40 Years of Discovery

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Editor: Arnie Heller
Designer: Amy Henke
Advisers: Bert Weinstein, Cindy Thomas

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40 Years of Accomplishments, Many More on the Way

Bert Weinstein

History is most interesting when seen through the eyes of those who lived it. In this 40th anniversary retrospective of bioscience research at Lawrence Livermore National Laboratory, we've asked 19 scientists to share their personal recollections about a major accomplishment in the program's history. We have not tried to create a comprehensive or seamless story. Rather, we've attempted to capture the perspectives of key individuals, each of whom worked on a research program that met significant milestones. We have focused particularly on programs and accomplishments that have shaped the current Biology and Biotechnology Research Program (BBRP).

In addition, we have included a timeline of biosciences at LLNL, a history of the directorate that appeared in the Laboratory's magazine, *Science & Technology Review,* in 2002, and a list of bioscience-related articles that have appeared over the years in *Science & Technology Review* and its predecessor, *Energy & Technology Review*.

The landscape of biological science today is stunningly different from 40 years ago. When LLNL bioscience began in 1963, we knew about the structure of DNA and that it was the carrier of genetic information. However, it would be another year before scientists would understand how DNA codes for the production of proteins and more than a decade before the earliest DNA sequence would be known. It is sometimes difficult to remember that it was only 15 years ago that the polymerase chain reaction, a synthetic method to amplify pieces of DNA was developed, and that only within the last half-dozen years has sequence data for entire organisms begun to be available.

In this publication, we have tried to capture some of the landmark and seminal research history: radiation effects studies, which were a major reason for founding the biological research program, and flow sorting and chromosome painting, which dramatically changed our ability to study DNA damage and enabled the creation of chromosome-specific clone libraries, a key step toward sequencing the human genome. Several histories relate to the Human Genome Project itself and surrounding technologies, and several to long-standing research themes such as DNA repair, food mutagens, and reproductive biology. Others describe more recent developments such as computational biology, health-care technologies, and biodefense research.

From the beginning, a characteristic of LLNL bioscience has been to apply technology to biological problems. Although we have taken a biological perspective for this history, many different parts of the Laboratory have been involved in these accomplishments. Computation, chemistry, engineering, and physics have all played strong roles. Over the course of four decades, bioscience has become far more quantitative and far more integrated with the physical sciences, engineering, and computer science. Today, a large and ever-growing fraction of biological research is done in front of a computer terminal, a dramatic difference from just a decade ago.

In a fast-paced world where scientific breakthroughs, many totally unexpected, occur regularly, it's dangerous (and possibly even foolish) to try to predict the future. However, extrapolating from the work going on today in BBRP laboratories, we can reasonably foresee several exciting developments over the next 10 to 20 years.

- It took us 15 years and $3 billion to sequence the human genome, with more than 90% of the actual data generated in the last three years. Sequencing costs are likely to continue to plummet to less than $1 million per mammalian genome (a few billion bases) and $1,000 per microbial genome (a few million bases). Comprehensive sequence data will become the expected starting point for essentially all biology research. In addition, it's likely that within a decade or two the parents of every baby will be given a list of the baby's significant genetic characteristics.

- Our research in radiation effects and molecular chemistry has the potential to increase the effectiveness of radiation treatment for cancers by a factor of 10 or more. This advancement can be accomplished by targeting the radiotherapy specifically to cancerous cells while simultaneously protecting healthy cells.

- Our computer modeling expertise, coupled to data obtained from experimental cell measurements, will make possible the development of 3D movies of biological processes with full quantum mechanical resolution. We can imagine watching simulations of molecular protein machines as they carry out DNA repair and studying simulations of cells' response to exposure to toxic chemicals, bacteria, and viruses.

- We can envision the diagnosis of infectious diseases becoming a routine part of a physical exam. Within 10 years, you may walk into your doctor's office and supply a saliva sample, and within a few minutes your doctor will have a readout of any infectious diseases to which you've been exposed, including possible bioterror agents.

- Finally, we could be using industrial production processes for chemicals, catalysts, and other materials based on biological processes that are thousands of times more efficient, specific, and rapid than many of our current chemical synthesis processes. For example, modeling the biochemistry of bacteria living in deep ocean vents could lead to efficient manufacture of hydrogen.

In the 20th century, the physical sciences transformed our life and society in innumerable ways. The 21st century will be marked by equally transforming advances in bioscience. We are just beginning to understand and utilize the tremendous diversity and creativity of living systems and apply them to our own benefit, such as improving health, maintaining the environment, producing energy, and ensuring our security. The next 40 years of bioscience at LLNL promises to be even more exciting.

I thank Arnie Heller for his sensitivity, skill, and persistence in conducting and recording these interviews. It is through his talent and dedication that these stories have been told.

Bert Weinstein is acting associate director of Biology and Biotechnology Research Programs.

1

Mort Mendelsohn: A-Bomb Survivors

Joining the Scientific Council was my first official involvement with the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki. This was in 1985 while I was associate director for Biomedical and Environmental Research. RERF is the primary organization that monitors the health of A-bomb survivors. They carry out periodic mortality studies and examine a subset of survivors every two years for intermittent changes in blood chemistry and health.

I think RERF asked me to join its Scientific Council because of Livermore's long-standing interest and experience in biodosimetry. They may also have confused me with another Mendelsohn from Livermore, John Mendelsohn, who was in the weapons program at the time. Around 1980 he and Bill Loewe had a major effect on RERF when they used radiation spectra and transport equations to show that the neutron doses then in use for the survivors were too high. Eventually, this led to DS86, a complete reconstruction of the A-bomb dosimetry.

From 1985 to 1995, Biomed and RERF were in close collaboration because RERF needed our biodosimetric technologies and we needed an accessible, exposed population. To collaborate effectively, we had to learn how to communicate across distance and culture and, above all, how to trust each other.

The first method we transferred was the Glycophorin A assay, a flow-cytometric method to determine in humans the frequency of mutant red blood cells. We invented this assay in the 1980s and demonstrated its usefulness in monitoring human somatic genetic damage. The assay seemed well suited for RERF because only small samples of blood were required, but it was anyone's guess what the assay would show in the survivors who were then around 40 years post exposure. Ron Jensen brought the technique over to Japan and was pleased to find the Japanese already proficient in immunochemistry and flow cytometry. In 1987, Biomed and RERF jointly published the first evidence that the assay detected residual mutations left by exposures four decades earlier. No other somatic mutation assay was able to reach back this far in time. RERF continued over the next decade to enrich the assay and apply it to the survivors.

Mort Mendelsohn

Chromosome painting was the second technology we brought to RERF. Years of cytogenetic work in Japan had shown the value of chromosome translocations as a radiation biodosimeter. However, the work was painstakingly difficult, making it impossible to apply at the scale that was needed. Livermore's invention was tailor-made for the efficient counting of translocations, but could it be transferred, and would it work on the aging samples in RERF's collection? Joe Gray and Joe Lucas made the transfer. The method caught on rapidly. It worked on the old samples, and it greatly accelerated the RERF studies. To this day, chromosome painting and the counting of translocations is still the primary biodosimeter in the survivors. As one good indication of its success, Joe Gray now sits on the RERF Scientific Council.

While I was serving on the RERF Scientific Council, an issue came up about how best to save and analyze DNA for studies on the heritability of radiation induced genetic damage. The plan was to identify exposed and control parents and to store blood samples from them and their children who were conceived after the bombing. In 1984, I obtained funds from the Department of Energy (DOE) and the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC), and asked Ray White of the University of Utah to help me organize a meeting in Alta, Utah, of about 40 molecular geneticists from around the world (including our own Elbert Branscomb). We brainstormed the potential ways to detect cross-generational mutations in the survivors' DNA. After several exciting days, we came up with a half-dozen potential methods, calculated their relative efficiency, and concluded that it was a bit too soon to go any further. Everyone was optimistic that the rapid advances in DNA methodology would soon make it feasible to estimate the background and the radiation-induced human mutation rate. One of the six methods was to sequence a significant part of the genome of both parents and children. This was an effort on the scale of sequencing the human genome, and in the eyes of many of the participants, the meeting, the analysis, and the excitement set the stage for the subsequent rapid development of the Human Genome Project.

RERF is a binational organization, and the National Academy of Sciences is responsible for the U.S. involvement, including the recruitment of American directors for the foundation. For years, they asked me to consider such a role, but I always felt that the AD role here at Livermore was too central to take sabbatical leave. When Tony Carrano became the new associate director in 1992, it gave me my opportunity to get out of the way here and to play an administrative role in Japan. In all, I stayed in Hiroshima for three years, first as an RERF director-at-large and then for two years as vice chairman. It was a challenging and wonderful experience, one that I will always cherish.

In retrospect, the 50-plus years of study of the A-bomb survivors is remarkable both as a compassionate gesture of remorse and a scientific approach to human biology. This has to be the largest and longest longitudinal study of human epidemiology. From it, we have greatly deepened our understanding of human cancer, embryology, immunology, and longevity. We have given the world a realistic assessment of radiation risk, and in the process have learned a tremendous amount about ourselves. Hopefully, the governments of Japan and the United States will see fit to continue the studies for the foreseeable future.

2

Virgie Shore: Lipoproteins

In the spring of 1963, John Gofman, the first Biomed associate director, was recruiting people to join the program, which had just started under Lab Director Mike May. John got his Ph.D. under Glenn Seaborg after receiving his M.D. He contacted some of his former biophysics graduate students from Lawrence Berkeley Laboratory (LBL), including my late husband, Bernard, to see if they would be interested in working at Livermore.

John had an interest in lipoproteins and heart disease. Bernard had done research on lipoproteins while working under John at LBL as a graduate student. I did my graduate study in biochemistry.

When we started at Livermore in July 1963, both Bernard and I worked in the same lab although he focused on the effects of low-level ionizing radiation. He worked with Paul Phelps and other people studying the uptake of atmospheric radioisotopes in animals.

Virgie Shore

The lipoprotein work was funded by the National Institutes of Health (NIH). It involved separating, purifying, and characterizing protein moieties of human plasma lipoproteins. We later got into proteins involved in lipid transport and metabolism. All the fats in the blood are transported in the form of lipoproteins. Lipids would be insoluble without proteins attaching to them. Each major class of lipoproteins has specific proteins attached to them called apoproteins. For example, so-called good lipoproteins like HDL have apoA1 and apoA2, with small amounts of apoCs. We were the first to isolate by column chromatography several apoproteins, including apoA1, apoA2, apoE, and the apoC's from human plasma.

We used isoelectric focusing to look at the various forms of apoproteins. It's a way of separating proteins in a gel base on the basis of small differences in their electric charges. Other investigators subsequently found that an increase in one form of apoE is associated with Alzheimer's.

In the only study we did involving animals, we fed rabbits a diet high in cholesterol. The diet caused high levels of apoE, which was associated with an increase in cholesterol esters. We also found increased apoE in Type III hyperlipoptoreinemia, which increases the risk for atherosclerosis and heart disease.

In the spring of 1963, John Gofman, the first Biomed associate director, was recruiting people to join the program, which had just started under Lab Director Mike May.

I participated in a group headed by Ronald Krauss, at LBL's Donner Lab, and Gary Nelson, at the Department of Agriculture. We looked at the human diet and the effects of fatty acids such as omega 3 and saturated fatty acids on the concentration of lipids in blood plasma. With these human studies, I became much more aware of the importance of minimizing certain fats in the diet.

In the early 1980s, I collaborated with scientists at University of California at San Francisco on studies of the role of apoproteins, particularly apoA1, as cofactors in lipid metabolism. I worked on lipoproteins, mainly with the LBL group, until my retirement in 1990.

3

Joe Gray: Cell Flow Sorting

I arrived at the Laboratory in 1972, having been recruited by Marv Van Dilla. I had just gotten my Ph.D. in physics from Kansas State University. Mort Mendelsohn had recruited Marv from Los Alamos, which had a major effort going in flow cytometry. Mort wanted to introduce more quantitative biology and image analysis in the department, and he also had a strong interest in building a cell sorter program.

Marv put together a multidisciplinary team of chemists, biologists, physicists, mathematical analysts, and engineers. Livermore was a convenient place to assemble such a team. Marv brought with him from Los Alamos a box of parts that helped us get a good start on a machine of our own design, one that we understood and could manipulate. Los Alamos was continuing with its own cell sorter program during this time.

Over the next decade, we kept improving the technology. We originally worked on whole cells, sorting them by the use of fluorescent dyes or by how they scattered laser light. Early in this period, at Mort's urging, Tony Carrano, I, and others figured out that we could process isolated chromosomes. We started with some hamster chromosomes that we "borrowed" from UC Berkeley, and we succeeded in labeling and sorting them as well. We were the first to use this approach to purify chromosomes. We later expanded to other species, including humans. Rich Langlois made important improvements in chromosome staining that further increased our ability to purify human chromosomes.

Joe Gray

Prior to that time, no technology existed that could purify sufficiently individual chromosomes of a single type for molecular analysis. Individual chromosomes could be recognized under the microscope, but there were no techniques to sort and purify them en mass. Once we were able to distinguish the chromosomes from one another with fluorescent dyes, we could sort them and purify them. So this gave molecular biologists a tool for obtaining nearly pure populations of a selected chromosome.

The demand for sorted chromosomes soon exceeded our sorting capacity, so Tony and Marv initiated an effort to clone the sorter-purified chromosome DNA. This effort eventually led to the Livermore–Los Alamos Gene Library Project in the early 1980s that cloned all the human chromosomes. The cloning project stimulated our development of high-speed cell sorters because researchers needed more purified DNA than our machines could provide. At the time, we were able to process a few thousand chromosomes per second. Eventually, we achieved about a tenfold increase in sorting speed.

By the mid-1980s, Dan Pinkel and I came up with the idea to attach fluorescent probes to our cloned chromosomes and use these as probes for fluorescence in situ hybridization. This process stained the target chromosomes with great specificity so it was possible to identify translocations involving these chromosomes. There was a lot of interest at DOE in techniques to assess radiation-induced genetic damage. One of the best ways to detect damage from ionizing radiation was to identify chromosome translocations. With chromosome painting, it was easy to count the translocations. We, in collaboration with Joe Lucas and Tore Straume, demonstrated the utility of this approach and eventually transferred it to the Radiation Effects Research Foundation in Japan for studying A-bomb survivors.

