources have applications in basic physics, material science and structural biology. The new technique of x-ray magnetic circular dichroism provides a unique probe of the magnetic properties of transition metals, rare earths, and actinides, and experiments involving differential scattering also appear promising. To provide a broad-band circular source for the Advanced Light Source, we propose to build a new elliptical wiggler beamline as a joint venture with national lab, university, and industrial participants. While the ALS beamline is under construction, the consortium will use bending magnet beam lines at the National Synchrotron Light Source, the Stanford Synchrotron Radiation Lab, and the Advanced Light Source.

The participants will add expertise in soft and hard x-ray spectroscopy, as well as several UHV chambers, detectors and a 6-Tesla magnet to the project. Funding is sought from the Department of Energy to build an elliptical wiggler, to complete the front end connections to the light source, and to develop beam lines and end stations over time. The facility will initially be operated as a participating research team at the Advanced Light Source, with access to outside users for material science and structural biology studies. This is an opportunity to bring together national laboratories, universities and private industry in a collaboration to build a unique facility.

SOFT X-RAY MICROSCOPY

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

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

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 *in vitro* and *in vivo* assays of DNA excision repair and the mutagenesis associated with this process.

After ten 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 8mer 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.

Our progress in the three main areas covered by the program is summarized below:

1) Molecular Matchmakers

In collaboration with Aziz Sancar of the University of North Carolina, the details of the mechanism of uvrABC excision repair has been established. UvrA, as a dimer, directs uvrB to the position of damage on the DNA. Following ATP hydrolysis, uvrB is bound tightly to the DNA at the damage site and the DNA is kinked by approximately 130 degrees. The union between the damaged DNA and uvrB is absolutely dependent on both the "Matchmaking" activity of uvrA and on ATP hydrolysis. UvrA is not a structural part of the final complex. The complex between uvrB and DNA, although metastable, has a half-life greater than several minutes. The kinked complex is then bound by uvrC, the 3' incision made by uvrB and then the 5' incision is made by uvrC. The "matchmaking" mechanism has been identified with many other protein-DNA binding systems. The "induced fit" between protein and DNA is realized through the catalytic role of the "matchmaker" and the energy provided by ATP hydrolysis. Clearly, conformations can be created by this mechanism which are not thermally accessible, suggesting that, at least in these cases, the lowest free energy states of interaction are not those utilized by the functioning cell.

2) Human Excision Repair

Human excision repair of psoralen monoadducts has 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.

3) 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.

BIOMOLECULAR STRUCTURE ANAYSIS BY NMR

Our work is aimed at understanding the relationship between the structure and function of biologically important molecules, and to use our knowledge of such relationships to design and/or select molecules with new functions. The primary tool used for structural studies is NMR spectroscopy, and some additional work goes into improving or extending the methodology.

There are a number of different types of systems under study. Proteins: we are analyzing the structure of several proteins which are involved in genetic regulation. This includes a bacterial repressor protein which responds to the iron level in the cell, transcription factor domains involved in sequence specific recognition of DNA and in activation through interaction with other proteins, and a sequence specific RNA

binding protein which alters splicing of a messenger RNA acting as a genetic switch. Understanding these regulatory proteins will allow us to understand how they function, how the functions can be manipulated externally (i.e., through drugs), and how to mimic the function in other systems. Similar experiments are being carried out on nucleic acids, including catalytically active RNA (a self-cleaving hammerhead sequence), on chemically modified DNA which are used in understanding repair of damaged DNA, and on molecules which bind to DNA in a sequence specific manner. Experiments are also underway to select for proteins with new DNA binding activity, by using a genetic library approach. Residues involved in contact with DNA are being varied, then selections for recognition of a new sequence is done through panning or growth selection. Additional structural characterization is being done of new 'bio'polymers being synthesized by other LBL groups, as well as natural 'bio'cofactors.

CENTER FOR BIOMOLECULAR DESIGN

The Center for Biomolecular Design bridges chemical science, biological science, and computer science by combining the expertise in LBL and the University of California, Berkeley. The Center catalyzes an interdisciplinary approach toward chemical biology and computational biology that merges a detailed understanding of biological systems with the ability to analyze and manipulate chemical structure. 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 LBL 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 have been constructed that "reprogram" the transmittal of information to cells. This research will aid in the therapy and understanding of disease including memory dysfunction and may ultimately lead to novel cellular based sensory or memory devices. Proteins from organisms that function under extreme conditions of pressure, temperature, and pH, known as extremophiles, are being isolated and characterized. These proteins can be redesigned in order to create new proteins with new catalytic properties functioning under extreme conditions and environment. 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.

One of the first manifestations of the Center's value is in the area of G-protein coupled receptors. Our research has expanded to include three G-protein coupled membrane receptors that are paradigmatic of the class of receptors that are responsible for neurotransmitter signaling in the brain and central nervous system. These include: human b_2-adrenergic receptor, fish olfactory receptor, and yeast a-factor receptor. To date, we have overexpressed the adrenergic receptor in a baculovirus overexpression system and a-factor receptor in a yeast overexpression system. Both proteins are now available in large supply. We are currently inserting the olfactory receptor into the baculovirus overexpression system.

In addition to the initial studies which use the techniques of x-ray and electron diffraction to determine the three-dimensional structure of this class of protein molecule, we are engineering the molecules with special properties. The engineered protein molecules maintain function but have added features that allow us to expand our knowledge into how dynamical information is transferred from one neuron to another across the neuronal cell membrane. These studies include site-specific spin-labeling the molecules for EFR studies and C^{13}/N^{15} labeling the molecules for solid-state NMR experiments. We are also creating chimeras of the three molecules in order to dissect the components important for signaling and specificity that is critical for neuronal signal transduction. Initial experiments include the swapping of the three cytoplasmic loops of one receptor with another for alternative G-protein signaling. We are also trying to determine the minimal architecture of each molecule that is necessary for molecular signaling.

STRUCTURAL BIOPHYSICS

There are two systems studied by structural biological methods. Both systems are central to the signal transduction pathway of cell growth and proliferation in normal and cancer cells:

(A) *ras* project: One of the most commonly found oncogenes in human cancer cells is the *ras* oncogene family. In genes of this family the difference between proto-oncogenes and oncogenes is often a point mutation caused by carcinogenic chemicals and radiations at or near residue 12 or 61 of the proteins encoded by the genes. It has been shown that the conformational changes from the GDP to the GTP bound state of *ras* proteins represent the molecular switch from the "off" to "on" state signaling cell proliferation. To understand the mechanism of the molecular switch, we have been comparing the three-dimensional structures of both GDP and GTP bound forms of the *ras* proteins, as well as comparing the structure of the proteins from the proto-oncogene and several oncogenes.

(B) Cell division cycle project: 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). 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 mentioned above, 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. A detailed understanding of these kinases will not only provide an improved understanding of normal cell cycle control but will also provide helpful clues about the abnormal cell cycle control mechanisms in the cancer cell transformed by radiation, chemical carcinogens, and environmental factors. 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. We have recently determined the structure of CDK2, and the purification of cyclin A is in progress.

PROTEIN ENGINEERING

Our research program has expanded to cover two principal areas: (a) the development of catalytic antibodies with novel specificities and reactivities and (b) an analysis of the signal transduction mechanism of *ras* p21 protein using as a tool methodology that enables us to site-specifically substitute into *ras* novel amino acids beyond those specified by the genetic code. Specifically, we are attempting to generate antibodies that catalyze stereospecific epoxide hydrolysis for use as bioremediation catalysts; antibodies that catalyze the selective conversion of prodrugs to cytotoxic drugs for cancer chemotheraphy; and antibodies that catalyze the stereospecific addition of HCN to a carbonyl group as a test of the ability of antibodies to carry out asymmetric catalytic reactions not currently possible via chemical approaches. Characterization of these antibodies also supplies new insight into the mechanisms of biological catalysis. Our work with *ras* focuses on the roles of amino acids in loop 1, 2 and 4 in signal transduction, and the mechanism of *ras* protein activation by GAP. Specifically we are examining residues Gly12, Gly13 and Lys16 in loop 1 function; Pro34, Thr35 and Ile 36 in loop 2 function and Ala 59, Gly60, Glu61 and Tyr 64 in loop 4 function. We are also attempting to site-specifically introduce flurophores, affinity and spin labels into *ras*. These studies should provide new insight into the mechanisms of cellular signal transduction as well as new information for the development of anticancer drugs.

MOLECULAR STRUCTURE OF MEMBRANE TRANSPORT SYSTEMS

Electron crystallography has developed into a powerful technique for molecular structure determination of membrane proteins. This crystallographic technique is now capable of providing structural information to near atomic resolution. The widespread use of this crystallographic technique will depend on the success of the further development of the crystallization of membrane protein forming two dimensional arrays. We have contributed to this success in the past and will continue to do so in the future.

In the last year, we have continued to make progress in the crystallization of cytochrome reductase for x-ray crystallographic studies. We obtained new crystal forms and will determine the diffraction qualities of our new crystals when we have synchrotron beam time at the Stanford Synchrotron Radiation Labora-

tory. It should be noted here that the crystallization of membrane proteins has been a major stumbling block in the progress of the structure determination of membrane proteins. Workshops organized recently by NIH have addressed this problem and recognized the need for increased support in structure determination of membrane proteins. We realize that a rapid progress on the structural work of cytochrome reductase will need a substantial increase in our effort. To achieve this, we clearly need the additional support from DOE or other funding agencies.

Substantial progress has been made in the purification of maltose transporter, a member of ABC (ATP-binding cassette) superfamily. Members of the superfamily can be found in organisms ranging from bacteria to mammalian cells and include the multidrug resistant protein, the peptide transporter that is involved in the antigen presentation, and the cystic fibrosis transmembrane conductance regulator (CFTR). The superfamily is also involved in many diverse functions such as cell division and DNA repair. Defects in these proteins have been related to a number of diseases such as cystic fibrosis. Knowledge about the structure of the maltose transporter will provide the molecular basis in understanding its functional mechanism. This understanding is expected to reveal the general principles governing the molecular mechanism of the ABC superfamily, and to provide insight into the structural basis for the causes of the diseases. We have established a new purification technique for obtaining homogeneous maltose transporter with high yield. The purified protein is adequate for screening the crystallization of this protein for electron and x-ray crystallographic structure studies.

Calmodulin-like protein is a calcium binding protein that can be found in the pseudostratified and stratified tissues of normal human breast, and its expression is down regulated when a stratified cell is transformed into a cancerous cell. Calmodulins and the related proteins play an important role in the epithelial growth and/or differentiation as well as in the breakdown of regulatory mechanisms for cells leading to pathological conditions and to uncontrolled proliferation. It is essential to obtain molecular structures of calmodulin-like protein as a basis for understanding its molecular mechanism. This understanding can be expected to provide an insight into the transformation of human breast cells, and to give a rational approach in combating the development of the breast cancer. Calmodulin-like protein has been cloned and overexpressed in *E. coli* by our collaborators, Drs. Martha Stampfer and Paul Yaswen. We have purified this protein to homogeneity that is suitable for crystallization. Crystals have also been obtained, but diffract to a rather limited resolution of about 3.0 Å. We have collected a complete native data set. The structure determination of this protein is currently in progress.

THREE-DIMENSIONAL STRUCTURE OF CATALYTIC RNA

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 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. Our objective is to determine the first three-dimensional structure of a catalytic RNA by using a combination of chemical probes, NMR spectroscopy, and x-ray crystallographic methods.

HUMAN GENOME PROJECT

The LBL Human Genome Center supports the national Human Genome Program with support from both DOE, OHER and the NIH National Center for Human Genome Research. The goal of work on human chromosomes has been focused chiefly on the Down Syndrome region of human chromosome 21. Work on mapping and sequencing the genome of the model organism, *Drosophila* , is another major component of the Center, supported by both DOE and NIH funds. DOE's support in this program is focused on the development of organism-independent techniques and technologies that can be applied to human sequencing. The LBL Human Genome Center has active programs in genomic DNA sequencing, quantitation of heterozygosity in human populations, physical mapping, and cDNA isolation and analysis. Each of these programs involves close interactions among the biology, instrumentation and automation, and informatics groups. We have chosen genomic DNA sequencing as our major focus for the coming years, while maintaining a balanced effort in the other three areas of genome research.

Our major instrumentation and informatics goal is to develop the degree of automation needed to produce an effective sequencing assembly line and to capture, assemble, analyze and distribute massive amounts of

sequence information. The automation of colony picking, polymerase chain reaction (PCR) set up, sequencing reaction set up, agarose gel loading, and gel analysis as well as the development of a large scale, extensible PCR thermocycler, and a large scale oligonucleotide synthesizer have permitted the Center to sequence over 700 Kb of DNA in 3kb fragments from both human and Drosophila.

The Instrumentation Group within the LBL Human Genome Center carries out a program of support for existing programs in mapping and sequencing together with longer-range development activities in the area of automation and advanced instrumentation development. During the past year, we have provided a number of enhanced capabilities for biologists involved in implementing a directed genome mapping and sequencing strategy. Included in these activities were the implementation of a digital image acquisition and analysis system adapted from large-scale PCR assays associated with STS mapping and transposon directed sequencing. In addition, a number of automation modules associated with commercial robots were developed and implemented. Among these were automated procedures for PCR reaction prep, agarose gel loading, and cycle sequencing chemistry.

A thermal cycler capable of performing 3 × 96 or 284 PCR reactions in a robotically supported environment was fabricated, tested and evaluated in day-to-day operation in the sequencing program. Details of the design allow for efficient thermal transfer to the reagent mixture allowing for faster thermal cycling than achievable by commercial thermal cyclers. Preliminary studies indicate that there are significant improvements to the quality of PCR results using the LBL thermal cycler. These results have significant implications for scale-up of the current approach to mapping and sequencing.

An automated oligosynthesizer capable of parallel synthesis of multiples of 12 oligos in a microtiter plate compatible format has also been developed. Experiments have shown that it is possible to synthesize 20-mers with greater than 99% coupling efficiency in the open dispenser format used in the system. The key to success is an Argon bath which surrounds the dispensers to prevent contamination of the reagents by oxygen and water vapor. The approach is amenable to an extended format in which multiples of 12 oligos up to a total of 96 per cycle can be automatically synthesized at high rate and at lower cost. The system has recently been used for the synthesis of oligos for an experimental study requiring 20 or more oligos per day.

Longer term developments include the study of mass spectrometry as an efficient method for the sizing of large macromolecules in a manner which would ultimately replace gel-electrophoresis in both mapping and sequencing and the development of an improved fluorescence sequencing platform capable of increased throughput relative to existing commercial systems. In the case of fluorescence sequencing, a system has been developed which exploits a combination of fiber optic coupling between the gel and a cooled-CCD camera in order to achieve a higher spatial resolution in the fluorescence detection channels. Preliminary data indicate the possibility of 100 lanes per run in a conventional acrylimide gel. Additional improvements include the use of thinner gels for more rapid sequencing in this highly parallel geometry.

The informatics support of the large scale mapping and sequencing work has received much benefit from a focus on the AceDB kernal and ancillary programs. It has permitted the Center to establish a public database, 21Bdb. 21Bdb is the first database with which one can visualize a map of Chromosome 21 populated with a variety of physical markers. One can also move markers on the map and observe the subsequent rearrangement. Even more extensive manipulations are possible with Flydb, the local database for Drosophila mapping and sequencing information. Public access to the Drosophila data is through FlyBase, maintained by collaborators at Harvard.

MASS SPECTROMETRY DETECTORS

Two major problems must be addressed before mass spectrometry can be routinely applied to the sizing of large DNA fragments. The size of fragments that can be ionized and vaporized must be increased because the current limit is about 100 bases. Our second focus has been the attempt to develop ion detectors that can respond to impacts of large ions. Future mapping and sequencing efforts employing mass spectrometry would benefit from the development of ion detectors that would be sensitive to ions larger than several hundred thousand daltons, the approximate upper limit reported for the mass spectrometric analysis of proteins.

We have approached these problems by constructing a mass spectrometer test stand that can be optionally adapted for DNA fragment ionization studies or detector testing. We have constructed a matrix-assisted laser desorption time-of-flight (MALD-TOF) mass spectrometer and an electrospray time-of-flight (ES-TOF) mass spectrometer. Each is equipped with a detector chamber in which custom-made or commercial detectors can be tested. The MALD-TOF system is linear and is used for low resolution studies, while the ES-TOF system is rectilinear and introduces ions at 90° to the flight tube.

We will continue to use these mass spectrometer systems both for the testing of alternative ion detectors and for preliminary experiments in which low resolution DNA sizing contributes to biological useful analyses.

YEAST GENETICS

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 LBL, that are involved in recombinational-repair of DNA lesions. He is also 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 cycle 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 lacks the *Mlu*I sites involved in cell cycle regulation. Plasmids have been made that lack the damage inducible region but which contain the *Mlu*I sites. Northern analysis will be used to determine whether or not DNA damage can be uncoupled from induction from cell-cycle regulation.

Expanding the scope of Dr. Mortimer's studies, during the last year, a new project was started on the genetics of wine yeast. This project seeks to develop superior wine producing yeast strains through genetic manipulations.

Medical Applications

THE CENTER FOR FUNCTIONAL IMAGING

Within the scope of the Center for Functional Imaging, positron emission tomography instrumentation, advanced radiopharmaceutical chemistry and data

analysis are joined with molecular biology for specific applications to fundamental medical science questions which are related to the human genome and gene expression.

The goals of the Center for Functional Imaging 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 on the application of positron emission tomography together with advanced mathematical techniques of analysis, chemical synthesis of specific tracers and detection instrumentation.

A major objective has been the elucidation of the characteristics of mental illnesses such as schizophrenia and dementias such as Alzheimer's disease and Parkinson's disease. While LBL, along with Brookhaven, developed a substantial body of knowledge regarding the cerebral metabolism in these diseases in the early 1980s, it has not been until recently that a distinct pattern representing subsets of schizophrenia and Alzheimer's disease were discovered at LBL using the very high resolution PET scanner built by LBL through OHER.

Cardiac disease studies in 1993 included 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 LBL during 1993 were 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 (≤ 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 (i) improved positron tomographs, (ii) silicon photodiodes, (iii) novel solid state photodetectors, and (iv) new, fast, high efficiency scintillation crystals. Technology 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.

Statistically based reconstruction algorithms for emission tomography are based on the knowledge of the statistics of the emission and detection processes and the *a priori* statistical, medical, and perceptual

knowledge at the time of reconstruction. At the end of FY 1992, algorithms based on statistical parameter estimation for image reconstruction from tomographic emission data were left at a point in which the advantageous characteristics of the Maximum Likelihood Estimator (MLE) method had become quite clear: In cases in which a lesion occurs in a low uptake region of the imaged organ in the presence of high uptake neighboring regions, a properly managed MLE reconstruction will result in substantially increased detectability of the lesion, when compared to a standard FBP reconstruction. In addition, preliminary work had been done toward incorporating prior knowledge about anatomy or other relevant facts into the algorithm in a Bayesian context. By the use of Visual Response Functions, a simple one-dimensional reconstruction exercise gave promising results.