Marv (Van Dilla) brought with him from Los Alamos a box of parts that helped us get a good start on a machine of our own design, one that we understood and could manipulate.

During this time, several Biomed researchers, including Ron Jensen, Brian Mayall, Frank Dolbeare, and Maria Pallavicini, were using cell sorters to study cancer cells. They investigated how cancer cells grew, proliferated, and responded to different chemicals.

In the late 1980s, Ger Van Den Engh and Barb Trask joined our group and further developed chromosome analysis and sorting technologies. During this time, Ger developed a well-engineered and flexible high-speed digital sorter. Cytomation eventually commercialized his design.

One of the keys to our success was the multidisciplinary nature of our work. Our sponsors, DOE and NIH, encouraged multidisciplinary research. I've noticed it's harder to maintain the multidisciplinary approach in academia than at the Laboratory. In my new job as Director for Life Sciences at Lawrence Berkeley National Laboratory, I plan to emphasize multidisciplinary projects. There's a real role for the national labs to continue large-scale, team research in the life sciences.

4

Dan Pinkel: Chromosome Painting

In the early 1980s, I was working with Joe Gray and a lot of other folks in a biophysics group headed by Marv Van Dilla. The group was developing cell sorting hardware and doing some imaging work, among other things. We were the physical sciences research group for Biomed. One of the things we were doing was improving the ability to separate the different human chromosomes. In fact, several people at Livermore had originally developed the technology to do it before I got to Livermore, including Joe, Tony Carrano, Rich Langlois, Phil Dean, Don Peters, and a few others.

DOE wanted to get more involved in molecular biology. A natural project was to use the unique chromosome sorting ability to make cloned libraries of all the human chromosomes. People had to develop more accurate and efficient techniques to stain chromosomes with fluorescent molecules, measure them, separate them, and isolate them to get enough DNA from each chromosome type to make the libraries. That effort eventually turned out to become a joint Livermore–Los Alamos project and was a precursor to the national labs' involvement in the Human Genome Project.

At the same time, people in several labs around the world were starting to show results with hybridizing DNA to cells and chromosomes and detecting the hybridization with fluorescence techniques. People in the field were meeting every couple of years and reporting good progress.

Dan Pinkel

Joe and I became interested in the potential of this technique and the possibility of using the chromosome libraries for hybridization. These could potentially be used to "paint" different chromosomes with specific fluorescent colors. We thought of all sorts of uses, for example, prenatal analysis and examining cancer cells. But the first application to attract funding was to improve biodosimetry in humans by measuring chromosomal aberrations. DOE wanted to be sure safe health standards were established for everyone exposed to radiation as part of their work environment. Thus, the department wanted to understand better the health effects of the A-bomb survivors from Hiroshima and Nagasaki. As people got better at calculating radiation doses, they got more concerned that the biological effects might be larger than first thought. So we thought about simplifying the process of estimating the radiation dose received by a person by measuring the chromosomal damage in white blood cells.

It's beastly hard to use standard cytological techniques to look at enough chromosomes through the microscope and find chromosomal aberrations in situations where doses are low enough to be of interest. You'd have to look at thousands of cells. One possible way to speed up the process was to paint chromosomes with the libraries. Previous research had shown that ionizing radiation caused translocations, an event whereby two different chromosomes break apart and then join together to make abnormal chromosomes that contain exchanges of the original parts. If we could make the normal chromosomes a different color, the translocations would be visible instantaneously because they would have portions with different colors. Perhaps then we could make a machine to detect the bi-color chromosomes.

We thought about simplifying the process of estimating the radiation dose received by a person by measuring the chromosomal damage in white blood cells.

There was a lot of DOE interest, and we were able to get funding. Solving our technical problems took a while, especially because of our lack of training in molecular biology. Fortunately, we had help from Jim Fuscoe in Tony's group, who provided molecular biology expertise.

In the mid-1980s, we published our solution to chromosome painting, and it has been widely adopted. The publication is one of the most highly cited to come out of Livermore. In the end, we didn't need a lot of different instruments to make the technology work. One of the keys is that both Joe and I are physicists, which helped us to consider the biophysical issues involved.

The technology is now sold by Vysis, a subsidiary of Abbott Pharmaceuticals. Vysis sells a wide range of chromosome painting products, including some that have been clinically approved by the Federal Drug Administration. The technology continues to move forward. Current applications for chromosome painting include measuring translocations either by whole chromosomes or by small probes targeted to important boundary points where two chromosomes come together, as in some leukemias.

Chromosome painting has become a standard technique in biological research, and we use it routinely at UC San Francisco. Its best use is when you're looking for a specific abnormality and have a specific probe to detect it. I'm currently working with our collaborators on microarray techniques to search the entire genome for sections of DNA that have been either duplicated or lost, which are common events in some cancers.

5

Jim Felton: Food Mutagens

In 1978, a story broke about a group of Japanese researchers who found mutagenic chemicals in cooked hamburger meat. At the time, a group of us was studying mutagenic chemicals that were produced by oil-shale retorting and coal gasification. It was a complex problem involving separating several organic fractions and assessing each fraction for its potential to initiate genetic damage.

One day, the National Institute of Environmental Health Sciences (NIEHS) asked Mort Mendelsohn if Livermore had the expertise to study food mutagens. Curiously, oil shale and food have a lot in common because some of the chemical processes are similar when both substances are heated. NIEHS gave us a multi-year contract to begin assessing the mutagenicity of hamburger meat. Our people working on the project included Fred Hatch, Hector Timourian, Brian Andresen, and Dan Stuermer.

The early research was difficult because we had to extract dozens of fractions of cooked meat and assess their mutagenicity. After a couple of years, a group headed by Mark Knize isolated a group of heterocyclic amines, all carcinogenic, that were present in low concentrations and that the Japanese hadn't found. These mutagens were produced at normal cooking temperatures in beef and other muscle meats when fried or broiled. We didn't find them in nonmuscle meats, such as liver, or in invertebrates, like shrimp.

Jim Felton

We discovered that frying hamburger at high temperatures produced the highest level of heterocyclic amines. One compound in particular, called PhIP, accounted for most of the mutagens. We also discovered something very important, that it's not the mutagens themselves that cause problems. Rather, metabolic enzymes try to eliminate them from the body by attaching OH groups to these water-insoluble compounds, a standard enzymatic mechanism for excreting drugs and chemicals from the body. Ironically, this reaction turns them into reactive compounds that bind to DNA.

We also studied different cooking methods to find ways of reducing the intake of the mutagens. We showed that cooking time and temperature significantly affect the amounts of mutagens generated. For example, reducing the frying temperature of hamburger greatly lowers the mutagenic activity. So does pretreating the meat with a microwave. Knize, Sue Healy, Robert Taylor, and Cyndy Salmon did a lot of this work.

One problem we faced was that mutagens are present in cooked foods at very low levels—about 0.1 to 50 parts per billion—which makes studying them at realistic exposure levels in laboratory animals rather difficult. One day, Jay Davis from Physics came by. He said he had a new instrument, yet to be finished, called an accelerator mass spectrometer (AMS) that used carbon tracing to detect extremely low levels of compounds of interest. Davis was curious if there were any biological applications for the AMS. Ken Turteltaub and I realized it might allow us to measure low levels of carbon-14-tagged mutagens given to test animals. This was during the time when saccharin studies were under attack because rats were being given enormous quantities. Using the AMS produced not only excellent data, which showed that the DNA damage was proportional to the dose, but it also gave birth to a new field: biological accelerator mass spectrometry.

Today, we have a collaboration with UC Davis under a long-term National Cancer Institute grant. One major focus is studying African-Americans' exposure to food mutagens because as a group, based on government studies, they tend to cook meat more well done than Caucasians. Scientists have been investigating for years why black American males have a higher incidence of prostate cancer. One hypothesis is that exposure to higher concentrations of food carcinogens may be responsible. Ken Bogen and Garrett Keating in Energy and Environmental Sciences are working with BBRP scientists to understand this problem.

We're also looking at what foods in the diet might counteract the harmful effects of food mutagens, or at least interfere in their activation to harmful agents by metabolic enzymes. We have a computational modeling effort under Mike Colvin to model in 3D these metabolic processes. We recently began using a new instrument in the Chemistry and Materials Science directorate, called a time-of-flight secondary ion mass spectrometer. We'll use it for mutagen metabolism studies and for imaging the carcinogens in a cell.

It's been a very successful program for 25 years, and it has earned us international recognition. I think we have been so successful because we have been able to apply several technologies that other labs didn't have. The bottom-line finding of our research is eat the food you want but cook it in a safe manner.

6

Larry Thompson: DNA Repair

The first time I remember hearing about the concept of DNA repair was shortly after I arrived at the University of Texas in Houston in 1964. To me it was a mind-boggling idea that cells could be so complex and sophisticated as to actually detect and repair damage in their DNA.

I started working on DNA repair in 1978, about five years after coming to the Lab. As part of my training in graduate school, I had studied cellular response to radiation damage, and I had learned procedures to isolate mutant cell lines. I hit upon the idea of looking for mutant mammalian cell lines that are deficient in their ability to repair damaged DNA.

We began screening for DNA repair mutants using a popular hamster cell line. These cells are easy to grow, almost like bacteria but much slower; they're genetically stable and widely used. Many of the cell lines were isolated at UC Berkeley and characterized at Livermore. Kerry Brookman, who worked with me for 20 years and then retired, helped start the mutant hunts.

We found two classes of mutants. The first was defective in nucleotide excision repair from ultraviolet and bulky chemical damage, and the second was defective in base excision repair. Both kinds of repair involve cut-and-patch mechanisms, but the proteins involved are completely different. Once we published our first paper in 1980, a lot of researchers jumped into the field.

Larry Thompson

We used rodent cell lines to isolate repair-deficient mutants because rodent and human cells are remarkably similar in terms of their DNA repair processes. By using rodent–rodent and rodent–human hybrid cells, we determined how many genes are involved in a repair process and identified the human chromosomes on which repair genes reside. In 1985, we used the first of several mutants to identify the location of a human gene that corrects a DNA repair deficiency.

We soon discovered that three repair genes mapped to chromosome 19 in humans. The presence of repair genes on human chromosome 19 helped to influence Tony Carrano to choose this chromosome for a detailed, cosmid-based physical mapping project as part of the Human Genome Project. We were among the first to assign repair genes to specific human chromsomes.

We then moved on to clone human repair genes. We published a paper in 1988 in which we reported the isolation and biological characterization of the second human repair gene ever to be cloned, ERCC2. This human gene corrects the repair defect and UV sensitivity of our first CHO mutant (UV5). ERCC2, now called XPD, is involved in the human cancer-prone disorder called xeroderma pigmentosum.

Gene cloning took off in the research community, and we entered a new era where we're kind of going in reverse. We're using cloned genes to make new mutants to study pathways. We're sticking with our hamster cell lines because we've gotten so much mileage from them.

To me it was a mind-boggling idea that cells could be so complex and sophisticated as to actually detect and repair damage in their DNA.

We've looked at several relatively rare diseases in humans that are associated with defects in repairing damaged DNA. In 1997, we cloned a gene associated with Fanconi anemia, a developmental disorder. People suffering from this disease have cancer predisposition and DNA damage sensitivity, which is caused by missing or mutated proteins in any of about 10 genes. The defect may not involve repair genes per se but instead processes that determine how damage is dealt with during DNA replication.

There are probably 200 to 300 genes and proteins involved in DNA repair. The list grows every week. There are perhaps 150 genes involved in directly repairing the damage and as many more involved with the recognition, signaling through complex phosphorylation cascades, and the cell-cycle checkpoint coordination of repair events.

Cells can't tolerate a double strand break in their DNA, and the efficiency of repair is amazing. A break is repaired more than 99 percent of the time. It's almost a perfect system. Looking back, I couldn't have imagined how complex and intricate DNA repair really is. What's so amazing is that so many proteins have more than one function; they interact with multiple other proteins, and each interaction has important biological significance. The repair machinery beautifully illustrates biological homeostasis at the molecular level.

About 15 BBRP people are now involved in gene repair. I've got nine people working with me; we're hiring two new people very soon. DNA repair is now a big field that is especially fundamental to cancer biology and cancer treatment.

7

Andy Wyrobek: Reproductive Biology and Health Effects Genetics

I was hired in 1975 by Bart Gledhill and Mort Mendelsohn as a member of the reproductive biology section. We were interested in how products from energy technologies such as coal and smoke affected human health, especially the reproductive system. We developed assays for sperm motion, shape, and fertilization ability, and that effort took us into the 1980s. Then we started to look at abnormalities in sperm chromosomes and link any abnormalities we found to observable birth defects.

In 1980, we were one of two labs in the world that could look at human sperm chromosomes using an artificial biological system. It turns out that when you incubate human sperm with a specially treated hamster egg, it accepts the sperm and opens up the sperm nucleus so you can see the individual chromosomes. Bragitte Brandriff and I worked for several years to make this assay work consistently.

In the mid-1980s, we started to develop assays that would detect chromosomal and DNA mutations in sperm, especially aneuploidy (extra or missing chromosomes). By 1990, we published several papers describing how we could see chromosomes inside sperm for the first time by using the FISH (fluorescence in situ hybridization) technique that was pioneered by BBRP's Dan Pinkel and Joe Gray. We matured the FISH technology and used flow cytometers and automated image analysis.

Andy Wyrobek

In the 1990s, our goal was to see if sperm could serve as biomarkers of paternally transmitted genetic defects and male-mediated developmental defects. If they could, then sperm assays would be much less expensive and much more efficient than conducting epidemiological studies to determine birth abnormal outcomes. For example, typical epidemiological studies would have to look at thousands of children to analyze the father's contribution to such events as birth defects and spontaneous abortions. However, you can detect comparable changes by analyzing the sperm from only 10 to 20 men.

By 2000, we developed a human sperm FISH assay for detecting chromosomal breaks, duplications, deletions, and aneuploidy in sperm. We can measure the frequency of sperm that would produce boys with Klinefelter's syndrome, girls with triple X syndrome, or Turner syndrome, and children with Down's syndrome.

We've used our human sperm FISH assay to test humans for sperm aneuploidies caused by exposure to ionizing radiation and to chemicals such as caffeine, alcohol, cigarette smoke, chemotherapy, benzene, and pesticides. We've also investigated various genetic and physiologic factors. We've also shown that certain cancer chemotherapies induced sperm aneuploidies and that age has an effect, with older men having higher levels of chromosomal damage than younger men.

In 1992, the National Institutes of Health asked for an animal model to test the mutagenicity of a variety of drugs, antifungal agents, and pesticides. For the past 11 years, we've been working to develop assays for mouse and rat chromosomal breakage. We've tested a number of chemicals for sperm aneuploidies with the mouse sperm FISH assay.

So we've developed the human and rodent tests, and now we're proposing a testing structure to determine risk factors for potential fathers. First, we propose that researchers do a rodent sperm FISH test to screen chemicals and build a priority list for human studies. Second, human sperm FISH tests would be performed on the top risk factors. Finally, epidemiological studies would be performed on those factors showing the highest mutagenicity from the human sperm FISH assay.

In the mid-1990s, we got interested in looking at the chromosomes in the early stages of the developing embryo. Francesco Marchetti joined the team and brought with him expertise to look at chromosomes in the embryo. The research showed that chromosomal aberrations in zygotes after paternal exposure to mutagens are predictive of the embryo's fate.

Last year, we showed a correlation between frequencies of aneuploidy in both germ and blood cells. Some men may carry mutations or genetic variants for genes that control common aspects of mitotic and meiotic chromosome segregation. I think this will prove to be a very important finding. We've discovered that some men have consistently high levels of aneuploidy in both their germ and somatic cells.

Recently, we've begun to investigate how eggs affect chromosomal damage in sperm. It's the next frontier for us. We know that the egg has stored in it messenger RNA and proteins involved with DNA repair and early development. Some eggs can repair all the damage in the sperm, while some eggs can hardly repair any damage. We're trying to understand the genetic basis of the differences. We've done some pilot work on this research topic. Matt Coleman joined the team and developed the capability to study gene expression in small numbers of cells using microarray technology. In the future, this research will be relevant for couples who might want to know, for example, if the woman's egg could repair chromosome damage caused by the man's smoking or other paternal exposures or lifestyle habits.

8

Tony Carrano: Human Genome

Many people don't realize that the first discussion about the needs for what came to be known as the Human Genome Project took place at a DOE-sponsored meeting on human mutation in Alta, Utah, in 1984. The following year, Bob Sinsheimer, the chancellor at UC Santa Cruz, convened a meeting to discuss whether it was feasible to sequence the human genome. Basically, the group told Bob that it was a great idea, but that technically we weren't ready.

Soon after, Charles DeLisi in DOE gave Mort Mendelsohn a phone call. Charles wanted to know what Livermore thought about sequencing the human genome. Mort called me (I was a group leader at the time), Joe Gray, and a few other people in and asked our opinions. In a conference call to Charles, we said sequencing the human genome was doable, but that certain other scientific tasks needed to be done as a prelude–for example, like assembly of a clone map. Moreover, we needed to develop a good strategy and better instrumentation.

DOE then began a series of meetings to get a consensus of the scientific community about the practicality of sequencing the human genome. The response was similar to ours. Yes, sequencing the human genome was worth doing, but we needed to ramp up the technology first.

Tony Carrano

Livermore and Los Alamos were in a unique position at the time because we had already begun assembling a unique collection of recombinant DNA clones for each of the human chromosomes. Our library of chromosomes was an outgrowth of the flow technology that BBRP pioneered. Livermore was the first lab to show that it was possible to identify and purify chromosomes using a flow system. The libraries of chromosomes made by Livermore and Los Alamos became essential for many labs until new cloning technologies came along, technologies that Livermore also played a major role in developing. We distributed our chromosome-specific recombinant DNA libraries to many institutions.

As soon as DOE formally initiated the program, I reprogrammed some of my own research funding to kickstart the human genome effort here, even before we got the first official funding. We brought in some talented people, especially molecular biologist Pieter de Jong, for mapping the human genome.

We already had a great team in place. Harvey Mohrenweiser, Anne Olsen, and Emilio Garcia led the gene finding effort. Greg Lennon managed the cDNA effort that eventually led to the NIH-DOE IMAGE project, the largest public collection of human genes. Informatics was first handled by Elbert Branscomb and then by Tom Slezak, who I consider one of the best people in the field. Jane Lamerdin did much of the early mapping technology development and also pioneered the pilot sequencing effort at Livermore. Linda Ashworth was a major internal facilitator for the effort, helping to keeping communication open between the various elements. Ray Mariella made important contributions to the instrumentation.

We had weekly meetings where we discussed which emerging technologies to adopt. We chose a fingerprinting technique developed in Britain. We reformatted it and automated it with fluorescent probes and high-throughput instruments.

We chose chromosome 19 to study for three reasons: we had already identified three human repair genes on it; we had discovered that it was rich in guanine–cytosine pairs, which implied that it contained a high number of genes; and it was a relatively small size. In retrospect, we made a wise choice because it is still more completely mapped than any other chromosome.

At the time we launched our mapping effort, there was a lot of good biomedical science going on in BBRP such as the work in DNA repair, toxicology, and biodosimetry. We didn't want the human genome effort to overrun the other BBRP programs. We wanted the work to complement the other research, and I am sure that the knowledge generated will continue to provide impetus to nongenome research.

Mapping chromosome 19 was a great time for our group. We were churning over ideas, pushing forward at a great pace. People were really excited, especially the postdocs we brought in. One concern at the start of the Human Genome Project was that it would turn off scientists because it would be mainly routine work. But no one in our group was turned off. We were making new discoveries and pioneering new things; it wasn't drudgery by any means.

We've gotten a lot of recognition for the prominent role we've played in the Human Genome Project and even before it started. We had a good, close-knit, young team. The foundations we laid were the beginning of our counterterrorism work in the early 1990s. We took the technology we had developed in genomics and applied it to DNA diagnostics and to the instrumentation for pathogen detection. These early forays in DNA forensics laid the foundation for the work being done today.

9

Tom Slezak: Bioinformatics

I came to the Lab in 1974 when I was a summer student with Business Services examining the Lab phone system. When I went into Brian Mayall's lab in Building 361, he was unpacking Biomed's first PDP-11 minicomputer. Brian pointed me to the Department of Applied Science (DAS), where I returned to get my masters in computer science. While at DAS I worked in Biomed (someone told me they had the most single women), where I got involved with an early color graphics display system. It cost about $50,000 and displayed in eight colors. I had to write the drivers and software for it.

In 1975 and 1976, Biomed had Modcomp computers for doing analysis of images from automated microscopes. In early 1976, we got our first network for hooking up the Modcomp computers. I left the Lab in 1977 but came back the following year. We got a PDP-11 with UNIX, which was still being developed by AT&T. That was the second or third UNIX machine on site. It belonged to Ted Young, who came from Massachusetts Institute of Technology to do image analysis.

Beginning in the early 1980s, we began to surf the computer technology wave. It was a wild ride. We were the first place at the Lab to use 16-bit microcomputers from Zilog. We had 50 terminals operating off three of the $50,000 Zilogs. Everyone else at the Lab was buying $400,000 VAX machines.

The arrival of the personal computer was a big event, especially the Mac in about 1984. It represented a huge shift, and people in Biomed flocked to it. This all started a few years earlier when Tony Carrano's lab budgeted for an Apple IIe, but he had to write a memo to justify it to Procurement because they worried that people would just use it for video games. They hadn't heard of someone using it for research in a lab.

In the mid-1980s, we installed Ethernet networking. We put in the cables through the ceilings at night. There's a couple-hundred-foot segment still in use. It was an amazing thing to transfer files between machines in 1985.

Tom Slezak

From 1982 to 1985, I spent half-time supporting Biomed and half-time as part of the Lab-wide UNIX support team. I remember we visited Sun when it was two weeks old. In May 1987, I began to work full time for Elbert Branscomb for what became the Human Genome Project. We were the first group at the Lab to use a Sun UNIX workstation for word processing. We then made a big switch to Sun for science use because we needed computers that were reliable and that didn't require us to buy naked computers and then buy parts and write software drivers. As the Human Genome Project scaled up, we bought more Sun workstations and servers and continued to ride the technology curves.

By 1988, we were mapping chromosome 19. I wrote some code that enabled us to use our Sun microprocessors in parallel. It was an example of the start of parallel processing at Livermore. Maximizing our microprocessors was an example of the dynamic tensions we've had here. The large expensive supercomputers, like the Crays, were all but inaccessible to Biomed. We didn't have funds to purchase time on them, and even if we had, we didn't have priority. Like many small Lab programs, we were forced to figure out how to do things with smaller computers. I got us down the parallel computing path and started us using industrial-strength databases.

When the Web appeared about 1994, we jumped on it. At the time we had Mac, PC, and Unix users, so we had to support three different interfaces. By the late 1990s, we developed some metadata-driven software databases that automatically generated interfaces with Web-based data input. That way, we could lower the cost of maintaining software.

When the Joint Genome Institute (JGI) opened, Mark Wagner and I helped to design networks and systems, together with LBL people, for the Human Genome Project sequencing effort. Working with my LBL colleagues was a satisfying part of the time I worked at JGI.

I returned from JGI in 2001 to work on countering bioterrorism. We pioneered the use of whole genome analysis for DNA signatures of pathogens. In the past, a researcher looked for signatures for one gene and then another. We said, let's have the data tell us what's unique about a particular pathogen by comparing it with other pathogen genomes and automating the process to drastically reduce the time. Now it takes less than two hours to come up with likely signatures for a new pathogen. Over the past few years, we have developed signatures for two dozen bacteria and viruses. Once we design a signature, it's validated by the Centers for Disease Control (CDC) and put into use by public health agencies.

The main focus of our bioinformatics group is supporting counter bioterrorism. We have 11 people: computer scientists, biologists, and mathematicians. We're the largest pathogen informatics group in the nation, and we collaborate with every federal group. This year, we were asked by the CDC to examine the SARS virus. More and more, we're being called in to assist people in the federal government who set policy. We're also doing bioforensics to uniquely identify particular strains of pathogens like smallpox and plague.

BBRP is always tracking the leading edge of science, and our computational support is part of that. When the emphasis was on the Human Genome Project, we knocked ourselves out to do our best on that. We had the best physical mapping group. Then we focused on sequencing, and JGI became the second or third most productive public sequencing center in the world. Now, we're using bioinformatics to help Livermore be the best in the world in pathogen signatures. We have an opportunity to really make a difference. Stumbling onto Biomed in 1974 turned into a most interesting career!

10

Christa Prange: IMAGE

In about 1993, we were focused on chromosome 19, mapping it and trying to find all its genes. My boss, Greg Lennon, had a sign in his office that read, "2000 by 2000." His goal was to map (find the precise physical location of) 2000 genes by the year 2000. We had to find genes one at a time, and it was obvious we weren't going to meet that goal. One of the reasons is that actual genes make up only about 2 percent of the total amount of DNA on any given chromosome.

We needed a much more efficient way to find all the genes. That's what the IMAGE (Integrated Molecular Analysis of Genomes and their Expression) Consortium does. IMAGE was conceived by four scientists: Greg, Charles Auffray from the French National Center for Scientific Research, Bento Soares from Columbia University (now at University of Iowa), and Michael Polymeropolous from National Institutes of Health (now at Novartis Corp.). They came up with the idea late at night after a DOE conference on genomics.

Their idea was to make a library containing all human genes and to share them with researchers around the world free of royalties, meaning that researchers would be free to develop new products based on those genes. They also wanted to provide people the sequencing, mapping, and expression data on the clones.

Christa Prange

They wanted to start with a specific tissue like heart and isolate all the genes that are turned on in heart tissue. All genes are present in all cells, but not all are functioning. For example, heart cells manufacture different proteins from liver cells. So different genes are turned on and off in different tissues. If you isolate all the active genes from all the different tissues, you have a complete set of genes. The concept was to make libraries of complementary DNA clones, which are made from DNA copies of messenger RNA that you've isolated from the tissue, and then replicate them in *E. coli* bacteria. After you completed the human gene libraries, you'd move on to other species for comparison.

Each of the four scientists had different research skills, and they decided to form a consortium. Bento contributed the first libraries, Charles did the first sequencing, Michael did the first mapping, and Greg did the cloning, distribution, and bioinformatics work.

Bento's first library was from human brain tissue. We took some plates that had 96 tiny wells filled with growth media, added *E. coli*, and placed a different gene (in *E. coli*) from Bento's library into each well. We added glycerol to the media to prevent the cells from being damaged from freezing because we store each plate at -80°C. In this way, we could archive the plates indefinitely, make new copies of the clones, and send those copies anywhere.

Along the way, we switched to 384-well plates and bar-coding each plate. Now, we make the plates robotically, with a lot faster throughput. In 1995, we set up a worldwide distribution network using several organizations. If someone wants a clone, they go to a distribution center, not us. The cloned genes are available royalty-free, for a small fee. We also send copies to a group that determines the sequence. We currently have about 12,000 plates—about seven million individual clones. It's the world's largest public collection of genes. Most are from different human tissues, but we also have mouse, rat, zebrafish, pufferfish, frog, and a few nonhuman primates such as Rhesus macaque.

We've also put on the Web data on the sequencing, mapping, and expression, as well as domain databases. Bioinformatics tools developed by Livermore's IMAGE team are also on the Web. There is a huge amount of data available free of charge.

Researchers can do several things with the clones. They can grow a cloned gene in *E. coli* to get an unlimited supply of the genes. They can analyze the proteins the genes make to see how a drug could bind to it. They can also make a mouse model by deleting the corresponding gene in a mouse and seeing if there is an effect when that protein is not present. Or they can study what happens when you add an extra copy of a gene, like what happens with Down's syndrome.

Including myself, there are four biologists at Livermore working on IMAGE plus three computational scientists doing data tracking and analysis. One of our goals is to determine the function of all the genes because we don't know what many genes do. Our bioinformatics programs allow us to visualize all the sequences that are similar between various species, which can give us clues as to what that gene might do. For example, we might know what a gene does in a chicken, so we can infer what the related gene will do in humans.