During FY 1993, we have continued to characterize the MLE reconstructions, particularly in view of results by other workers in which advantageous characteristics similar to those of the MLE have been found for FBP when the filter for the latter is made space variant taking into consideration first and second order statistics. Work has been initiated in collaboration with J. Fessler, of the University of Michigan, who is the principal developer of those results. The first half of the project comparing results is completed: in the cases in which statistical differences were expected to be found between FBP with space invariant and space variant filters, they were not found. Data is now being generated for the case in which the differences would be expected to be seen if the space variant filter performs as Fessler's initial results seemed to indicate.

The one-dimensional theory for Bayesian reconstruction has now been extended to two-dimensions and it has been demonstrated that the complexity of the calculations does not increase as the square of the side dimension of the image, but instead, it only increases as two times that side dimension. Computer programs to exploit that characteristic are now being written.

The possible role of Wavelets analysis in tomographic image reconstruction has been investigated in some detail. The new techniques, which are very successful in image compression, for example, may have two areas of applicability: 1) non-linear filtering of the very sharp, but very noisy, images that result when the MLE process is allowed to go to convergence and 2) small scale filtering between iterations of the MLE method. The latter has already been shown to lead to apparently excellent images in non-tomographic reconstruction (Hubble telescope images) by a French group.

Radiation Effects of Vascular and Blood Diseases

LBL's program dedicated to investigating vascular diseases, blood platelets, and megakaryocytes in laboratory animals has two prominent goals. The first is to analyze vascular diseases by positron emission tomography (PET) and other detection systems. The types of vascular lesions that are of interest are atherosclerosis, deendothelialization, and radiation-induced tissue damage. Identification of appropriate radioisotopically labeled probes may permit localization of vascular abnormalities by noninvasive imaging and, also, modulation of radiation induced tissue damage. The second goal is to characterize the regulation of blood platelet production, with emphasis on processes that are endogenous to the marrow.

Heavy-Ion Radiosurgery

Since the closure of the Bevalac facility in February 1993, LBL has initiated the transfer of the heavy-charged-particle radiosurgery technology to a hospital-based environment at the Loma Linda University Medical Center (LLUMC), Loma Linda, California. The charged-particle Bragg-peak radiosurgery program at LBL has become recognized as the leader in the field, and the scientific and professional staff at LBL have gained considerable experience in radiosurgery-related imaging techniques, target delineation, the role of associated therapeutic procedures (e.g., endovascular embolization, microsurgery) and patient management.

LLUMC has a state-of-the-art hospital-based proton treatment facility which, when fully operational, will be able to serve more than 1000 patients per year. This facility has the world's only currently operational proton gantry, thereby permitting reliable beam delivery from multiple angles. For the past 2 years, the scientific and professional staff at LLUMC has been applying proton irradiation to the treatment of patients with various cancers.

Charged-particle beams have uniquely advantageous physical properties for application to focal intracranial irradiation (stereotactic radiosurgery). These properties include: sharp lateral and distal borders, and enhanced dose in the Bragg ionization peak relative to

the entrance plateau ionization region. Over the past 13 years, LBL has developed Bragg-peak radiosurgery for the treatment of inoperable intracranial AVMs. AVMs are congenital collections of abnormal blood vessels in the brain which are prone, without treatment, to cause lethal or recurrent disabling brain hemorrhage, progressive neurologic impairment, seizures refractory to medical treatment, and intractable vascular headaches. Symptoms typically appear before age 40 and occur in otherwise healthy and productive young people. Bragg-peak radiosurgery is used to induce permanent thrombosis (occlusion) of the abnormal blood vessels of the AVM and thereby remove the risk of hemorrhage and reverse other associated disabling symptoms. Results to date for AVM patients treated at LBL are excellent. Cure rates greater than 90% have been achieved with charged-particle radiosurgical treatment of small AVMs, and with a low risk of treatment-related complications. The technical developments and the dose-volume-response data obtained at LBL are now ready for application in the hospital-based environment.

In collaboration with LLUMC, LBL staff will review and provide expertise on all aspects of patient evaluation, management and treatment with proton radiosurgery. LLUMC will provide clinical collaboration from the faculty and staff of the Departments of Radiation Medicine, Neurosurgery and Diagnostic Radiology. LLUMC will also make available to the collaborative program their state-of-the-art proton treatment facility. Currently, one fixed horizontal beamline and one gantry beamline are fully operational; two additional gantry beamlines, scheduled for completion in 1993, will dramatically increase patient capacity for additional cancer patients and for the new program in proton radiosurgery. LLUMC will develop (in consultation with LBL) hardware and software necessary to adapt the LLUMC facility for charged-particle radiosurgery. Prospective patients referred to LBL and/or LLUMC will be entered into a collaborative evaluation and radiosurgical treatment protocol. Procedures will be carried out jointly by LBL and LLUMC staff to facilitate refinement of the charged-particle radiosurgery method at the LLUMC proton facility. By the end of the 2-year project, LLUMC staff will be capable of treating patients with small AVMs without further LBL assistance. Because of the unique and complementary expertise at LBL and LLUMC staff, a timely and successful completion of this project is anticipated. This project represents an ideal opportunity to secure a successful long-term dividend to the U.S. taxpayer for DOE's investment in this area of research.

IMAGING OF APOLIPOPROTEIN E-BINDING RECEPTORS *IN VIVO*

The long-term goal of this research project 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. Radiopharmaceutical receptor imaging agents will be extremely valuable tools for determining physiologic, pathologic, and therapeutic effects on lipoprotein metabolism and cardiovascular disease risk. Among the possible candidates for such an agent, one based on the dimeric or trimeric repeat of a high affinity receptor-binding peptide sequence of apolipoprotein (apo) E appears to be the most promising.

With the availability of a receptor binding peptide probe, it will be possible to determine the extent of specific binding to low density lipoprotein (LDL) receptors, which are rate-limiting for the hepatic clearance and irreversible catabolism of plasma cholesterol. LDL receptor-deficient cells and a rabbit model of LDL receptor deficiency will be used to test the specificity of binding. Physiologic and pharmacologic manipulation of hepatic LDL receptors can then be used to optimize conditions for quantitating receptor function by positron emission tomography (PET). Up-regulation of LDL receptors is achieved by LDL-lowering therapy, including low-fat, low-cholesterol diets and treatment with bile-acid binding resins and hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Conversely, down-regulation can be produced by feeding high-cholesterol, high-saturated fat diets. By systematically varying LDL receptor activity in the rabbit model and ultimately in humans, these maneuvers will allow assessment of PET imaging as a clinical tool for quantitating function of hepatic LDL receptors in a variety of conditions affecting cardiovascular disease risk.

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.

E. Progress Accomplishments and Research Highlights

E. Program Accomplishments and Research Highlights

Analytical Technology

KP-01-01

CHARACTERIZATION OF AIRBORNE RADON CONCENTRATIONS (GADGIL, SEXTRO)

- Used a previously validated model of radon transport and entry to elucidate the role of soil and gravel permeabilities in the effectiveness of the two commonly used sub-slab ventilation techniques for mitigation of indoor radon in high radon houses.

- Used the previously validated model of radon transport and entry to quantify the increased heating energy costs from the two standard sub-slab ventilation techniques, as a function of soil and gravel permeabilities.

- Conceptually developed, and quantified the effectiveness of an approach to place a sub-gravel impermeable membrane layer under the basement slab for improved performance of sub-slab ventilation systems for indoor radon mitigation.

KP-01-02

DETECTOR MATERIALS, INSTRUMENTATION (JAKLEVIC, HALLER)

- Very large area (75 mm diameter) lithium-drifted silicon detectors can now be, and have been, fabricated using a recently developed technique to getter defects in float-zone silicon. Gettered silicon material can now be used for a wide variety of large area Si(Li) detectors, with areas previously unattainable with ungettered material.

- Amorphous germanium entrance window contacts yield germanium detectors with superior peak-to-

background ratios and thinner dead layers than previously observed in Si(Li) or Ge x-ray detectors. These contacts allow Ge to be used for x-ray detection in the low energy x-ray regime previously difficult to access with standard Ge detectors.

- Fabricated the first prototypes of large area, position-sensitive p-type silicon drift detectors. P-type material is a more desirable detector material than the conventionally used NTD n-type Si due to its lower cost, simpler detector processing and potentially greater resistance to radiation damage.

Environmental Research

KP-02-01

AEROSOL & CLOUD CHEMISTRY (NOVAKOV)

- We have shown, as a part of our ongoing project on the modification of cloud optical properties by aerosols on El Yunque peak, that CCN concentrations cannot be explained by sulfate concentrations alone.

- To account for the "missing" CCN we have performed measurements with the objective to quantify the contributions of sulfate, seasalt and organic aerosol mass concentrations to the CCN number concentrations.

- Our results obtained on El Yunque indicate that at this marine site about 37% of the CCN number concentrations can be accounted for by sulfate and 63% by the organic aerosol mass concentration. The approach used was to convert the measured mass size distributions to number size distributions of sulfate, seasalt and organic aerosol from which the contributions of these species to the CCN and total aerosol number concentrations could be estimated.

- These conclusions, if confirmed at other locations, may be of particular relevance to the question of CCN-cloud droplet relationship because theoretical studies have shown that the production of new sulfate CCN is not the major pathway for the conversion of anthropogenic sulfur dioxide emissions to sulfate. The reason being that anthropogenic sulfate mass is believed to be principally produced by SO_2 oxidation in cloud droplets, i.e., a pathway would not produce new sulfate CCN, only larger particles formed on pre-existing aerosols. In contrast the organic aerosol is not formed in cloud droplets, but is derived either from direct emissions of primary particles, or produced by condensation of low vapor pressure products of gas-phase reactions of reactive hydrocarbons. Therefore, it can be expected that the number concentration of organic aerosol, including the fraction that serves as CCN should be more strongly related to the emissions of anthropogenic pollutants.

KP-02-03

RADON TRANSPORT THROUGH SOILS AND INTO HOUSES (SEXTRO, FISK)

- Air permeability of soil is a critical determinant of radon entry into buildings. A new technique has been developed to measure air permeability of soil, using a sinusoidally-varying pressure signal emitted from a source probe and measuring the time lag at a detection probe . This two-probe technique allows the distance and orientation between the source and detector probe to be changed, thus permitting investigation of permeability as a function of path length and examination of differences between horizontal and vertical components.

- Because houses interact with soils at a scale of up to several meters, soil measurements at commensurate distances are necessary. Scale dependence of air permeability has been observed in the soil at the Ben Lomond small structures site, with an apparent increase of about a factor of 6 for a scale of about 2.5 to 3 m compared with the previous single-probe measurements. In addition, vertical permeability was found to be about a factor of two smaller than that measured horizontally.

- Radon entry rates have been measured as a function of the size of the opening to the soil in our two basement structures, which differ only with the presence of a gravel layer below the slab in one structure. These experiments demonstrate that beyond a minimum opening size, the radon entry rate is nearly independent of the size of the opening for the structure with the subslab gravel, as compared with the structure without the gravel, where the entry rate depends upon the size of the opening. These results have important implications for understanding radon entry and control in buildings.

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

- Determined the first stability constant of Np(V) with NTA at different ionic strengths (I = 0.5, 1.0, 2.0, 3.0 M) using spectrophotometry (Nitsche et al.).

- Determined stability constants of Pu(IV) and Pu(VI) with NTA at 0.1 and 1.0 M ionic strength respectively using spectrophotometry (Nitsche et al.).

- Determined first stability constant of Pu(V) with NTA at 0.1 M ionic strength by two independent methods simultaneously: spectrophotometry and potentiometry (Al Mahamid et al.).

- Determined stability constants of Co(II) with NTA at 0.1 M ionic strength using potentiometry (Al Mahamid et al.).

- Studied the dissolution of Pu(IV) polymeric form by adding excess amount of NTA (NTA/Pu(IV) = 45/1) (Al Mahamid et al.).

- Studied the complexation behaviors of Pu(III), Pu(IV), Pu(V), and Pu(VI) with NTA at macroscopic concentrations (10^{-3} M) using spectrophotometry and at environmental concentration level (10^{-7} M) using a solvent extraction technique (Al Mahamid et al.).

FIELD HETEROGENEITY (MAJER)

- Defined the hierarchy of physical characteristics that control fluid flow and can be imaged by means of in situ application of high-resolution seismic imaging in dispersive and "fast-path" environments.

- Determined the dominant physical characteristics that can be imaged in situ and related to hydrologic properties using data from controlled laboratory and in situ soil block experiments (up to several meters).

RADIONUCLIDE CHEMISTRY (PERRY)

- Prepared and isolated several solid phases of co-contaminants involving cobalt with organics, including cobalt (II)-EDTA.

- Isolated an interface system in one synthetic system that involved the aminocarboxylic compound ethylenediaminetetra acetic acid (EDTA) organic compound as a substrate onto which was bonded a transition metal complex of EDTA.

- Conducted x-ray photoelectron and other spectroscopic studies on several of the cobalt-EDTA phases; this work will be extended to the use of the Advanced Light Source at Lawrence Berkeley Laboratory.

Health Effects

KP-03-02

AIR POLLUTANT EXPOSURES IN BUILDINGS (NERO, DAISEY)

- Examined correlation between indoor concentrations and local radium concentrations using data from New York and Minnesota, finding substantial predictive power, comparable to earlier results for Minnesota and supporting the success of this approach generally. Further developed the computer-analytical systems needed for implementing this approach at various geographic scales, including county and census tract.

- Established a general way of properly estimating the power of the correlation models to predict the distribution parameters of the actual indoor radon concentrations, rather than of the monitoring data presently available. We found, for example, that the radium data alone were able to predict (true) county geometric concentrations for Minnesota with an R2 of 0.6, noticeably greater than the R2 with respect to the mean of the limited monitoring data presently available.

- Developed and applied a toxicokinetic model for the disposition of benzene for humans exposed to airborne benzene at occupational and environmental levels. The model predicted slight dose-rate effects for formation of primary metabolites of benzene at occupational exposure levels but not at typical environmental exposure levels.

- Completed field measurements of volatile organic compounds, nicotine, respirable particles, and air exchange rates in selected California office buildings with smokers.

MOLECULAR MECHANISMS OF CELLULAR EFFECTS OF HEAVY IONS (BLAKELY)

- Continued experiments with DNA-protein crosslink-sensitive cell lines to examine dose-and LET-dependent survival and yield of lesions after exposure to high-LET 32 MeV/u nitrogen ions accelerated at the LBL 88" cyclotron.

- Continued the evaluation of particle effects on the cell-cycle dependent DNA-double-strand break repair deficient mutant cell, XR-1, using its parent line as a control.

- Continued the scoring and analysis of the particle fluence- and LET-dependent yield of human #2 chromosome PCC fragments, and the repair-modified increase in chromosomal misrejoinings and correlate with residual damage in daughter cells.

- Participated in initiating an X-ray microscopic imaging study of chromatin fibers in mitotic and synchronized cells at the ALS.

BIOLOGICAL EFFECTS OF MAGNETIC FIELDS (LIBURDY)

- Recently demonstrated that microwave fields can disrupt calcium ion bridges which stabilize liposome bilayers. This finding provides important insight into how structural stability of the phospholipid bilayer is influenced by microwaves.

- Developed the first, well-characterized, shielded electromagnetic fields exposure system for cells in long-term culture. Through use of mu-metal shielding and an optimized coil design we evolved a standard system for uniform field exposures. We have also extended this approach to the development of a well-characterized exposure system for use in single-cell, digital imaging microscope studies.

- With the Imaging Technology Group (P.I., W. Johnston; LDRD) we began the development of a digital imaging microscope system to monitor changes in cell function and shape for rapid visual servoing. This digital imaging microscope will be one of the first at LBL to use fiber optics for visual servoing on living cells.

- Extended our breast cancer studies to established a dose-threshold between 6–12 mGauss (0.6–1.2 uTesla) for onset of the blocking effect of environmental-level, sinusoidal magnetic fields on melatonin's natural inhibition of human breast cancer cell growth in culture. Magnetic fields in this range are commonly encountered in the work place and environment. Pulsed field exposures are planned that simulate MRI/NMR and other waveforms.

MOLECULAR CARCINOGENESIS (BISSELL)

- Applied the newly developed rapid assay for distinguishing normal and malignant cells to study tumor suppressor genes. WE now have evidence that overexpression of nm23, a putative tumor suppressor gene exerts a dramatic effect on the rate of growth via formation of basement membrane.

- Showed in a final chapter on our work on tumorigenicity of rous sarcoma virus in the embryo that a chondroitin sulfat protoglycan is responsible for the apparent normal phenotype of the infected cells in ovo. In culture, addition of basement membrane restores this function.

- Solved the mystery of regulation of whey acidic protein (WAP): cells in tissue culture make copious amounts of TGF-a. This in turn inhibits cells and cell and cell junctions, and down regulates WAP. Studies in TGF-a transgenic mice confirm this finding. Extracellular matrix down regulates TGF-a and thus upregulates WAP.

- Showed that highly utilized viral promoters and enhancers (MMTV, SV40, CMV) are strongly regulated by ECM. MMTV and CMV are analogous to the ECM-response element (BCE1) we isolated and characterized from the bovine b-casein promoter in that they are dramatically upregulated by ECM. SV-40 is analogous to TGF-b and a in that it is down regulated.

- Showed that the active molecule in ECM action is laminin and determined that a peptide in chain A of laminin interacts with the integrin receptor to start the signal transduction pathway.
- Showed in collaboration with Judy Campisi that the Id gene (a transcription factor HLH class) down regulates differentiation. Experiments with sense and antisense stable transfection showed suppression and super-induction of b-casein respectively.

- Showed in collaboration with Zena Werb, UCSF, that virgin transgenic mice with low expression of a functional stromelysin-1 gene before pregnancy "think" they are pregnant: the gland buds and there is measurable b-casein. Thus here is clear evidence that ECM-degrading elements are involved in development and branching morphogenesis.

- Determined that tenascin, and ECM molecule, is an inhibitor of tissue-specific genes in the mammary gland and may be a native modulator of function in vivo.

IN VITRO TRANSFORMATION OF HUMAN MAMMARY EPITHELIAL CELLS (STAMPFER)

- Found that the calmodulin-like protein (CLP) encoded by NB-1 differs functionally from authentic calmodulin, and may alternatively activate or inhibit calmodulin dependent enzymes in normal HMEC.

- Identified a 110 kD protein which binds *in situ* in a calcium dependent manner to CLP in HMEC extracts.

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.

- Began construction of a human cDNA expression library.
- Identified a novel β-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.