Another focus has been finding full-length versions of genes. Because many of the same genes are expressed in many different tissues, we get a lot of duplicate genes or broken-off pieces. Our goal is to get a full gene contained in one clone as well as get rid of duplicates.

The main benefit of IMAGE is that researchers do not have to waste valuable resources trying to find a gene; they can just order it and spend their time studying what it does.

11

Irene Jones: Chernobyl

When Mort Mendelsohn was associate director, one of his goals was to develop biodosimeters for radiation exposure. By the mid-1980s, Livermore had developed two indicators of exposure to ionizing radiation: the Glycophorin-A assay, which used red blood cells, flow cytometry, and monoclonal antibodies to study changes in a single gene; and chromosome painting, which looks for rearranged chromosomes in lymphocytes using fluorescent DNA. Both of these techniques were applied in studies of the Japanese survivors of Hiroshima and Nagasaki.

When the nuclear reactor at Chernobyl in the Ukraine blew up in April 1986, we said let's see what we can do. The Glycophorin-A assay was up and running, but chromosome painting was not much deployed yet. Dick Albertini, from the University of Vermont, had developed a third assay that looked at the HPRT gene in lymphocytes. We thought it might also be useful. Chernobyl gave us a unique opportunity to look at a population with varied exposure to radiation and see how well the assays could do in detecting radiation effects.

Of course, Chernobyl was half the world away in a country that was distressed by a lot of internal events. The Soviet Union came apart during the time we did the study, and that presented opportunities and challenges, as old connections faltered and new ones had to be made.

The group led by Ron Jensen (now at UC San Francisco) developed a proposal to the National Institutes of Health (NIH) to compare the three biomarkers in people exposed to ionizing radiation and also study genetic changes in children born after the accident. The team included Jim Tucker, who specialized in chromosome painting; Rich Langlois, who was expert in Glycophorin-A; Harvey Mohrenweiser, who looked for mutations inherited from the father, since most of the exposed workers at Chernobyl were male; and myself, who knew the HPRT assay. Dave Nelson, a statistician, joined us in later years.

Irene Jones

It took us several years to get the proposal funded. In the meantime, we started work and kept the team together under LDRD funding. I'm told that our Laboratory Directed Research and Development (LDRD) study was among the most highly leveraged LDRDs ever because a small investment resulted in well over $10 million in NIH funding.

The first thing we had to do was to identify a Russian champion of the project to pull things together. Pavel Pleshanov, a biophysicist in Moscow, was the man. His research involved flow cytometry so he and Rich Langlois had a common interest. Pavel got things done. Bart Gledhill and Ron Jensen went to Russia to meet with him and set up human subject review panels before we started. Pavel found collaborators in the Ukraine and Russia and gave us access to people with ionizing radiation exposure.

In the beginning, our intention was to study the small group of people who were there when Chernobyl blew up and shortly after, who received big doses. They mostly lived in the Ukraine. But there were hundreds of thousands of cleanup workers, also called liquidators, mainly from the Army, who drove trucks, washed roads, cooked meals, and did all kinds of jobs. They didn't wear dosimeters; they served for a certain period and then were told to go home.

In 1990, the Soviet Union broke up, and some of the coherence of our study was lost as nationalities and politics came into play. Our research became a study of Russians. Pavel recruited new Russian collaborators when connections to the Ukrainian population ended.

Pavel identified clinics where liquidators were receiving care. People who had been at Chernobyl were interested in our study, but getting a control group to participate was very difficult. People were skeptical that Americans would be interested in them if nothing was wrong with them. On top of that, just getting samples here and through customs in a timely fashion was a challenge. Outfits like FedEx didn't exist in Russia in the early years. Later, we delighted in tracking shipments online.

We learned we had to be culturally sensitive with the questionnaires we asked our subjects to fill out. An important question was distinguishing between traditional hand-rolled cigarettes, which are extremely strong, and regular store-bought cigarettes. Questions about how the subjects prepared their meat, of such interest to Livermore because of the toxic chemicals we identified in meats cooked at high temperatures, were laughed at by the Russians because American practices like barbecuing and eating a lot of meat were not part of their lives.

We sent our Russian collaborators blood-drawing equipment and other supplies. As soon as samples arrived at Livermore, everyone convened to divvy things up, and scientists went back to their labs. It often took weeks or even months to get results, and then we'd gather together to discuss results.

We were funded twice by the NIH. It was a real coup to have Pavel here in 1996 for our NIH review. Other Russian researchers visited us from time to time, often on their first trip outside Russia. We learned a lot from their reactions to things we took for granted. Several of us traveled to Russia. Rich and Jim saw Chernobyl. One time, Harvey and I visited a medical institute in the city of Tula, south of Moscow. We were the first Americans ever to visit; the region had been closed to outsiders. When we arrived, the staff had gathered on the front steps waiting for us, and TV cameras were recording the event.

I took over the study from Ron in 1995. We completed the project when our last paper was published in 2002. It provided a good benchmark for low-dose radiodosimetry. Chromosome painting still remains the gold standard assay, Glycophorin A is good for higher doses, and HPRT has good sensitivity but adds little to information from chromosome painting.

The study reflected the kind of science you'd like to see at the Lab because so many different disciplines were working together. It was a group effort. There were no egos involved. Success was the goal.

12

Ken Turteltaub: Center for Accelerator Mass Spectrometry

It was about 1987 when Jay Davis and Ivan Proctor from Physics made a presentation to BBRP about an accelerator mass spectrometer (AMS) that they wanted to build on site. I remember a viewgraph that Jay showed that said something like, "Beware of nuclear physicists bringing solutions to problems you never knew you had."

Davis and Proctor were exploring possible Livermore applications for an accelerator mass spectrometer. At the time, about five or six labs around the world were using the technology, but there wasn't a lot of thought about finding biological applications. The technology underlying accelerator mass spectrometry had been published in back-to-back papers in *Science* in 1978 by a group from the University of Rochester and a group from Simon Fraser University in Canada. Earle Nelson from Simon Fraser did a sabbatical at Livermore and helped us to get our first machine going. Two of his people, John Vogel and John Southern, also came to work here.

Ken Turteltaub

I remember a viewgraph that Jay (Davis) showed that said, "Beware of nuclear physicists bringing solutions to problems you never knew you had."

At the time of Jay's presentation, Jim Felton and I were trying to understand the dose response of carcinogens such as food mutagens. A lot of scientists were concerned about the practice of giving rats massive doses of a suspected carcinogen, like an artificial sweetener, looking at the results, and then extrapolating the results downward. The question was whether that approach was appropriate for determining the risk to humans at small or moderate ingested dose levels. Perhaps there was a threshold level, or maybe the response was linear. There was no technology good enough to measure extremely low levels—nanograms—except, apparently, with the accelerator mass spectrometer. We in BBRP were intrigued at the technology's potential and decided to give it a shot.

The Center for Accelerator Mass Spectrometry began as a Director's Initiative under the Physics, Chemistry and Materials Science, and BBRP directorates. Jay bought the parts and built it in 1988 with a lot of help from Physics people.

At first, there was a lot of confusion about setting up the machine because physicists and biologists speak different languages. I remember one meeting where the physicists emphasized the importance of zero degree magnets, and I wondered why they would need to bring the temperature down to zero degrees. I finally learned that they meant an angle of zero degrees. By the same token, lots of physicists didn't understand our discussions of biological issues. But we all finally came together.

When the first data came off the machine, I got so excited because we were detecting levels that we couldn't even approach before. The physicists were worried when they saw our data because they didn't realize that biologists rely on statistics. We'll make 10 measurements and calculate the mean. Physicists will take one measurement and be very confident about that measurement.

The machine worked so well that it hit me that this technology had a big future. I could see a lot of applications, and I was fully committed. We were here night and day and on weekends, having a great time and doing some great work. We published our first paper, in 1989, in the *Proceedings of the National Academy of Science*. The research was on how the body transforms food mutagens so that they bond to DNA. It was the first reviewed scientific paper that used AMS for biological research.

There was a really good response to the paper and to a number of talks that we gave. It led to an entire effort within the biological sciences to use AMS, one that the National Institutes of Health now funds. In our first year of operation, we had about 10 collaborations. Now 30 groups around the world have used it.

We wrote several patents that have become licensed. One licensee is working to serve pharmaceutical companies in Britain.

As AMS has become more useful to biology, we've understood a lot more about it. We've also added a second, smaller machine, that does carbon dating exclusively. We have physicists, biologists, engineers, and nuclear chemists who make the center work, which is one of the center's strengths. It's changed a lot of careers.

13

Fred Milanovich: Rapid PCR

Development of a way to rapidly identify DNA via polymerase chain reaction (PCR) was a breakthrough event that launched Livermore's biodefense program. It began in the mid-1990s, when Allen Northrup and others had a vision of revolutionizing PCR by dramatically shrinking the size of PCR instrumentation.

At the time, PCR was a well-established technique for identifying specific regions of DNA. PCR works by making multiple copies of a particular segment (referred to as the amplicon) of the DNA in the sample. By heating the sample, the double-helix DNA separates into single complementary strands. When the sample is cooled, single strands of DNA called primers attach to the ends of the region to be amplified. Subsequently, an enzyme using nucleotides provided as a reagent replicates that region of DNA. With each heating–cooling cycle, the amount of DNA doubles. Eventually, the amount of DNA is great enough that it can be detected and identified.

In the early 1990s, PCR instruments were large and slow. The heart of these instruments was a thermal cycling unit that was typically a large aluminum block that took time to heat up and cool down. The amplified DNA product was then analyzed by gel electrophoresis. The whole process took many hours.

Northrup reasoned that the key to rapid PCR was to speed up the thermal cycle. And the way to do that was to use a tiny sample chamber with as little mass as possible that would heat and cool very quickly. He also reasoned that we could use fluorescent tags and lasers to detect the DNA right in the thermal cycling chamber, thereby eliminating the need to make gels.

Fred Milanovich

The Lab's Microtechnology Center had lots of experience making miniature devices for many Lab programs. They fabricated inch-long silicon chambers, just thick enough for a tiny disposable polypropylene test tube, with a window on each side. Laser or other light could then be directed through the window to excite the fluorescent tags and monitor the amplification of DNA in real time.

We knew that portable detectors would be a tremendous advance for both the military and for civilian first responders investigating any suspected biowarfare incident. We obtained LDRD funding to demonstrate our technologies.

During that time, U.S. Senator Sam Nunn from Georgia visited the Laboratory. He was one of the first in Washington to take note of the nation's vulnerability to biological terrorist attack. We all recognized that a major limiting factor was the current state of biodetector technology and the length of time required for analysis. We showed him what we were doing to develop a portable detector that could rapidly amplify and identify DNA.

About a year later, in 1996, the Nunn–Lugar–Domenici Act was passed, and it included substantial funding for civilian biodefense. Thanks to our LDRD funding, we had the clear lead in the biodetection field, and we captured a large share of initial funding. We subsequently developed several prototype instruments with multiple PCR chambers.

In September 1997, we successfully field-demonstrated the first portable, battery-powered, multichamber PCR instrument, which provided results in roughly 30 minutes. In February 1998, this instrument was deployed by the U.S. Army to the Middle East.

In April 1999, we published an article in *Science* reporting seven-minute detection of key biological agents, a breakthrough in PCR analysis. In July 1999, we demonstrated the first truly handheld PCR instrument, and in December 1999, we unveiled a 24-chamber suitcase-sized portable instrument. The handheld device was extensively beta-tested in 2000 in such applications as testing imported fish for bacterial contamination and analyzing blood samples for malaria in Africa.

Recognizing the tremendous potential of these instruments, we engaged in aggressive technology transfer efforts, licensing the multichamber technology to Cepheid and the handheld technology to Environmental Technology Group (ETG) of Smiths Industries. The Cepheid Smart Cycler is now the standard in the business and forms the heart of biodetection systems like BASIS (Biological Aerosol Sentry and Information System), which was deployed at the 2002 Winter Olympics in Salt Lake City. ETG's handheld detector, released early this year, is in use in the military and in some public health departments.

We're now working on an instrument that's completely autonomous, functioning automatically from sample collection through sample preparation, DNA amplification, analysis, and reporting of results. This technology was just licensed to MicroFluidics Systems, Inc.

The development of rapid PCR has opened new horizons for biological research, clinical practice, public health, and biodefense. It's the type of advance that I believe could only happen at a place like Livermore, where biologists, engineers, laser physicists, and computer scientists work side by side. And rapid PCR is only the beginning. We're deep into R&D on even more advanced and exciting biodetection instruments and systems.

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Pat Fitch: Health-Care Technologies

In the early 1990s, Lab Director John Nuckolls formed a working group to determine just how many medical-related projects were going on at the Lab. Tony Carrano, the AD for BBRP, chaired the group, which included people from every directorate. At the time, I was leader of the Engineering Research Division. Other people in the group who later played important roles included Ralph Jacobs and Dennis Matthews from Lasers, Allen Northrup from Engineering, and Bart Gledhill from BBRP.

We decided to make a master list of medical-device projects at the Lab. To our surprise, the list of projects was amazingly long. Every directorate had at least one medical-related project, and a few, especially Lasers and Engineering, had several projects. Livermore people were working on these projects because they saw a way that our technologies could change the nature of care people received at the doctor's office or at a hospital.

Pat Fitch

Based on the long list of projects, we decided that Livermore could play an important role in improving health care. Nuckolls said that with so much going on, we ought to be coordinated Lab-wide, so he formed the Center for Health Care Technologies and placed it in BBRP. I was director and Dennis was deputy. We reported to Tony, but every AD kept an eye on how we were proceeding.

Our goal was to get technologies to patients. This requires companies to make the devices or other products. We met with a lot of companies to find out what they needed to take our technologies to market. We knew, for example, that for a stroke-related product to be successful, it had to work effectively, be approved by the FDA, appeal to a physician, and be reimbursable by an Health Maintenance Organization or Health Care Financing Administration.

We discovered that there is a big chasm between the benchtop results at research institutions and the medical devices that were being tested and marketed by companies. The biggest cultural thing the Lab had to learn was that a company has to make enough money from a new device to recoup its initial investments and to sustain its continued operations. Companies have to pay for FDA approval, for marketing and sales staffs, and for their facilities. To succeed, they need to price the device at a lot more than the parts cost.

We wanted real advice so we had an advisory board composed of physicians and venture capitalists. We got earfuls of advice from a lot of different people. For example, we had lunch with one of the founders of Boston Scientific. He told us companies like his have a hard time knowing how to interact with the Lab. He said big companies would be happy to license new patents, but they were more used to simply buying a company that has a team in place already working to bring something to market. He told us we should be talking to venture capitalists and new, small companies if we have something that is at an early stage, even if it's going to revolutionize the medical device field.

After that discussion, we shifted our emphasis more to venture capital firms and small medical companies. That decision ended up working out well because small companies will focus everything they've got on one device. However, some companies saw us as a government behemoth and found it difficult to deal with the Lab because of all the large company processes we have.

There was amazing productivity in those early years. We published a lot and gave a lot of talks. We had no problems communicating with and getting the interest of physicians. Our projects were highly multidisciplinary.

After about three years, we were seeing a lower funding commitment from DOE than we had anticipated, For me, the Human Genome Project was a big attractor. Dennis took over and crafted a newer entity, the Medical Technology Program, which involved Physics, Engineering, Defense and Nuclear Technologies, and BBRP. As I look back on the center and Dennis's program, I think one of our greatest legacies for the Lab is the talented new people we've attracted to Livermore.

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Dennis Matthews: Health Care Technologies

In the early 1990s, there was interest around the Lab, primarily in Engineering and BBRP, in doing medical instrumentation. My job was working for Mike Campbell, associate director for Lasers, and Mike asked me to develop commercial applications for laser technology. I realized right away one of the most promising opportunities for laser spinoffs was in medicine.

Pat Fitch put together an initiative to found the Center for Health Care Technologies, and I became his deputy. Lab Director John Nuckolls decided the center belonged in BBRP. One of our first major thrusts was a strategic initiative to develop technologies to treat stroke. From that, we launched all kinds of things, including some important partnerships with companies.

Doctors came to us and essentially asked rocket scientists to develop medical devices. At first we said, are you kidding? But we soon realized that doctors would love to be scientists. They can't do experiments. They have to follow protocols; if they deviate from a protocol they're slapped with a lawsuit. In getting to know doctors, I went to UC San Francisco to witness minimal invasive surgery, which uses little portals instead of making large cuts into the body.

Dennis Matthews

I think the seminal event took place around 1995 when we sponsored a workshop at Wente's. We called it Stroke Summit, and we invited doctors who treated stroke patients to meet with Livermore engineers, biologists, and physical scientists. Out of that workshop, we received over the years between 10 and 15 patents, and we spawned several startup companies with Livermore technology. For example, Endovasix commercialized our clot removal system; we continue to receive royalties on the patent. We came up with an idea to treat an acute stroke with a catheter-based system to remove the clot. Prior to this, if you had a stroke, medicine couldn't do much for you. We also developed several biosensors such as the continuous glucose sensor, which was transferred to Minimed.

Clint Logan and Laura Mascio Kagelmeyer also developed a digital mammography system with Fisher Engineering. The system took advantage of the Lab's x-ray detectors. We built an x-ray catheter to treat arterial plaque; the device tapped the Lab's microtechnology capability. We also developed, under Christine Hartmann-Siantar, the PEREGRINE system for radiation treatment protocol.

Eventually, the Center for Health Care Technologies went away. In 2002, I formed the Medical Technology Program, a partnership among Defense and Nuclear Technologies, Engineering, Lasers, and BBRP. We've had a lot of collaborations with other universities and medical research centers such as UC San Francisco and Davis, State University of New York at Buffalo, and the Mayo Clinic.

Recently, we've become more involved with infectious disease because of homeland security. We're building artificial organs such as an artificial retina with Oak Ridge National Laboratory and the University of Southern California. We're also developing an artificial kidney that can be worn on the outside of the body like a fanny pack. It uses a Lab invention, aerogels, to filter the blood.

The recognition by NCI in 2002 of the joint UC Davis/LLNL cancer center was a significant event. We're in the hardest of all areas in which to make significant contributions, and that is developing technology to detect and treat cancer. We've commercialized the Bioluminate probe for detecting breast cancer, and we're working to commercialize Lifewave, which would use the Lab's microwave impulse radar technology to monitor vital signs while someone is on a gurney.

The development that may have the greatest effect is the sensitive detection of biomarkers, either genes or proteins, which would indicate a cancer or precancerous condition. Imaging can't detect the kinds of tiny changes that biomarkers would indicate. We're also trying to spin off proton radiography, which was developed for stockpile stewardship.

I recently formed the Center for Biotechnology, Biophysical Sciences, and Bioengineering (CBBB). It involves BBRP, Physics and Advanced Technologies, Engineering, and Chemistry and Materials Science. Our emphasis is on developing new initiatives and concepts in the biosciences and medicine with an emphasis on multidisciplinary efforts. The Medical Technology Program, which still exists, is focused on developing new medical devices.

The fundamental things I've learned is that medical researchers have not historically been involved in interdisciplinary projects. The Lab to me personifies team science, and there's a lot we can do for the health industry.

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Rod Balhorn: Structural Biology

By 1994, much of our research had developed to the point that BBRP decided it was time to establish a program in high-resolution structural biology. This program would provide the tools and expertise at Livermore for us to determine with atomic resolution the detailed structures of proteins, DNA, and their complexes.

Most of the previous structural work at BBRP had been limited to performing biochemical, spectroscopy, or microscopy studies of very large macromolecular complexes, such as chromosomes and whole cells. Several groups within the program were studying the proteins involved in sperm DNA packaging, the metabolism of food mutagens, and the process of DNA repair, and their work had evolved to the point of needing detailed structural information. New staff, such as Michael Thelen, had been hired, who focused their research on the interactions between proteins, rather than genetics.

In the mid-1990s, we set up a program under an LDRD Strategic Initiative to establish a high-resolution structural biology capability. It consisted of three parts: computational biology, x-ray crystallography, and nuclear magnetic resonance (NMR).

The program needed capabilities using both x-ray crystallography and NMR. X-ray crystallography provides structural information about molecules trapped in a crystalline (static) state, but it offers very high resolution, usually in the range of 1 to 3 angstroms. NMR also provides high-resolution data but offers the capability of obtaining information about protein motion and the dynamic aspects of its interactions with other molecules. Using NMR, we can study a protein floating in solution and determine how different parts of its structure change over time and as a result of its binding to other molecules.

The first part of the structural biology effort was the computational biology work. Krzysztof Fidelis was hired to establish a protein structure prediction group and then Mike Colvin arrived from Sandia to provide a capability in quantum chemistry and molecular dynamics simulations at the atomic level. Since that time, our computational biology group has grown significantly. An internationally used structure prediction facility was set up a few years after Krzysztof Fidelis arrived, and the facility's ongoing protein structure prediction "contest" is now used routinely by the international community as a mechanism for developing structure prediction methods and assessing how well they work. Since the contest's inception, it has had a major impact on the field and has changed the way people predict protein structures.

Rod Balhorn

During this time, we also established our x-ray crystallography facility under the direction of Bernhard Rupp. Bernhard and his group established very early a collaboration with the Gladstone Institute at UC San Francisco to study apolipoproteins involved with atherosclerosis. This collaboration helped us quickly move from structural analyses of small molecules into the world of protein structure determination. The group began working with other universities and laboratories, and it joined a consortium devoted to speeding up the process for protein structure determination. Their contribution to the multilaboratory effort has been to automate the process and improve the efficiency of crystallizing proteins.

In 1995, Monique Cosman was hired to set up our biomolecular NMR facility and establish a research effort studying the structural aspects of DNA damage and the function of proteins that interact with DNA. She extended the research on benzo[a]pyrene DNA adducts she had been carrying out at Sloan-Kettering and began examining how proteins interact with these adducts and recognize damage. These adducts are formed when an individual is exposed to cigarette smoke, automobile exhaust, and other combustion products. In collaboration with Ken Turteltaub, she determined the structure of the adduct formed by the food mutagen PhIP. She also began collaborating with other BBRP groups to determine the structures of proteins involved in DNA repair and the structure of complexes of DNA with proteins that inactivate and protect the sperm cell genome.

The BBRP NMR group recently joined forces with Robert Maxwell's group in Chemistry and Materials Science and established the Center for National Security Applications of Magnetic Resonance in Building 151. Last fall, Monique also brought together representatives from all the Bay Area NMR spectroscopy groups to respond to a call from the National Institutes of Health to establish a very high field (900 MHz) NMR instrument as a center to serve Bay Area users. The Bay Area groups combined forces to apply for the development of a northern California facility to be located at UC Berkeley, with Livermore as a backup location. We recently learned that this facility will be funded, so we will have a local state-of-the-art capability.

These new capabilities in structural biology have had a major impact on BBRP research, and they have allowed us to move quickly to respond to national needs in biodefense through the development of threat agent detection reagents. Using a combination of all three structural biology resources (computational modeling, NMR, and x-ray diffraction), the group's first efforts have focused on developing small molecule reagents for use in detecting botulinum and related neurotoxins. This work is being extended to developing molecules that bind selectively to the surface proteins on pathogenic bacteria and viruses. These new reagents will complement the efforts of others at Livermore involved in developing DNA-based detection reagents and methods. The new reagents will provide a second type of detection response that can be used to analyze samples, thereby increasing the certainty that a particular agent is present or not.

Viruses have DNA or RNA molecules that mutate so quickly it's often hard to find sequences that can be used to identify them. But their proteins stay relatively constant; if they didn't, the virus couldn't continue to function. Using computational methods to look at protein sequences, the structural biology group has begun to develop synthetic molecules for virus and bacterial detection. A similar approach is being used to develop prototype therapeutics to counter exposure to anthrax and other kinds of biotoxins.

We're also using our structural biology capabilities to develop targeting reagents for cancer therapy. We're working with UC Davis researchers to help design small molecules that can be used to carry radionuclides directly to tumor cells and treat patients with lymphomas. In collaboration with Julie Perkins in Chemistry and Materials Science, we've recently synthesized two molecules that bind to the surface of human lymphoma cells.

So the major efforts of the structural biology group involve basic science, supporting biodefense, and developing reagents for health care. We also have the potential to contribute to other areas of Laboratory research. For example, our NMR capabilities can help the National Ignition Facility Programs to evaluate polymer laser targets and the Forensics Sciences Center to identify unknown materials.

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Mike Colvin: Computational Biology

I started at the Laboratory as a postdoc in the newly formed Institute for Scientific Computing Research in 1986 and then joined the Physics directorate. I moved to Sandia in 1990, where I started a small computational biology group. After about four years, I started talking with Ken Turteltaub and Elbert Branscomb, who were working on projects that involved computational modeling. Rod Balhorn had started a new program in structural biology with an LDRD Strategic Initiative in NMR and X-ray crystallography. Rod was already working with Nick Winter, a computational chemist from Physics, and his students. Through a collaboration with this group, I got involved in some BBRP projects.

BBRP had hired Krzysztof Fidelis as a postdoc to do protein modeling. Krzysztof and others organized the Critical Assessment of Techniques for Protein Structure Prediction (CASP) in 1994. It was designed as a contest to test different modeling methods via the process of blind prediction. Ever since, CASP has been held every two years. Krzysztof sends out a protein sequence, and people submit their best predictions of the protein's 3D structure. Researchers doing the modeling don't know the true structure because it is still in the process of being experimentally derived. Over the past 10 years, CASP has grown to be an enormous event in which hundreds of groups compete, and it has brought a lot of recognition to the Laboratory.

Mike Colvin

In 1997, Associate Director Tony Carrano decided it made sense, given the growing role of computational sciences, to create a formal computational biology effort. He hired me as group leader to build on the existing modeling work and lay the groundwork for what has now become the Computational and Systems Biology Division. I hired two postdocs, Dan Barsky and Felice Lightstone, who are now group leaders in this division.

With LDRD funding, we started a collaboration to simulate biological systems using Livermore's teraflops-scale supercomputers and first-principles molecular dynamics simulation techniques that were being developed by Francois Gygi at the Center for Applied Scientific Computing and Giulia Galli in Physics and Advanced Technologies.

With this seed funding and capabilities, we started looking for appropriate biology projects; we even taught a one-day class on computational biology for BBRP experimentalists. Quite quickly, a number of BBRP experimentalists joined us in collaborations. Based on these initial interactions, over the past six years we have published a large number of collaborative papers on topics including the mechanisms of food mutagens, the repair of damaged DNA, the binding of synthetic antibodies to toxins, and the action of anticancer drugs.

The Computational and Systems Biology Division is continuing to grow and address new research problems. Krzysztof now is working on assembling a protein model database that will be the official repository of all the best predicted protein structures submitted by researchers worldwide. It could be even bigger than CASP and become a critical part of the protein structure community.

We are also riding the wave of increasingly fast computers that are arriving at the Laboratory. I think the next generation of 100-plus teraflops computers will have a huge impact on biochemical simulations. Right now, we can afford to do our most accurate simulations on systems of only 200 atoms for only 1 picosecond. The new supercomputers will allow us to simulate thousands of atoms for tens of picoseconds and address many biochemical mechanisms. Thus, in the next four to five years, there will be a tremendous wealth of problems we can go after. Of course, in addition to doing simulations on these very large "capability" computers, we also do lots of simulations on the Lab's "capacity" computers; for many problems, we need to run medium-scale simulations on a very large set of biochemicals.

In another new direction, we are exploring how novel experimental capabilities at the Lab can provide data that will lead to better biological simulations. To this end, we've just inaugurated the LLNL Physical Biosciences Institute (PBI) to develop ways to lower the barriers to collaborations between biologists and physical and computational scientists. The PBI is really a training program, in which we've hired a number of very good postdocs who will be using experimental capabilities from around the Laboratory to make accurate measurements or manipulations of different biological phenomena at the cellular or molecular level. For example, one PBI postdoc is collaborating with people in Chemistry and Materials Science on special techniques to measure the pH in specific areas within the cell. Another postdoc is collaborating with Physics to use very fast laser pulses to do "surgery" within a single cell. I believe the PBI will help to continue the Laboratory's strong history of bringing new technical capabilities to important bioscience problems.