INTERSPECIES EXTRAPOLATION AND RISK ASSESSMENT (GOLD)

- In order to select chemicals for carcinogenicity testing, we have ranked potential human hazards on an index, HERT, that uses LD50 and concentrations in food to approximate possible human carcinogenic hazard. We have found exposures for approximately 78 chemicals with concentrations greater than 10 ppm in 25 common foods. Some of those that rank the highest are: caffeine (from coffee, tea and chocolate), alpha-chaconine and alpha-solanine (from potato), trigonelline (from coffee), theobromine (from chocolate), geraniol (from carrot), 2-methylpropanoic acid (from strawberry jam), and furfural (from heating sugars).

- To rank possible carcinogenic hazards in the workplace, we use an index, PERP (Permitted Exposure/Rodent Potency), which expresses the worker permitted lifetime exposure (mg/kg/day) as a percentage of the lifetime dose that induces tumors in 50% of rodents (TD50 in mg/kg/day). The PERP values for 75 rodent carcinogens to which workers are exposed differ by more than 100,000-fold from each other. For nine chemicals the permitted worker exposure is greater than 10% of the tumorigenic dose rate in rodents (TD50).

- Examined interspecies extrapolation between monkeys and rodents for 16 rodent carcinogens for which 20-year-long monkey bioassays were recently completed by NCI. Seven of the 16 rodent carcinogens (44%) were not considered carcinogenic in monkeys by NCI. For the nine chemicals that are carcinogenic in both rats and monkeys, there is good target site concordance between the species; the liver is the most common target site in monkeys and in rats.

- Analyses of the potential impact of a reduction in the standard protocol for cancer bioassays from 4 sex-species groups to 2, suggest that further consideration should be given to reducing the protocol: 90% of rodent carcinogens that are identified by 4 groups could be identified by one test in each species; a 2-group protocol also identifies as multiple-site and 2-species carcinogens those that are so identified by a 4-group protocol; potency values are also similar.

BONE MARROW STEM CELLS (NARLA)

- Permanent and efficient repopulation of the erythroid elements in non-irradiated thalassemic mice has been accomplished. Work has begun on the biology of embryonic stem cells for use as donors of hematopoietic stem cells. These findings should into the mechanisms by which stem cell repopulation occurs.

KP-03-03

RADIOLOGICAL PHYSICS (CHATTERJEE)

- Using the concept of 'faithful' repair, a theoretical model has been developed to relate the production of double strand breaks with translocation and dicentric yields induced by imaging radiation in human cells. This mechanistic model allows damage assessment at low doses.

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.

GENETICS OF GROWTH CONTROL (BARTHOLOMEW)

- Demonstrated that a mutant T-antigen, defective in transformation, has an altered phosphorylation state. This mutant T-antigen is phosphorylated like the wild T-antigen primed to initiate DNA synthesis; and in fact, the mutant T-antigen stimulates *in vitro* DNA synthesis more efficiently than wild type T-antigen.

MUTAGENESIS: FUNDAMENTAL CHEMISTRY (HEARST)

- Described a new mechanism for the interaction of proteins with nucleic acids. Molecular Matchmakers are the ATP requiring enzymes which promote the binding of regulatory proteins to DNA.

- Described the synthesis of large quantities of DNA oligomers containing monoadducted psoralens. These oligomers are receiving considerable attention as agents for promoting site directed mutations in vivo.

- Determined the solution structures of the DNA duplex containing either a psoralen monoadduct or a psoralen crosslink.

X-RAY ABSORPTION SPECTROSCOPY (CRAMER)

- Developed plans for the sorption elliptical wiggler beamline at the Advanced Light Source. This will provide high intensity linear and circularly polarized x-rays beams for biological spectroscopy and diffraction.

- Demonstrated site specific x-ray absorption using high resolution fluorescence detection on a mixture of Mn oxidation states.

MOLECULAR STRUCTURE OF MEMBRANE TRANSPORT SYSTEM (JAP)

- Obtained new crystal forms of cytochrome reductase for x-ray crystallographic studies.

- Made progress in the purification of maltose transporter, a member of ABC (ATP-binding cassette) superfamily.

- Established a new purification technique for obtaining homogeneous maltose transporter with high yield. The purified protein is adequate for screening the crystallization of this protein for electron and x-ray crystallographic structure studies.

- Purified calmodulin-like protein to homogeneity suitable for crystallization. Crystals have been obtained, and we have collected a complete native data set.

BIOMOLECULAR STRUCTURE ANALYSIS BY NMR (WEMMER)

- Applied a new 'soft-pulse' NMR method to the accurate determination of coupling constants in nucleic acids. This approach improves the accuracy of the measurements, and also provides a direct method for analysis of error limits.

- Completed studies of psoralen monoadducted and crosslinked DNA oligomers, yielding structures which are providing insight into both the photochemical reaction mechanism, and also features which might be recognized by repair enzymes.

- Isotope labelled the activation domain of the transcription factor c-jun for dynamic studies. Relaxation measurements show that although this 'domain' is not highly structured, the dynamic features are uniform throughout and less flexible than a fully random coil peptide would be.

- Determined resonances of the DNA binding domain of the heat shock transcription factor have been assigned, and a low resolution structure. The solution structure is very similar to the crystal structure just determined for this protein, showing a similar fold to the CAP protein. This work provides a basis for further studies of the basis for sequence specific DNA binding.

- Determined the resonances and secondary structure of the second RNP domain of the sequence specific RNA binding protein S¥l. The secondary structure is consistent with the general motif found for this large class of proteins. The full structure determination is under way and will provide the basis for determining the basis for sequence specific recognition of single stranded RNA.

- Determined the mode of DNA binding of peptides from a transcription factor. Showed that the se-

quence Pro-Arg-Gly-Arg-Pro is critical to the binding in A-T rich DNA sequences, and that the shape as well as the charge of the Arg sidechain is important. This is the first structure analysis of this type of DNA binding motif.

- Prepared site specifically tritium labelled DNA, on which high resolution heteronuclear NOE studies carried out. This demonstrates feasibility of such studies providing a mechanism for detecting specific structural features in large nucleic acids.

- Used ATP tritiated at the 2-position to probe the interaction of ADP with the myosin S1 fragment in solution. Showed that there are two conformations present, which change populations with temperature, and interchange at a relatively rapid rate.

- Labelled of a self-cleaving hammerhead RNA with ^{15}N, and collected heteronuclear correlation spectra. This is allowing us to extend the analysis of proton NMR data, which we had previously used to determine the secondary structure of this type of RNA.

- Identified a new chemical linkage in a bilin from a Cryptophycean Algae. The new bonding scheme shows larger diversity than previously known in this class of light-harvesting protein.

STRUCTURAL BIOPHYSICS (KIM)

- Three-dimensional structure of a mutant human M-CSF has been determined at 2.4 Å resolution. Macrophage-colony stimulating factor (M-CSF) is one of a group of at least 18 glycoproteins, collectively known as hematopoietic growth factors, that regulate the growth and differentiation of blood cells and enhances their ability to kill infectious microorganisms and tumor cells.

- Determined the crystal structure of histidine binding protein of the *Salmonella typhimurium* with and without histidine. This protein recognizes amino acid histidine, binds to it, then interacts with a membrane complex to transport it inside the cell.

- The final decision of a cell to divide or not to divide is made by a class of proteins called CDK. We have determined the crystal structure of one such protein, CDK2. The structure revealed the features that suggest how this class of proteins are activated and deactivated to allow cells make commitment to enter the cell division cycle.

ARTIFICIAL ENZYMES (SCHULTZ, WEMMER, AND KIM)

- Probed structural and catalytic roles of *ras* mutations commonly associated with oncogenic activation. Properties of wild-type and transforming *ras* are retained by mutants containing a variety of unnatural amino acids. These functional mutants suggest a role of loop L4 residues in switch function and participation of loop L1 in activation of *ras* by effector molecules.

- 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, HEARST, KIM, AND WEMMER)

- Pursued NMR studies of self-cleaving "hammerhead" RNAs from plant virusoids; labeled RNAs to make resonance assignments possible. Subsequent enzymatic synthesis of a slowly cleaving sequence allows the detection of bases in the conserved region, and reveals slow dynamic processes present for these bases. Created plamids enabling the expression of RNA sequences in bacterial cells, allowing for stable isotope labeling.

- Studied small, independently folding domains of the *Tetrahymena* intron by NMR. The three-dimensional spectra contains essentially the same information as the two-dimensional spectra. However, homonuclear cross peaks will be separated in different planes according to the chemical shift of the carbon bound to either of the protons originating each cross-peak.

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

YEAST GENETICS (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.

- Initiated a Technology Maturation project, in conjunction with the California wine industry, to conduct a genetic study of wine yeast strains used in natural fermentations.

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

KP0404

THE HUMAN GENOME CENTER (RINE, SPENGLER, JAKLEVIC, MARKOWITZ, ZORN)

Biology

- Developed improved minimal plasmids for transposon-facilitated DNA sequencing.

- Continued development and implementation of high-throughput DNA sequencing, including the completion of more than 700 kb of assembled and edited 3 kb subclones of DNA from both human and Drosophila sources.

- Developed, in the chromosome 21 P1 mapping project, a P1 screening strategy which allows quick identification of P1 clones in a large segment of human chromosomes. This strategy contains three steps: In step one, inter-Alu DNA fragments are hybridized DNA filters containing pools of a human P1 library. In step two, the 12 P1 clones from each positive pool are re-arrayed, filters made and . hybridized with inter-Alu DNA fragments from step one localizing the hybridized P1's to an overlapping region of the selected YAC's. In step three, end fragments and inter-Alu fragments generated from each P1 are cross-hybridized to determine their overlaps. In approximately three months, we have identified over 600 P1 pools containing DNA hybridized by the inter-Alu fragments from YAC's which cover approximately 14 Mb stretch of chromosome 21. We have further localized 166 single P1's to YAC's which were previously mapped to the three million base pair region between markers CBR and ETS2. This region is likely to contain genes responsible for the major phenotypes of Down's syndrome (DS) including mental retardation.

- Examined, in the chromosome 21 cDNA project, a set of 69 anonymous STS's previously mapped to chromosome 21 for their ability to amplify fetal brain polyA$^+$ RNA in the RT-PCR (reverse transcription-polymerase chain reaction) assay. As a result, 25 STS's generated fragments of predicted sizes. Of 11 fragments used in Northern hybridization, 6 hybridized with specific RNA bands indicating that these sequences are indeed transcribed. This work is currently in press in *Genomics*. As a result of the chromosome 21 cDNA selection, we have also isolated and partially sequenced 175 chromosome 21 cDNA. These sequences including 140 new cDNAs have been deposited in the GenBank. The mapping result of these cDNAs has been submitted to *Genomics* for publication. Recently, the cDNA isolation and mapping effort has been focused on the 3 Mb region which contain genes responsible for the major Down Syndrome phenotypes. The goal of this project is to accumulate over 200 cDNAs in this region. To date, we have already isolated and mapped 51 cDNAs in this region. Since the cDNA project started over a

year ago, the accumulated number of new chromosome 21 cDNA has reached approximately 210, *tripling?* the number of cDNAs.

- A proposed transgenic project was funded through Laboratory Director Research and Development Program as a joint effort with Dr. Edward Rubin's group in the Life Science Division. The goal of this project is to carry out phenotypic studies of transgenic mice created by introducing defined segments of human DNA from the DS region of chromosome 21.

- Completed approximately 25% of the physical map of the Drosophila genome with the mapping of more than a thousand sequence tag sites (STSs) and enhanced notation.

- Generated new phage lambda-based cDNA cloning vectors that ease the elucidation of the sequence of full-length cDNAs.

- Initiated experiments aimed at the development of novel technology for the generation of cDNA libraries that correspond to the 5' end of individual mRNA molecules.

Instrumentation and Automation

- A high-throughput, microtiter plate compatible thermal cycler capable of integration with existing robotic handling systems has been constructed and successfully tested. Preliminary results indicate that the faster cycling speeds attainable with the system provide significant advantages for PCR assays required for the directed sequencing strategy currently being used in the center.

- An automated, large-scale DNA synthesizer capable of generating 96 oligos in a microtiter plate format has been designed and tested. Using a 12 channel reaction chamber as a building module, we have demonstrated the successful synthesis of 20-mers with a 99% coupling efficiency among the bases. The large-scale, cost-effective synthesis of large-numbers of custom oligos is possible using this instrument.

- A Beckman Biomek robot application to load four agarose gels was designed, completed and tested.

- We began development of a high-throughput, automated DNA preparation machine including major design, purchasing, and initial assembly stages.

- Completed construction and engineering testing of a three-station Rapid Thermal Cycler. Results from biological testing are very encouraging: five-fold increase in speed, substantially reduced background and improved specificity. The machine is now in routine production use for large scale sequencing.

- Demonstrated 12-channel automated oligosynthesis using a dispensing geometry capable of scale-up to a 96-well microtiter plate format.

Informatics

- Developed and released to the community the mapping and sequence database 21Bdb. 21Bdb, based on ACEdb, is the first database with which one can visualize a map of Chromosome 21 populated with a variety of physical markers. One can also move markers on the map and observe the subsequent rearrangement.

- Developed object-oriented programs to assist in the LBL directed sequencing strategy that permit integration of the products of gel analysis with data on clones, the map, sequences, etc. These modules in turn will fit into an overall laboratory data management program being developed in collaboration with Baylor College of Medicine.

- Developed new programs in Smalltalk for submitting data to Genbank and GDB. The program supports the use of a template in which only minor changes need be made for repeated submissions of data that are similar and part of an overall project. This is part of a larger project that permits metadata to directly create the workinguser interface.

- Finished the first version of BioPoet, which utilizes parallel processing for increased performance of time-consuming sequence matching algorithms by accessing multiple processors, e.g., workstations.

- Development of the database Flydb to support the physical mapping of *Drosophila*. Flydb provides a graphical representation of the physical map, digitized versions of the microphotographs of the regions being mapped and an integrated contig assembly program (see below).

- Developed and implemented an algorithm for the assembly of contigs using the *Drosophila* P1 library and clone-limited strategy employed at LBL.

- Completed the automation of data flow for the *Drosophila* physical map project, in collaboration with the Instrumentation Group.

- Completed a program for interactive analysis of gel images. This program assists in locating positive hits from PCR screening, resolves the result to locate plate-row-column, reports the results to Flydb and writes a log of the activity.

- Released new versions of several widely used data management tools, including ERDRAW and SDT. New versions of ERDRAW (Extended Entity-Relationship Diagram Drawing) and SDT (Extended Entity-Relationship Schema Design) tools, together with other utilities and the first version of ERVALS (Extended Entity-Relationship Value Set Editor) tool have been completed and released at the end of December 1993. This release improves the previous versions of ERDRAW and SDT. ERVALS is a new graphical editor that allows specifying or modifying user-defined value-sets (data types).

- Developed two new utilities, syb2sdt and orac2sdt that generate SDT files from Sybase and Oracle database definitions, respectively. We have also started porting our tools to new platforms that are available at LBL. Thus, we will provide versions of our tools for Sun/Solaris, on SGI, on HP workstations and on DEC's Alpha workstation. The speed of porting will depend on the availability of required compilers (C, C++) and window managers (motif) on these machines at LBL. Currently, there are versions of SDT available for Sun/Solaris.

Medical Applications

KP0601

VASCULAR AND BLOOD DISEASES (EBBE, TAYLOR)

- Performed studies in hypercholesterolemic rabbits to identify possible agents for imaging atherosclerosis. Various unlabeled porphyrin analogs, and metallo-porphyrin complexes were found to selectively accumulate in atherosclerotic lesions following intravenous injection.

- Developed methods to radiolabel porphyrin-type compounds with positron-emitting isotopes, and performed preliminary *in vitro* uptake experiments incubating excised rabbit aortas with radiolabeled agents,

- Oxidized and radioiodinated low density lipoprotein (LDL) for testing as an imaging agent for atherosclerosis. This compound, unlike native LDL, did not localize to atherosclerotic plaques in hypercholesterolemic rabbits.

- Found that an alteration in kidney function after exposure of mice to radiation appears to result from a combination of direct radiation effects on the kidney and also from systemic effects of irradiation of other organs.

EFFECTS OF HEAVY PARTICLE RADIATION ON TUMORS/ HEAVY ION RADIOSURGERY (LEVY)

- Initiated the transfer of the heavy-charged-particle radiosurgery technology to a hospital-based environment at the Loma Linda University Medical Center.

EXPERIMENTAL MEDICAL, CLINICAL, AND DIAGNOSTIC ISOTOPES (BUDINGER)

- Developed quantitative whole body PET studies in breast, prostate and lung cancer which show disease distribution better than x-ray CT and magnetic resonance methods.

- Successfully deployed the 47-level commercial PET in dynamic heart perfusion kinetic studies using the Rb-generator developed in the past by LBL.

- Developed software specification of three-dimensional volumes of interest from 3-D datasets at arbitrary orientations.

- Extended existing software developed by LBL for region of interest analysis (two-dimensional) to volume imaging tomographs.

- Developed software to evaluate the statistical correlation in time-activity curves acquired from dynamic PET due to attenuation compensation.

EXPERIMENTAL MEDICAL DEVELOPMENT OF RADIONUCLIDES (VAN BROCKLIN)

- Achieved routine production of [^{18}F]fluorodeoxyglucose for in-house nonhuman primate studies and clinical research patient studies of breast cancer, lung cancer and myocardial viability.

- Developed and began to biological evaluate a fluorine-18 labelled mitochondrial imaging agent - fluorodihydrorotenone.

- Discovered a new mechanism of tracer fluoride incorporation into medicinally important organic mol-

ecules. Fluoride ion was introduced into model compounds with a nitro substituent in what was believed to be an unreactive position. This unprecedented reaction will be applicable to a broad series of compounds with the nitro group in a similar position. Additionally, discovereda new chemical rearrangement reaction while producing one of the model nitro compounds for the fluorination study.

- Loaded the LBL Sr-82/Rb-82 generator with 200 mCi of Sr-82 from Brookhaven National Labs. Studied twenty-five patients with suspected heart disease to assess myocardial blood flow.
- Synthesized and evaluated halogenated atipamezole analogs- α_2-adrenergic receptor ligands *in vitro.*

- Accessed mini-cyclotron for development of novel fluorine-18 labeling techniques and production of PET radiopharmaceuticals.

- CRADA established between CFI and Somatix for the development of a dopaminergic tracer, fluoro-meta-tyrosine, to enable studies of dopaminergic neuronal activity following reinervation with gene therapy. This gene therapy technique may be utilized in the reversal of the onset of Parkinson's disease.

POSITRON EMISSION TOMOGRAPHY INSTRUMENTATION (DERENZO)

- Demonstrated a positron tomograph detector module using a 2 × 2 array of bismuth germanate (BGO) crystals and room temperature silicon photodiodes.

- Demonstrated the measurement of depth of interaction in a 3 × 3 × 20 mm BGO crystal using a silicon photodiode array and a photomultiplier tube.