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Elbert Branscomb: Founding of JGI

In the 1985 to 1986 time frame, Charles DeLici, then the director of what is now DOE's Office of Biological and Environmental Research (OBER), initiated the Human Genome Project (HGP) by establishing three "genome" centers at the Lawrence Berkeley, Lawrence Livermore, and Los Alamos national laboratories. This move set loose a firestorm of angry, scornful controversy and opposition. The elite of research biology had already agreed that sequencing the genome was a stupid idea. It would cost way more than it was worth, it would displace much single investigator research with a monster "big science" project, it would bury us in a mountain of data we wouldn't know how to deal with, and so forth. And the suggestion that this bad idea would be undertaken by a completely wrong agency (the DOE) only served to greatly compound the crime.

After a failed effort to block DOE's initiative, health science leaders turned successfully to ensuring that the National Institutes of Health would dominate and control the project (properly so, in my mind). The legislation (in 1988) that began the HGP as a national, rather than merely a DOE program, split funding about 60:40 between NIH and DOE (this ratio has widened steadily ever since). A 5-year plan (fiscal years 1991–1995) detailing the goals of the U.S. Human Genome Project was presented to members of congressional appropriations committees in mid-February 1990.

Elbert Branscomb

Of course, as the HGP is crossing its finish line, it is widely and justly celebrated as one of the greatest and most important scientific achievements ever. It has thrust life science research into a bright new golden age. Among the many who had argued furiously against the project, it is now amusingly hard to find any who are ready to acknowledge the fact.

By the mid-1990s, the HGP was dominated by the U.S. NIH and British Wellcome Trust efforts, and the DOE effort had become a relatively minor player. At this point,the NIH and Wellcome Trust decided to switch from the preliminary stage of genome mapping and sequence technology development to an aggressive charge at sequencing the human genome. To effect this, they made a quick and dramatic increase in investment in high-throughput sequencing.

In the context of these facts, in March 1995, Ari Patrinos took over as director of OBER. This included responsibility for the DOE's HGP, which had become that office's top priority. Patrinos quickly concluded that unless the DOE effort was radically reorganized, it would soon become an irrelevant historical footnote and that his office's genome funding, and most probably its funding for all life sciences research, would be lost. He decided that DOE needed to mount, very rapidly and under a fixed budget, a large-scale sequencing effort that would compete with the much more advanced programs under NIH and Wellcome Trust. This would require that DOE put all the money it had been investing at the three genome centers into high-throughput sequencing and also achieve an increase efficiency of at least 10-fold virtually overnight. Yet, at that point, only Lawrence Berkeley National Laboratory (LBNL) had made sequencing a primary focus.

After consulting with experts, his DOE leadership, and the three laboratory directors, Patrinos concluded with them that the only hope of meeting this challenge lay in putting all of the budget into one centrally controlled, high-throughput "industrial" sequencing effort located at a single facility. This idea was hotly opposed by almost all of the people at the centers. For one thing, the three centers had been fierce competitors from their founding. For another, they had enjoyed autonomous authority over their unchallenged one-third of the available budget. Also, each had its own unique approach to sequencing and felt that it alone had the best technology and people. Each center felt certain that if the investment was to be centralized, it should be done at its own center. Finally, many of the staff at the centers rejected the idea that any radical change was needed. It was widely believed, both inside and outside the laboratories, that the idea of forming a "Joint Genome Institute" (JGI) out of the three existing centers was doomed to fail. Undeterred, Patrinos persisted.

In spring 1996, the concept for the JGI was accepted in principle by the lab directors and by DOE management. In late 1996, a Memorandum of Understanding on operating the JGI was agreed to between the three labs, and I was named the JGI's initial director. The JGI was officially launched on January 1, 1997. Given the stakes and the risks involved, there is no doubt that launching it represented a great, do-or-die gamble for DOE.

The story of what then transpired is predominantly the history of the JGI's sequencing productivity, as measured in output, quality, and cost. In FY 1997, as the JGI was forming, all three labs together sequenced about 2 million bases of human DNA. In the spring of 1997, I stated (shooting blindly in the optimistic dark) that our goal for FY 1998 was to produce 20 Mb. For this, we were publicly ridiculed by James Watson and several other eminencies. No sequencing effort had previously achieved even a threefold increase in output in one year, and the just-forming JGI seemed especially handicapped. Yet, we slightly exceeded this goal and established ourselves as one of the Big Five in the public sequencing effort. Currently, the JGI is producing nearly 2 billion bases of raw sequence per month—more in three days than it did in its famous first year—and at less than 1 percent of the cost. In quality and efficiency, it is now without equal.

In that critical first year, many Livermore employees played key roles: Tony Carrano acted as the JGI's field commander for sequencing, Jane Lamerdin and Paula McCready led Livermore's sequencing teams, and Tom Slezak managed the informatics teams and the data flowing from the sequencers.

At the end of 1998, we moved our main sequencing efforts, and the teams from LLNL and LBNL, into the first of two buildings in Walnut Creek, California. Trevor Hawkins, who was experienced in industrial approaches to high-throughput sequencing, joined the staff as my deputy and head of sequencing in March 1999. We were both convinced that to stay competitive, we needed to focus increasingly on strict industrial methods and gamble everything on new technical approaches. Sequencing output soared while a large fraction of the staff, including almost all of the senior staff, left for other positions. Soon thereafter, the Stanford Human Genome Center, under Rick Myers, was added to the JGI as its primary finishing facility. In the fall of 2000, I resigned as director in favor of Trevor, and about the same time, Susan Lucas, an LLNL employee, became the head of the sequencing production operation at the Walnut Creek facility. Sequencing productivity and quality rose steadily and dramatically under her direction and has continued to do so ever since. Trevor served for about a year before leaving. Eddy Rubin of LBNL then stepped in and is now permanent director. To Jim Watson's credit, he openly praised us for our successes on several occasions and served for a time on the JGI's Policy Board.

DOE can take pride in having made quite a few key contributions to the completion of the HGP, but the essential core of these is JGI's work on sequencing human chromosomes 5, 16, and 19. We owe a great measure of this success to three remarkable Livermore employees. Sarah Wenning started as a supplemental labor employee working as my administrative assistant and is now head of JGI's Operations Department. Laurie Gordon, whose role in the mapping efforts on the JGI chromosomes, especially during the final rush to completion, has been both essential to success and a virtuoso in skill and dedication. With respect to chromosome 19, Laurie's work was of course building on the exceptionally successful foundation in mapping that chromosome earlier laid down by Anne Olsen and Bragitte Brandriff. Susan Lucas, starting in an entry-level job in Livermore's sequencing team in November 1997, and armed only with a B.S. degree, emerged rapidly as a gifted leader and manager. I believe she has no equal in the business.

Given the stakes and the risks involved, there is no doubt that launching it represented a great, do-or-die gamble for DOE.

Throughout this history, however, the dominant factor in the JGI's success, and much more as well, has been its guiding hand, Marvin Frazier. As director of OBER's Life Sciences Division, Marv has been the heart and mind of all of DOE's genomic sciences efforts. Under his guidance, the JGI has gone on to sequence the genomes of many other creatures: a fish, a primitive chordate, some funguses, several protozoans, and many bacteria. It is now doing a tree, a frog, several plant pathogens, and many more bacteria. The JGI has also established important efforts in evolutionary, functional, and computational genomics.

There is little doubt that the success of the JGI saved DOE's genome bacon; but in doing that it achieved a lot more. On the basis of JGI's record and capabilities, DOE has mounted a number of aggressive new basic and applied science initiatives, most notably and importantly the Genomes to Life program. In these programs DOE is establishing itself as a major factor in the basic scientific enterprise of figuring out how life works.

Was forming the JGI a good idea? Was it really necessary? Was it done the right way? History, blessedly perhaps, is famous for refusing to disclose its alternatives.

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Bert Weinstein: Putting "Bio" in Biodefense

I first got involved in biodefense in the fall of 1996 when I was in NAI (Nonproliferation, Arms Control, and International Security directorate) and serving as a member of DOE's Science Council, an advisory board to its Nonproliferation and National Security Program. Don Prosnitz and others in NAI made a bold proposal for DOE to take a strong leadership position and develop technology for countering bioterrorism. At the time, protecting civilian populations from bioterrorism was not much on the nation's radar screen. The military had its own solutions, but they were not appropriate for protecting civilians. A small group, mainly from Livermore, Los Alamos, and Sandia, planted the idea in DOE and Congress for a strong national development effort.

The seeds of Livermore's technical contributions were in development at the time. We had developed rapid PCR (polymerase chain reaction), and the first field test took place at the Army's Dugway test center in the fall of 1996. It was very successful, got people's attention, and put Livermore on the map as having something to contribute.

Bert Weinstein

Early on (and even now to some extent), there was a tendency for people to think all the challenge was in technology development—if you could do rapid PCR amplification of DNA, then detecting pathogens was solved. But in reality, the biology is very complex. A PCR reaction amplifies about 1/20,000th of a microbe's DNA. Which tiny piece should be targeted? Will naturally occurring microbial backgrounds interfere with detecting pathogens? How hard is it to find DNA segments that aren't shared with other microbes? How hard is it to find DNA that's included in every single strain of a pathogen? These were all questions about which we knew very little and are still just getting a handle on.

So it was important to get an investment in biology as well as technology. Fortunately, a few far-sighted people recognized this and started the ball rolling. Janet Dorigan, from one of the famous "three letter agencies," started a program in the early 1990s to develop DNA signatures. People in BBRP, like Jane Lamerdin, Jackie Stillwell, and later Gary Andersen and others, used our capabilities in DNA sequencing and PCR to develop primers that could detect pathogens. Looking back, these now seem like extremely crude products. Only tiny amounts of sequence data were available, and candidate primers were chosen at random for testing. Very little was known, and there were few tools, but it was cutting-edge for the time.

In 1996, the Nunn–Lugar–Domenici bill established the first DOE funding for countering biological attacks. There were huge communication gaps between the scientists and the program leaders at DOE and other agencies. Communication with the biologists was a particular problem. None of the program people understood biology, and few research biologists had experience working for a product-focused organization. Fortunately, both the biologists and the program managers believed that biology was important to successful biodefense, but we lacked the ability to construct a good plan for the program managers with milestones and priorities.

We were fortunate that the DOE leadership, especially Page Stoutland (at the time on assignment from Los Alamos and currently a Livermore employee), pushed for a long-term road map for how scientists would support a national program. Page provided time for the planning to evolve as biologists learned to communicate to program managers and vice versa.

I helped the biologists couple better to the program. We organized a series of visits, meetings, and workshops with biological researchers from Livermore, Los Alamos, and other centers. This started a planning process that lasted about two years and was one of the most constructive interactions between the national labs and Washington I've seen. Together, we figured out what was needed, and the scientists had time to redirect their research to the right goals. By 1999, we had articulated a road map for providing the scientific foundation to underpin a strong bioterrorism defense. A greatly expanded Chemical and Biological Nonproliferation Program began in late 1999.

In parallel with this planning dialogue with the DOE program, people in BBRP, NAI, and other Livermore directorates continued working to link biology and technology. Technology development efforts were focused on handheld detectors and autonomous air–sampling systems. Several of us, Fred Milanovich and I in particular, had many discussions about what was needed from the biologists to make these technologies successful. Eventually, we learned how to formulate and articulate the science goals in a way that technologists, biologists, and program managers could all understand. The ideas seem pretty obvious in retrospect, but at the time, they provided a tremendously important clarification of the objectives and the roles of the different science elements. A few years later, Holly Franz from the Air Force made a remark that really captured the relationship between the technologists and the biologists. She had been touring the detector development work and met some of the biologists. "Oh," she said, "so these are the people who put bullets in your guns."

With experience and the addition of more smart people, we refined the effort. Paula McCready joined us in 1999 and brought her knowledge of high-throughput genomic methods. She focused on developing and validating assays and getting them into the field. This led to the BASIS demonstration project and put us on the map on the biology side as well as the technologies side.

By 2001, we had enough experience to tie all the pieces together into a clear, coherent plan. In particular, we figured out how to use newly abundant sequence data and informatics as the feedstock for assay development. Tom Slezak and his group worked with Paula and created a terrific computational pipeline for assay development.

So why were we successful? It wasn't apparent that we would be, and in the beginning, we received a lot of skeptical comments and questions. Looking back, we were successful for two reasons: we had the right people, and we made the right partnerships.

DOE had people at the national labs with extensive experience in the Human Genome Project and decades of biological research. We had people who could pull together resources from many disciplines and who could construct and run a product-oriented program with basic research as a component. We had topical experts on specific pathogens such as anthrax, and we were comfortable working on national security problems.

Looking back, we were successful for two reasons: we had the right people and we made the right partnerships.

The second success factor has been the partnerships we've made with other organizations. Early on, there was much debate about which federal organization would be in charge in the event of a domestic bioterror incident. The debate is not over. We tried hard not to take sides and to work with everyone, but we did make some choices. My conviction was that when there was an incident it would be, first of all, a public health problem. It might become a criminal investigation, it might have national security implications, but it would start as a public health issue. So we decided to work closely with the Centers for Disease Control (CDC). I arranged a visit to CDC by DOE leadership in March 1999, and when the expanded CBNP started in the fall, Paula took the lead and really developed the relationship beautifully. CDC validates our products and distributes them to the public health network. This partnership has proved invaluable in gaining acceptance and credibility for our work.

Since fall 2001, with 9/11 and the anthrax attacks, biodefense has changed dramatically. When we started in the mid-1990s, it was a cottage industry. Now, there's a huge push to develop a nationwide infrastructure with a multi-billion-dollar investment. The number of players in the field has skyrocketed. It's nice to see that our original vision was pretty much on target and became the starting point for the vision for the Department of Homeland Security. It has also put us in a good position to build on that vision.

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Biological Research Evolves at Livermore

As the Laboratory celebrates its 50th anniversary, its biological research program begins its 40th year. Established in May 1963 by the Atomic Energy Commission, the program's original mission was to investigate the effects of ionizing radiation on humans.