- Developed an integrated circuit with 16 charge amplifiers and shaping circuits in a 2 mm^2 area.

- Used a recently-completed a table-top pulsed x-ray system to measure the rise time and fall time of a number of scintillators with unprecedented accuracy—a timing resolution of 100 picosecond.

- Measured high resolution decay times of CuI and Yb_2O_3, which establish them as among the fastest inorganic scintillators known.

- Established research collaborations with Brigham Young University (Salt Lake City, Utah) and with Lawrence Livermore National Laboratory in the computation of molecular wavefunctions in crystals.

STATISTICALLY BASED ALGORITHMS (LLACER)

- Demonstrated the feasibility of the extension of one-dimensional Bayesian reconstruction with Visual Response Functions as carriers of prior information to two-dimensional work. An adequate first order theory shows that the complexity of the calculations increases only as two times the side dimension of the image field.

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

- Obtained evidence that there is an abnormal apolipoprotein (apo) E-containing low density lipoprotein particle in subjects with a common heritable metabolic phenotype associated with increased coronary disease risk. The presence of this particle may indicate altered function of an apoE receptor in this disorder.

Selected 1993/94 Awards, Honors and Editorships

James C. Bartholomew

- Outside reviewer, Lawrence Livermore National Laboratory, Biomed Division "Institutional Research and Development Grant Program"

Mina J. Bissell

- 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*
- Vice-chair, Science Advisory Board Committee on Indoor Air Quality and Total Human Exposure, U.S. Environmental Protection Agency
- Treasurer, International Society of Exposure Analysis
- International Advisor, 6th International Conference on Indoor Air Quality and Climate
- Member, DOE Laboratory Directors Environmental and Occupational/Public Health Standards Steering Group
- Member, DOE/EPA Laboratory Directors Focus Group on Risk Assessment; Editor of Report to DOE and EPA on potential areas of research collaboration
- Member, Advisory Committee of the Health Effects Component of the University of California Toxic Substances Research & Teaching Program

Stephen Derenzo

- IEEE Nuclear and Plasma Sciences Society Merit Award

William Fisk

- Member, Advisory Council of the Bay Area Air Quality Management District, Chair of the Technical Committee
- Member, Editorial Advisory Board, *Indoor Air*

Ashok Gadgil

- Recipient, Pew Scholars Award in Conservation and the Environment

Karina Garbesi

- Recipient, 1993 Alexander Hollaender Distinguished Postdoctoral Fellowship

Robert M. Glaeser

- Elizabeth Roberts Cole Award, Biophysical Society
- Associate Editor, *Journal of Structural Biology*

Lois Gold

- Member, Society of Toxicology

John E. Hearst

- Executive Editor, Nucleic Acids Research
- Member, Editorial Review Board, Molecular Toxicology
- President, American Society for Photobiology
- Chair, Grants and Awards Committee, American Society for Photobiology
- Program Chair, Annual Meeting, American Society for Photobiology
- Sigma Xi National Lecturer

William Jagust

- Editorial Board, Journal of Neuroimaging

Bing Jap

- Member, NIH Biophysical Study Section

Sung-Hou Kim

- Member, Editorial Board, *Current Opinion in Biotechnology*
- 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

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, LBL
- 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

Robert P. Liburdy

- Member, American College of Radiology, Committee on Research and Technology Assessment of the Commission on Neuroradiology and Magnetic Resonance. Subcommittee on MR Biological Effects of the Committee on Research and Technology Assessment.
- Member, IEEE Committee on Man and Radiation (COMAR).
- Member, NIH Radiation Sciences Study Section, Reserve Reviewer, Division of Research Grants, NIH.
- Member, IEEE, Committee on Electromagnetic Field Standards.

Ernest Majer

- NRC Panel for Annual Review of Rock Mechanics

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 Nero

- Member, OHER Radon Principal Scientist Advisory Committee
- Chair, Forum on Physics and Society, American Physical Society
- Member, International Academy of Indoor Air Sciences

Dale Perry

- Member, Corporate Participation Committee, Materials Research Society.

- Member, Panel for the Restoration and Remediation of the Fernald, Ohio, DOE Uranium Processing Plant.
- Head of Solid State Materials and Engineering Chemistry Subpanel, Division of Industrial and Engineering Chemistry, American Chemical Society.

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, National Academy of Sciences
- 1993 Rochester Section's Harrison Howe Award, American Chemical Society
- H. Smith Broadbent Lectures in Organic Chemistry at Brigham Young University
- 7th Paul Ehrlich Lecture, FAES, National Institutes of Health
- 1994 Peter J.W. Debye Lecturer, Cornell Section of the American Chemical Society
- NRC Distinguished Lecturer
- Monsanto Lectureship, Purdue University
- Merck Lectureship, Cambridge University
- Member, Editorial Advisory Board, *Biocatalysis*
- Member, Editorial Advisory Board, *Catalysis Letters*
- 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
- Senior Editor, Encyclopedia of Bioorganic Chemistry
- Editorial Board, Perspectives in Drug Discovery and Design
- Editorial Board,Chemistry and Biology
- Editorial Advisory Board, Protein Science
- Chairman, Affymax Research Institute, Palo Alto, California

Richard Sextro

- Member, Radiation Advisory Committee of the Science Advisory Board, U.S. Environmental Protection Agency

Martha Stampfer

- Associate Editor, *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

- LBL Outstanding Performance Award

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.

F. Program Orientation

F. Program Orientation for FY 1994–1996

Analytical Technology

During next two years, the Indoor Atmosphere Program studies on radon progeny behavior will concentrate on experimental and theoretical research on two fronts: the deposition of radon progeny on to indoor surfaces, and driving mechanisms for entry of radon-bearing soil gas into buildings. In the research on deposition, we aim to resolve the large (about a factor of two to four) discrepancy between model predictions and experimental estimates for rates of removal of radon progeny by deposition on surfaces. In the research on driving forces for radon entry, we aim to look for and investigate driving forces other than steady-state indoor-outdoor pressure difference. Some of these, such as the interaction of wind with the ground surface near a building, we have recently discovered to be significant and important. Investigations of other mechanisms, such as entry driven by "pumping" action of wind pressure fluctuations will be initiated.

In FY 1995, we will 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.

* * *

In an effort to provide a broad base of detector and instrumentation development for a wide range of x-ray, gamma-ray and particle detection applications, we will continue to focus our work towards current areas of technology that could greatly benefit from improved detector instrumentation. In particular, the detection of x-rays from synchrotron sources and the development of new materials for detectors for room

temperature, field portable instruments for environmental survey applications, will be emphasized. The mechanism of gettering of defects in float-zone silicon will be systematically studied in order to develop a reliable and reproducible technique for production of large area Si(Li) detectors. Future work on the GaAs will focus on materials characterization and further purification of the epilayers in order to achieve the purity required for device application. A patent disclosure has been filed for a new detector design that would greatly improve the charge collection, and thus greatly improve the energy resolution, of high efficiency II-VI semiconductors, which are very useful for room temperature gamma-ray spectroscopy applications. If successfully developed, this new detector design will find a wide range of applications in many disciplines, such as radioactive waste management, treaty verification, nuclear medicine, and low level environmental waste monitoring.

* * *

Environmental Research

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.

* * *

The knowledge of thermodynamic and kinetic parameters that influence the complexation of radionuclides with organic materials is necessary to predict and model the interaction and mobility of co-contaminants and their maximum release concentrations in the subsurface. When the behavior of the organic-radionuclide wastes is understood, it will be possible to develop technologies that minimize the migration of radionuclides and stabilize them under an insoluble form.

The future work will be focused on collaborative research with other investigators of the co-contaminant chemistry subprogram. The following studies will be conducted:

- use of a direct technique such as Photoacoustic Spectroscopy to study the speciation of Pu in the Pu-NTA system at 10^{-6}–10^{-7} M, with a metal to ligand ratio of 1/100.
- stability of plutonium with NTA and microbes.
- stability of plutonium with NTA and Ca/Al/Fe and microbes.
- complexation of cobalt with NTA and Fe_2O_3-coated sand.
- complexation of cobalt with EDTA and Fe_2O_3-coated sand.

* * *

In our subsurface waste program we are continuing our study of thermodynamic complexation constants, solution speciation, solubility and the reaction kinetics of complexes and compounds that can form between radionuclides and organic constituents of mixed organic radionuclide wastes. The study focuses on the reactions of plutonium and cobalt with organic facilitators and microbes. Facilitators are organic compounds that interact with the radionuclides and modify their geochemical behavior; for example, aliphatic and aromatic organic acids and chelating agents. Microbial activity can also modify the geochemical transport behavior. Aqueous complexation with these organic co-contaminants and natural organic materials can lead to increased or decreased contaminant solubility and can result in increased or decreased sorption and transport behavior for metal contaminants that may be very different from those of the uncomplexed metal ions. The effects of complexation are very important in mixtures of organic waste materials coming from extraction and decontamination activities with radionuclides and non-radioactive heavy metals.

Existing thermodynamic databases used as input for predictive modeling of radionuclide behavior in the subsurface are either incomplete or completely lack data for complexation, speciation, and solubility of most mixed-waste complexes and compounds. To determine these missing thermochemical constants and the redox stabilities, we use organic ligands. Potentiometric titrations and spectrophotometric

measurements will aid in determining the complex formation constants and the kinetics of these metal-organic systems.

Health Effects

LBL's prominent program in indoor environmental risk entails investigations into the influence of radon, combustion pollutants and volatile and semi-volatile organic pollutants. In an effort to develop a methodology for identifying high-radon areas of the U.S., we have begun using large scale data sets to examine on what scale statistically-based methodologies can be successful using data from Minnesota, New York and Washington. The objective is to estimate concentration estimators at the census tract level (about 4000 people). Such a methodology could then be applied across the U.S. to localize areas where people are receiving the highest exposures. We anticipate that this approach can also be applied to other geographically linked parameters.

Analysis of the field data on exposures to toxic volatile organic compounds from environmental tobacco smoke in office buildings, which are being made in collaboration with the California Department of Health Services, will be completed. We will also continue our research to develop health-linked metrics of exposures to complex environmental mixtures and mechanism-based risk assessment models that utilize the latest discoveries in mechanisms of carcinogenesis.

We also plan to initiate efforts to model indoor exposures to toxic VOCs, polycyclic aromatic hydrocarbons, and respirable particles from environmental tobacco smoke using the data from the population-based U.S. radon survey and the emissions factors that we have recently measured for these species in our room-size environmental chamber. This effort should enable us to identify subpopulations (e.g., children, by geographic area) with the greatest exposures to these species and most at risk for health effects associated with exposures to ETS, including respiratory illnesses and asthma.

* * *

The plan for studying the molecular mechanisms of cellular effects of heavy ions consists of the following objectives:

- Complete experiments with DNA-protein crosslink-sensitive cell lines to examine dose- and LET-dependent yield after high-LET ions from the 88" cyclotron.
- Continue gas chromatography-mass spectrometry analysis to characterize DNA-protein crosslinks induced by high-LET radiations.
- Examine cell-cycle dependence of chromatin breaks, DNA-protein crosslinks and DNA-DSB yields following a high-LET radiation exposure.

* * *

Resource for Molecular Cytogenetics

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 WCPs coupled with automation may facilitate monitoring of DOE workers for genetic damage as required by recent legislation; 2) Identification of genetic aberrations in human tumors. 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) Genetic 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 LBL Genome Center, will provide probes for these studies.

Partnerships are critical to the success of the Resource for Molecular Cytogenetics. Resource staff collaborate with numerous groups throughout the United States and the World. Active collaborations include:

UCSF/LBL

Dr. Judith Campisi, Life Sciences Division, LBL, 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. Fahrid Chehab, University of California, San Francisco. Mapping mouse YAC probes for obesity gene.

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

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

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 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. A. 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. Helen Donis-Keller, Division of Human Molecular Genetics, Dept. of Surgery, Washington University. Mapping of YACs on human chromosome 7.

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. Mortimer Mendelsohn, Radiation Effects research Foundation. Hiroshima, Japan. Investigation of radiation-specific genetic changes in human breast cancers.

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.

* * *

With the development of our rapid assay to distinguish normal breast cells from their malignant counterparts, we have embarked on an ambitious new program to determine the possible underlying mechanisms of this fundamental difference. As this new emphasis will require a great deal of manpower and funds, we have decided to reduce, and to eventually eliminate, our efforts with retroviruses in the chicken model. But the refined techniques and cumulative

knowledge acquired in that project will be invaluable in studying human breast cancer. In this model, we have also succeeded in demonstrating the significance of the three-dimensional environment and the role of extracellular matrix molecules in regulation of supressor genes such as nm 23. 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.

* * *

The *in vitro* transformation of human mammary epithelial cell (HMEC) project will continue to characterize the product of the NB-1 gene which is downregulated in malignantly transformed HMEC. Like calmodulin, this protein most likely functions by binding and regulating key cellular enzymes and/or structural proteins. In the coming year, we will concentrate our efforts on the purification and characterization of the 110 kD protein to which CLP binds in a calcium dependent manner. We will compare the protein to known calmodulin binding proteins in this size range. Using standard chromatographic techniques, we will attempt to obtain sufficient quantities of the 110 kD protein for sequencing and inoculation of host animals for polyclonal antibody production. We will also analyze the ability of the protein to phosphorylate itself, and other common substrates in the presence and absence of CLP, calmodulin, +/– calcium.

We are also initiating a new line of investigation designed to determine how local cellular microenvironment influences the growth and differentiated status of normal and malignant HMEC. Studies in other tissues and cell types indicate that growth and differentiated properties depend upon a complement of general and cell type-specific transcription factors, which are influenced, in turn, by extracellular factors such as cell shape, cell-cell interactions, and cell-extracellular matrix interactions. By examination of the factors which regulate expression of a differentiated gene product in normal and malignant breast epithelial cells, we hope to identify changes which are causally related to the loss of coordinated growth and differentiation observed during development and progression of breast cancer. Expression of the lactoferrin gene has previously been shown to be downregulated or absent in a large proportion of human breast tumor specimens. Because lactoferrin is expressed at most functional stages of normal mammary development, its expression can be examined in HMEC from breasts of non-pregnant, non-lactating individuals. We will use lactoferrin gene expression as a model system to identify regulatory factors which behave differently in normal and transformed HMEC.

* * *

During the next two years 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 b-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.

* * *

The objectives the Risk Assessment—Carcinogenic Potency Project are to improve methods of identifying carcinogenic hazards to humans, compare possible hazards from a wide variety of exposures in order to broaden the perspective on risk, and make the best possible use of available bioassay data to investigate interspecies extrapolation in carcinogenesis. In several papers we have discussed that current regulatory policy is based on the idea that rodent carcinogens are potential human carcinogens; however, the chemicals tested for carcinogenicity in rodents have been primarily synthetic. Our ranking of possible carcinogenic hazards (HERP) for known rodent carcinogens indicated that when viewed against the large background of naturally-occurring rodent carcinogens in typical portions of common foods, the residues of synthetic pesticides or environmental pollutants rank low.

Since only a small proportion of the many chemicals to which humans are exposed will ever be tested in carcinogenesis bioassays, it is important to identify those that might be of greatest possible hazard to humans at prevailing levels of human exposure. One strategy for choosing chemicals to test is to prioritize chemicals according to how they might rank in terms of possible hazard if they were to be identified as rodent carcinogens. We have shown that estimates of carcinogenic potency from rodent bioassays are constrained to a narrow range about the high dose tested (MTD). Thus, knowing the dose at which a chemical is toxic can be used to approximate carcinogenic potency, and we are using an index HERT (ratio of Human Exposure/Rodent Toxicity) to identify chemicals for carcinogenicity testing. HERT uses readily available LD50 values to measure toxicity. We have found exposures for approximately 78 chemicals that occur in concentrations greater than 10 ppm in 25 foods commonly consumed in the U.S. and that have not been adequately tested for carcinogenicity. Some of those that rank highest are: caffeine (from coffee, tea and chocolate), alpha-chaconine and alpha-solanine (from potato), trigonelline (from coffee), theobromine (from chocolate), geraniol (from carrot), 2-methylpropanoic acid (from strawberry jam), and furfuryl alcohol (from cooked foods).

In keeping with our program goals, we have begun a collaborative, LDRD-funded project with 3 other divisions at LBL, to develop a methodology for integrating health risk assessments and economic analyses into the decision-making process that establishes remediation priorities and selects technologies for clean-up at federal sites. The product of this work will be a methodology that integrates scientific understanding of contaminant exposure pathways and human health effects with economic analyses and site remediation planning. Our integrated approach is aimed directly at reducing risk rather than merely at meeting concentration targets for water, soil, or air. This methodology will be used to build a workstation-based information network in an object-oriented format to be used as a management tool and a medium for informing the public about relative risks, benefits, and options. LBL and McClellan Air Force Base have formed a partnership to achieve the project objective. In the first year of this project we will use our exposure estimates at a McClellan test site to develop estimates of possible carcinogenic hazards (HERP), and then compare these with the other exposures in our ranking in order to help guide priority-setting. For example, we will rank possible carcinogenic hazards from pollutants that migrate from the site into homes, then compare these with possible hazards from other chemicals or from exposures to the same chemicals in indoor air from U.S. homes and offices.

General Life Sciences

LBL's Human Genome Center is developing into one of the world's premier incubators of sequencing technologies. As the Center increases its focus on genomic sequencing, the integration of automation, instrumentation, and informatics becomes even more critical. With initial construction money for the Human Genome Laboratory in the FY 1994 budget, ground breaking is anticipated in October 1995 and completion scheduled for 1998. Until this space is available, production sequencing will come online in renovated LBL space in Building 64, with beneficial occupancy scheduled for Fall 1994. Engineering laboratories and informatics space have been occupied in Building 74, helping to provide integration of key personnel and also easing the transition of new instrumentation from design prototype in Engineering to an experimental prototype used by biologists in Building 74. This intermediate step enables minor design changes to be incorporated before the instrumentation comes into production. We have used this step-wise process successfully for our major instrumentation, including the image acquisition system, the colony picker, and the large-scale PCR apparatus.

In order to position the Center for continued expansion in sequencing capability, it is critical to be actively involved in developing new technologies, as well as providing direct support for the current production facilities. Strategies and techniques that will permit sequencing of 50 to 100 Mb over several years are still not adequate to sequence the entire human genome of 3 Bp. Our strategic approach will assure that advanced methods for sequencing and mapping will be efficiently incorporated as they are perfected.

Robotics and automation support for mapping and sequencing scale-up will continue. A major development issue in the near term involves the integration of various automation modules into systems capable of performing specific functions, e.g., PCR gel assays. A key issue in this effort will be the implementation of procedures to acquire and track data associated with the large number of samples encountered during a normal work cycle. An important innovation will be the use of bar-codes as a method for sample identification and tracking.