Today, Livermore's biological research extends far beyond studying the effects of radiation. A primary emphasis is countering the terrorist threat that grips our nation. The anthrax scares in the fall of 2001 alerted us to the danger of bioterrorism and heightened the need for fast, accurate, inexpensive methods to detect biological warfare agents. Fortunately, long before last fall, Livermore was a leader in developing innovative methods and technologies for early detection of bioterrorism threats. Since the attack, the Laboratory has intensified its efforts in this area so vital to national security.

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Radiation effects and bioterrorism response have more in common than might at first be apparent. The link is DNA, the genetic code of all living things. Technologies developed during Livermore's studies of how radiation affects DNA contributed to the founding of the Human Genome Project, the largest biological research project ever undertaken. Since the working draft of the human genome was completed in 2000, the genomes of many other animals and microbes have been sequenced. Sequencing the DNA of bioagent microbes supplies the basis for DNA signatures that are being put to work in new detectors.

Livermore's early analysis of DNA damage has evolved into long-term research in several areas important to human health. Research on radiation exposure resulted in new assays that were first used to evaluate genetic changes in atom bomb survivors in Japan and later applied to understanding the exposures incurred by workers who cleaned up the Chernobyl nuclear power plant after the 1986 accident. Several of these tools have broad application in bioscience. Another research area focuses on how DNA repairs itself. One project analyzes the ways that damaged DNA affects sperm during critical stages of reproduction. Another examines how cooking certain foods produces chemicals that damage DNA. Along the way, Livermore bioresearchers have pioneered many new tools and methods for bioscience research, often collaborating with physicists, chemists, engineers, and computer scientists.

In 1972, Roger Batzel, then Laboratory Director, said, "I personally view Bio-Med as an area which could well grow. It's been a relatively small program, but I think it could develop into one of the strengths of the Laboratory."

Batzel could hardly imagine how dramatically Livermore's nascent biomedical program would grow and change. The recent proposal to establish a homeland security center of excellence at Livermore owes much to the distinguished efforts over the years of many Livermore biological research scientists.

Of Chromosomes and DNA

Biological studies at Livermore have two major origins. One was the advent of thermonuclear testing in the Pacific Ocean during the mid-1950s. The other was Project Plowshare, which was devoted to the peaceful uses of nuclear weapons for stimulating underground natural gas production, mining, blasting out harbors, and perhaps even creating a new Panama Canal. Testing in the Pacific and in the Soviet Union had made radioactive fallout a major public issue. With Plowshare's vision of nuclear explosions near populated areas for routine engineering tasks, nuclear contamination became a more direct concern.

John Gofman, a professor of medical physics at the Donner Laboratory of the University of California at Berkeley, was recruited to set up the new program. As it happened, Project Plowshare was largely shelved by the time Gofman started working. "But he studied the dose to humans anyway, with an emphasis on radiation safety," says Mort Mendelsohn, who followed Gofman as leader of the biomedical research program.

By 1963, the scientific community suspected that DNA was the cellular part most sensitive to radiation damage. Gofman had already become involved in cytogenetics, the study of chromosomes, a field that was making major advances at the time. According to Mendelsohn, "Gofman wanted to measure chromosomes for a reason that was way ahead of its time." Many researchers were growing

During the 1983 celebration of the 20th anniversary of biomedial research at Livermore, then Laboratory Director Roger Batzel, Associate Director Mort Mendelsohn, and former Program Director John Gofman viewed the work of bioscientist Laurie Gordon.

We count it as a privilege to do everything we can to assist our medical colleagues in the application of these new tools to the problems of human suffering.

Ernest O. Lawrence, in his acceptance speech for the 1939 Nobel Prize for Physics, speaking of practical applications for his cyclotron.

cancer cells in culture, and Gofman suggested examining the chromosomes in these cells to see what changes they had in common. He developed a method of analyzing chromosomes by measuring their length. It proved to lack adequate sensitivity, but his work set the stage for future cytogenetics progress at Livermore.

In 1974, two years after Mendelsohn's arrival, Livermore scientists made history when they successfully measured and sorted hamster chromosomes using flow cytometry. In humans and other complex organisms, DNA is packaged into chromosomes. Humans have 23 pairs, or 46 total. With flow cytometry, researchers could for the first time automatically identify and sort individual chromosomes or whole cells for subsequent assessment.

During the 1970s and 1980s, the Laboratory made rapid advances in flow cytometry and was for many years a premier institution for cytometric research. In fact, Mendelsohn and other Livermore scientists founded the Society for Analytic Cytology, now the International Society for Analytic Cytology. The journal *Cytometry*, first issued in 1980, was published from Livermore for many years. More recently, Livermore engineers miniaturized flow cytometry in microfluidic systems that support medical devices and detectors for biological and chemical agents. (See *S&TR*, November 1999, pp. 10–16.)

By 1979, scientists had learned how to sort human chromosomes, which are much smaller and more varied than the hamster's. By 1984, says Mendelsohn, "We had increased our proficiency and confidence in flow cytometry such that we could separately identify and study each of the human chromosomes." This ability, combined with worldwide developments in recombinant DNA technology, led to the Livermore–Los Alamos project to build human chromosome-specific DNA libraries.

"The development of chromosome-specific libraries was important," continues Mendelsohn. "At that time, sequencing technology was slow and primitive. The thought of sequencing the entire human DNA was overwhelming. But when the sequencing process could be broken down into smaller pieces—chromosomes—it became a possibility."

At a 1984 meeting, molecular geneticists from around the world brainstormed the potential for DNA-oriented methods to detect heritable mutations in the children of people who survived the atom bombs in Japan. Many of the questions were so challenging that large-scale, detailed genomic sequence analysis would be needed to even attempt to answer them. (To this day, the basic question of how often heritable mutations occur remains unanswered.) Recognizing the classes of problems that require large-scale, detailed sequence data helped inspire the idea of sequencing the entire human genome.

In 1986, the Department of Energy launched a major initiative to completely decipher the human genetic code. A year later, Livermore researchers began to study chromosome 19, which they had earlier learned was home to several genes important for DNA repair. DOE joined forces with the National Institutes of Health in 1990 to kick off the Human Genome Project.

In 1992, Anthony Carrano became associate director of biomedical research. Carrano, who had been studying chromosomes and DNA since arriving at Livermore in 1973, was instrumental in building the Laboratory's human genome efforts, particularly sequencing. In 1996, he helped form the Joint Genome Institute (JGI). This collaboration of the Livermore, Berkeley, and Los Alamos national laboratories pooled resources to form a production facility to sequence human chromosomes 5, 16, and 19 for the international Human Genome Project.

During the 1990s, sequencing technologies matured, becoming ever more automated. Sequencing speed increased rapidly. A working draft of the three chromosomes was completed in April 2000, a year ahead of a greatly accelerated schedule set just 18 months earlier. (See *S&TR*, April 2000, pp. 4–11.) This accomplishment was a major step toward understanding DNA and its functions and a significant contribution to the completion of draft sequences of the entire genome in June 2000.

Still Much to Learn

In the excitement over the completed sequence of the human genome, it is easy to forget that this step is just a prologue. The next step is to identify all of our genes and determine what they do and how they do it. Comparative genomics—in which the genomes of different species are compared—is helpful. Mouse DNA is useful because about 99 percent of a mouse's genes are similar to human genes. Comparing how these genes work in mice and how they are activated under different conditions tells us much about our own genes. A JGI team led by Livermore biologist Lisa Stubbs compared human chromosome 19 with similar sections of the mouse DNA to understand the functional significance of DNA sequences. (See *S&TR*, May 2001, pp. 12–20.) Stubbs notes, "Imagine taking human chromosomes, shattering them into pieces of varying lengths, and putting them back together in a different order. That's what mouse chromosomes look like." The Japanese pufferfish (fugu) has also been sequenced because its genome is a compact version of our own.

Another outgrowth of the Human Genome Project is proteomics, the study of the 100,000 or so proteins that are generated by our DNA. Proteins are the building blocks of our cells and of the molecular machinery that runs our tissues, organs, and bodies. Understanding how proteins operate is essential to understanding how biological systems work.

X-ray crystallography and nuclear magnetic resonance spectroscopy are two tools Livermore is using to determine the three-dimensional structure of proteins at the atomic level. From that structure, computational methods can attempt to model a protein's function. But determining the structure protein by protein would take years of research to complete. Instead, Livermore scientists are using the minimal data available in computational models to try to predict a protein's structure.

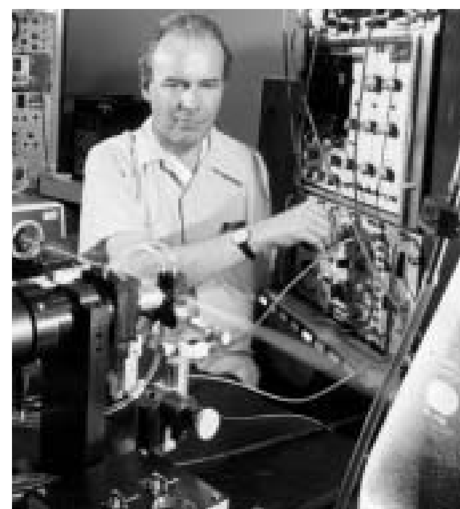
Measuring Radiation Effects

In the first 10 years of Livermore's biological research program, scientists searched for biological measurements that would indicate the radiological dose to which an individual had been exposed. Livermore developed several biological dosimeters to detect and measure changes in human cells, significantly advancing the study of human radiation biology and toxicology. The first was the Glycophorin-A assay that detects residual mutations in human red blood cells from exposure to radiation decades earlier. Its first use was on atom bomb survivors in Japan.

Work on the Glycophorin-A assay begat one of Livermore's first biotechnology projects. In the late 1970s, Laboratory biologists needed antibodies that recognize the subtle distinction between normal and mutant red blood cells. Researchers rolled up their sleeves and began to produce these and many other made-to-order monoclonal antibodies (antibodies derived from a single cell) with a range of potential uses—from detecting sickle cell anemia to evaluating how fast cancer cells are growing. Livermore is no longer in the production mode, but many of its monoclonal antibodies were commercially produced and used by others.

Another important technology developed at Livermore in the mid-1980s is chromosome painting. Scientist Dan Pinkel was instrumental in developing this technology, and the patent for this work has been one of the most lucrative in

Bioscientists Anthony Carrano, who later became associate director, and Larry Thompson in 1978. They had just developed a quick and efficient test to detect damage to genes. The test was based on a finding by Livermore scientists that there is a direct relationship between hard-to-spot gene mutations and an easily recognized process that occurs during cell division. Today, Thompson performs research on DNA repair processes.



Marv Van Dilla, an expert in flow cytometry, came to Livermore from Los Alamos in 1972. Shown here in 1973, Van Dilla was instrumental in establishing the Laboratory's preeminence in cytometric research. Livermore was the first to use flow cytometry to sort chromosomes.

Researcher Laura Chittenden is shown with a mouse. Mouse DNA, 99 percent of which is similar to human DNA, is being compared with human DNA to help uncover clues to gene regulation and control.

Chromosome painting is the process scientists use to fluorescently label small pieces of DNA from a chromosome-specific library. These chromosome-speciific fluorescent probes bind to complementary sequences of the target chromosome and, when viewed under a microscope using fluorescent light, can reveal a targeted gene along a chromosome. This photo is of chromosomes from one-day-old mouse embryos. The chromosomes are chromosomes 1, 2, 3, and X. The circled chromosome is Y.

Livermore's patent portfolio for the past several years.

When first developed, chromosome painting was used to identify DNA damage in which the ends of two chromosomes break off and trade places with each other. These "reciprocal translocations" are one of the distinguishing effects of radiation damage to DNA. Using chromosome painting, scientists can see and count translocations between two differently painted chromosomes to determine a person's likely prior exposure to ionizing radiation. This method of identifying translocations is 10 to 100 times faster than it was before, with greatly increased reliability.

A third dosimetry method measures the frequency of mutations in the hypoxanthine phosphoribosylantransferase (HPRT) gene in lymphocytes. This assay was developed elsewhere, but since the 1980s, researchers led by biological scientist Irene Jones have greatly expanded understanding of the assay's ability to detect DNA damage from ionizing radiation.

Immediately after the 1986 Chernobyl nuclear accident, the glycophorin-A assay was put to work to screen cleanup workers for their exposures. Years later, bioscientists used the HPRT assay and chromosome painting to measure mutations and alterations in lymphocytes to reconstruct the doses received. (See *S&TR*, September 1999, pp. 12–15.)

To Your Health

A natural extension of studying the effects of ionizing radiation on humans was to explore how radiation and chemicals interact with human genetic material to produce cancers, mutations, and other adverse effects.

In the face of damaging toxins, DNA is able to repair itself—up to a point. How DNA repairs itself has been a focus of ongoing research under bioscientist Larry Thompson almost since the Laboratory began to study DNA damage. Livermore chose to sequence chromosome 19 as part of the Human Genome Project because its properties suggested that it was gene-rich, which proved to be an accurate prediction. Chromosome 19 has the highest gene density of any human chromosome. It was also an apt choice because Livermore researchers had earlier discovered that three genes on chromosome 19 are involved in the repair of DNA damaged by radiation or chemicals. In studies of the Chernobyl cleanup workers, a goal has been to understand why the same dose of radiation has different effects on the cells of individuals. Identifying the differences in DNA repair gene sequence and function for different individuals is key.

In the 1970s, Livermore's growing expertise in flow cytometry enabled researchers to analyze and sort sperm for the first time. Using this approach, scientists could begin to study the effects of pollutants on DNA during critical stages of sperm formation. Under the leadership of biophysicist Andrew Wyrobek, Livermore has developed several powerful molecular methods to visualize individual chromosomes in sperm and to detect genetic defects in embryos. (See *S&TR*, November/ December 1995, pp. 6–19.) These research methods, combined with animal models, have broad implications for screening males for chromosomal abnormalities and genetic diseases,

for studying the effects of exposure to mutagenic agents, and for assessing genetic risks to embryos and offspring.

Even the food we eat can damage our DNA. Both 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyrinidine (PhIP) and 2-amino-3,8-dimethylimidazol [4,5-f] quinoxaline (MeIQx) are heterocyclic aromatic amines that appear in meat when it is cooked at high temperature. These compounds and others produced when they are digested form adducts, which are molecules that attach to DNA strands and may interfere with their function. Jim Felton, who is now deputy associate director for Biology and Biotechnology Research Program (BBRP), led a group studying food mutagens for almost two decades.