Efforts in the area of enhanced hardware for fluorescence sequencing will continue with an emphasis on exploiting the larger potential throughput by mating the sequencer with specialized hardware for sample loading. In addition, more sophisticated software for data acquisition and base-calling will be developed. Applications in the areas of large-scale genetic mapping will be explored in conjunction with the biologists.

Second generation versions of the thermal cycler and oligosynthesizer are currently being planned for eventual fabrication and testing. Finalization of these plans will depend upon results obtained from extensive testing of the existing prototypes.

* * *

As a spin-off of the forty-year investment in research on yeast genetics at LBL, Dr. Mortimer has begun to pursue an opportunity of great potential value to the wine industry of California, as well as to the rest of the world's prominent wine producers. Recent studies in California and Europe have established that the flavors of wines are at least partially dependent on the strain or strains of yeast carrying out the fermentation of the grape must. Dr. Mortimer has demonstrated that it is possible to cross natural strains and thus establish a breeding program to obtain more optimum yeast strains. During the past two years Dr. Mortimer has worked on a new project in collaboration with a group in Italy. These investigators have isolated over 100 strains of yeast from natural fermentations in various wineries in the region of Emilia Romagna and from other regions of Italy. They have characterized these yeast strains for production of compounds of interest to the wine industry such as H_2S, SO_2, acetoin, higher alcohols, acetic acid and acetaldehyde. Dr. Mortimer has carried out detailed genetic analyses on 28 of these strains. He has shown that all are diploid and homothallic. About half have excellent spore viabilities and are homozygous for all the markers. The remainder have lower spore viabilities and are heterozygous at one to seven loci. He proposes that sporulation and rediploidization by homothallic switching can lead to completely homozygous strains that are free of accumulated genetic defects, a process we call "Genome Renewal" (Mortimer *et al* 1994). Most of the strains are able to ferment sucrose and maltose yet the sugars in grape must are >98% glucose and fructose. Even though all 28 strains come from a relatively small region of Italy, they are all genetically unique.

During 1993, Dr. Mortimer again spent several months in Italy on the wine yeast project. He constructed several crosses between natural strains and these were analyzed by gas chromatography for the production of certain compounds in wine. Two strains that differed more than 20-fold in the production of acetoin were crossed and this difference segregated 2:2 in 27 asci analyzed. High levels of acetoin were found to be recessive (Romano *et al.* 1994). This experiment demonstrated the feasibility of crossing natural strains and of breeding new strains based on naturally-occurring differences. Dr. Mortimer hopes to develop these studies to the point that superior wine strains can be made by crossings between various natural wine yeasts. Dr. Mortimer was awarded a RAISA fellowship from the Italian government to support his stay in Italy during 1993.

During the fall of 1993, Dr. Mortimer made collections of fermenting musts from nine California wineries and has analyzed the *Saccharomyces* yeast strains involved. Dr. Mortimer has found that even in a single fermentation, several strains of *Saccharomyces* participate. There is growing belief that this may be a contributing factor in developing the complexity associated with wines made by natural fermentation. Dr. Mortimer has been in contact with members of the UC Davis Department of Viticulture and Enology and have provided them with some strains. Dr. Mortimer is also in contact with nine California winemakers that have expressed great interest in his findings. Dr. Mortimer was recently awarded a Technology Transfer grant by LBL to continue this wine yeast research targeting the production of optimized wine yeast strains for California wine producers.

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Our major research activities in structural studies of membrane proteins, specifically membrane transport systems, have been supported by DOE. We have come to a point where we need an increase in support if major programs on structural and function of membrane proteins are to be established in LBL. We currently have leading edges in this growing and important field. In the following year, we have received support from LDRD to initiate a project on the crystallization of cystic fibrosis transmembrane conductance receptor. In addition, we anticipate receiving support from the private sectors for our research activities in the crystallization and structural studies of selected membrane proteins that are known to have clinical importance. We will also seek support

through NIH grants when we have obtained some preliminary results, which are needed for successful grant applications. We hope to receive additional support from DOE, which has long recognized the importance of research activities.

* * *

In our program of biomolecular structural analysis, the emphasis will remain on determining solution structures of biologically important molecules by NMR spectroscopy. The focus will be in three areas: 1) proteins involved in genetic regulation (particularly transcription factors); 2) self-cleaving hammerhead RNA; and 3) biologically engineered molecules prepared with specific designed/selected binding activity. In particular the structures of the HSF DNA binding domain, and the Sxl RNA binding domains will be completed. The dynamic analysis of c-jun will also be finished. More extensively isotope labelled hammerhead RNAs will be prepared, and 3D NMR will be applied to the determination of the solution structure. As engineered/genetically selected molecules become available they will be examined by NMR. The only significant area equipment need is an upgrade for the 500 MHz NMR spectrometer console. The majority of the funding has been requested from the DOE URIP but there may be some additional components to be purchased from other funds.

* * *

The Mutagenesis: Fundamental Chemistry 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.

* * *

A new project has been initiated to develop position-sensitive multi-element array detectors with integrated pulse processing channels, to provide instrumentation that is capable of fully exploiting the intense flux and specialized x-ray beams available at new and existing synchrotrons. The detector and pulse-processing electronics will be made using planar CMOS silicon technology, and will be designed for low noise, high count rate applications. Such planar devices will be geared toward biological EXAFS and protein crystallography applications. This project requires detector and electronic development in several stages, and requires a serious commitment to adapting the multi-element strip detectors, used by the high energy physics community, to x-ray applications. In addition to developing the planar silicon detectors and associated signal-processing electronics, work will continue to address the myriad of materials and processing issues which effect solid state radiation detectors.

Mass spectrometric capabilities and developing new detectors improvements are also being targeted. For the detector work, improved commercially available detectors will be tested along with the new detectors we are designing. Discrete dynode aluminum oxide ion multipliers and lead oxide based ion multipliers will be incorporated into a detector module so that their efficiency for large ions can be compared. Bolometric detectors measure the temperature jump that occurs when the kinetic energy of an ion is converted to heat during impact. New bolometers reportedly display a timing resolution of about 100 ns, a marked improvement over older versions. Several triboluminescent materials have been identified for use as substrates for molecular impacts. In this sensor, light is emitted from the triboluminescent crystal when it is fractured by an impacting ion. We anticipate that, as improvements in the mass spectrometer are achieved, a multitude of biological applications will be explored.

Medical Applications

THE CENTER FOR FUNCTIONAL IMAGING

The major future direction of the positron and single-photon-emission tomography studies is the application of new PET tracers of the neurochemistry of the brain using newly developed radiopharmaceutical agents and the high resolution PET. Specifically we will demonstrate patterns of chemical imbalance associated with mental illnesses including behavior disorders, drug abuse and craving. This program has broad implications for society including nutrition and educational strategies. We have incorporated high resolution PET with magnetic resonance for these studies and the subjects under study will expand from our present program in Alzheimer's disease and schizophrenia to Parkinson's disease, epilepsy and mental disorders such as depression. We have established strong links with the UCSF medical center in neurology and psychiatry in addition to our links through formal appointments in radiology. This brings to the LBL program the clinical experts in mental diseases to augment our present neuroscience program led by Dr. William Jagust, Neurologist, who is now an investigator at LBL.

With the siting of the biomedical isotope cyclotron at LBL in 1994, 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. New methods include placing labels at different positions on molecules and development of probes of mitochondria function, probes of messenger RNA in addition to probes of neurochemistry.

The medical applications program of CFI has included magnetic resonance applications from the standpoints of very high field studies to evaluate human cerebral cortex micro structures as well as to reveal chemical composition of the normal and abnormal tissues. This program started with an evaluation of the safety of human studies at very high fields and is now embarked on studies at 4 T with collaborators at the Univ. of Minnesota, Univ. of Alabama and Univ. of Illinois. The direction of the future will be to combine the attributes of magnetic resonance with those of high resolution PET.

In the area of PET instrumentation, the following objectives are targeted:

- Collaboration with the optical crystal growth department of LLNL on the technology maturation of scintillator crystals, and technology transfer to commercial scintillator crystal growers.

- Assembly of two working PET detector modules. Imaging of phantoms on computer-controlled test table.

- Design and construction of a 60-image-plane, 20,000-crystal positron tomograph that represents a significant advance in spatial resolution, event rate, and sensitivity. Installation at LBL in support of the Medical Science research program.

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Future directions in the studies of vascular and blood diseases center on the development of atherosclerosis imaging agents with an anticipated milestone of selection and utilization of an agent for noninvasive imaging. Other directions include the adoption of new animal models, such as transgenic or gene knockout mice, for screening imaging agents, and cytochemistry studies of megakaryocytes.

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Central to the research aims of the Heavy Ion Radiosurgery program is the effective transfer of charged-particle radiosurgery technology to medical and research centers developing the technology of stereotactic radiosurgery, particularly charged-particle radiosurgery, in the United States (e.g., Stanford University, University of California San Francisco, Loma Linda University, Harvard University, University of Washington), Canada (e.g., University of British Columbia, McGill University), Europe (e.g., University of Uppsala, Sweden; Radiological Institutes and ITEP, Moscow and Leningrad, U.S.S.R.; University of Cologne and G.S.I., Germany; P.S.I., Switzerland), and Japan (N.I.R.S., Chiba).

Emphasis for new research initiatives include investigations of dose comparisons of radiosurgical modalities and incorporation of research data obtained from the LBL heavy-ion radiosurgery program into three-dimensional treatment planning, investigation of beam delivery systems (including raster scanning and variable Bragg peak modulation), and incorporation of biophysical models into physical dose-distribution calculations.

Collaboration continues with more than 150 hospital centers throughout the world (North America, Europe, South America, and Japan) and is assured for the coming years; several hundred referring neurological physicians and surgeons serve as a referral base for our clinical research program. The multi-institutional protocol has been interrelated with the neuroscience protocols of Stanford University, University of California San Francisco, the VA Medical Center in Palo Alto, California, and the Merritt-Peralta Medical Center, Oakland, California.

A collaborative multi-institutional program has been initiated with Loma Linda University Medical Center and Stanford University Medical Center to transfer the proven technology and expertise required for charged-particle radiosurgery of AVMs to the hospital-based-accelerator environment.

Due to the death of Program Principal Investigator Jacob I Fabrikant, M.D., Ph.D. on May 2, 1993, direction of the program now continues under Richard P. Levy, M.D., Ph.D.

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

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

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

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

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

Finally, ongoing research in lipoprotein structure and metabolism, both in our laboratory and other institutions, holds promise for the development of other specific receptor ligands that would be of considerable interest as PET imaging agents. Examples include probes for atherogenic lipoprotein species, such as oxidatively modified apoB-100 and apo(a), and anti-atherogenic components, such as the major HDL protein, apoAI.

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Major Foreign Meetings

Blakley:

COSPAR (Committee on Space Research) 1994, 10-22 July, 1994, Hamburg, Germany.

Budinger:

BAT-SHEVA Seminar on Functional Brain Imagining, 5–10 June 1994, Tel Aviv University, Israel.

4th Int. Symposium in Technetium in Chemistry and Nuclear Medicine, 12–14 Sep 1994, Bressanone, Italy .

Osteoarthritis Symposium, 5–7 October 1994, Montreal, Canada.

Campisi:

International Symposium on Aging, Immortalization and Neoplastic Transformation of Human Cells. April 21–23, 1994. Okayama, Japan.

Derenzo:

"Physical Processes in Fast Scintillators," September 1994, St. Petersburg, Russia.

Huesman:

ECAT (positron tomography) Technical Users' Meeting, 13–16 April 1994, Brussels, Belgium.

Krauss:

International Atherosclerosis Society meetings, October 1994, Montreal, Canada.

Wemmer:

International Conference on Magnetic Resonance in Biological Systems, Summer 1994, The Netherlands.

Bissell:

ICOB; 14–15 March 1994, Victoria, BC.

Hinterzartener Kreis fur Krebsforschung; 24–27 April 1994, Starzacha-Weitenburg, Germany.

EMBO: Growth & Differentiation of Epithelial Cells; 10–14 September 1994, Genova, Italy.

British Society for Cell Biology, 18–21 September 1994, Manchester, England.

Int'l Society for Differentiation, 22–26 October 1994, Hiroshima, Japan.

G.
Work for Others

G. Work for Non-OHER Organizations

The success of DOE life and environmental sciences programs at LBL 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 LBL 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 chlorophylls 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 Conservation and Renewable Energy (CRE)

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 LBL 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. This work is complementary to OHER-sponsored studies in analytical technology and health effects related to indoor exposures to radon and other 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 LBL.

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 LBL'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, LBL 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 LBL 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 LBL 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 LBL 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 LBL to assess the health risks of space exploration. The nation's need for LBL 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 LBL 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 LBL its Specialized Center for Research and Training (NSCORT) in Radiation Health. 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 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 excel-

lent 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 LBL 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. LBL 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.

LBL 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.

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

Analytical Technology

J. Daisey, L. Gundel	EPA Annular Denuders to Collect Semi-volatile Organics	14,200
E. Haller	NASA, Far-Infrared Semiconductor Detectors and Materials	$180,000
E. Haller	NASA Evaluation of Pyroelectric Materials	$50,000
E. Haller	NASA, Far-Infrared Advanced Photoconductors	$96,000
A. Hodgson	CPSC Volatile Organic Chemical Emissions from Carpet Cushions	$58,000
N. Madden	NASA Various Germanium Detectors for Space Applications	$470,000
R. Sextro	EPA Radon Entry into Florida Homes	$103,400
J. Walton	NASA Various Special Silicon Detectors	$244,000

Health Effects

E. Alpen	NASA Tumorigenic Potential of HZE Radiations	$143,554
A. Chatterjee	NASA Specialized Center of Research and Training	$650,281
S. Curtis	NASA Deep Space Flight Radiation Risk Assessment	$61,111
S. Curtis	NASA Risks to Normal Organ Function from Solar Particle Events	$69,016
S. Dairkee	EPA grant Mammary Epithelial Cell Transformation as Defined by Monoclonal Antibodies	$86,806
J. Daisey	NIH grant Environmental Tobacco Smoke: Physico-Chemical Properties	$265,600

J. Daisey	UCB/NIEHS Soil Gas Transport: A Mechanism of Indoor Exposures to VOC	$272,200
J. Daisey	CARB Toxic Volatile Organic Compounds in Environmental Tobacco Smoke	$118,600
J. Daisey, R. Sextro	UC Tobacco-Related Disease Research Program, Characterization of Particulate-Phase ETS in Differing Environments	$169,900
P. Durbin	NIH grant Biological Evaluation of New Actinide-Chelating Agents	$94,388
R. Goth-Goldstein	EPRI New Strategy for Coal Tar Remediation	$55,230
A. Kronenberg	NIH grant Heavy-Ion Mutagenesis	$46,878
A. Kronenberg	NASA Mutagenesis in Human Cells with Accelerated H & Fe Ions	$96,065
R. Liburdy	NIH grant High Field NMR Bioeffects: Lymphocyte CA2+ Metabolism	$71,800
J. Miller	NASA Experimental Study of Nuclear Interactions Relevant to High Energy HI Transport	$243,056
R. Schwarz	CRADA - Amgen Tendon Repair Factors	$52,276
M. Stampfer	NIH grant Characterization of Human Mammary Cells	$222,162

General Life Sciences

M.H. Barcellos-Hoff	NIH grant Stromal Influence on Expression of Preneoplasia	$73,109
J. Bastacky	UC Tobacco-Related Disease Research Program. Effects of Tobacco on Rat Lung Alveolar Lining Liquid	$121,604
J. Bastacky	Subcontract from MIT	$8,333

E. Berry	NIH grant 3-D Crystals of Cytochrome Reductase: A Membrane Protein	$77,319
M. Bissell	UC President's Postdoctoral Fellowship Program - Z. Pettway	$30,220
M. Bissell	UCSF NIH subcontract Role of Metalloproteinases in Mammary Gland Remodeling	$86,477
Z. Cande	NIH grant A West Coast Facility for IVEM	$22,801
J. Campisi	NIH grant Cellular Senescence and Control of Gene Expression	$148,496
J. Campisi	NIH grant Growth Requlation in Normal and Transformed Cells	$124,590
J. Campisi	NIH grant Senescent- and Longevity-Modulating Genes	$152,133
J. Conboy	NIH grant Red Cell Band 4.1: Dev. Changes in RNA Splicing	$140,818
K. Downing	NIH grant Tubulin Structures by Electron Crystallography	$101,990
R. Glaeser	NIH grant Membrane Proteins: High-Resolution EM	$466,980
E. Golder	AHA Maturation of HDL by LCAT in Transgenic Mice	$16,500
T. Head-Gordon	USAF grant Neural Networks as a Predictive Tool for Constrained Optimization Protein Folding	$35,727
M. Maestre	NIH grant Physical Structure of Viruses	$202,318
R. Mortimer	NIH grant Molecular Analysis of X-Ray Damage and Repair in Yeast	$152,287
M. Narla	NIH grant Red Cell Deformability *In Vitro* and Survival *In Vivo*	$127,186

M. Narla	NIH grant Red Cell Membrane Studies	$974,266
M. Narla	Univ. British Columbia Subcontract Rheological and Adherence Properties of Sickle Cells	$144,434
M. Narla	UCSF/NIH grant Cellular Consequence of Hemoglobin Membrane Interaction	$61,770
M. Palazzolo	UCB subcontract (NIH) Drosophilia Genome Center	$1,142,603
M. Palazzolo	NIH grant Drosophilia Genome, Phase III, Sequencing Technology	$127,814
E. Rubin	AHA grant Analysis of HDL Atherosclerosis in Transgenic Mice	$61,400
E. Rubin	UCSF/NIH subcontract Construction of a Transgenic Mouse Model for Sicle Cell Anemia	$184,704
G. Shyamala	NIH grant Normal and Neoplastic Growth: Role of Estrogens and HSP90	$151,123
S. Scherer	NIH grant Mobile DNA in Candida Albicans Biology and Epidemiology	$99,825
B. Singer	NIH grant Alkylation of Polynucleotides *In Vitro* and *In Vivo*	$147,125
B. Singer	NIH grant Biochemical Mechanisms of Vinyl Chloride Carcinogenesis	$214,117
L. Stoltzfus	NIH grant In Vivo Effects of Apolipoproteins AI Variance on HDL	$31,200
D. Wemmer	NIH grant National Tritium Labeling Facility	$616,544
P. Yaswen	NIH grant Calmodulin - Related Gene and Epithelial Transformation	$134,265

Medical Applications

J. Alonso	NIH grant Design Study for UCD Proton Facility	$250,521
J. Bielicki	AHA grant Extracellular HDL Assembly with Apolipoprotein AI Variants	$21,600
T. Budinger	NIH grant Cardiovascular Flow and Metabolism	$659,426
T. Budinger	NIH grant Cerebral Blood Flow Patterns in Alzheimer's Disease	$204,621
T. Budinger	NSF subcontract New Technology for High Field NMR Imaging and Spectroscopy	$20,255
T. Budinger	NIH training grant Quantitative Cardiovascular Research	$101,400
M. Callow	AHA grant Atherogenesis in Mice Expressing Lp(a)	$21,600
J. Castro	NIH grant Treatment of Cancer with Heavy Charged Particles	$262,559
P. Coxson	NIH Grant Statistical and Modeling Uncertainties in Dynamic PET	$72,598
S. Derenzo	NIH grant Search for Ultrafast Heavy Atom Scintillators	$197,254
T. Forte	NIH training grant Lipoprotein Methodology, Structure and Function	$115,771
T. Forte	UC Tobacco-Related Disease Research Program, Effect of Cigarette Smoke on High Risk Lipoprotein Profiles	$100,820
B. Ishida	NIH grant Pre-Beta Migrating High Density Lipoproteins	$77,971
W. Jagust	NIH grant Alcohol and Memory: A PET Study	$123,755

R. Krauss	NIH grant Lipoprotein Subclasses: Structure, Origin, and Metabolism	$1,135,720
R. Krauss	Children's Hospital Subcontract Genetic Influences on Lipoprotein and Atherogenic Responses to Dietary Fat	$494,234
R. Krauss	Sequoia Hospital Subcontract Metabolic Atherosclerosis Magagement	$324,416
R. Marshall	NIH grant Fluorodeoxyglucose Kinetics in Myocardium	$165,431
W. Moses	NIH grant High Resolution PET Dectors Using Solid State Photonics	$164,466
A. Nichols	NIH grant Apolipoprotein-Specific HDL and Cholesterol Tansport	$209,401
T. Nordahl	NIH grant Hippocampal Metabolism in Schizophrenia	$94,264
M. Roos	NIH subcontract Development of NMR Assisted Cryosurgery	$52,043
T. Sargent	NIH grant Methyl Carbon Pathway in Psychosis	$50,350
D. Tribble	UC Tobacco Research Cigarette Smoke Exposure and Antioxidant in Blood	$46,000
P. Williams	NIH grant Effects of CHD Prevention on Lipoprotein Subclasses	$49,370
P. Williams	NIH RCDA Effects of Exercise, Diet and Fat Lost on Lipoproteins	$60,580
P. Williams	NIH grant Weight Set Point and HDL Concentration in Runners	$188,310

Laboratory Directed Research and Development

W. Chu	Biophysical Modeling for Optimization Treatment Plans	$95,000
T. Forte	Environmental Air Pollutants and Oxidative Stress	$87,000
T. Head-Gordon	Comprehensive Theoretical Approach to Protein Folding	$100,000
M. Maestre	Single DNA Molecule Chemistry	$50,000
J. Rine	Genetic Map of Canis Familiaris	$100,000
M. Roos	Head Probe for Nuclear Magnetic Resonance Imaging and Spectroscopy at 10 T	$50,000
E. Rubin	Creation of Transgenic Mice	$190,000
D. Tribble	Variations in Susceptibility to Environmental Oxidants as Studied Using Transgenic Mice Models	$125,000

H.
Critical Issues

H. Critical Issues

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, LBL Director

This summarizes also the mission of LBL's Biological and Environmental Research Program.

LBL's Vision 2000 establishes the overarching goals of the Laboratory's strategic planning effort. It focuses on the DOE as our primary sponsor, but also establishes a commitment to reaching out and forging new partnerships with industry, academia, and other national laboratories to create value for the economy, enhance education, and contribute to the community. The four major goals of Vision 2000 are as follows:

Distinguish ourselves as a premier DOE multiprogram national laboratory by performing research of the highest scientific quality. Build on our educational and technical resources to gain a competitive advantage for addressing problems of national significance and advancing the mission of the DOE.

Create value for the economy, enhance education, and contribute to the community through partnerships with industry, universities, and other laboratories.

Make LBL the location of choice for facilities and programs. Our operational, administrative, and technical resources will integrate seamlessly with the research and engineering programs to make an LBL that works. All of our activities will be conducted with full regard for the environment, health, and safety.

Commit to developing our people to their fullest potential. We value and seek diversity in our work force. We will create an environment that respects the individual, encourages leadership, stimulates innovation, fosters integrity, and demands excellence.

To achieve these goals, LBL must understand the context of its research programs within the framework of DOE, other national laboratories, and the nation, and must incorporate this information into our strategic planning effort. LBL's mission identifies biology and material sciences as the two major areas of emphasis and growth.

Based on its core competencies, LBL can make major contributions to the mission of the Office of Health and Environmental Research. In particular, LBL'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. LBL's Human Genome Center is a national source of expertise in automated techniques for mapping human chromosomes, sequencing selected human-gene DNA fragments, and improving data analysis and interpretation. The Advanced Light Source will give biologists access to the world's brightest source of soft x-rays and new beam lines for x-ray crystallography, strengthening DOE's national position in structural biology. LBL's research medicine programs provide new insights using imaging and molecular nuclear medicine research techniques and developing the highest-resolution diagnostic systems in the world. Cell and molecular biology research involves pioneering research in understanding differentiation and malignancy in selected areas which complements the expertise in Berkeley campus. Environmental research capabilities in pollution sensing, transport, effects, and cleanup promise to reduce costs for environmental remediation problems.

Trends and Opportunities

ANALYTICAL TECHNOLOGY

Because of the gradual shift in OHER priorities over the past several years, this category is being reevaluated in terms of its relevance to the OHER mission. Budget constraints have forced the airborne radon project at LBL to continue at a reduced level of funding and constrained scope of work.

OHER is beginning to recognize the importance of detector development for effective synchrotron research. LBL researchers are working closely to put together a coordinated program in detector development.

HEALTH EFFECTS

Continued support is available to improve information on public health risk from mining and residential radon exposures, while expanded support is needed for studies of molecular mechanisms involved in radon-induced lung cancer. Increased funding for new molecular-based tools for health surveillance and biological dosimetry are being sought while DNA repair and genetic susceptibility research have been identified as critical areas for development. There is also a need for the development of human cell culture models to facilitate the study of cancer initiation and progression. LBL has developed a rapid and accurate assay system for distinguishing normal and malignant breast cancer cells.

GENERAL LIFE SCIENCES

Along with the advances emanating from LBL's Human Genome Center comes the need for significant expansion of support—particularly the support for LBL's Human Genome Laboratory construction. Groundbreaking is scheduled for mid-1995 with completion by January 1998. Within the next five years, LBL will seek a doubling of the current level of funding. We anticipate an increased emphasis on our unique multi-disciplinary capabilities—large-scale sequencing with advanced instrumentation and computational support systems. Genome management is considering a redirection of our megabase sequencing effort to include chromosomes 5 and 20.

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 being formulated at LBL to develop the potential structural biology resources at the Advance Light Source. To ensure the success of LBL's Structural Biology Initiative, additional support is required for beamline construction and upgrades at the ALS Structural Biology Support Facility.

A coordinated program in computational biology is needed to link the advancements of biological sciences with computer and information science at LBL. Such an endeavor crosses all biological and biomedical research activities sponsored by OHER and will encompass databases of human and model organism 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, and new computational simulations. Although no funding is expected in FY 1995, LBL will be seeking on the order of $2 million from OHER to launch a computational biology initiative in FY 1996.

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 Nuclear 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 cyclotron will aid in the development and production of these reagents.

The National Biomedical Tracer Facility (NBTF) will maintain U.S. research infrastructure for stable and radioactive tracers that are of vital need in biomedical research including metabolic studies, diagnostic imaging, and other applications. The NBTF will benefit from LBL's strengths in accelerator systems design and engineering, biomedical applications of isotopes, and radiopharmaceutical chemistry. The facility would be based on a proton accelerator with a maximum energy of 100 MeV and a current of 500 to 750 μA. The facility would include isotope processing and radiochemical laboratories and supporting infrastructure. The facility will take advantage of many of the Laboratory's existing resources, including utilities, shielding, and some of the components of the recently closed Bevalac accelerators. Once established, the NBTF will serve as a center for a consortium of institutions invested in this important national project. OHER is interested in developing programs in Molecular Medicine and LBL is in a strong position to compete. We already have received new funding for this effort and intend to expand the program.

Strategic Partnerships

Partnerships with other national laboratories, government agencies, and state governments will contribute to successful strategic planning for the national laboratories. LBL'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. LBL'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 LBL's role can be facilitated by DOE management policies that enable the national laboratories to work more effectively with industry and with other government agencies, such as the National Institutes of Health, the Environmental Protection Agency, the National Aeronautics and Space Administration, and the Defense Advanced Research Projects Agency. LBL has identified the following issues that affect our ability to fulfill the laboratory's mission:

- Implement DOE's mission for technology research. DOE programs need to more fully support research that is broadly responsive to the long-term economic vitality of the nation. LBL is working with DOE and other national laboratories to develop programs that make full use of current technical capabilities to benefit the nation.

- Enable industrial R&D partnerships. In the past, DOE organizational structures and policies have impeded the timely development of cooperative research with industry. 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. LBL 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 Berkeley campus 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. For example, effective utilization of the Advanced Light Source will require additional beamlines and the development of the second-floor offices and laboratories for users. Utilization of these capabilities is required to gain the full benefit of the investment in these unique national facilities.

Lost Technology Transfer Opportunities

Opportunities for fostering productive partnerships between LBL's biosciences researchers and the U.S. biotechnology industry are being unjustifiably constrained. Funding to seed these collaborative relationships is not available in timely allocations. Also, too much emphasis is placed on existing technology transfer mechanisms which are geared toward conveying to the marketplace "on-the-shelf" technologies developed at the Laboratory. We must transfer technology earlier in the development equation to more effectively contribute to the viability of small biotech businesses.

Out of the nation's top one hundred biotechnology companies, only five are currently profitable. Most biotechnology companies are smaller concerns unable to develop in-house the higher risk enabling technologies necessary to bring their products to the marketplace. The role of the national laboratories can be made more effective by providing the funds to leverage our expertise and unique facilities to complement the needs of this nascent industry—thus aiding the advancement of small biotechnology enterprises toward profitability. DOE must begin to recognize the value of investing in biotechnology partnerships at an earlier precommercial stage. We can then work together to develop new, more appropriate mechanisms so that we may play a more significant role in preserving the nation's preeminence in the biotechnology industry. Integrated planning between the Energy Research-Laboratory Technology Transfer office and OHER may enable a more effective means of tapping into biotechnology partnerships.

Constraints on New Initiatives

LBL'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.

LBL 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 LBL's support services while maintaining its high level of research capabilities and performance.

Limited DOE resources set constraints on the range and scope of LBL'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. Examples from three key DOE program areas illustrate the Laboratory's priorities for the Office of Health and Environmental Research. LBL's Human Genome Center and the Advanced Light Source give biologists access to advanced technology needed to address fundamental life science challenges: to understand the genetic and structural basis of energy-derived and environmentally derived health issues. Fundamental studies in cell and molecular biology complement and complete our quest to understand gene expression.

NIH Redux—Good News/Bad News

A significant portion of Life Sciences funding at LBL comes from the National Institutes of Health: some $14 million for FY 1993, compared to $12.4 million from OHER. The good news is that since last year's writing, a memorandum of understanding (MOU) has been signed waiving the 3.2% added factor and depreciation for all NIH grants. However, the bad news persists: LDRD costs will be allowed, but will not be funded by NIH. This means that the investigators must absorb the cost. The MOU issue needs to be revisited with the new NIH head.

DOE is currently considering a 7% overhead charge on capital equipment, including equipment purchased on NIH grants and other work-for-others projects. This would present yet another impediment to our significant partnerships with NIH. Because of the integral role NIH plays in support of the Life Sciences at LBL, it is critical that we at LBL move now to begin negotiations with DOE and NIH. We cannot effectively pursue our innovative research without adequate funding for capital equipment, particularly in support of new starts and expanding programs.

Developing Alternative Funding Sources

The major critical constraint to the further development of several OHER projects at LBL, 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. For example, the senescence-specific marker we recently discovered is an ideal candidate for a small grant from AFAR, the American Federation for Aging Research ($25,000–35,000, no indirect costs paid). Even this small amount of funds could make a difference in single investigator-driven research. DOE is unlikely to be interested in this marker until we have some preliminary data indicating that it can be used to isolate novel tumor suppressor genes. We are of course planning to obtain preliminary data of this sort, but, in the absence of dedicated funding, progress will be necessarily slow.

Other small 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 is an example of being penny-wise and pound-foolish. Moreover, it places economics above scientific considerations. With their 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.

Improving the Scientific Basis for Risk Assessment

Risk assessment is being used increasingly to make decisions regarding environmental policies and relative risk has been proposed as a more rational and cost-effective means to manage our resources for the protection of society from the most significant environmental risks to human health, welfare and ecological resources. Although this is a very reasonable approach, it often founders on a lack of critical information on the two major components of risk assessment: (1) Exposures assessment for environmental pollutants and physical agents, and (2) Health risks at low exposure levels.

DOEs energy policies can have a very significant impact on the environmental health of the population. The most significant environmental health risks of the population in general are from pollutants generated by energy production, use and conservation, e.g., combustion of fuels for transportation, electrical generation and transmission, space heating and cooling. Examples of such pollutants include airborne respirable particulate matter, ozone, polycyclic aromatic hydrocarbons, PCBs, radionuclides. Impacts of energy-related pollutants on the global environment, e.g., the ozone layer, as well as exposures to and risks from electromagnetic fields associated with energy transmission and use are also of concern. These widespread exposures of he population also provide the background risks against which DOE must set priorities for its environmental restoration activities.

As concerns about the health of our global environment grow, increasing demand will be made upon DOE to evaluate the environmental risks associated with its energy policies and to develop national energy policies that minimize such risks. DOE/OHER already conducts research on many energy-related exposures and risks. However, in view of changes in the mission of DOE, it would be timely to undertake a focused effort to reevaluate the environmental agents of most concern to DOE and to establish an *integrated and targeted* research program to fill the gaps in the data required for risk assessment and for establishing sound and effective energy (and environmental) policies. A targeted research program on risk assessment should not be confused with research in which the scope and methods of the effort are dictated in advance by research managers of an agency (Office of Technology Assessment, 1993). Such research should also not be restricted to simple

categories of basic or applied. A targeted and integrated research program on risk assessment would require identification of goals and development of a research plan to support these goals.

We suggest (and would strongly support) an effort by DOE/OHER to develop a targeted and integrated research program to improve the scientific basis for risk assessment. Such an effort would require identification of the environmental problems and agents of most concern to DOE, identification of the key gaps in our scientific understanding, development of a set of strategic goals, and an integrated research plan to provide the scientific information needed to reduce the uncertainties in assessment of risk for the most important environmental agents. Such an effort could involve a collaboration of DOE research managers, scientists from the DOE laboratories, and even other federal agencies, e.g., EPA, with responsibilities for environmental policies and regulations.

Toward Mitigating Bureaucracy

DOE, LBL, and UC should work toward eliminating redundancy of reviews and direct their efforts to improve performance within a well-defined management framework. DOE should work to implement proposed rules that clarify implementation and reduce redundant paperwork presently required for the National Environmental Protection Act (NEPA) compliance and should conduct NEPA document reviews and issue determinations in a timely manner. The system of DOE orders should be revised to reduce redundant paperwork requirements and to clarify and improve the efficiency of operations:

- **Paperwork reduction.** The existing framework for achieving DOE goals for partnership and environmental compliance is counterproductive and results in delay and indecision. The Cooperative Research and Development Agreement paperwork chills the relationship with industrial partners and NEPA paperwork defers the achievement of program goals rather than supporting effective decision making.

- **Excessive reviews.** LBL program and support activities are subject to more than 300 reviews annually. These costly reviews reduce productivity and defer necessary actions. DOE should streamline its review action by emphasizing biennial and triennial reviews.

- **Redundant oversight.** LBL is subject to review by the General Accounting Office, the DOE Inspector General, headquarters, and Field Operations, other federal, state, and local agencies, the University of California, and its own internal auditing and review procedures. The current contract between the University of California Regents and the Department of Energy calls for the elimination of redundant oversight. There is no evidence in the development of DOE oversight functions that this requirement has been addressed.

DOE, LBL and UC need to work to better understand the drivers of indirect costs. Opportunities for consolidating redundant and/or non-value-adding activities need to be identified. Overlap in DOE Orders is causing inefficient use of indirect funding while the DOE controller's office is working to reduce costs. Rigorous implementation of the new DOE Directives Process is needed to ensure that the cost impact and usefulness of proposed orders are identified before they are issued as final requirements. Better coordination between DOE Offices of Principal Interests (OPI) is also needed to eliminate conflicting and overlapping requirements.

Working Together to Enhance Communications

As the Secretary of Energy undertakes a critical assessment of the national laboratories, it becomes even more crucial for LBL to demonstrate the value of its basic scientific and technology development endeavors. That burden falls on LBL to aggressively promote its achievements, not only throughout the scientific and industrial communities, but also amongst the wider community of the tax-paying lay public. As important, is the need for LBL to convey its achievements back to the OHER Program Directors and the wider DOE audience in a timely manner. This represents an area of significant opportunity for LBL. We recognize that we must improve our performance here. To be most effective, a commitment must be made to establish an on-going dialog between the Laboratory and OHER, so that quality communication becomes systematic.

I. Resource Quantitation

I. Resource Quantitation

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 1992		FY 1993a		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[b]	30.1	59.0	31.5	93.0	33.0	97.7
Post-doctorates	3.4	5.5	4.2	15.0	3.0	24.2
Technicians	18.7	41.0	20.4	64.0	26.6	83.3
Animal care	0.7	2.2	3.2	3.2	0.5	2.2
Total direct personnel	52.9	107.7	59.3	175.2	63.1	207.4
Professional (adm.)	6.6	8.0	11.0	15.0	11.6	15.8
Clerical, adm. support	6.1	9.3	15.0	17.0	16.0	18.1
Maintenance	4.6	5.2	—	—	—	—
Total indirect personnel	17.3	22.5	26.0	32.0	27.6	33.9
Visiting scientists[c]	29.0	53.0	10.0	17.0	23.5	40.0
Graduate students[c]	23.0	34.0	9.0	17.0	11.0	20.8
Undergraduates[c]	48.0	100.0	20.0	58.0	22.3	64.8

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	60	279	117
Chapters, reviews	13	7	22
Books, proceedings (edited)	3	—	22
Reports/documents	0	8	30
Presentations at technical meetings	121	202	105
Meetings organized	0	—	0

[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:

4 M.D.
51 Ph.D
24 MS/BS
1 D.V.M.

[c]Data given as head count (number of participating individuals).