PhIP and MeIQx have been shown to cause cancer in laboratory animals when administered at high doses. More recently, researchers wanted to know whether DNA and protein adducts can be detected in laboratory animals and humans when they take in a smaller, more typical dietary amount of these substances. In numerous experiments using carbon-14-tagged PhIP and MeIQx molecules, the team has

Biology Meets the Computer—The Early Days

Throughout its 50-year history, the Laboratory has pioneered the use of powerful computers to solve complex scientific problems. Challenges in biological research were no exception.

In the mid-1960s, new work on the dynamics of cell multiplication made use of computer codes first developed for Livermore's weapons program. Part of an effort to design an optimal radiation dosage program for cancer therapy, the study included an ingenious calculation system using computer codes to simulate cell activity.

A remarkable combination of an electron microscope and a computer in 1968 produced dramatic three-dimensional images of organelles, tiny working parts within the cell nucleus. Using essentially the same process the human brain uses to produce three-dimensional images from two flat pictures—one taken with each eye—the computer took 12 electron microscope shots, integrated the information, and created three-dimensional images of the organelles that were 50,000 times their real size. The feat had never before been accomplished.

By 1973, Livermore's cytophotometric data conversion system (CYDAC) was attracting interest when it showed that it could measure the DNA in individual chromosomes to great sensitivity. CYDAC studies showed unsuspected small differences in chromosomal DNA content among supposedly normal individuals.

In its first clinical application in 1974, CYDAC confirmed a suspected chromosome abnormality in a patient with chronic myelogenous leukemia (CML). In the early 1960s, scientists discovered that CML was invariably associated with a loss of genetic material from a portion of chromosome 22. This aberration was rarely found otherwise. About 10 years later, researchers at the University of Chicago found an excess of chromosomal matter on chromosome 9 in the same patients. They suspected that the lost material from chromosome 22 had been captured by chromosome 9. It took CYDAC's unprecedented precision to confirm that hypothesis and set cancer researchers on the track of other DNA translocations.

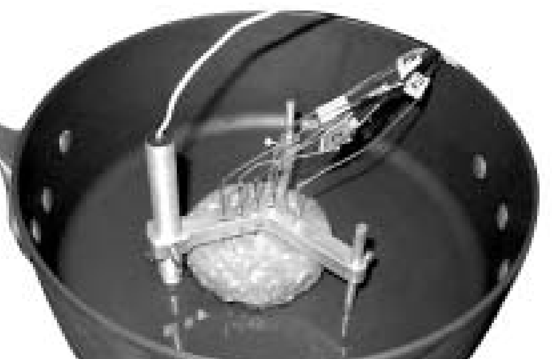
Bioengineers at Livermore combined mechanical skills with an understanding of biology to design the cytophotometric data converter (CYDAC), a highly sensitive diagnostic instrument that measures the amount of DNA in chromosomes. In this 1976 photograph, bioresearcher Linda Ashworth uses CYDAC to scan chromosomes from a mammalian cell.

confirmed not only that adducts can be detected at low doses, but also that humans may be more sensitive to these substances than mice or rats.

Such experiments would not have been possible without Livermore's Center for Accelerated Mass Spectrometry. Physics-based accelerator mass spectrometry (AMS) is so sensitive that it can find one carbon-14 atom among a quadrillion other carbon atoms. It can observe the interaction of mutagens with DNA in the first step in carcinogenesis. Livermore is one of just a few institutions in the world using AMS routinely for biomedical and pharmaceutical applications, and it is a recognized leader in the field. (See *S&TR*, July/August 2000, pp. 12–19.)

Continuing a long tradition of collaboration with universities, Livermore joined forces with the University of California at Davis Cancer Center in October 2000 to fight cancer, the nation's second leading killer. Together, they are researching cancer biology, prevention, and control as well as new cancer detection and treatment techniques. In July 2002, the center attained National Cancer Center status from the National Cancer Institute. AMS is a key technology in this collaboration's research.

Meat cooked at high temperatures produces mutagens, which are compounds that can damage DNA. Here, a fully instrumented hamburger patty is fried to determine its temperature as a function of depth as well as the corresponding concentrations of food mutagens. The data are used to develop computer simulations of the cooking process and to predict the formation of mutagens.



Putting the Computer to Work

Computers have played an integral role in biological research at Livermore for years (see the box on p. 52). In fact, the biomedical program was the first one at Livermore to purchase a personal computer for scientific use. The Procurement Department looked on this purchase with considerable suspicion, viewing a personal computer only as a means to play "Pong." But that little PC automated what had been a tedious manual cell-counting process, and it is impossible to imagine the Laboratory without desktop computers today.

Using both mainframe and personal computers, the Laboratory has pioneered many new ways to use the computer in a biological research setting. Bioinformatics is an area of special strength. In bioinformatics, computer scientists organize the results of molecular biologists' work, developing databases and new analytical tools so that the data can be put to good use. Livermore's leading role in the Human Genome Project would not have been possible without the efforts of BBRP's bioinformatics team. Computer scientist Tom Slezak started this group almost 25 years ago and still leads it.

"Our work is 'bottom of the iceberg' stuff and invisible to most people," says Slezak. "But it's really important. In sequencing the human genome, the flood of data was enormous. As other organisms are sequenced and as the field of comparative genomics takes off, we try to leverage our computational capabilities to stay a step or two ahead."

Computational biology, a relatively recent research area, builds on the Laboratory's strength in computations. According to Michael Colvin, who leads the Computational Biology Group at Livermore, "The emerging explanation of biological functions in terms of their underlying chemical processes is creating an important role for predictive chemical simulations in biological research."

Livermore scientists are at the forefront of integrating computation and experiment in bioscience. Ongoing computational biology projects include studying the action of anticancer drugs, DNA-binding properties of mutagens in food, the binding of ligands to selected sites on proteins, the mechanisms of DNA repair enzymes, and the biophysics of DNA base pairing. (See *S&TR*, April 2001, pp. 4–11.)

A particularly exciting tool in computational biology is first-principles quantum mechanics methods to describe the electronic structure of atoms and their chemical properties. Computerized quantum simulations permit researchers to "see" inside biochemical processes to learn how reactions are taking place on a molecular and even atomic level. Such simulations are highly intensive computationally and had to await the arrival of massively parallel computers before they could be performed. (See *S&TR*, April 2002, pp. 4–10.)

Fighting Bioterrorism

Bacteria, viruses, biological toxins, or genetically altered organisms could be used to threaten urban populations, destroy livestock, and wipe out crops. These agents are difficult to detect and to identify quickly and reliably. Yet, early detection and identification are crucial for minimizing their potentially catastrophic human and economic cost. At Livermore, developing technologies to detect agents of biological warfare has been under way for a decade. Livermore researchers pioneered technologies for rapid detection of tiny amounts of DNA. Equally important has been identifying specific DNA sequences that can be targeted with our detectors. With the recent anthrax attacks and the resulting awareness of bioterrorism threats, Livermore has stepped up its efforts to optimize stationary and portable equipment to detect biological agents.

The foundation for this research was laid during the early years of the program and studies of DNA. For example, by computationally comparing the DNA sequence of *Yersinia pestis*, the bacterium that causes bubonic plague, with the sequence of its close relatives and other bacteria, Livermore has been able to develop unique DNA signatures that allow *Y. pestis* to be quickly detected. (See *S&TR*, May 2000, pp. 4–12.)

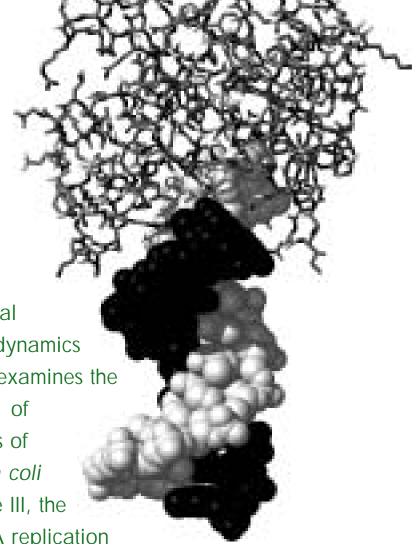
An entirely new sequencing analysis technique, developed by Livermore's bioinformatics team, recently won one of two 2002 Lawrence Livermore Science and Technology Awards. Using their experience from many years on the Human Genome Project, the team members found a novel way to perform whole genome analysis to compare genomic sequences. With it, they can rapidly determine unique DNA signatures of biowarfare pathogens. They are the first to apply whole genome analysis to pathogens.

Several DNA-detection technologies have been licensed to industry, most recently the Handheld Advanced Nucleic Acid Analyzer (HANAA). Some of these devices depend not only on accurate DNA signatures but also on microfluidics—the miniaturization of piping systems through which fluids flow. In a collaboration with Los Alamos National Laboratory, Livermore's DNA analysis capabilities were used to develop the analysis core of the Biological Aerosol Sentry and Information System, which was deployed at the 2002 Winter Olympics in Salt Lake City, Utah.

Another technique for detecting biological agents focuses on detecting the proteins that DNA generates. Protein detection techniques are typically fast and easy to use but are not as sensitive and specific as DNA detection methods. Livermore is designing seek-and-destroy, antibodylike molecules, called high-affinity ligands, that target specific proteins in biological agents. The development of ligands for detecting tetanus toxin is almost complete. This detection methodology promises to be fast and easy to use as well as highly sensitive and specific. (See *S&TR*, June 2002, pp. 4–11.)

Physics to Biology

Many threads link physics advances and bioresearch progress. Ernest O. Lawrence, founder of the Laboratory, set the precedent for applying tools developed in the course of physics research to fighting human disease. After Lawrence built the cyclotron, he put it to use as a medical tool as quickly as he could. In 1937, Lawrence's mother, Gunda, was told by many specialists



This classical molecular dynamics simulation examines the motion of 1 of 10 proteins of *Escherichia coli* polymerase III, the major DNA replication enzyme in E. coli bacteria. This protein's function is to "proofread" a newly synthesized DNA strand by excising any incorrect bases immediately after they are added to the DNA. The goal of this simulation is to understand the chemical mechanism of the proofreading function. Shown as sticks is the proofreading protein. The yellow and green spheres simulate the double-stranded DNA being proofread.

The Handheld Advanced Nucleic Acid Analyzer can detect biological pathogens in the field. It examines the DNA of a sample and compares it with the known DNA sequence of various pathogens such as anthrax and plague. Rapid detection of agents of biological warfare could help save lives because the diseases resulting from many such pathogens are highly treatable if detected early.

that she had an inoperable tumor. But her life was saved by radiation treatment with the only megavolt x rays then available in the world, using a device developed by her son. She was still living in Berkeley when he died 21 years later.

In this tradition, Livermore recently developed an innovative tool for analyzing and planning radiation treatment for tumors. In the early 1990s, researchers began combining Livermore's huge storehouse of data on nuclear science and radiation transport with Monte Carlo statistical techniques. The result was PEREGRINE, a radiation planning technology that has been licensed to a private company and was approved for use by the U.S. Food and Drug Administration in September 2000. (See *S&TR*, June 2001, pp. 24–25.)

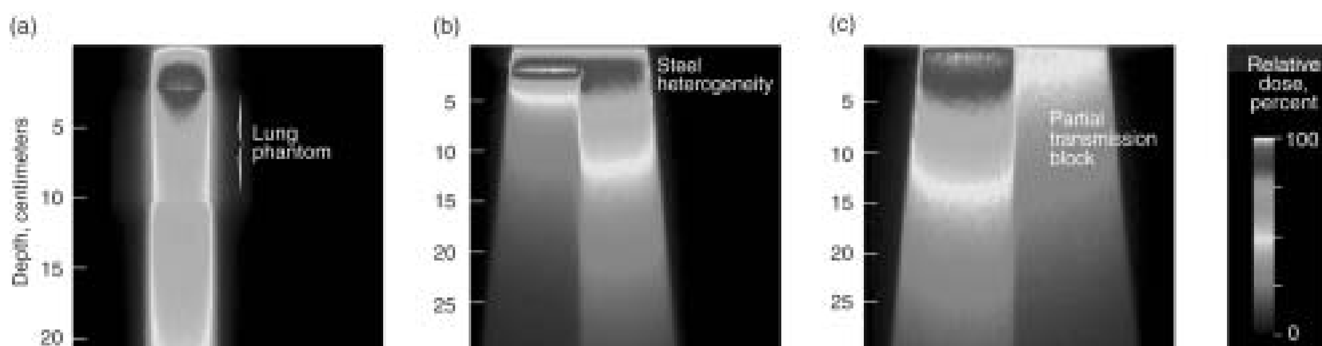
Mrs. Lawrence's treatment and PEREGRINE bring the results of physics research to bear on a pressing medical challenge. Weapons materials have also been used in artificial hip joints designed at Livermore. X-ray tomography developed to examine the inner components of nuclear weapons has revealed the bone weakening of osteoporosis. Quantum simulations, a physics tool that can describe the fundamental interactions of weapons materials, are exposing the inner workings of biochemical processes important to human health. X-ray diffraction using synchrotron light sources, another physics tool, illuminates proteins to help define their function.

The next step in biological research will depend on another tool made possible by advanced physics research—even more powerful computers than are available today. "Where we're going next," says Bert Weinstein, acting associate director for BBRP, "is to understand the whole system of genes. Not just genes as individual parts but as an integrated, intermeshed set of molecular machines, working together to produce the miracle of life."

—Katie Walter

For more information about Biology and Biotechnology Research Program Directorate:
www-bio.llnl.gov/

For details about the history of biology research at Livermore:
www-bbrp.llnl.gov/50_year_anniversary/



PEREGRINE is an innovative radiation planning technology developed at Livermore. Taken by the staff at the University of California at San Francisco, these images of PEREGRINE measurements demonstrate how effectively PEREGRINE can handle different materials and shapes, including (a) heterogeneous materials such as soft tissue and air in the lung, (b) a steel prosthesis, and (c) a partial transmission block that protects healthy tissue from radiation treatment.

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