Structural Biology

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	3.0	25.0	5.0	25.0	5.0	26.0
Post-doctorates	1.5	25.0	1.0	26.0	1.0	26.0
Technicians	0.5	2.0	0.5	2.0	3.0	6.0
Animal care	0.0	0.0	0.0	0.0	—	—
Total direct personnel	5.0	52.0	6.5	53.0	9.0	58.0
Professional (adm.)	0.5	1.6	0.5	0.5	0.5	0.5
Clerical, adm. support	1.0	5.0	0.5	5.5	0.0	5.5
Maintenance	0.2	1.4	0.2	1.4	1.4	1.4
Total indirect personnel	1.7	8.0	1.2	7.4	1.9	7.4
Visiting scientists	0.0	1.0	0.0	2.0	—	2.0
Graduate students	6.0	40.0	5.5	47.0	10.0	32.0
Undergraduates	10.0	15.0	5.0	15.0	9.0	17.0

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	73	65	37
Chapters, reviews	0	—	—
Books, proceedings (edited)	0	—	—
Reports/documents	10	10	1
Presentations at technical meetings	21	32	25
Meetings organized	0	—	—

[a]Number of OHER-sponsored scientific professionals by highest degree:

10 Ph.D
4 MS/BS

Earth Sciences

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	3.5	3.5	3.7	3.7	3.7	50.0
Post-doctorates	0.5	0.5	0.8	0.8	0.8	5.0
Technicians	0.5	0.5	0.5	0.5	0.5	10.0
Total direct personnel	4.5	4.5	5.0	5.0	5.0	65.0
Professional (adm.)	0.0	0.0	—	—	—	5.0
Clerical, adm. support	0.2	0.2	0.3	0.3	0.3	3.0
Maintenance	0.0	0.0	—	—	—	—
Total indirect personnel	0.2	0.2	0.3	0.3	0.3	8.0
Visiting scientists	0.1	0.1	—	—	0.2	20.0
Graduate students	0.5	0.5	0.7	0.7	1.25	25.0
Undergraduates	0.2	0.2	0.2	0.2	0.2	15.0

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	6	6	3
Chapters, reviews	0	0	0
Books, proceedings (edited)	0	0	0
Reports/documents	0	3	3
Presentations at technical meetings	2	3	4
Meetings organized	0	1	2

[a]Number of OHER-sponsored scientific professionals by highest degree:

8 Ph.D.
2 MS/BS

Energy and Environment

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	3.7	68.1	4.3	94.0	5.2	118.0
Post-doctorates	0.8	21.0	2.1	9.0	1.1	11.0
Technicians	1.3	66.9	1.5	77.0	1.3	71.0
Animal care	0.0	0.0	0.0	—	—	—
Total direct personnel	5.8	156.0	7.9	180.0	7.6	200.0
Professional (adm.)	0.2	14.1	0.2	11.0	0.2	11.0
Clerical, adm. support	1.2	28.9	1.4	19.0	1.3	20.0
Maintenance	0.1	0.0	—	—	—	—
Total indirect personnel	1.5	43.0	1.6	30.0	1.5	31.0
Visiting scientists	1.3	91.0	0.3	85.0	0.3	85.0
Graduate students	1.8	28.4	2.2	22.0	2.5	21.0
Undergraduates	0.0	7.9	0.3	10.0	—	—

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	4	19	20
Chapters, reviews	0	1	2
Books, proceedings (edited)	1	—	18
Reports/documents	11	26	6
Presentations at technical meetings	4	10	29
Meetings organized	0	—	—

[a]Number of OHER-sponsored scientific professionals by highest degree:

11 Ph.D
3 MS/BS

Engineering

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	5.0	9.0	5.0	9.0	5.0	9.0
Post-doctorates	0.0	0.0	—	—	—	—
Technicians	2.0	4.0	2.0	4.0	2.0	4.0
Animal care	0.0	0.0	—	—	—	—
Total direct personnel	7.0	13.0	7.0	13.0	7.0	13.0
Professional (adm.)	0.0	0.0	—	—	—	—
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	0.0	0.0	—	—	—	—
Graduate students	1.0	1.0	1.0	1.0	1.0	1.0
Undergraduates	1.0	1.0	1.0	1.0	1.0	1.0

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	11	11	15
Chapters, reviews	0	1	0
Books, proceedings (edited)	1	0	1
Reports/documents	14	10	20
Presentations at technical meetings	12	8	15
Meetings organized	0	0	1

[a]Number of OHER-sponsored scientific professionals by highest degree:

5 Ph.D.
2 MS/BS

Information and Computing Sciences

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific, Ph.D.)[a]	0.5	0.5	2.0	2.0	6.8	6.8
Post-doctorates	0.0	0.0	—	—	—	—
Technicians	1.5	1.5	1.5	1.5	—	—
Animal care	0.0	0.0	—	—	—	—
Total direct personnel	2.0	2.0	3.5	3.5	6.8	6.8
Professional (adm.)	0.0	0.0	—	—	—	—
Clerical, adm. support	0.0	0.0	—	—	—	—
Maintenance	0.0	0.0	—	—	—	—
Total indirect personnel	0.0	0.0	—	—	—	—
Visiting scientists	0.0	0.0	—	—	—	—
Graduate students	0.0	0.0	—	—	—	—
Undergraduates	0.0	0.0	—	—	0.6	0.6

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	0	—	—
Chapters, reviews	0	—	—
Books, proceedings (edited)	0	—	—
Reports/documents	0	—	—
Presentations at technical meetings	0	—	—
Meetings organized	0	—	—

[a]Number of OHER-sponsored scientific professionals by highest degree:

1 Ph.D.
2 MS/BS

Summary Total

Personnel (FTE)	FY 1992		FY 1993		FY 1994	
	BER	(T)	BER	(T)	BER	(T)
Professional (scientific)[a]	58.9	194.6	59.6	214.7	58.7	307.5
Post-doctorates	7.7	36.0	15.0	35.8	5.9	66.2
Technicians	29.5	146.9	26.2	89.9	33.4	174.3
Animal care	0.7	2.2	8.2	8.2	0.5	2.2
Total direct personnel	96.8	379.7	109.0	348.6	98.5	550.2
Professional (adm.)	8.3	30.7	13.0	27.6	12.3	32.3
Clerical, adm. support	10.0	48.9	21.0	42.0	18.6	47.6
Maintenance	5.4	7.1	1.9	1.9	1.9	1.9
Total indirect personnel	23.7	86.7	35.9	71.5	32.8	81.8
Visiting scientists	42.4	168.1	10.5	104.0	24.0	147.0
Graduate students	34.8	82.0	22.4	56.7	25.8	99.0
Undergraduates	53.4	126.7	21.5	69.2	33.1	98.4

Information Transfer (OHER Sponsored Research)

	FY 1991	FY 1992	FY 1993
Journal articles (peer reviewed)	206	380	192
Chapters, reviews	23	9	24
Books, proceedings (edited)	17	—	41
Reports/documents	50	57	60
Presentations at technical meetings	235	255	178
Meetings organized	0	1	3

[a]Number of OHER-sponsored scientific professionals by highest degree:

4 M.D.
86 Ph.D.
1 D.V.M.
37 MS/BS

Appendix 1
Publications

Appendix 1. Publications

Analytical Technology

Rossington, C.S., Fine, P.M., and Madden (1993) N.W., Large Area, Low Capacitance Si(Li) Detectors for High Rate X-Ray Applications, *IEEE Trans. Nucl. Sci.* **40**(3/4):354–359.

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Appendix 2
Research Highlights

Appendix II. Selected Research Highlights

The following brief narratives describe significant research highlights of OHER-sponsored research at LBL during FY 19923 This selection also reflects the diversity of research interests at the Laboratory and represents efforts in three research divisions—Life Sciences, Structural Biology, and Energy and Environment. While diversified, the research described here points to four broad areas of research emphasis at LBL: molecular cytogenetics, structure-based drug design, methodology for human genome mapping, and the environmental transport and impact radon. The titles of the narratives follow.

- Probing the Genetic Basis for Breast Cancer
- Cancer Drugs: The Next Generation
- Mutation Detection Using a Mismatch-Binding Protein, MutS.
- cDNAs Mapped to P1 Contig on Chromosome 21
- New Insights for Radon Transport and Entry into Buildings

Probing the Genetic Basis for Breast Cancer—Molecular cytogenetics leads to improved methods for studying gene function

(Joe Gray, 415-476-3461; Dan Pinkel, 415-476-3659)

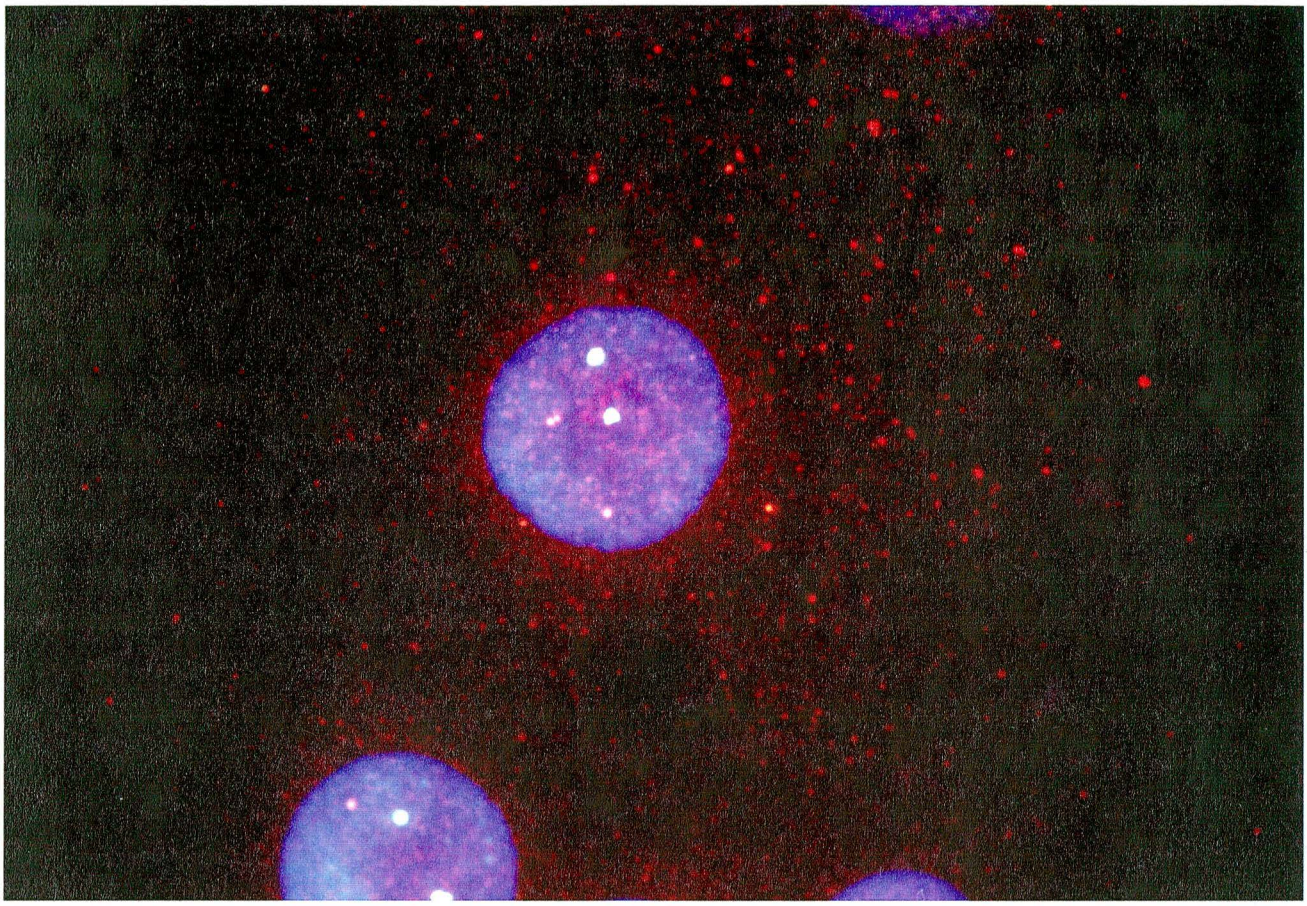
Applying the massive amount of information being generated by the Human Genome Project is an important mission of the Resource for Molecular Cytogenetics. The Resource, a joint effort between LBL and UCSF, is pioneering improved methods of detecting and studying genetic abnormalities in cancer and other genetic diseases, including those found prenatally and in newborn infants.

An LBL/UCSF team led by Resource principal investigators Joe Gray and Dan Pinkel and UCSF scientist Fred Waldman is applying these methods to study genetic changes implicated in breast cancer progression. With support from the Bay Area Breast Cancer Program, the genetic basis for breast cancer is gradually unfolding.

In recent work, a technique called comparative genomic hybridization (CGH) was applied to detect and determine the chromosomal location of genetic aberrations in a large number of breast cancers. Many of the tumors showed loss of material on chromosome 16. Probes to this chromosome obtained from the Los Alamos National Laboratory were used to define the precise region involved on chromosome 16. The gene coding for the cell adhesion molecule E-cadherin was located in the region indicated as being abnormal by these studies. This information, plus evidence from others suggesting that E-cadherin function is frequently lost in advanced cancers, prompted a more detailed investigation of the E-cadherin gene.

First, the investigators, in collaboration with the LBL genome project, isolated a YAC (Yeast artificial chromosome) probe for E-cadherin and used this probe to measure the number of copies of the E-cadherin gene in breast cancer cell lines. These studies suggested a strong association between loss of one allele (normal cells have two) of E-cadherin and loss of E-cadherin function. Next, the investigators obtained information about the E-cadherin DNA sequence from the human genome project DNA sequence database and used this information to study the remaining allele. This work showed that the allele was mutated. Thus, loss of E-cadherin function in breast cancer seems to be caused by a physical deletion of one allele and mutation of the other. Loss of function in this gene, which normally produces a protein that binds cells together, may lead to increased ability to invade and metastasize.

The next step is to study E-cadherin in tissue isolated from actual tumors of the breast and other tissues, such as the bladder, ovary and prostate, to determine whether similar mutations and deletions occur there. Identification of E-cadherin mutations and deletions in human cancers may improve prognosis in these cancers, and may further our understanding of how cells escape from their normal location and spread through the body.



Loss of E-cadherin expression, associated with an increased ability for a breast tumor to metastasize, is illustrated by fewer copies of the E-cadherin gene (green spots) than the reference section of the chromosome (orange spots).

Cancer Drugs: The Next Generation—Scientists use x-ray crystallography to decipher the structure of a key protein involved in cell division

(Sung-Hou Kim 510-486-4333)

In simple terms, cancer can be described as cell division run rampant. Cell division is driven by a cascading signaling pathway, each event catalyzed by an enzyme of a unique conformation. Thus, one strategy for impeding the destructive course of the disease would be to design drugs that lock one such enzyme in the inactive form.

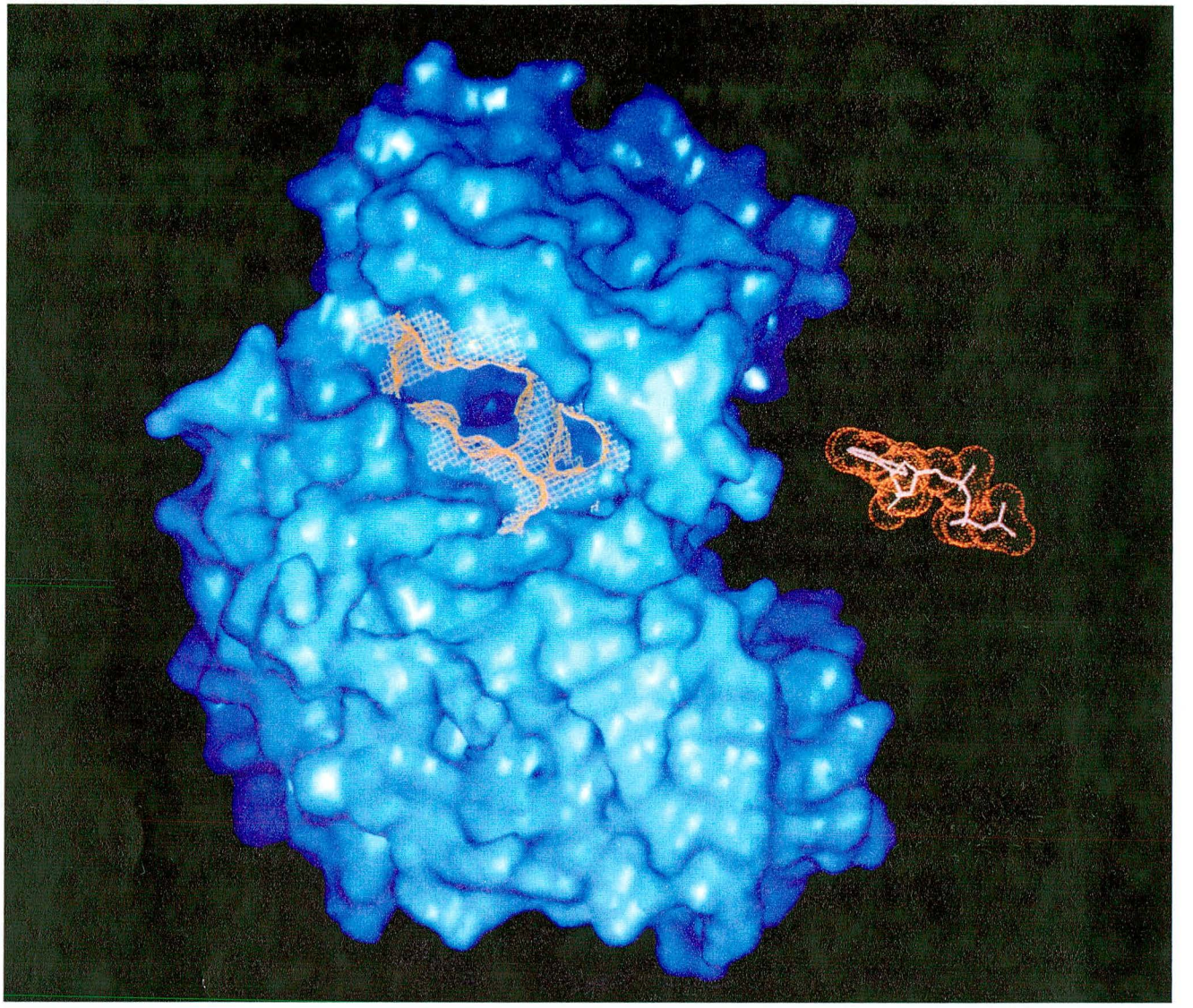
Scientists from LBL's Structural Biology Division, in collaboration with colleagues at UC Berkeley and UC San Francisco, have achieved a dramatic step toward realizing this strategy—by solving the three-dimensional structure of a key protein that triggers the events culminating in cell division. This is the first time the structure of a protein involved directly in controlling cell division has been determined.

Cyclin-dependent kinase 2 (CDK2) is a member of a large family of protein kinases that initiates the principal transitions of the eukaryotic cell cycle. Kinases attaches a phosphate group onto a succeeding enzyme. Various types of kinases are involved in the chain of reactions that follow cell stimulation by a growth factor. These events include *Start*, the critical step when the cell becomes committed to a new division cycle. It is at this stage when cell division can go awry—leading to cancer.

The three-dimensional structure of CDK2 was determined by Sung-Hou Kim's x-ray crystallography group at LBL—an effort led by Hendrik L. De Bondt with Jarmila Jancarik and Sung-Hou Kim. David O. led the San Francisco group in isolating and purifying the protein. A recent issue of the journal Nature featured this collaborative effort.

X-ray crystallography merges art with science by growing a crystal from the protein of interest, then shooting it with an x-ray beam. Distinct patterns are generated that, when analyzed by computational methods, a molecular structure emerges. Now that the three-dimensional structure is determined, several research groups, including some biotechnology companies, are beginning to design potential drugs that bind and inactivate this protein.

This powerful tool, soon to be enhanced by beams of incomparable brightness generated by the Advanced Light Source, will provide promising opportunities for industrial linkages, furthering the investigation of structure-based drug design.



The shape of the protein provides the key information in the design of an inhibitor drug. Using x-ray crystallography in combination with computerized image analysis, scientists determined the crystal structure of cyclin-dependent kinase 2 (CDK2), the protein believed to send the signal that irrevocably commits a cell to divide. Liver and thyroid cancers have been linked to a (CDK2) genetic mutation. Drugs are being designed to block the site where the molecule is activated.

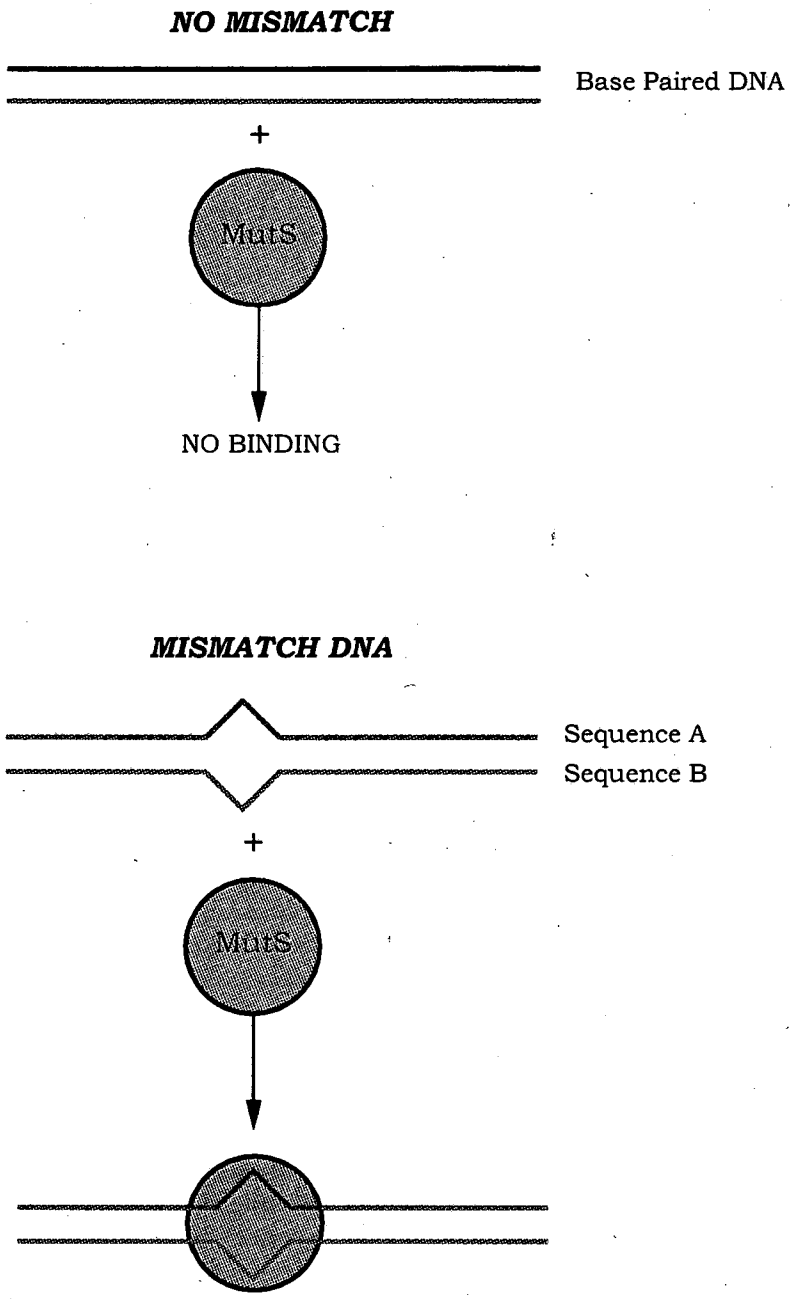
Mutation Detection Using a Mismatch-Binding Protein, MutS.

(Alla Lishanski, 486-7332; and Jasper Rine, 642-7047)

Variation in human DNA occurs at the level of 0.1% of the DNA, 3 million base pairs. Some of this variability, or polymorphism, is the source of genetic disease, with the "disease" variation or allele only slightly different from the gene that has no disease impact.

MutS protein is a member of the family of *E. coli* methyl-directed mismatch repair enzymes. It recognizes and binds base pair mismatches in DNA, e.g., at places where C is opposite A or T rather than G, as well as longer mismatches from unpaired bases. These abilities were used to develop a technique that would test for heterozygosity at a specific locus in the two chromosomes of an individual. Genomic DNA samples are extracted and the region of interest is amplified by the polymerase chain reaction (PCR). PCR products are heat-denatured and reannealed. In the case of a homozygous allele only homoduplexes are generated upon reannealing of the PCR products. If the target DNA is heterozygous at the test locus, reannealing yields four different DNA species: two homoduplexes and two heteroduplexes. Depending upon the nature of the difference between the two alleles, resulting heteroduplexes contain various single-base pair mismatches or small bulges in one of the strands. The MutS protein binds to mismatched areas (PART A), and formation of the MutS-mismatch complex is detected by a gel mobility-shift assay.

We applied this experimental approach to PCR products from two regions of the human cystic fibrosis (CFTR) gene that harbor the most frequently occurring mutations within this gene: a 3-bp deletion, DF508 in exon 10, and point mutations G542X, G551D and R553X in exon 11. PART B shows preferential binding of MutS protein to CTT and GAA bulges in heteroduplex DNA molecules. This test allowed correct detection of all 5 heterozygous carriers of the DF508 mutation among the 15 individuals tested. The method also detected point mutations in exon 11 of CFTR. MutS protein detects mismatches on PCR fragments as large as ~ 500 bp in length. This method is much faster than detection of carriers by sequencing and may be amenable to large-scale typing.



MUTS DETECTION OF HETEROZYGOTES

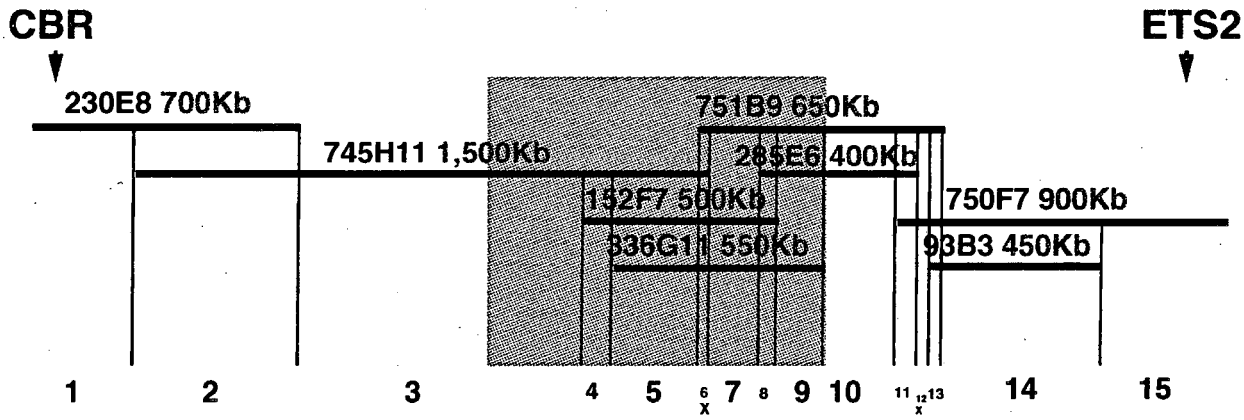
The action of the protein, MutS, on mismatches in DNA can be used to detect polymorphisms in individuals as well as to detect carriers of disease genes. Genomic DNA is amplified by PCR, denatured, reannealed and then mixed with MutS. The protein recognizes and binds the bulge from single and multiple base-pair mismatches that occur in heterozygotes. The protein bound complex shifts its mobility in gel assay, moving more slowly than free DNA. Any of the possible mismatches can be detected, although the G-T and A-C mismatch are illustrated on the right side as heteroduplexes.

cDNAs Mapped to P1 Contig on Chromosome 21

(Jan-Fang Cheng, 510-486-6590)

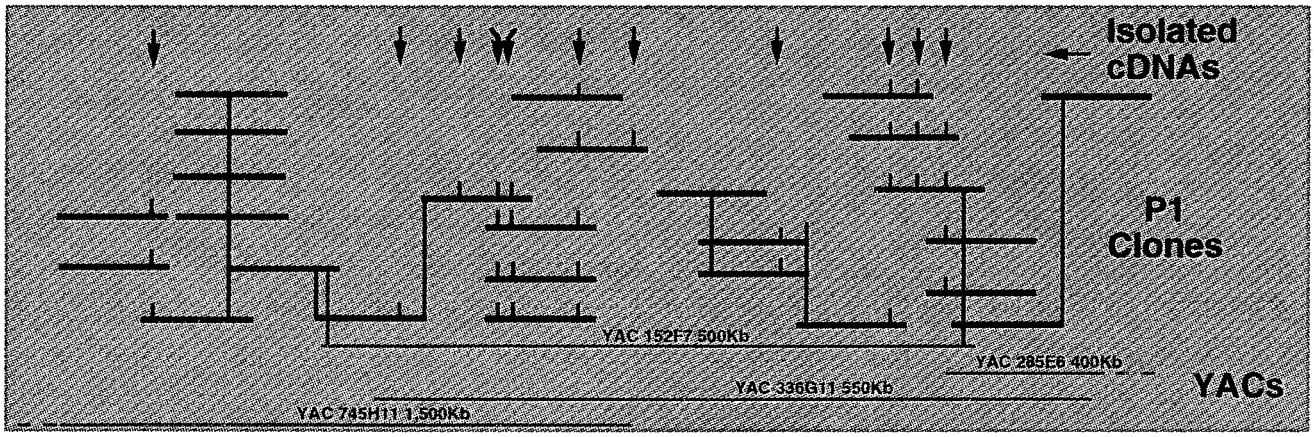
Down Syndrome (trisomy 21) is the most common birth defect, affecting 1 in 700 liveborn infants. The Down Syndrome (DS) region comprises approximately 3 Mb of DNA extending from D21S17 to ETS2 on chromosome 21, and contains approximately 50-100 clones. The P1 mapping project provides a set of overlapping genomic fragments that are ideal substrates for gene isolation and mapping, as well as for large scale sequencing. The series of overlapping clones (a contig) covering the DA region (Fig. A) was generated through a hybridization-based, clone-limited approach that minimizes the number of steps necessary to cover a region of the genome. Two steps identify particular P1 clones in the region and cross-hybridization by end probes provides order. The second part of the study utilizes our cDNA selection method to isolate and sequence an abundance of cDNA sequences (approximately 200 to 400) from this region. These partial cDNA sequences are then used to amplify 3' and 5' flanking sequences for the assembly of full length cDNA sequences. Isolated cDNAs are mapped to the individual P1s to generate a gene map (Fig. B). In all, we have isolated and mapped 45 independent cDNAs in the major Down Syndrome region and over 140 new cDNAs on chromosome 21, more than tripling the number mapped to this chromosome. In the next year, the gene map will be correlated with a phenotypic study of transgenic mice. We anticipate that these mice, which have been created through microinjection of mapped P1s, will permit identification of genes responsible for major DS characteristics.

The 3 Mb Contig of The Critical Region of Down's Syndrome is Flanked by CBR and ETS2 Markers



A. A contig of P1 clones aligned along a larger contig (3 Mb) of Yeast Artificial Chromosomes (YACs) spanning the Down Syndrome Region of chromosome 21. Individual probes select the P1s from a pooled set, and a second probing selects the individual P1. Orientation and alignment of the P1s is done in a cone-limited way using end probes from each of the P1s.

Expressed Genes in The Down Syndrome Region



B. A gene map of the heart of the DS region on chromosome 21. This blowup is from the same colored section in Part A. Isolated cDNAs are mapped both to P1s and thus the YACs. These genes will be tested in transgenic mice in the coming year.

New Insights for Radon Transport and Entry into Buildings

(Richard Sextro 510-486-6295)

Radon is the largest single source of radiation exposure to members of the general public. Although radon is found in all homes and buildings, average concentrations vary from building to building. One of the objectives of the radon research program undertaken by scientists in the Indoor Environment Program at LBL has been to investigate the factors that influence radon entry and concentrations in buildings, and in particular, to examine the causes of elevated indoor radon concentrations observed in some buildings.

Radon entry into actual buildings and the resulting indoor concentrations are often highly variable, the product of complex interactions between the building and the external environment. In order to examine these effects under more controlled—and hence more manageable both experimentally and theoretically—conditions, we have constructed and instrumented two small basement structures at a site in the Santa Cruz (CA) mountains. The two structures are identical except that the East basement rests directly upon the undisturbed native soil while the floor of the West basement rests upon a 10-cm-thick layer of gravel. Both situations are typical of common house construction practices.

One of the first results of this work was the discovery that the measured radon entry rate into the West structure was about 8 times higher than what we had predicted using a very detailed model for radon transport through soil. Further investigation revealed that the air permeability of the soil—a major factor controlling radon movement—increases with length scale. Using a soil permeability value based on the length scale of the experimental basement as input to the model substantially improves the agreement between the predicted and observed radon entry values. Such scale effects have important implications for soil gas transport of other contaminants, including trace gas fluxes from the soil into the atmosphere. We are currently investigating this phenomenon in other soil systems.

Another important result has been experimental verification that the presence of gravel below the floor slab enhances radon entry into the building. In addition, it creates a very non-linear entry system so that even small openings between the interior and the soil (above a minimum size) permit almost as much total radon entry as do larger openings. On the other hand, in the structure without the underlying gravel, radon entry does depend on the size of the opening. Results such as these have important implications for reducing radon concentrations in existing buildings and for designing and constructing radon-entry-resistant new buildings.

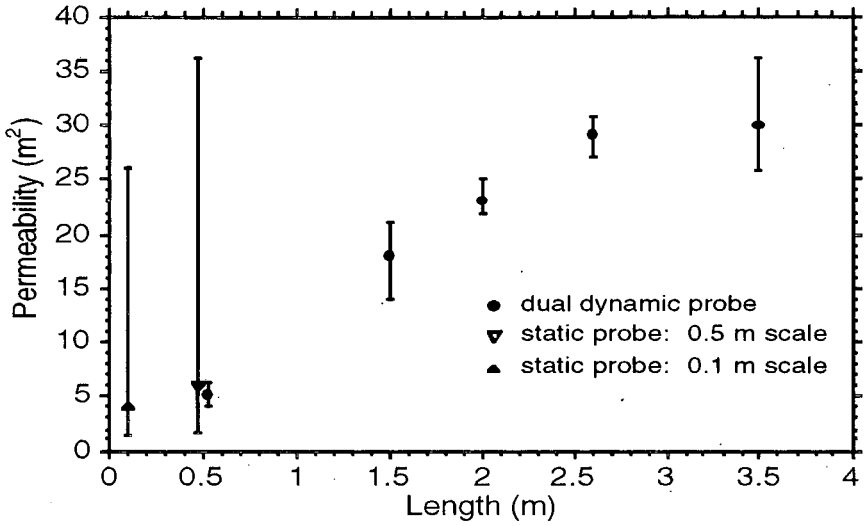


Figure 1. Air permeability of soil as a function of length scale, combining both static- and dual-dynamic probe measurements. These results demonstrate that the mean permeability is strongly scale dependent, with permeability increasing by a factor of about 10 as the length scale increases from 0.1 to 3.5 m. Results from the static probe measurements are indicated by the geometric mean while those from the dynamic probe measurements are given as the arithmetic mean. In each case, the full range of measured values is indicated.

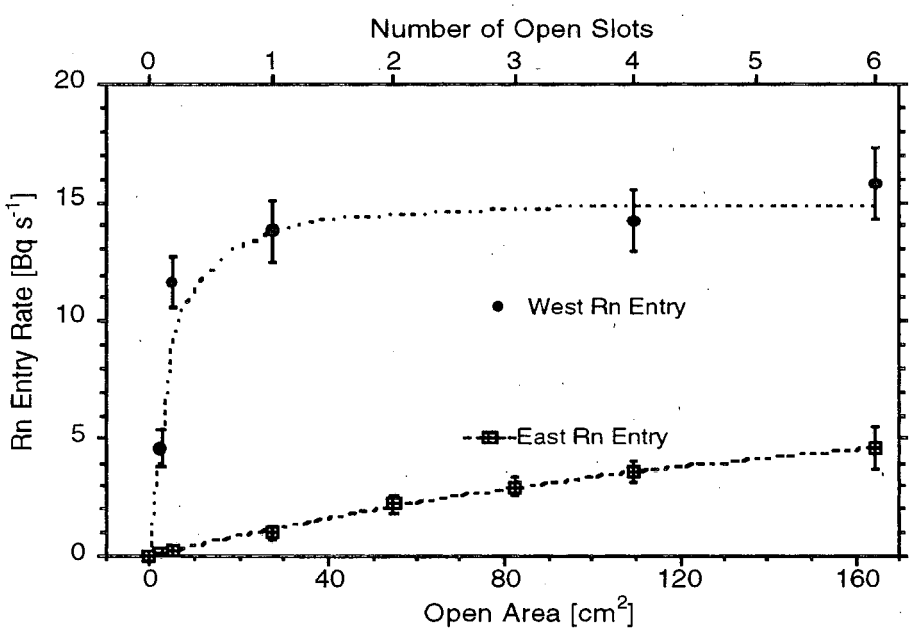


Figure 2. A comparison of steady-state advective radon entry rate as a function of open area, measured at –20 Pascal structure depressurization. In the West structure, which has the subslab gravel layer, the radon entry rate rapidly increases with increasing open area, quickly reaching a maximum entry rate of 16 Bq s^{-1}. In contrast, in the East structure the radon entry rate slowly and continually increases with increasing open area. When all six slots are open the advective radon entry rate into the West structure is almost 4 times greater than the radon entry rate into the East structure. The dashed lines through the data are to guide the eye.